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August 1987

DoE 1579-M

CRITICAL ASSESSMENT OF HEALTH ASPECTS OF ALTERNATIVE DISINFECTANTS TO CHLORINE (EHT 9276 SLD)

Final report to the Department of the Environment

DoE Contract Reference: PECD 7/7/199
Contract Duration: June 1986 to June 1987

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On 1 June 1986, the Department of the Environment placed a contract (DoE Contract Reference: PECD 7/7/199) with the Water Research Centre to undertake a critical assessment of the potential health risks associated with use of ozone, chlorine dioxide, chloramine and UV irradiation.

The original contract ended on 31 March 1987 but was extended for a further 3 months, finally ending on 30 June 1987.

This report completes the work undertaken for this contract.
There has been concern over the by-products formed during the use of chlorine as a drinking water disinfectant. The data on the organic and inorganic by-products of alternative disinfectants to chlorine are reviewed and the health implications for consumers discussed.

The alternative disinfectants have been less comprehensively studied than chlorine and there is very little data which relates to the formation of by-products under conditions typical of water treatment. Ozone has been most studied, chlorine dioxide to a lesser extent and chloramine and U.V. hardly at all. However, the data indicate that the organic by-products are, on the whole, likely to be of less concern than those from chlorination.

In general, they appear to produce less mutagenicity than chlorination but clear comparative studies are required to confirm this.

There remain some uncertainties about the possible effects of the inorganic reaction products of chloramine and in particular chlorine dioxide on vulnerable sections of the population.
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1. INTRODUCTION

Chlorine has been used to disinfect drinking water since the beginning of this century. However, with the advent of sensitive methods of analysis it has become evident that this procedure results in the formation of chlorinated organic byproducts, including trihalomethanes (Rook 1974, Bellar et al 1974). Since many of these chlorinated compounds are suspected of posing a risk to health in the long-term, several chemical, epidemiological and toxicological studies have been conducted throughout the world to evaluate these risks.

Many chemicals which have been identified in chlorinated water have been shown to be toxic to man although at much higher concentrations than those normally encountered in drinking water. In addition, concentrated extracts of chlorinated drinking water have been shown to be mutagenic in vitro. Whilst epidemiological studies to date have been inconclusive with regard to relating water chlorination to chronic health effects they have indicated that any risk posed by water chlorination is small. Despite this, there are moves to switch from chlorination to other forms of disinfection which utilise ozone, chloramine, chlorine dioxide or ultraviolet (UV) irradiation. These alternatives have already been used to various extents in different parts of the world (including the UK). However, before any of these alternative disinfectants can be used to replace chlorine it is important that some estimation of the efficiency of disinfection is made together with the determination of any byproducts, organic or inorganic, which may themselves pose a risk to health as great, or greater than, the risk posed by chlorination byproducts.

The objective of this study which began in June 1986 is to critically assess the potential health
risks associated with the use of ozone, chlorine dioxide, chloramine and UV irradiation. This has been done by collating data on the organic and inorganic byproducts associated with these disinfectants. Where byproducts have been identified from the literature search, data on the toxicology of these compounds was also collected and an assessment of the health risks was made.

2. CRITICAL ASSESSMENT OF THE PRODUCTION OF ORGANIC BYPRODUCTS

2.1 Introduction

Under typical water treatment conditions the ratio of disinfectant to total organic carbon lies in the range 1-10 (wt/wt). It should be noted that under these conditions disinfectant is usually added to provide a concentration at the point of application of between 0.1 and 5 mg l\(^{-1}\). Since individual organic compounds are usually present at very low concentrations (1 µg l\(^{-1}\) or less) – except during pollution incidents – the ratio of disinfectant to each individual substrate is very high (∝10).

Many organic compounds are present in water and the usual disinfectants, chlorine, ozone, chlorine dioxide and chloramine, can react with both organic and inorganic substances. In practice such reactions are competitive and thus for any particular substrate only a fraction of the total amount of disinfectant present will be available. Therefore, in the case of reactions of individual substances, an accurate estimation of the effective disinfectant to substrate ratio is difficult to make.

Potentially, pH is a major factor in reactions of disinfectants under aqueous conditions. The range of pH in water disinfection is not large (between pH 5 and pH 9) and only in specific cases will it exert significant effects on the extent rate and byproducts of reaction.
Overall the majority of studies reviewed was not carried out under appropriate conditions of potable water disinfection since they did not address the following:

i) realistic concentrations of individual organic substrates;

ii) modifying reactions of inorganics.

Inorganic anions influence the course and rate of reactions between organic compounds and disinfectants. Bicarbonate anions (HCO$_3^-$) slow ozonation reactions by acting as free radical scavengers and frequently influence the course of reaction by permitting one of the direct reaction mechanisms to dominate. In contrast, bromide (Br$^-$) is rapidly converted to hypobromous acid (HOBr) by chlorine, ozone, chlorine dioxide and, under some circumstances, chloramine. Hypobromous acid then reacts with organic substrates to form brominated products.

The remainder of this review will be confined to a discussion of the chemistry of byproducts of ozone, chlorine dioxide, chloramine and ultraviolet irradiation. No attempt will be made to discuss the byproducts of chlorination.

2.2 Ozonation

The products of aqueous chlorination and ozonation have been reviewed as part of a DoE-funded project entitled, "Effects of Disinfection on Organic Substances in Water" (Fielding et al 1987b). This review discussed the products formed by the action of ozone (and chlorine) on a range of substrates including humic and fulvic acids, aromatic compounds such as phenol and aniline, polyaromatic hydrocarbons, fatty acids, alcohols, heterocyclic compounds, amino acids, surfactants and pesticides.
From this review it is evident that ozone can react with all of the above compound types. However, it was also evident that the experimental conditions used to study the formation of byproducts, during ozonation, exerted a major influence on the reactions that occurred and hence on the byproducts formed. A summary of the experimental conditions used in the ozonation studies reviewed is presented in Table 1.

Ozone may react with organic compounds in the following ways:

i) dipolar cycloaddition
ii) electrophilic attack
iii) nucleophilic attack
iv) radical reactions
   (indirect reactions)

Examples of these are given in Figures 1-4 respectively. The direct reactions (i) (iii) tend to be specific and occur at relatively slow reaction rates. Radical reactions occur as a result of the decomposition of ozone in water, predominantly to produce hydroxyl (OH⁻) radicals. Reactions of hydroxyl and other radicals occur at relatively fast rates and are less specific than direct reactions.

Recent studies that have examined the effect of ozonation on organics in water under realistic conditions have not usually addressed the formation of products from specific organic substrates (Lykins et al 1986, Rachwal et al 1987, Huck et al 1987). However, Lykins et al (1986) and Rachwal et al (1987) have reported that after ozonation (but before slow-sand filtration) the concentrations of aliphatic aldehydes, ketones and carboxylic acids were increased. Furthermore, Lykins et al (1986) reported that ozonation reduced the concentration of atrazine, alachlor, total alkyl benzenes, total
<table>
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<tr>
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<th>Substrate concn</th>
<th>Ozone concn</th>
<th>pH</th>
<th>Contact time</th>
<th>Temp °C</th>
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<td><strong>HUMIC SUBSTANCES</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Lawrence et al (1980)</td>
<td>Fulvic acid</td>
<td>500 mg l⁻¹</td>
<td>(0.2 g h⁻¹: 0.3-1.0 mg O₃ per mg fulvic)</td>
<td>-</td>
<td>15-60 min</td>
<td>Ambient</td>
</tr>
<tr>
<td>Gilbert (1983)</td>
<td>Humic acid</td>
<td>67 mg l⁻¹</td>
<td>-</td>
<td>3.7</td>
<td>70 min</td>
<td>-</td>
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<tr>
<td>Rechow and Singer (1984)*</td>
<td>Fulvic acid</td>
<td>ca 4.0 mg l⁻¹ TOC</td>
<td>9 mg l⁻¹</td>
<td>7.0</td>
<td>0-60 min</td>
<td>21</td>
</tr>
<tr>
<td>Legubé et al (1985)*</td>
<td>Fulvic acid</td>
<td>5 and 10 mg l⁻¹</td>
<td>0.3-4 mg l⁻¹</td>
<td>7.5 and 7.0</td>
<td>120 min or more</td>
<td>20</td>
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<tr>
<td>Watts (1985)</td>
<td>Humic acid</td>
<td>ca 35 and 200 mg l⁻¹</td>
<td>ca 125 mg l⁻¹</td>
<td>6 and 7</td>
<td>60 min</td>
<td>Ambient</td>
</tr>
<tr>
<td>Fulvic acid</td>
<td>ca 25 and 200 mg l⁻¹</td>
<td>ca 125 mg l⁻¹</td>
<td>6 and 7</td>
<td>30 min and 60 min</td>
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<tr>
<td>Natural water</td>
<td>11 and 13 mg l⁻¹</td>
<td>3.5-4 mg O₃ per mg Org C</td>
<td>6 and 7</td>
<td>20 min</td>
<td>Ambient</td>
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<tr>
<td>Doré et al (1987)</td>
<td>Fulvic acid</td>
<td>5 mg l⁻¹</td>
<td>1 mg mg⁻¹ (COD)</td>
<td>7.5</td>
<td>120 min</td>
<td>20</td>
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<td><strong>AROMATIC COMPOUNDS (excluding hydrocarbons)</strong></td>
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</tr>
<tr>
<td>Doré et al (1980a)</td>
<td>Phenol</td>
<td>18.8 mg l⁻¹ (2 x 10⁻⁴ M)</td>
<td>* 5 (200 mg h⁻¹)</td>
<td>-</td>
<td>0-40 min</td>
<td>-</td>
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<td></td>
<td>Catechol</td>
<td>22 mg l⁻¹ (2 x 10⁻⁴ M)</td>
<td>* 5 (200 mg h⁻¹)</td>
<td>-</td>
<td>0-40 min</td>
<td>-</td>
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<tr>
<td></td>
<td>Phenol</td>
<td>0.9 mg l⁻¹ (10⁻⁵ M)</td>
<td>* ca 70</td>
<td>-</td>
<td>0-10 min</td>
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Table 1 (continued)

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<th>Temp °C</th>
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<tr>
<td>Brunet et al (1980)</td>
<td>Phenols</td>
<td>2 x 10^-4 M</td>
<td>ca 150 mg h^-1</td>
<td>3.0-11.2</td>
<td>0-60 min</td>
<td>Ambient</td>
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<td>Chrostovski et al (1983)</td>
<td>Phenols</td>
<td>10-50 mg l^-1</td>
<td>1.5 mg min^-1</td>
<td>-</td>
<td>0-180 min</td>
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<td>Gilbert (1980)</td>
<td>trans,trans-muconic acid</td>
<td>142 mg l^-1</td>
<td>10 mg min^-1</td>
<td>3</td>
<td>0-180 min</td>
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<td>Decoret et al (1984)</td>
<td>Phenol</td>
<td>235-470 µg l^-1</td>
<td>2 x 10^-5 M l^-1</td>
<td>4 and 6</td>
<td>0-90 min</td>
<td>1 and ambient</td>
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<td>Benzaldehyde</td>
<td>265-530 µg l^-1</td>
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<td>4 and 6</td>
<td>0-90 min</td>
<td>1 and ambient</td>
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<td>Acetophenone</td>
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<td>4 and 6</td>
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<td>El Dine et al (1984)</td>
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<td>Caprio et al (1984)</td>
<td>Nitrobenzene</td>
<td>307 mg l^-1</td>
<td>max 20 x 10^-3 M l^-1</td>
<td>0-150 min</td>
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<td>Legubé et al (1981a)</td>
<td>Phenol</td>
<td>38-2820 mg l^-1</td>
<td>-</td>
<td>Natural</td>
<td>-</td>
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<tr>
<td></td>
<td>Aniline</td>
<td>930-27 000 mg l^-1</td>
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<td></td>
<td>Phenoxyacetic acid</td>
<td>760 mg l^-1</td>
<td>5 x 10^-3 M</td>
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<tr>
<td>p-Cresol</td>
<td>1080 mg l⁻¹ (10 x 10⁻³ M)</td>
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<tr>
<td>2-Methyl-4-</td>
<td>500 mg l⁻¹ (2.5 x 10⁻³ M)</td>
<td>-</td>
<td>Natural</td>
<td>-</td>
<td>Ambient</td>
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<tr>
<td>chlorophenoxy-acetic acid (MCPA)</td>
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<tr>
<td>Legubé et al</td>
<td>Phenol 18.8 mg l⁻¹ (2 x 10⁻⁴ M)</td>
<td>56.4 mg l⁻¹ (3.6 l h⁻¹)</td>
<td>-</td>
<td>0-120 min</td>
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<tr>
<td>(1981b)</td>
<td>Hydroquinone 22 mg l⁻¹ (2 x 10⁻⁴ M)</td>
<td>62.7 mg l⁻¹ (3.7 l h⁻¹)</td>
<td>-</td>
<td>0-120 min</td>
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<td></td>
<td>Aniline 18.6 mg l⁻¹ (2 x 10⁻⁴ M)</td>
<td>49.4 mg l⁻¹ (3.5 l h⁻¹)</td>
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<td>2,4-Dichlorophenoxyacetic acid 22.1 mg l⁻¹ (1 x 10⁻⁴ M)</td>
<td>61.8 mg l⁻¹ (3.4 l h⁻¹)</td>
<td>-</td>
<td>0-120 min</td>
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<td>MCPA 30 mg l⁻¹ (1.5 x 10⁻⁴ M)</td>
<td>61.8 mg l⁻¹ (3.4 l h⁻¹)</td>
<td>-</td>
<td>0-120 min</td>
<td>Ambient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legubé et al</td>
<td>Styrene 11.4 mg l⁻¹ (1.1 x 10⁻⁴ M)</td>
<td>197 mg l⁻¹ (12.8 l h⁻¹)</td>
<td>5.0</td>
<td>ca 10 min</td>
<td>-</td>
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<tr>
<td>(1983)</td>
<td>Benzaldehyde 22.4 mg l⁻¹ (2.11 x 10⁻⁴ M)</td>
<td>238 mg l⁻¹ (12 l h⁻¹)</td>
<td>4.2</td>
<td>60 min</td>
<td>-</td>
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<tr>
<td></td>
<td>Methylbenzene 32.4 mg l⁻¹ (2.53 x 10⁻⁴ M)</td>
<td>212 mg l⁻¹ (12.8 l h⁻¹)</td>
<td>5.9</td>
<td>ca 30 min</td>
<td>-</td>
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<td>Diethylphthalate 35.5 mg l⁻¹ (1.6 x 10⁻⁴ M)</td>
<td>251 mg l⁻¹ (12 l h⁻¹)</td>
<td>6.3</td>
<td>150 min</td>
<td>-</td>
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<td>Reference</td>
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<td>Substrate concn</td>
<td>Ozone concn</td>
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<td></td>
<td>Ethylbenzene</td>
<td>20.5 mg l⁻¹</td>
<td>197 mg h⁻¹</td>
<td>6.3</td>
<td>120 min</td>
<td>-</td>
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<td></td>
<td></td>
<td>(1.93 x 10⁻⁴ M)</td>
<td>(13.3 l h⁻¹)</td>
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<td></td>
<td>Chlorobenzene</td>
<td>18.5 mg l⁻¹</td>
<td>142 mg h⁻¹</td>
<td>6.3</td>
<td>150 min</td>
<td>-</td>
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<td></td>
<td></td>
<td>(1.65 x 10⁻⁴ M)</td>
<td>(13.3 l h⁻¹)</td>
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<tr>
<td>Gilbert and Zinecker (1980)</td>
<td>4-Aminobenzene sulphonic acid</td>
<td>173 mg l⁻¹</td>
<td>10 mg min⁻¹</td>
<td></td>
<td>15-20 min</td>
<td>-</td>
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<td></td>
<td></td>
<td>(1 x 10⁻³ M)</td>
<td>(24 l h⁻¹)</td>
<td></td>
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<td>4-Aminobenzoic acid</td>
<td>137 mg l⁻¹</td>
<td>10 mg min⁻¹</td>
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<td>15-20 min</td>
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<td>(24 l h⁻¹)</td>
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<td></td>
<td>4-Amino-2-nitrophenol</td>
<td>154 mg l⁻¹</td>
<td>10 mg min⁻¹</td>
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<tr>
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<td>(1 x 10⁻³ M)</td>
<td>(24 l h⁻¹)</td>
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<td></td>
<td>4-Chloro-aniline</td>
<td>127 mg l⁻¹</td>
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<td>(1 x 10⁻³ M)</td>
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<td></td>
<td>2-Aminophenol</td>
<td>109 mg l⁻¹</td>
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<td>(1 x 10⁻³ M)</td>
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<td></td>
<td>4-Amino-5-hydroxy naphthalene-sulphonic acid</td>
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<td>15-20 min</td>
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<tr>
<td></td>
<td>mono-sodium salt</td>
<td>(1 x 10⁻³ M)</td>
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<tr>
<td>Gilbert (1983)</td>
<td>Aniline</td>
<td>93 mg l⁻¹</td>
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<td>15 min</td>
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<td>(1 x 10⁻³ M)</td>
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<td>Benzenesulphonic acid</td>
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<td>60 min</td>
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<td>(1 x 10⁻³ M)</td>
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<td></td>
<td>4-Chloro-o-cresol</td>
<td>142 mg l⁻¹</td>
<td>10 mg min⁻¹</td>
<td>5.5</td>
<td>40 min</td>
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<td>(1 x 10⁻³ M)</td>
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<td>Substrate concn</td>
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<td>Contact time</td>
<td>Temp °C</td>
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<tr>
<td>2-Nitro-p-cresol</td>
<td></td>
<td>153 mg l(^{-1}) (1 x 10(^{-3}) M)</td>
<td>10 mg min(^{-1})</td>
<td>5.9</td>
<td>50 min</td>
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**HYDROCARBONS**

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<th>Contact time</th>
<th>Temp °C</th>
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<tbody>
<tr>
<td>Butkovic et al (1983)</td>
<td>Pyrene</td>
<td>50.5 µg l(^{-1}) (2.5 x 10(^{-7}) M)</td>
<td>10(^{-4}) M</td>
<td>7</td>
<td>less than 5 mins</td>
<td>25</td>
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<td>Phenanthrene</td>
<td>279 µg l(^{-1}) (1.57 x 10(^{-6}) M)</td>
<td>10(^{-4}) M</td>
<td>7</td>
<td>less than 5 mins</td>
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<td>Benzo[a]pyrene</td>
<td>176 µg l(^{-1}) (7 x 10(^{-7}) M)</td>
<td>10(^{-4}) M</td>
<td>7</td>
<td>less than 5 mins</td>
<td>25</td>
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<tr>
<td>Helleur et al (1979)</td>
<td>Fluorene</td>
<td>1.2 mg l(^{-1}) (7.2 x 10(^{-6}) M)</td>
<td>* 2-4</td>
<td>5-8</td>
<td>0-20 mins</td>
<td>-</td>
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<tr>
<td></td>
<td>Fluoren-9-one</td>
<td>6.0 mg l(^{-1}) (33.3 x 10(^{-6}) M)</td>
<td>* 2-4</td>
<td>5-8</td>
<td>0-20 mins</td>
<td>-</td>
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<tr>
<td>Fielding et al (1987)</td>
<td>Toluene</td>
<td>10 µg l(^{-1}) (1.06 x 10(^{-7}) M)</td>
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</tr>
<tr>
<td></td>
<td>o, m, p-Xylene</td>
<td>10 µg l(^{-1}) (9.4 x 10(^{-8}) M)</td>
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<tr>
<td></td>
<td>Naphthalene</td>
<td>10 µg and 1 mg l(^{-1}) (7.8 x 10(^{-8}) and 7.8 x 10(^{-6}) M)</td>
<td>5 mg l(^{-1})</td>
<td>Ambient</td>
<td>30 mins</td>
<td>10</td>
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<td></td>
<td>Fluorene</td>
<td>10 µg and 1 mg l(^{-1}) (6.0 x 10(^{-8}) and 6.0 x 10(^{-6}) M)</td>
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<td>Contact time</td>
<td>Temp °C</td>
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<tr>
<td>Phenanthrene</td>
<td>10 µg and 1 mg l⁻¹ (5.6 x 10⁻⁸ and 5.6 x 10⁻⁶ M)</td>
<td>5 mg l⁻¹</td>
<td>Ambient</td>
<td>30 mins</td>
<td>10</td>
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<td>Fluoranthrene</td>
<td>10 and 200 µg l⁻¹ (5 x 10⁻⁸ and 1 x 10⁻⁶ M)</td>
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<tr>
<td>Pyrene</td>
<td>10 and 200 µg l⁻¹ (5 x 10⁻⁸ and 1 x 10⁻⁶ M)</td>
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NITROGEN AND/OR SULPHUR-CONTAINING COMPOUNDS

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<th>pH</th>
<th>Contact time</th>
<th>Temp °C</th>
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<tbody>
<tr>
<td>Faujour et al (1980)</td>
<td>Ethylamine</td>
<td>1.4 g l⁻¹ (31 x 10⁻³ M)</td>
<td>3.6 g h⁻¹</td>
<td>Basic</td>
<td>0-50 min</td>
<td>-</td>
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<tr>
<td></td>
<td>Dimethylamine</td>
<td>1.4 g l⁻¹ (31 x 10⁻³ M)</td>
<td>3.6 g h⁻¹</td>
<td>Basic</td>
<td>0-50 min</td>
<td>-</td>
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<td></td>
<td>Trimethylamine</td>
<td>1.4 g l⁻¹ (24 x 10⁻³ M)</td>
<td>3.6 g h⁻¹</td>
<td>Basic</td>
<td>0-50 min</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ethylmercaptan</td>
<td>50 mg l⁻¹ (0.8 x 10⁻³ M)</td>
<td>3.6 g h⁻¹</td>
<td></td>
<td>0-120 min</td>
<td>-</td>
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<tr>
<td></td>
<td>n-Butylmercaptan</td>
<td>50 mg l⁻¹ (0.56 x 10⁻³ M)</td>
<td>3.6 g h⁻¹</td>
<td></td>
<td>0-120 min</td>
<td>-</td>
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<tr>
<td></td>
<td>n-Propylmercaptan</td>
<td>50 mg l⁻¹ (0.66 x 10⁻³ M)</td>
<td>3.6 g h⁻¹</td>
<td></td>
<td>0-120 min</td>
<td>-</td>
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<tr>
<td>Elmaghri-Tabib et al (1982)</td>
<td>Methylamine</td>
<td>70 g l⁻¹ (2.26 M)</td>
<td>2.6 g h⁻¹</td>
<td>Basic</td>
<td>0-40 min</td>
<td>-</td>
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<td>Reference</td>
<td>Substrate</td>
<td>Substrate concn</td>
<td>Ozone concn</td>
<td>pH</td>
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<td>Temp °C</td>
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<tr>
<td>Ethylamine</td>
<td>70 g l⁻¹ and 14 mg l⁻¹ (1.56 M and 0.3 x 10⁻³ M)</td>
<td>2.6 g h⁻¹</td>
<td>Basic</td>
<td>0-40 min</td>
<td>-</td>
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<tr>
<td>Propylamine</td>
<td>70 g l⁻¹ (1.2 M)</td>
<td>2.6 g h⁻¹</td>
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<td>0-40 min</td>
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<td>Cyclohexylamine</td>
<td>70 g l⁻¹ (0.71 M)</td>
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<td>Basic</td>
<td>0-40 min</td>
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<td>Dimethylamine</td>
<td>70 g l⁻¹ (1.56 M)</td>
<td>2.6 g h⁻¹</td>
<td>Basic</td>
<td>0-40 min</td>
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<tr>
<td>N-Methylbutylamine</td>
<td>70 g l⁻¹ (0.8 M)</td>
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<td>Trimethylamine</td>
<td>70 g l⁻¹ and 64 mg l⁻¹ (1.2 M and 1.1 x 10⁻³ M)</td>
<td>2.6 g h⁻¹</td>
<td>Basic</td>
<td>0-40 min</td>
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<td>Triethylamine</td>
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<td>Basic</td>
<td>0-40 min</td>
<td>-</td>
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<tr>
<td>Duguet et al (1980)*</td>
<td>Glycine</td>
<td>150 mg l⁻¹ (2 x 10⁻³ M)</td>
<td>ca 30 mg min⁻¹</td>
<td>6.1 – 10.7</td>
<td>0-180 min</td>
<td>20</td>
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<tr>
<td>Yamada and Somiya (1980)</td>
<td>Glycine</td>
<td>100 mg l⁻¹ (1.3 x 10⁻³ M)</td>
<td>14-140 mg min⁻¹</td>
<td>4, 7 and 10</td>
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<td>Leucine</td>
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<td>Phenol</td>
<td>100 mg l⁻¹</td>
<td>14-140 mg min⁻¹</td>
<td>4, 7 and 10</td>
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<td>(1.06 x 10⁻³ M)</td>
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<tr>
<td>Glucose</td>
<td>100 mg l⁻¹</td>
<td>14-140 mg min⁻¹</td>
<td>4, 7 and 10</td>
<td>20-80 min</td>
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<td>(0.55 x 10⁻³ M)</td>
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**OXYGEN-CONTAINING COMPOUNDS**

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<tr>
<td>Lutton et al</td>
<td>EDTA</td>
<td>29.2-146 g l⁻¹</td>
<td>54 mg min⁻¹</td>
<td>-</td>
<td>6 h</td>
<td>25 and 60</td>
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<tr>
<td></td>
<td></td>
<td>(0.1 - 0.5 M)</td>
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<tr>
<td>Chang and Chian</td>
<td>Methanol</td>
<td>15-35 mg l⁻¹</td>
<td>13 mg l⁻¹</td>
<td>9</td>
<td>-</td>
<td>21-25</td>
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<tr>
<td>(1981)</td>
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<td>(0.47-1.1 x 10⁻³ M)</td>
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<tr>
<td>Fielding et al</td>
<td>Decanoic acid</td>
<td>10 µg l⁻¹</td>
<td>5 mg l⁻¹</td>
<td>Ambient</td>
<td>30 min</td>
<td>10</td>
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<tr>
<td>(1987)</td>
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<td>(5.8 x 10⁻⁴ M)</td>
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<td></td>
<td>Dodecanoic acid</td>
<td>10 and 500 µg l⁻¹</td>
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<td>(5 x 10⁻⁸ and 2.5 x 10⁻⁶ M)</td>
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<td></td>
<td>Tetradecanoic acid</td>
<td>10 µg l⁻¹</td>
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<td>(4.4 x 10⁻⁴ M)</td>
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<td>Hexadecanoic acid</td>
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<td>(3.9 x 10⁻⁴ M)</td>
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<td>Hexadec-9-enoic acid</td>
<td>10 and 500 µg l⁻¹</td>
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<td></td>
<td>(3.9 x 10⁻⁴ and 2.0 x 10⁻⁶ M)</td>
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<td>Heptadecanoic acid</td>
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<td>(3.7 x 10⁻⁴ M)</td>
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Table 1 (continued)

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<th>pH</th>
<th>Contact time</th>
<th>Temp °C</th>
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<tr>
<td></td>
<td>Octadecanoic acid</td>
<td>10 μg l(^{-1}) (\text{~}(3.5 \times 10^{-8} \text{ M}))</td>
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<td></td>
<td>Octadec-9,12-dienoic acid</td>
<td>10 and 500 μg l(^{-1}) (\text{~}(3.6 \times 10^{-8} \text{ and } 1.8 \times 10^{-6} \text{ M}))</td>
<td>5 mg l(^{-1}) Ambient</td>
<td>30 min</td>
<td>10</td>
<td></td>
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</tbody>
</table>

**SURFACTANTS**

| Joy et al (1980) | p-Toluene Sulphonate acid | 172 mg l\(^{-1}\) \(\text{~}(1 \times 10^{-3} \text{ M})\) | 24 and 11 mg min\(^{-1}\) | 3 and 12 | 0-180 min | 25      |

**PESTICIDES**

<table>
<thead>
<tr>
<th>Legubé et al (1981)</th>
<th>2,4-D and MCPA</th>
<th>see aromatics section</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doré et al (1980)</td>
<td>2,4-D</td>
<td>22 mg l(^{-1}) (\text{~}(1.0 \times 10^{-4} \text{ M}))</td>
</tr>
<tr>
<td></td>
<td>MCPA</td>
<td>22 mg l(^{-1}) (\text{~}(1.5 \times 10^{-4} \text{ M}))</td>
</tr>
<tr>
<td></td>
<td>Phenoxyacetic acid</td>
<td>30 mg l(^{-1}) (\text{~}(1.5 \times 10^{-4} \text{ M}))</td>
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</tbody>
</table>

- = not specified
* = ozone to substrate ratio (moles per mole)
+ = the effects of the presence of some inorganics were studied
Figure 1. Reaction scheme for 1,3-dipolar cyclo-addition. (Döre, 1985).
Figure 2. Reaction scheme for electrophilic attack. (Döre, 1985).
Figure 3. Reaction scheme for nucleophilic attack. (Dóre, 1985).
Figure 4. Indirect reaction (action of OH on phenol).

\[
\begin{align*}
\text{OH} & \quad \xrightarrow{\text{OH}^+} \quad \text{phenol} \\
\text{pH} & > 7 \\
\end{align*}
\]
alkanes, total phthalates and total chlorobenzenes relative to unozonated water but did not specify the products which were formed during ozonation.

In contrast, Fielding et al (1907a) have shown that unsaturated hydrocarbons and unsaturated fatty acids readily react with ozone under conditions typical of water treatment. Furthermore, these authors have shown that the products of these reactions include aldehydes, fatty, acids, aldehydo-acids and di-acids.

Other studies which examined the formation of byproducts were conducted under conditions atypical of water treatment (Fielding et al 1987b). However, these studies were useful since they permitted the reactivities of likely water contaminants to be assessed as well as allowing the products of many of the reactions to be identified. Generally, it was found that substituent groups influenced both the rate of reaction of the compounds with ozone and the site of attack. Electron-donating substituents resulted in rapid reaction to form ortho and para-substituted products whereas, electron-withdrawing groups led to slow reactions forming meta-substituted products. Ozone reacted with aromatic compounds to form hydroxylations, oxidation, condensation and ring-cleavage products. Condensation products were favoured by the unrealistically high substrate concentrations used in some studies. Consequently caution is needed when relating the results to drinking water treatment practice.

Amino acids reacted with ozone to form hydroxylamines, N-oxides, oximes, amides, acids and aldehydes. Only a little work on the ozonation of pesticides has been reported. Pesticides containing phosphorus-sulphur bonds were shown to be susceptible to oxidation to result in the
formation of phosphorus-oxygen bonds. Some of the data reported for the ozonation of pesticides have significant inconsistencies and need to be verified. For some compound types which frequently occur in source waters used for potable water abstraction little information on ozonation byproducts exists, eg surfactants.

In conclusion, it is evident that there are areas where insufficient knowledge exists to allow prediction of the product formed during disinfection of water with ozone. Further work is needed to determine the effect of ozonation on many types of organics which commonly occur in source waters.

2.3 Chlorine dioxide

Little is known about the reaction mechanisms by which chlorine dioxide reacts with organics, under aqueous conditions, except that the mechanisms are very complex and almost certainly involve one electron-type oxidation (EPA 1979, Stevens 1982, Aieta and Berg 1986), ie free radicals, eg $\text{ClO}_2 + e^- \rightarrow \text{ClO}_2^-$.

Recent studies (Lykins et al. 1986, Huck et al. 1987) have examined the effects of chlorine dioxide disinfection on natural waters using typical treatment conditions. Lykins et al. (1986) reported that chlorine dioxide did not appear to affect the concentrations of atrazine, alachlor, total alkanes, total phthalates, total chlorobenzenes, total nitrobenzenes or total alkyl aldehydes with respect to undisinfected water. Huck et al. (1987) observed that some changes in the composition of organics analysable by gas chromatography (GC) occurred after treatment with chlorine dioxide. However, these authors did not identify any of the products.
Other studies have shown that chlorine dioxide, reacts with a wide range of organic compounds (albeit under conditions atypical of water treatment) including amines, aldehydes, ketones, phenols, alkenes, aromatic hydrocarbons, nitrogen heterocyclic compounds, unsaturated fatty acids, amino acids and pesticides. Reaction of these compound types with ClO₂ generally resulted in the oxidation of the substrate (Ben-Amor et al 1984). In some instances chlorinated products were observed. It has been proposed that the chlorinated products resulted from the byproduct formation of hypochlorous acid during the oxidation of some substrates, notably phenol. Other possible explanations include the formation of chlorine from the subsequent reaction of chlorite (ClO₂⁻) with the organic substrate (EPA 1979, Simmon et al 1979, Lindgren and Nilsson 1973). Alternatively it is possible that the ClO₂ was contaminated with chlorine (Aieta and Berg 1985, Ben-Amor et al 1984, Masschelein 1967).

Since chlorine dioxide appears to act as a one electron oxidising agent many of the reactions can be expected to proceed via free radical mechanisms. Evidence to support this was given by Chen et al (1982) who demonstrated that compounds bearing a benzylic site such as ethylbenzene, indan, tetralin, diphenylmethane and fluorene readily oxidise at pH 3.5 at the benzylic position to yield mainly alcohols and ketones (Figure 5) although some chlorinated products were also detected when granular activated carbon was present in the reaction mixture.

Quinones, hydroquinones, chloroquinones, formic, oxalic and maleic acids have been observed after the oxidation of phenol with ClO₂ (Wajon et al 1982, Simmon et al 1979, Ben-Amor et al 1984). Wajon et al (1982) studied the kinetics of
oxidation of phenol and postulated a mechanism to account for the formation of chlorinated products. Simmon et al (1979) surveyed the reactivities of a range of organic compounds including humic, carboxylic and amino acids, heterocyclic amines and aldrin. All of these compounds reacted with ClO$_2$ but only a few gave identifiable products (Table 2).

Table 2. Products formed by reaction of ClO$_2$ with selected substrates (Simmons et al 1979)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>phenol</td>
<td>hydroquinone, chlorohydroquinone, mono-, di and trichlorophenols, fumaric acid</td>
</tr>
<tr>
<td>hydroquinone</td>
<td>chlorohydroquinone</td>
</tr>
<tr>
<td>benzene</td>
<td>hydroquinone, catechol</td>
</tr>
<tr>
<td>1,1,-diphenylhydrazine</td>
<td>diphenylamine, diphenyl-N-hydroxy(-amine), dihydroxydiphenylamine, mono- and dichlorodiphenylamine</td>
</tr>
<tr>
<td>diethylamine</td>
<td>acetaldehyde</td>
</tr>
<tr>
<td>phenylalanine</td>
<td>benzoic acid, phenyllactic acid</td>
</tr>
</tbody>
</table>

Taymaz et al (1979) have also investigated the reactivity of phenylalanine as well as glycine with ClO$_2$. These authors report that phenylalanine gives rise to phenylacetic acid, phenylacetaldelyde, benzoic acid and benzaldehyde, whereas glycine is oxidised to formaldehyde and carbon dioxide.

The effect of ClO$_2$ on a range of nitrogen heterocycles was studied by Lin and Carlson (1984) and is summarised in Table 3. Most of the substrates were consumed by ClO$_2$ to yield oxidised and, in some instances, ring-ruptured products.
Table 3. products formed from reaction of ClO₂ with heterocycles (Lin and Carlson 1984)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>indole</td>
<td>oxindole, isatin</td>
</tr>
<tr>
<td>3-methylindole</td>
<td>3-methyloxindole, o-formamidoacetophenone</td>
</tr>
<tr>
<td>2-phenylindole</td>
<td>2-phenyl-3-oxoindole, 2-hydroxy-2-phenyl-3-oxodihydroindole, 2-benzamidobenzoic acid</td>
</tr>
<tr>
<td>N-phenylpyrole</td>
<td>mono- and dioxophenylpyroles, hydroxy mono- and dioxophenylpyroles</td>
</tr>
<tr>
<td>carbazole</td>
<td>1-hydroxy-3-oxocarbazole</td>
</tr>
<tr>
<td>phenanthridine</td>
<td>6-oxophenanthridine</td>
</tr>
<tr>
<td>acridine</td>
<td>9-oxoacridine</td>
</tr>
</tbody>
</table>

Other studies have shown that oxidation of organic substrates with ClO₂ frequently gives rise to products similar to those obtained by ozonation.

For example the reaction of secondary and tertiary amines to form aldehydes (Ben-Amor et al 1984) or the conversion of parathion to paraoxon and p-nitrophenol (Rav-Acha 1984 cf Laplanche et al 1984).

The effect of ClO₂ on the proteins and nucleic acids of F2 virus were determined by Noss et al (1986) and Hauchman et al (1986) who showed that chemical groups within these polymeric molecules could react with ClO₂. However, no products were identified.

Generally, trihalomethanes are not formed when ClO₂ reacts with organic material. Additionally using ClO₂ in conjunction with aqueous chlorine results in less THM being formed than when chlorine is used solely (Aieta and Berg 1986, Suh and Abdel-Rahman 1985, Rav-Acha 1984, Rav-Acha et al 1983, Stevens 1982, Noack and Doerr 1980).
It should be noted that most of the studies discussed above were conducted under conditions quite atypical of water treatment practice in terms of disinfectant and substrate concentrations, pH and contact time. Additionally, the chemical composition of the disinfectant, i.e., degree of contamination (if any) with other oxidants was generally not determined. Therefore it appears that there is a requirement to study the effects of ClO₂ on organics in water taking recognition of the conditions likely to occur during water treatment.

2.4 Chloramines

Chloramination was originally used to avoid the occurrence of chlorophenolic and iodoform tastes that occasionally occur during drinking water chlorination. Subsequently, chloramines were found to more stable than free chlorine yet were capable of preventing microbial regrowth in the distribution system. Currently, there is renewed interest in using chloramines for disinfection since chloramination does not produce appreciable quantities of THMs (Robertson and Oda 1983, Mitcham et al 1983).

In aqueous solution, chlorine reacts with ammonia to form chloramines by the following series of reactions (Jolley 1973, Wolfe et al 1984, White 1986).

\[ \text{Cl}_2 + \text{H}_2\text{O} \rightarrow \text{HOCl} + \text{HCl} \]

\[ \text{HOCl} + \text{NH}_3 \rightarrow \text{NH}_2\text{Cl} + \text{H}_2\text{O} \]

\[ \text{HOCl} + \text{NH}_2\text{Cl} \rightarrow \text{NHCl}_2 + \text{H}_2\text{O} \]

\[ \text{HOCl} + \text{NHCl}_2 \rightarrow \text{NHCl}_3 + \text{H}_2\text{O} \]

The reaction products are dependent on the pH and relative concentrations of the reactants. Jolley
(1973) reports that the formation of NCl$_3$ occurs at pH values below 4 and therefore it is unlikely to occur during water treatment. However, Palin (1950) has reported the occurrence of nitrogen trichloride at neutral pH in the presence of excess free chlorine.

The redox potentials ($E^0$) of monochloramine under acidic and basic conditions have been calculated to be 1.45 V and 0.75 V respectively. From this it is evident that under acidic conditions monochloramine is a stronger oxidant than bromine ($E^0 = 1.09$ V) whilst under basic conditions it is a weaker oxidant than hypochlorite ($E^0 = 0.89$ V).

In contrast, relatively little is known about dichloramine, but it is known that it can oxidise iodide to iodine ($2e^-I_2 \rightarrow 2I^-; E^0 0.54$ V).

As with the other disinfectants, few studies of byproduct formation have been conducted under realistic water treatment conditions. Lykins et al (1986) reported that no significant change in concentration was observed for atrazine, alachlor, total alkanes, total phthalates, total chlorobenzenes, total nitrobenzenes or total alkylaldehydes after disinfection with chloramine. Huck et al (1987) observed that chloramination produced little change in the organics found in raw water. In contrast, Arbor et al (1985) reported that use of chloramination resulted in the formation of various quantities of THMs, TOX, organic chloramines and dichloroacetonitrile depending on when and how chloramination was applied during water treatment.

Other studies which examined the formation of byproducts from specific precursors were generally conducted under conditions removed from normal water treatment practice. Monochloramine has been
shown to react with primary amines (including amino acids) and secondary aliphatic amines to form the corresponding organic chloramines. Additionally, it has been shown that it can react with tertiary aliphatic amines to form aldehydes and dialkylchloramines (EPA 1979, Issac and Morris 1983, 1985). Monochloramine has been shown to react with thiols (including the amino acid, cysteine) to form disulphides (eg cystine) or sulphenamides.

\[
R{-}SH + Cl{-}NH_2 \rightarrow R{-}S{-}S{-}R + R{-}S{-}NH_2
\]

thiol \hspace{1cm} \text{disulphide} \hspace{1cm} \text{sulphenamide}

Other studies have shown that monochloramine reacts with fulvic acid (Jensen \textit{et al} 1985). Although no products were identified in this study, the authors observed that non-purgeable, total organic halide was formed and this material was different from that formed by chlorination of fulvic acid. The effect of chloramination on phenolic acids has been studied by Carlson and Lin (1985). These authors demonstrated that with high concentrations (0.1–0.3 mM) prolonged contact times (24 h) monochloramine reacts with 4-hydroxybenzoic, 2,4-dihydroxybenzoic, 2,4,6-trihydroxybenzoic and 4-hydroxy-3-methoxybenzoic acids to yield mono- and dichloro products plus chlorinated decarboxylation products, ie mono-, di- and trichlorophenols. With 4-hydroxy-3-methoxycinnamic acid, these authors reported that chloramination yielded both the chlorinated and the decarboxylated products (Figure 6).

The same authors (Lin and Carlson 1984) showed that monochloramine did not react with a range of aromatic heterocyclic compounds (see Table 3) under similar conditions.
Aldehydes and ketones have been shown to react with monochloramine under aqueous conditions to form chlorimines which then readily eliminate hydrogen chloride to yield nitriles (Hauser and Hauser 1930). In the presence of sodium hydroxide, chloramination of ortho-hydroxyaromatic aldehydes results in the formation of ortho-hydroxyaromatic amides (March 1985).

Kanno et al (1982a, 1982b) found that chloramination of a range of aromatic hydrocarbons and their derivatives; benzene, toluene, xylene, naphthalene, phenols, aniline, naphthol and naphthalamines resulted in the production of cyanogen chloride. (It must be stressed that the studies by Kanno et al (1982a, 1982b) and Hauser and Hauser (1983) were conducted under conditions of very high concentration of substrates and oxidants.)

Lin et al (1984) also showed that naphthalene was consumed by monochloramine but in the presence of bromide, at pH 4.5 to yield naphthaquinone and bromonaphthaquinone as well as mono- and dibrominated naphthalene. In contrast biphenyl did not react with monochloramine in either the presence or absence of bromide nor did naphthalene react with monochloramine at pH 7.5 (Figure 7).

It is evident that from the data presented there is a requirement for further work to be done to determine the reactivities of organics likely to occur in surface waters with chloramines and to identify the products of these reactions.

2.5 Ultraviolet irradiation

Ultraviolet (UV) irradiation has been used to a small extent for the disinfection of drinking water since the First World War (Groocock 1984). Subsequently it was observed that the bactericidal
action of specific wavelengths of UV light were related to the UV absorption maxima of nucleic acids (Grocock 1984, Meschner 1987). The UV emission spectrum maximum of low-pressure mercury vapour lamps occurs at 254 nm, very close to the absorption maximum of DNA. Consequently, low pressure mercury vapour lamps have been used for disinfection of high quality waters (i.e., waters with low turbidity) particularly in rural areas or where small volumes are processed.

In many of the studies comparing UV irradiation with chemical disinfection it was emphasised that UV irradiation does not result in the formation of byproducts, e.g., chlorinated hydrocarbons formed during chlorine disinfection. However, UV irradiation relies on photochemical reactions in order for disinfection to occur and this results in photochemical byproducts.

Minimum standards for the intensity of radiation at all points in a disinfecting chamber of 16 mW sec cm⁻² have been set by the US Department of Health. This standard was set to allow a safety factor of two for the destruction of Escherichia coli, since the UV dose required to destroy E. coli totally is 6.6 mW sec cm⁻² (Collentro 1986, Grocock 1984). However, Mechsner (1987) has proposed that 25 mW sec cm⁻² is necessary for effective drinking water disinfection. At these intensities, length of time that the water is irradiated can be from a few tens of seconds to a few minutes, depending on the flow rate of water through the contactor.

Several studies of the effect of UV irradiation on organic compounds in aqueous solution have been made. Generally the conditions employed in these studies were far removed from the condition likely to be encountered during water treatment.
Armstrong et al (1966) demonstrated that the following compounds were totally consumed during prolonged (3 h) irradiation with UV; pyridine, 2,2-bipyridine, adenine, ethanol, methanol, glucose, glucosamine, formic, acetic, oxalic and palmitic acids, dimethylalanine, casein glycerol, phenylalanine and humic acid. All of these were presumed (on the basis of residual organic carbon measurements) to have been mineralised. In contrast, urea was found to be more resistant to decomposition since only 50% was consumed even in the presence of hydrogen peroxide. Zoeteman et al (1982) and Jolley et al (1983) found organic byproducts of UV irradiation after treatment of river water and wastewater respectively.

Suzuki et al (1982, 1983, 1985) reported the formation of mutagens after the irradiation of compounds such as: benzene, naphthalene, biphenyl, anthracene, pyrene, phenol, chlorobenzene, aniline, toluene, sodium benzoate, sodium benzenesulphate, theonine, tryptophan, phenylalanine and tyrosine in the presence of either sodium nitrate or nitrite. Presumably the reaction involved the formation of reactive nitrogen oxide species.

Sundstrum et al (1986) and Glaze et al (1987) have reported the effect of using UV irradiation in conjunction with strong oxidants such as hydrogen peroxide or ozone. This produces hydroxyl free-radicals which then react with organic substrates. These authors have shown that compounds considered to be unreactive even with ozone such as tri- and tetrachloroethylene are rapidly consumed resulting in the quantitative conversion of organic chlorine to chloride.

As with the other disinfectants discussed, it is evident that more work needs to be done to establish the reactivities of organic substrates.
2.6 References


KANNO S, NOJIMA K and OHYA T (1982A) Formation of cyanide ion or cyanogen chloride through the cleavage of aromatic rings by nitrous acid or chlorine. IV. On the reaction of aromatic hydrocarbons with hypochlorous acid in the presence of ammonium ion. Chemosphere 11(7), 663-667.

KANNO S, NOJIMA K and OHYA T (1982b) Formation of cyanide ion or cyanogen chloride through the cleavage of aromatic rings by nitrous acid or chlorine. V. On the reaction of aromatic amines or phenolic compounds with hypochlorous acid in the presence of ammonium ion. Chemosphere 11(7), 669-673.


3. CRITICAL ASSESSMENT OF THE TOXICITY OF ORGANIC BYPRODUCTS BY OZONATION

3.1 Introduction

Most of the studies in which products of ozonation have been identified have used model compounds as substrates. This imposes a number of limitations in the prediction of the toxicity of ozonated water. For example, the ozonation of organics in water and the products formed are influenced by a number of contributory factors, including ozone concentration, the nature and concentration of organic substrate, pH and contact time. It has also been demonstrated that the products resulting from ozonation of aqueous solutions of model compounds may differ in the presence and absence of humic acids. Therefore the conditions experienced during ozonation of water may be difficult to simulate in the laboratory, and acceptance of the products resulting from model studies as exemplary of those produced in water sources may be questioned.

Ozone has a short half-life in water and will not reach the consumer. No consideration has been given to the toxicity of ozone per se, or to occupational exposure of water treatment workers.

3.2 Toxicity of ozonated water samples

There have been very few studies performed in which the toxicity of ozonated water has been tested in vivo. Lykins et al (1986) performed several in vivo tests on ozonated water samples in parallel with water treated using other means of disinfection. Administration of ozonated samples, concentrated by reverse osmosis, in the drinking water of CD-1 mice (10 males and 10 females) for 30 days, resulted in no gross pathological changes at necropsy. Similarly, no gross pathological changes were observed in mice after administration of 0.3 ml of XAD concentrated ozonated samples at two
dose levels (4000x and 2000x) by oral gavage, 3 times per week, for four weeks.

Concentrated extracts of ozonated water samples have been tested in short-term assays designed to detect either tumour initiating potential or the ability to promote the activity of other initiators of tumour formation. Lykins et al (1986) carried out a mouse adenoma assay as one means of determining the tumour initiating potential of the samples. Male and female mice were administered 0.25 ml of XAD concentrated samples (4000X or 2000X) by oral gavage, 3 times per week, for eight weeks. After an additional 16 weeks, no increased incidence of lung adenomas over controls was observed in samples treated by ozonation or any other disinfectant used.

In skin initiation-promotion assays with Sencar mice, 0.5 ml of sample was administered orally 3 times per week for two weeks, followed two weeks later by topical administration of the promoter 12-tetradeconylyphorbol-13-acetate (TPA) (1.0 µg, 3 times per week for 20 weeks). None of the disinfected XAD concentrates (4000X) possessed skin tumour-initiating activity (Lykins et al 1986).

Lykins et al (1986) also carried out a rat liver foci assay, in which the XAD concentrates were administered orally to partially hepatectomised rats for 7 days, followed by administration of 500 mg/l sodium phenobarbitol in the drinking water for a further 56 days. The animals were sacrificed after a total of 70 days. None of the concentrates were able to initiate an increased incidence in rat liver of gamma glutamyl transpeptidase positive foci over controls. These studies highlighted the difficulties experienced using in vivo tests on water samples. The concentration techniques used are highly selective, concentrating only a small
proportion of the total organic matter present. In addition, the nature and concentration of the compounds present in the concentrated extracts are largely unknown and there are difficulties in both calculating appropriate dose levels and assessing the true significance of those actually used. This is particularly important in many short-term bioassays which rely on doses close to or at those producing toxicity in order to obtain effects even with positive control compounds.

3.3 Mutagenicity of ozonated water samples

It has been shown that chlorination of water as a means of disinfection may result in the production of mutagens (Water Research Centre 1985, Cheh et al 1980, Kool et al 1982, Maruoka and Yamanaka 1983). A number of studies have been carried out to examine the effect of ozonation in the mutagenicity of water samples.

The ozonation of water has been shown to both reduce the mutagenicity of existing compounds in water samples and to create new mutagenic compounds. Van Hoof (1982) studied the effect of ozonation on mutagenic activity in drinking water. It was found that post-ozonation (2 mg/l) removed direct-acting, frameshift mutagens (in Salmonella typhimurium TA98) formed as a result of chlorination, and reduced the activity of direct-acting, base-pair substitution mutagens in strain TA100. However, the production of direct-acting, base-pair substitution mutagens was detected when the ozonated fractions were eluted from the XAD columns at pH 2, although this effect was inactivated by microsomal enzyme addition (S9). Therefore, the results of mutagenicity tests on water samples may be dependent on the extraction procedure used.
Unpublished studies at WRc using freeze-dried concentrates, demonstrated that ozonation of water led to a reduction of the mutagenic activity in *S. typhimurium* strain TA98, in the presence of rat liver S9. Pre-ozonation of water followed by slow sand filtration also reduced the mutagenic activity in TA98, but had little effect in TA100, compared with non-ozonated samples. Denkhaus et al (1980) similarly demonstrated that the mutagenic activity of samples from a wastewater reclamation plant (concentrated by solvent extraction and rotary evaporation, and extracted at acidic, neutral and alkaline pH) was decreased by ozonation treatment, as detected by Ames tests using five strains of *S. typhimurium*. Conversely, Gruener (1978) demonstrated an increase in frameshift and base-pair substitution mutagens in recycled wastewater (low pressure, low temperature distillation concentrates) due to ozonation. These mutagens did not require activation by microsomal enzymes, although the activity in strain TA98 (detecting frameshift mutations) increased in the presence of rat liver S9 fraction. Van Hoof et al (1985) demonstrated that pre-ozonation of surface water also resulted in the production of a direct-acting, frameshift mutagen, using XAD-concentrated extracts of neutral and acidic samples. This mutagenic activity was reduced, but not eliminated by the addition of microsomal enzymes.

Jolley et al (1982) investigated the mutagenicity of samples from different wastewater plants. They demonstrated that ozone could both produce and destroy mutagenic constituents in a single treatment plant. Considerable variability in the mutagenicity of ozonated samples, taken from the same plant at different times in the year, was also observed.
Lykins et al (1986) compared the mutagenicity of water following various disinfection treatments. Ozonated samples, concentrated either by reverse osmosis or by macroreticular resins (pH 2), showed no significant increase in mutagenicity in Ames tests compared with non-disinfected samples. Monochloramine-, chlorine- and chlorine dioxide-treated concentrates, prepared by resin adsorption, all exhibited an increase in mutagenic activity in comparison with the ozonated or non-disinfected samples.

The effect of pre-ozonation on the mutagenicity of raw water was also studied by Bourbigot et al (1986), using XAD concentrated samples. Ozonation at higher doses did not lead to the creation of new mutagens, and some existing mutagens were destroyed. However, at lower doses, ozonation resulted in the production of weak mutagens, although these could be removed by GAC filtration. Similarly, Kool and Hrubec (1986) demonstrated that XAD concentrates (7000X, eluted at acidic and neutral pH) of samples from 5 different water sources ozonated with 3 mg/l showed a slight increase in mutagenic activity, whereas doses of 10 mg/l reduced or removed the mutagenic activity observed in the untreated samples. Cognet et al (1986) also demonstrated the variation in mutagenic activity of ozonated raw water (concentrated by XAD resins, at pH 2-3) with the dose of ozone applied. It was found that conditions of ozonation resulting in minimum mutagenicity may be defined for a given water source, but that such an optimum is subject to the variations in organic content of the water.

Kowbel et al (1982) investigated the effect of ozonation on the mutagenic activity of six extracts of fulvic acids, derived from soil or water. All but one extract gave negative results for mutagenicity in the Ames test, using five strains
of *S. typhimurium*, either with or without S-9 activation. One highly ozonated soil extract showed weak mutagenicity in strain TA100 which was reduced with metabolic activation.

From these studies it is difficult to draw any definitive overall conclusions since there is no consistent pattern in the results obtained. In general, where ozonation does produce mutagenicity this tends to be at relatively low ozone doses. At high doses, the compounds responsible for this mutagenicity appear to be destroyed. There is also some indication that where mutagens are produced, these may be detected following sample concentration at acidic pH.

### 3.4

**The effect of ozonation on the mutagenicity of model compounds**

A number of studies investigating the effect of ozonation on the mutagenicity of specific model compounds have been performed. Caulfield *et al* (1979) demonstrated, using Ames tests, that ozonation inactivated the mutagenicity of aflatoxin B1, the pesticides, captan and Daxon (active ingredient fenamidone), and also certain alkylating agents including sodium azide and bis(2-chloroethyl)amine. Other alkylating agents (Beta- propiolactone, propane-sultone and N-methyl-N’-nitro-N-nitrosoguanidine) did not show any alteration in mutagenicity due to ozonation. However, ozonation was shown to convert three chemicals (dimethylhydrazine, 2-hydroxyethylhydrazine and benzidine) to direct-acting mutagens, although only dimethylhydrazine produced a stable mutagen.

Cotruvo *et al* (1977) investigated the effect of ozonation on the mutagenicity of 28 organic compounds either identified, or likely to occur, in drinking water or wastewater. The ozonation products of seven of these compounds (ethanol,
phenol, hydroquinone, 1,1-diphenylhydrazine, benzidine, nitrilotriacetic acid and 2,4-dinitrotoluene) showed weak, mutagenic activity in *Saccharomyces cerevisiae*. However, this activity was not dose-related, and the results were not reproducible in most cases. The only compound giving positive mutagenicity results in the Ames test was benzidine.

Burleson *et al* (1979) used Ames tests to determine the mutagenicity of selected mutagens after ozonation in water. The mutagenicity of some polyaromatic amines (acriflavine, proflavine and B-naphthylamine) and polycyclic aromatic hydrocarbons (benzo(a)pyrene, 3-methylcholanthrene and 7,12-dimethyl-benz(a)anthracene) was inactivated by short periods of ozonation. Burleson also studied the effect of ozonation of the carcinogens 7,12-dimethylbenz(a)-anthracene and 3-methylcholanthrene upon tumour induction in mice. Topical administration of the ozonated compounds resulted in a lower incidence of tumours than administration of the non-ozonated compounds.

### 3.5 Effect of ozonation on trihalomethane formation

The formation of halogenated organics, in particular trihalomethanes (THMs), as a consequence of water chlorination, has initiated several studies investigating the effect of ozonation as a means of reducing trihalomethane precursors (THMPs) or the actual THMs. Lykins *et al* (1986) demonstrated that the ozonation of raw water did not significantly reduce the THM concentration, compared with non-disinfected water. However, ozonation compared favourably in this study with the use of chlorine dioxide, chloramine or chlorine, since parallel streams treated with these disinfectants all showed an increase in THM concentration. Samples from several water sources were ozonated by Amy *et al* (1986), who also showed
that high ozone doses could reduce the THMP content of the water. However, the extent of inactivation varied with water source, and as only a portion of the total precursors were inactivated in each sample, even at high doses, it is possible that only some of the THMPs are affected by ozonation.

Glaze et al (1982) demonstrated that the removal of THMPs from two US water sources was appreciably more effective if a combination of UV and ozonation was used, rather than ozonation alone.

Other workers have investigated the effect of pre-ozonation (ozonation performed prior to chlorination) on THMPs and THM formation. Van Hoof et al (1986) demonstrated that THMP concentrations in surface water samples were lower after pre-ozonation, although the extent of reduction varied between 10% and 70%. However according to Glaze and Wallace (1984), pre-ozonation of water followed by granular activated carbon treatment did not significantly reduce water THMP concentrations, compared with unozonated water. Veenstra et al (1983) investigated the effect of pre-ozonation of raw water on THM production over a period of 11 months. The reduction in THMs observed due to pre-ozonation was not consistent through the different seasons and on some occasions ozonation actually increased the concentration of THMs in the water. Rice et al (1981) also demonstrated that pre-ozonation resulted in elevated concentrations of THMS over those formed upon chlorination alone.

Sierka and Amy (1985) studied the concentrations of THMPs following oxidation of a humic acid solution by ozone, with added catalytic effects of ultraviolet light (UV) and/or ultrasound (US). The most effective reduction in THMP levels was obtained by the use of a combination of ozone-UV-US, which was further enhanced by addition
of bicarbonate ions. These conditions were also the most effective in the removal of non-volatile total organic carbon.

Yamada et al (1986) studied the formation of THMPs due to pre-ozonation of certain aromatic organics. Ozonation was shown to be capable of controlling THMP formation from compounds which usually act as high THM precursors, for example, resorcinol, aniline, salicylaldehyde, phenol and p-hydroxybenzoic acid. However, ozonation of other compounds, which usually show a low capacity for the production of chloroform, resulted in raised concentration of THMPs. Such compounds included hydroquinone, salicylic acid, methoxybenzene, benzaldehyde and benzoic acid. In each case, the level of ozone consumed was an important factor determining the concentration of THMPs produced. It was also found that pre-ozonation of humic acid was not as effective at reducing its THMP as similar pre-ozonation of the aromatic compounds.

The effect of post-ozonation on the trihalomethanes themselves has also been studied. Aeration of chlorinated water with high ozone/air mixture to water ratios (6:1) reduced the trihalomethane content by up to 50%, but lower doses (up to 25 mg/l ozone for 4 to 5 minutes) were not able to show oxidation of these compounds (Love et al 1976). According to Symons et al (1981), ozone alone does not possess sufficient oxidising capacity to remove THMs, but its efficacy can be enhanced by combination with UV.

It may be seen from these studies that the action of ozone on the levels of THMs and THMPs does not produce a consistent response, and that concentrations of these compounds may be elevated or reduced as a result of ozonation performed in place of, before or following chlorination. Such
variation may be expected, since differences in humic substances and organic chemicals in different water sources may account for differences in THM levels. However, it is possible that the efficacy of THM destruction may be enhanced by the use of ozonation in combination with added catalysts or other disinfectants, although further studies may be needed to confirm this. Since the effect of ozonation on THM levels is so variable, it may be necessary to evaluate the suitability of this disinfectant for use in each individual water supply.

The reaction between bromide occurring naturally in water and ozone has not been considered in this report, but deserves mention. Bromide ions may be oxidised by ozone to ultimately form bromate, a reaction which proceeds via formation of hypobromous acid (Haag and Boigne 1984, Cooper et al 1986). Hypobromous acid can react with humic substances to form brominated products including bromoform, hence contributing to an increase in trihalomethane concentration. Halogenated organics may also be formed as a result of the reaction between hypobromous acid and various other organic compounds in water. Such brominated organic products are not well characterised and therefore no comment on their toxicity can be made here. Therefore it would be desirable to have a greater knowledge of the reactions occurring in waters containing bromide upon ozonation, with respect to the formation of brominated organics.

3.6 Products of ozonation

3.6.1 Carboxylic acids

Carboxylic acids have been identified as major products of ozonation of humic substances (Lawrence et al 1980, Killops 1986). The monocarboxylic
acids identified included valeric, heptanoic, lauric, myristic and palmitic acids. The ozonation of domestic wastewater has also been shown to produce fatty acids (Chappell et al 1981). These acids exist naturally or are used as additives in foodstuffs. Their toxicity has been poorly studied. A recent review (Barnett and Tilling 1987) reports these compounds to be of low-acute oral toxicity. There is very little data on chronic toxicity. No mutagenic effects have been shown by those acids tested, and the only evidence of carcinogenic activity has been shown by lauric acid, which was reported to induce leukaemic lymphomas and subcutaneous sarcomas in rats upon repeated subcutaneous injection (Swern et al 1970). The relevance of this route of administration to exposure to lauric acid in water is not clear.

Formic acid has been identified as a product of ozonation of styrene and benzaldehyde (Legube et al 1984), phenol (Niki et al 1978), and amino benzoic acids (Gilbert 1976). Studies of repeated oral administration of formic acid have indicated this compound to be of low toxicity. The only adverse effects observed when rats were fed 8-360 mg/kg formic acid in the drinking water for 2-27 weeks was a decrease in body weight gain (Hallony 1969).

No data concerning any potential carcinogenic activity was found but formic acid has shown mutagenic activity in studies using E. coli and Drosophila (Freese et al 1967).

A number of dicarboxylic acids have been identified as ozonation products of humic and fulvic acids (Lawrence et al 1980, Watts et al 1984, Watts 1985) the majority of which are saturated aliphatic diacids. The major toxic effect of these, particularly shown by the shorter chain acids, is irritancy to the skin, eye and mucous membranes
(Fassett 1963, Sax 1975). However, this is unlikely to be of any significance at the concentrations which will be encountered in drinking water. Little other information concerning the toxic effects of diacids exists, but some of these, including succinic, maleic, adipic and citric acids, are incorporated into intermediary metabolism, and therefore it seems unlikely that these will exert any toxic effects.

Oxalic acid has been identified as a product of ozonation of phenols (Gould and Weber 1976, Niki et al 1978) and non-phenolic aromatics, including styrene, benzaldehyde, and naphthalene (Legube et al 1983), aniline (Gilbert 1983), 4-chlororesol and 4-aminobenzoic acid (Gilbert 1976) and certain herbicides (Struif et al 1978). Only large doses of oxalic acid are toxic to man with a mean lethal dose of 15-30 g in the adult human, although 1.2 g was sufficient to cause death in one case (Gosselin et al 1984). No data on the chronic toxicity of oxalic acid in animals was found. Oxalic acid complexes with calcium (Fassett 1973) depleting the levels of ionised calcium in body fluids and depositing insoluble calcium oxalate in the liver, kidney and other organs. This may lead to systemic and haemostatic effects, and tissue damage may occur as a result of ischaemic necrosis due to vascular stasis.

Little information was available on the toxicity of the hydroxyacids found on ozonation of humic and fulvic acids (Killops 1986). Hydroxyethanoic acid (glycolic acid) is reported to act as a severe irritant on rabbit eyes and may also cause skin irritation. Hydroxypropanoic acid (lactic acid) is a normal intermediate of mammalian metabolism. Few toxic effects have been attributed to chronic administration of lactic acid, although birds fed a diet of 10% lactic acid developed polyneuritic crises (FAO/WHO 1974).
No toxicological information was available on indane-1,7-dicarboxylic acid, indane-1-formyl 7-carboxylic acid and 1-indanone-7-carboxylic acid, which have been identified as products of ozonation ofacenaphthene (Chen et al 1979).

It is unlikely that any toxic effects would occur following long-term exposure to the low concentrations of these compounds which are likely to be found in drinking water.

3.6.2
Dihydroxybenzenes and benzoquinone

Benzene and phenols have been identified in both raw and drinking water (Fielding et al 1981, US EPA 1980, Akiyama et al 1980,) and may also be found associated with humic substances. Ozonation of these compounds in aqueous solution results in formation of hydroquinone, pyrocatechol and benzoquinone (Kuo 1984, Decoret et al 1984). Further oxidation of these products may produce paraquinone and cause opening of the benzene ring (Legube et al 1980).

Chronic exposure of man to benzene causes degradation of bone marrow and aplastic anaemia, and has also been implicated as a human leukaemogen (Snyder et al 1981). Benzene is metabolised to hydroquinone, benzoquinone and catechol. These metabolites are thought to be responsible for benzene toxicity, since they accumulate in the bone marrow, where they bind covalently to macromolecules (Rickert et al 1979, Greenlee et al 1981, Schwartz et al 1985). Therefore, ozonation of benzene and phenol in aqueous solution may convert these substrates to their toxic metabolites. The WHO guideline value for benzene is 10 μg/l (WHO 1984a).

Administration of hydroquinone by oral gavage (500 mg/kg body weight, 101 times in 151 days)
resulted in death of half of a test group of 16 rats within two months (Carlson and Brewer 1953).

Administration of a total of 0.5 g hydroquinone in the feed of 10 pregnant rats during the gestation period, resulted in a significant increase in the number of resorptions over non-treated controls (Telford et al 1962).

Little information on the toxicity of benzoquinone was found. However, benzoquinone was not mutagenic in Ames' tests using S. typhimurium strains TA97, TA98 and TA100 (Sakai et al 1985).

The results of mutagenesis assays for both hydroquinone and pyrocatechol appear to vary with the test system used. Hydroquinone has been shown to be non-mutagenic in the Ames test, using strains TA97, TA1537, TA98 and TA100, either with or without metabolic activation (Florin et al 1980, Sakai et al 1985). However, Gocke et al (1981) reported that hydroquinone gave both positive and negative mutagenicity results using strain TA1535 and rat liver S-9 mix with different media. Positive mutagenicity results have been obtained with hydroquinone in Saccharomyces cerevisiae (Cotruvo et al 1977), and in the HeLa DNA synthesis test (Painter and Howard 1982). Hydroquinone also induced sister chromatid exchanges in human lymphocyte cultures (Morimoto and Wolff 1980) but not in cultured Chinese hamster V79 cells (Wild et al 1981).

Negative mutagenicity results have been obtained for pyrocatechol in the Ames test using strains TA1535, TA1537, TA98 and TA100 (Florin et al 1980, Rapson et al 1980, Bjeldanes and Chew 1979, Nazar et al 1981) and also in the E. coli DNA polymerase assay (Rosenkranz and Leifer 1980). However, weak mutagenic activity was exhibited by pyrocatechol in
S. cerevisiae (Kunz et al 1980) and this compound also induced an increase in the number of chromatid breaks and exchanges in Chinese hamster ovary cells (Stich et al 1981). The frequency of sister chromatid exchanges was increased in human lymphocyte cultures due to exposure to $1.6 \times 10^{-6}$ to $2.0 \times 10^{-4}$ m (Morimoto and Wolff 1980), but not in Chinese hamster V79 cells following exposure to $0.5-2.0 \times 10^{-5}$ m (Wild et al 1981).

3.6.3 Methylglyoxal

Ozone has been shown to produce methylglyoxal as a result of its reaction with toluene and other compounds of similar structure in municipal water supplies. Methylglyoxal has been found to exert mutagenic activity in a number of test systems, including S. typhimurium TA104 (Harnett et al 1985), the hypoxanthine-guanine phosphoribosyl-transferase locus in V79 cells (Cajelli et al 1987) and dose-dependent sister chromatid exchanges in Chinese hamster ovary cells (Faggin et al 1985). However, its general toxicity has been poorly studied. Peters et al (1978a) showed the acute oral LD50 to range from 0.53 g/kg in the newborn rat to 1.99 g/kg in the adult male rat. The toxicity of methylglyoxal in pregnant rats was also investigated by Peters et al (1978b). The pregnant rat appeared to possess some protective mechanism against methylglyoxal toxicity that was absent in the non-pregnant rat, according to the comparative LD50s obtained, but the exact nature of this mechanism is unknown.

3.6.4 Epoxides

Ozone reacts with unsaturated organic compounds in water to form ozonides as intermediate products. However, if this reaction is hindered, for example by a large functional group, it is thought that epoxide formation may then occur (Carmichael et al 1982).
Epoxides tend to be unstable compounds which are often converted to more stable products of ozonation (Henschler 1977). This creates problems in their detection and therefore the evidence for their formation is limited. In addition the number of epoxides which could be formed by ozonation of the many organic compounds existing in water is vast, and thus it is difficult to predict the toxicity of ozonated water with respect to these compounds.

Epoxides are a group of compounds which may act as alkylating agents by their reactions with nucleic acids and proteins, and hence are potential mutagens (Wheeler 1962, Weil et al 1963). Canter et al (1986) tested 37 aliphatic epoxides, comprised of various subclasses, for mutagenic activity using the Ames test. A total of 28 compounds showed mutagenic activity in one or more of five strains of S. typhimurium. Of the eight unsaturated aliphatic epoxides tested three were mutagenic in strain TA100. However 1,2-epoxyhexadecane, 1,2-epoxydodecane, 1,2-epoxytetradecane, 1,2-epoxyhexadecane and 1,2-epoxyoctadecane, of which the unsaturated precursors have been identified in drinking water in the UK, showed no mutagenicity in any of the Salmonella strains tested. Despite this, 1,2-epoxyhexadecane has been shown to induce squamous carcinomas in mice following skin application (Van Duuren et al 1967).

All four halogenated aliphatic epoxides tested by Canter et al (1986) (epichlorohydrin, epibromohydrin, 1,2-epoxy-3,3,3-trichloropropene and 1,2-epoxy-4,4,4-trichlorobutane) were mutagenic in TA100 without activation. Henschler (1977) demonstrated that the epoxides formed by oxidation of trichloroethylenes, 1,1-dichloroethylene and monochloroethylene were also mutagenic.
Wester et al (1985) demonstrated the action of epichlorohydrin as a carcinogen on administration to rats, (0, 0.002 and 0.01 g/kg body weight, 5 days/week for 2 years) by oral gavage. A high incidence of squamous cell carcinomas were observed in the forestomach of animals exposed for greater than 18 months, compared with controls, and this effect appeared to be dose related.

The insecticide heptachlor is also oxidised to the epoxide by ozone (see Section 3.5.13).

3.6.5 Phthalate esters

Phthalate esters have been identified in ozonated wastewater (Jolley et al 1982) and as products of ozonation of fulvic acids (Lawrence et al 1980). These compounds have not been identified as products of ozonation in studies with model compounds, and are not consistent with products of oxidation reactions. Therefore it seems likely that phthalates are released from the matrix of fulvic acids during ozonation, rather than being formed as a result of an oxidation reaction. It is possible that the phthalates released may be degraded by further ozonation, since it was demonstrated that ozonation of raw water resulted in a decrease in the phthalate concentration existing before disinfection (Lykins et al 1986).

Phthalate esters are generally of low acute oral toxicity although some have shown subacute toxic effects on the liver and testes of rodents at high doses (Cater et al 1977, Oishi and Hiraga 1980, Foster et al 1981). Testicular degeneration and depression of body weight gain are the main toxic effects reported following chronic exposure of rodents to phthalates (Shaffer et al 1945, Gray et al 1977). However, evidence suggests that such effects may not occur in other species (Rhodes et al 1983, Jackh et al 1984).
Mutagenicity studies using bacterial test systems suggest that the phthalate esters are at most only very weakly mutagenic (Seed 1982, Kozumbo et al 1982, Zeiger et al 1982, Simmon et al 1977). However, carcinogenicity studies in rodents have shown signs of carcinogenic potential. In a US National Toxicology Program study (1982), di-(2-ethylhexyl) phthalate was shown to induce liver tumours in rodents and an increased incidence of myelomonocytic leukaemia in female rats was induced by butyl benzyl phthalate (US National Toxicology Program 1981). However, the mechanism of the carcinogenicity of these phthalate esters may be epigenetic, probably related to peroxisome proliferation.

High doses of di-(2-ethylhexyl)phthalate and di-n-octyl phthalate have been observed to act as teratogens in rodents, and di-(2-ethylhexyl)phthalate also showed foetotoxic effects, causing such effects as lowered foetal weight, increased resorptions and increased skeletal abnormalities (Singh et al 1972, Nikonorov et al 1973, Shoita et al 1980, Yagi et al 1980).

3.6.6 Halogenated hydrocarbons

Lawrence et al (1980) ozonated fulvic acid extracted from soil, and showed the production of certain halogenated hydrocarbons. However, ozonation of a water sample containing high fulvic acid concentrations, by the same workers, did not show these compounds as products. Halogenated hydrocarbons are unlikely to be products of oxidation by ozone, and thus it seems probable that these compounds were released from the matrix of the soil-derived fulvic acid. Since halogenated hydrocarbons from anthropogenic sources are known to occur in water, it is possible that they may be released in this way upon ozonation of fulvic acids in some water sources. Some comment on their toxicity is therefore appropriate.
The halogenated hydrocarbons identified by Lawrence et al (1980) consisted of four polychlorinated benzenes: tetrachlorobenzene, pentachlorobenzene, hexachlorobenzene and tetrachloromethoxybenzene. Little toxicological information, however, is available on pentachlorobenzene and tetrachloromethoxybenzene. Of the three tetrachlorobenzene isomers, 1,2,4,5-tetrachlorobenzene appears to have the longest biological half-life and shows the greatest toxicity. Rats fed 500 ppm of this isomer in the diet (equivalent to 50 mg/kg body weight per day) for 28 days showed histopathological changes in the liver, thyroid, kidneys and lungs (Chu et al 1983). Similar effects were observed in rats fed this dose for 90 days (Chu et al 1984).

Villeneuve and Newsome (1975) administered hexachlorobenzene orally to rats and guinea pigs (500 mg/kg body weight) for 16 days. The guinea pig appeared to be the more susceptible species to hexachlorobenzene toxicity, killing all 14 animals during the dosing period. Of 11 male and 11 female rats dosed, 8 and 5 died during dosing, respectively.

The toxic effects of hexachlorobenzene in humans has been well documented since the accidental consumption by approximately 4000 people in Turkey of up to 4 mg/kg/day, for as long as 2 years. The major toxic effects observed were porphyria cutanea tarda, hepatomegaly and cirrhosis. A number of other neurological, visceral, arthritic and urinary effects were also observed (Peters 1976, Cam and Nigogosyan 1967). The induction of porphyria by hexachlorobenzene does not, however, occur in some experimental species, for example, the beagle dog (Gralla et al 1977) and rhesus monkey (Rozman et al 1977).
All three isomers of tetrachlorobenzene were found to be non-mutagenic in S. typhimurium either with or without rat or hamster liver S9 activation (Havorth et al 1983). Hexachlorobenzene was found to be non-mutagenic in a dominant lethal test using rats dosed orally with 20, 40 or 60 mg/kg body weight for 10 days (Khera 1974).

No information on the carcinogenicity of tetrachlorobenzene could be found. Hexachlorobenzene has been shown to act as a carcinogen in mice and hamsters. Administration of hexachlorobenzene (12–24 mg/kg body weight/day for 101–106 weeks) in the diet of mice was found to induce liver cell tumours. No such tumours were observed in control mice (Cabral et al 1978, 1979). Hamsters fed 4–16 mg/kg body weight/day hexachlorobenzene in the diet showed an increase in the incidence of hepatomas, liver haemangioendotheliomas and thyroid adenomas over control animals (Cabral et al 1977).

Studies have indicated that tetrachlorobenzene is not teratogenic in rodents (Kacev et al 1984, Kitchin and Ebron 1983a). However, embryotoxic effects were observed in rats given oral doses of 300 mg/kg/day on days 9–13 of gestation. These included decreased crown-rump length, head length and yolk sac diameter, and inhibition of growth (Kitchin and Ebron 1983b). Hexachlorobenzene was found to be teratogenic in mice fed 100 mg/kg hexachlorobenzene on days 7–16 of gestation (Courtney et al 1976). Foetal body weight was decreased, and an increased incidence of cleft palate and kidney malformations over controls was produced.

3.6.7
Methyl phenol

Methyl phenol (cresol) was one of the products of ozonation of a fulvic acid solution (Lawrence et al...
1980), although it was not stated which isomer was identified. Adverse toxic effects were not observed when minks and ferrets were given o-cresol at concentrations of up to 2520 and 4535 ppm, respectively, in the diet for 28 days (Hornshaw et al 1986). Minks fed up to 1600 ppm in the diet for 6 months did not exhibit any adverse effects on reproduction. Pool and Lin (1982) showed that o-, m- and p-cresol were all non-mutagenic in the Ames test, using five strains of S. typhimurium, either with or without rat liver S-9 activation.

Little other toxicological information was found to be available on cresols.

The presence of cresols may cause tainting problems in water, since the taste and odour thresholds are reported to be 0.002-0.003 mg/l and 0.2-1.4 mg/l respectively (Dietz and Traud 1978).

3.6.8 Aldehydes

Ozonation of humic substances and various water sources has resulted in the production of a number of aldehydes as products and intermediates, including benzaldehyde, formaldehyde, nonanal and other aliphatic aldehydes (Lawrence et al 1980, Legube et al 1983, Killops 1986, Yamada and Somiya 1980, Decoret et al 1984, Chappell et al 1981). Aldehydes may also be formed as products of ozonation of hydroxyazobenzene dyes (Kerzhner et al 1985). Since aldehydes may be further oxidised by ozonation, the concentrations of these products may depend on the ozone dose used.

Little information is available on the toxicity of higher aliphatic aldehydes but it has been suggested on the basis of existing evidence that these compounds are of low acute toxicity (Hunt and Oven 1985). A number of aldehydes have been tested for mutagenicity in the Ames test, giving negative
results, either with or without metabolic activation by rat liver S-9 (Florin et al 1980, Sasaki and Endo 1978).

Benzaldehyde is rapidly metabolised to hippuric acid upon oral ingestion in experimental animals (Bray et al 1951, Teuchy et al 1971), and is of low acute oral toxicity, with LD50 values reported as 1.3 g/kg and 1.0 g/kg in the rat and guinea pig, respectively (Jenner et al 1964). It is also readily oxidised to benzoic acid in air.

The effects of subchronic exposure of rats and mice to benzaldehyde was studied by Kluwe et al (1983). Doses of 800, 600 and 1200 mg/kg/day for 90 days resulted in depression of body weight gain in male rats, male mice and female mice, respectively. In addition, encephalopathy and nephropathy was observed in both sexes of rats given 800 mg/kg/day, and male mice administered 1200 mg/kg/day also developed nephropathy. No toxic effects were observed in rats given 400 mg/kg or less, or in mice given 300 mg/kg or less. There appeared to be little information available on chronic exposure to benzaldehyde.

Benzaldehyde was not mutagenic in the Ames test using several strains of Salmonella, either with or without metabolic activation (Havorth et al 1983, Sasaki and Endo 1978, Florin et al 1980). However, it was found to act as a weak inducer of chromosome aberrations in Chinese hamster cells in vitro (Kamasaki et al 1982).

Formaldehyde is very soluble in water, but most of the toxicity data available are concerned with the effects of formaldehyde inhalation, and few studies of the toxicity following oral ingestion of the solution have been performed.
Formaldehyde is metabolised rapidly by formaldehyde dehydrogenase in the liver and blood, producing formic acid, which may then be further oxidised to carbon dioxide and water (Eells et al 1981). Formaldehyde is highly toxic upon acute inhalation by experimental animals, primarily as a consequence of its irritancy.

Acute oral LD50 values using aqueous formaldehyde solutions (2%) have been found to be 800 mg/kg and 260 mg/kg in the rat and guinea pig, respectively (Smith et al 1941). Acute ingestion of formalin (37% solution of formaldehyde) by humans is reported to cause severe irritation to the mucosal surfaces of the gastrointestinal tract (Allen et al 1970), and in some cases formalin consumption has proved to be fatal (Zipf and Bartscher 1933, Levison 1904, Ely 1910). Solutions of formaldehyde are irritant to the skin of experimental animals and humans. It has been shown to act as a potent skin sensitiser in the guinea pig maximisation test (Maurer et al 1979), and causes contact dermatitis in occupationally exposed workers (Rostenberg et al 1952, Sneddon 1968, O’Quinn and Kennedy 1965, Berrens et al 1964, Schorr et al 1974).

Til et al (1987) recently performed a sub-acute oral study of formaldehyde using rats. Formaldehyde was administered in the drinking water at doses of 5, 25 and 125 mg/kg body weight per day, for four weeks. Toxic effects were observed only in the highest dose group, and these included fur discolouring, decreased plasma protein and albumin levels, thickening of the limiting ridge and hyperkeratosis in the forestomach and occasional slight focal gastritis in the glandular stomach.

A study in which rats, guinea pigs, rabbits, monkeys and beagles were continually exposed
(24 hours) to 4 ppm formaldehyde vapour for 90 days showed that none of the test species exhibited signs of toxicity during the exposure period. However, some inflammatory lung changes were noted at autopsy (Coon et al 1970).

Formaldehyde has given conflicting results in mutagenicity tests using bacterial systems. Negative results were obtained in the Ames test, using five strains of S. typhimurium including TA98 and TA100, and formaldehyde concentrations of up to 2 μM/plate (Gocke et al 1981). Sasaki and Endo (1978) detected weak mutagenic activity in TA100 using limited concentrations, but this activity was abolished in the presence of a microsomal enzyme fraction. However, Marnett et al (1985) showed that S. typhimurium strain TA104 (which has a deleted UVrB gene and hence is deficient in an error-free DNA excision-repair mechanism) was much more sensitive to formaldehyde-induced mutagenesis than TA100, TA97 or TA98. Mutagenic activity due to formaldehyde has also been demonstrated in E. coli (Nishioka 1973). Formaldehyde has exhibited mutagenic activity in a number of other test systems. An increase in intragenic recombinants was produced by treatment of S. cerevisiae cells in the exponential growth phase with 6-60 mM formaldehyde (Chanet et al 1975). It was also shown that the lethal effects of formaldehyde on S. cerevisiae were enhanced using strains with deficient mechanisms for excision repair of UV-induced pyrimidine dimers (Chanet et al 1976). Induction of weak mutagenic activity by formaldehyde has also been demonstrated in Neurospora crassa (Kolmark and Westergaard 1953).

Drosophila larvae, fed a diet prepared with 0.1-0.25% aqueous formaldehyde solution, showed an increased incidence of sex-linked lethals over controls at all dose levels. Examination of
salivary gland chromosomes showed that the most common mutagenic events were small deficiencies and duplications (Auerbach et al 1977, Kaplan 1948).

There are little data available concerning the carcinogenicity of formaldehyde except by inhalation. Svenberg et al (1980) reported the occurrence of nasal carcinomas in rats following exposure to formaldehyde at 15 ppm, 6 hours/day for 18 months. No such tumours were observed in animals exposed to 2 or 6 ppm, or in controls. Albert et al (1982) also demonstrated the induction of squamous cell carcinomas of the nasal cavity in rats, following inhalation exposure to 14 ppm formaldehyde, 6 hours/day, 5 days/week for life. However, hamsters exposed to 10 ppm, 5 hours/day, 5 days/week for life showed no tumours of the respiratory tract, although an increase over controls in hyperplastic and metaplastic areas of the nasal epithelium was observed (Dalbey 1982). These finding are complicated by the severe irritancy of formaldehyde vapour.

The only evidence found for a correlation between formaldehyde and cancer in occupationally exposed workers was given in an epidemiology study by Olsen and Asnaes (1986). An increase in the risk of squamous cell carcinoma and adenocarcinoma of the nasal cavity and paranasal sinuses was demonstrated in men exposed to gaseous formaldehyde, compared with those never exposed.

The teratogenicity of formaldehyde in mice has been studied by Marks et al (1980). Pregnant mice were dosed with 74, 148 and 185 mg/kg on days 6-15 of gestation. The highest dose was lethal to 22 of 34 dams, and the number of resorption sites was increased in the surviving animals. However, no evidence for teratogenic effects was found in foetuses of surviving animals at any of the dose levels used.
No reproductive or teratogenic effects were observed when formaldehyde was administered in the diet of beagles at 125 and 375 ppm on days 4-56 of gestation (Hurni and Ohder 1973).

3.6.9 Ketones

Several ketones have been identified as products of ozonation of humic substances (Lawrence et al 1980, Watts 1985). Most of these are complex multisubstituted molecules, for which no toxicological information appears to be available, for example trimethyltetrahydrobenzofuranone, 1-hydroindole-2,3-dione, 4-methyl-3-oxtene-2-one, 1,5,5-trimethylcyclohexane-3-one, 3,6,6-trimethylbicyclohexane-2-one and isobenzofurandione. Limited toxicological information is available on the products 3-octanone and 2,6-dimethyl-2,5-heptadiene-4-one. The intraperitoneal LD50 of 3-octanone in mice was reported to be 406 mg/kg (Krasavage et al 1982), and this compound is moderately irritant to rabbit skin at 500 mg/24 hours (Opdyke 1975). However, no sensitization reactions were detected in guinea pigs, using a modified Draize test (Sharp 1978), and no irritation was produced in 25 humans subjected to a 40 hour closed patch test with 2% 3-octanone in petrolatum (Kligman 1973). No information on the mutagenicity of 3-octanone was found, but 2-octanone was reported to be non-mutagenic in the Ames test, using several strains of Salmonella either with or without metabolic activation (McMahan et al 1979).

Ozonation of acenaphthene was found to produce 1-indanone, 7-formyl-1-indanone and 7-hydroxyindanone (Chen et al 1979), but little toxicological information is available on any of these compounds. Florin et al (1980) showed that 1-indanone was non-mutagenic in the Ames test using four strains of Salmonella either with or without rat liver S-9.
It is clear that there is insufficient data available for an assessment of the toxicity of the ketones identified as ozonation products to be made.

3.6.10 Aliphatic hydrocarbons

A range of C-15 to C-30 alkanes were reported as products of humic-acid ozonation and were also identified in ozonated samples of upland waters (Killops 1986) and domestic wastewater (Chappell et al 1981). Lykins et al (1986) demonstrated the reduction of alkane concentration in raw water due to ozonation, although the products formed in this study were not identified. Therefore it is possible that alkanes may be degraded by further ozonation.

Alkanes appear to be readily absorbed from the gastrointestinal tract and incorporated into lipids (El Mahdi and Channon 1933, Stetton 1943, Albano and Fishbein 1970, Tulliez and Bories 1977). Very little information is available on the toxicity of the alkanes identified as ozonation products, although they have been shown to be irritant at very high concentrations (Motoyoshi et al 1979).

The only data available for the mutagenicity of alkanes are for n-eicosane, shown to be non-mutagenic in the Ames test (Florin et al 1980), and hexadecane, which failed to induce ouabain-resistant mutants in V79 Chinese hamster cells (Lanka et al 1978).

Bingham and Nord (1977) demonstrated the cocarcinogenic effect of n-alkanes and ultraviolet light on mice. The incidence of malignant skin tumours was increased due to application of 50 μl n-decane, n-dodecane or n-tetradeacne prior to exposure to UV (254 nm, 290–320 nm or greater than 350 nm), three times per week. No tumours were
observed in mice treated with either alkane in the absence of UV.

Hexadecane has been shown to influence the pharmacokinetics of hexachlorobenzene in rats, enhancing its excretion from the large intestine (Rozman and Rozman 1983, Scheufler and Rozman 1984). It has also been reported (Rozman 1984) that hexadecane may potentiate the toxicity of other halogenated hydrocarbons, such as TCDD. Rats administered 60 μg/kg TCDD intraperitoneally, were fed a diet supplemented with 5% hexachlorobenzene for 10 days after dosing. Mortality was increased from 60% in controls to 100% in hexachlorobenzene-treated animals.

On the basis of the available information, it seems unlikely that the alkanes produced during ozonation of water represent any significant health hazard to humans. However, the ability of long-chain alkanes to influence the toxicity of other organic products may require further study.

3.6.11 Esters

The following esters have been identified as products of ozonation of humic and fulvic acids (Killops 1986, Lawrence et al 1980):

dibutyl butene-1,4-dioate
dimethyl azelate
dimethyl malonate
dimethyl pimelate
dimethyl sebacate
dimethyl suberate
ethyl hexanoate
ethyl dimethyl azelate
methyl benzoate
methyl-2-ethyl-hexanoate
methyl heptanoate
methyl hexanoate
methyl-4-oxo-pentanoate
methyl palmitate
methyl phenyl acetate.

Some of these products may result from use of a certain extraction process or solvent (Killops 1986) rather than being ozonation products. Little toxicological information is available on most of these esters. However, the toxicity of a number of esters of carboxylic acids been reviewed by and Barnett (1987). These are reported to have low acute and chronic toxicity but little information concerning the mutagenicity or carcinogenicity of the esters was found. It is likely that the esters listed above are similarly of low toxicity.

3.6.12 Other compounds

The ozonation of fulvic acid resulted in the formation of several compounds for which no toxicological information was found to be available. These included trimethoxy methane, nicotinonitrile and 4-ethyl resorcinol (Lawrence et al 1980). Limited toxicological data are available for 2-methyl resorcinol, although it is not known whether it may be assumed that this compound shows similar toxic effects to 4-ethyl resorcinol. Rats dosed orally with 0-80 mg/kg 2-methyl resorcinol, five times per week for 12 weeks, showed no signs of toxicity at autopsy (CTFA 1984a). Negative mutagenicity results were obtained for 2-methyl resorcinol in the Ames test, in cultured Chinese hamster cells and in vivo in CD-1 mice (CTFA 1984b). No clinical evidence of irritation or sensitisation was found in humans subjected to two repeated insult patch test studies, using a 3% solution of 2-methyl resorcinol (TKL Research 1984).
Ozonation of water may result in the release of pesticides from the matrix of humic substances (Killops 1986), or in the formation of new products as a result of oxidation of the pesticides themselves.

Rice et al (1901) reported that the pesticides phosalone and aldrin are readily oxidised to destruction by treatment with small amounts of ozone. However, a number of other pesticides, including dieldrin, chlordane, lindane, DDT, polychlorinated biphenyls and pentachlorophenol, were only slightly reactive when ozonated in water under conditions typical of those in a drinking water treatment plant.

Ozonation of certain pesticides has been found to actually increase their toxicity. For example, the organophosphorus compounds malathion and parathion are converted to the intermediates malaoxon and paraoxon, respectively before further oxidative degradation (Rice et al 1981). These metabolites are thought to be responsible for the toxicity exerted by the pesticides acting via inhibition of cholinesterase. Chronic exposure to malathion and parathion may lead to neuro-psychiatric disorders and peripheral neuropathies or myopathies in mammals (Gosselin et al 1984). Major intoxications from any route of exposure may result in respiratory distress due to central nervous paralysis, and possible death.

The insecticide heptachlor has been identified in US river and drinking water (Shackelford and Keith 1976, Sandhu et al 1978). Ozonation of heptachlor, produces the toxic compound heptachlorepoxide (Rice et al 1981) which is stable to metabolism by microsomal epoxide hydratase, the enzyme normally responsible for epoxide metabolism in the mammalian
liver. In addition, this epoxide is also stable to further ozonation.

Heptachlor epoxide is a major metabolite of heptachlor in mammals, which has been shown to persist in adipose tissue, and has been identified in human milk in high concentrations (Savage 1976, Miller et al 1979). A review of the literature on teratogenicity studies and the effects of heptachlor epoxide on reproduction (WHO 1984b) concluded that this compound was not teratogenic, but that higher exposure levels may result in a decrease in litter size and the lifespan of offspring.

Heptachlor epoxide was found to be nonmutagenic in the Ames test, using S. typhimurium strains TA1535, TA1536, TA1537 and TA1538, either with or without metabolic activation (Marshall et al 1976). Negative mutagenicity results were also demonstrated in the dominant lethal test in mice (Arnold et al 1977). However, unscheduled DNA synthesis was induced in SV40 transformed human cells in vitro, following exposure to 10, 100 or 1000 μM heptachlor epoxide for 8 hours, in the presence of rat liver S9 (Ahmed et al 1977).

Reviews on the carcinogenicity of heptachlor and heptachlor epoxide have concluded that there is sufficient evidence that these compounds are carcinogenic in mice, but that only limited data exist for the carcinogenicity in other experimental animals (WHO 1984b, IARC 1979). The WHO guideline value for heptachlor/heptachlor epoxide is 0.1 μg/l (WHO 1984a).

3.7 Discussion

The data on the likely byproducts of ozonation are limited which in turn severely limits the assessment of potential health effects of
identified compounds. However, from the data available so far there is nothing to suggest that ozonation will produce compounds which are likely to be of major concern with regard to human health. Epoxides which may be formed are usually mutagenic but there is no evidence to suggest that these are likely to survive in distribution.

Evidence on the mutagenicity of ozonation byproducts is mixed but indicates that ozone produces less mutagenicity than chlorine. The mutagens which may be formed have not been identified or closely characterised so no comment can be made on the potential hazard or risk to consumers from these compounds. However, unlike chlorine, as the ozone dose is increased the mutagenicity appears to be reduced.

Ozone appears to release some compounds which are trapped in the humic and fulvic acid matrices. The significant of this is not immediately apparent since they may normally pass through water treatment within the matrix. They would appear to be no different to other micropollutants from anthropogenic sources encountered in drinking water.
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4. CRITICAL ASSESSMENT OF THE TOXICOLOGY OF THE ORGANIC BYPRODUCTS OF CHLORINE DIOXIDE

4.1 Introduction

The action of chlorine dioxide on organic byproducts is primarily by oxidation although the organic reaction products of chlorine dioxide disinfection are even less well studied than for ozone. In addition chlorine dioxide breaks down to chlorate and chlorite which are present at relatively high concentrations in the disinfected water, a combined concentration equivalent to approximately the dose of chlorine dioxide applied.

4.2 Mutagenicity of chlorine dioxide-treated water samples

A number of studies have been made of the mutagenicity of water samples following treatment with chlorine dioxide.

Backlund et al (1985) used the Ames test to determine the mutagenic activity of XAD-concentrated samples (pH 2.1) of humic water and alum flocculated humic water treated with chlorine dioxide. Samples of the treated water were adjusted to pH 2.1 prior to concentration on XAD 4/8, and the concentrates were tested for mutagenicity in S. typhimurium strains TA97, TA98 and TA100. Use of a combination of chlorine dioxide with chlorine resulted in a significantly decreased level of mutagenic activity in comparison with chlorine alone. No mutagenicity was observed in water treated with chlorine dioxide alone.

The potential of chlorine dioxide to exhibit substantially reduced mutagenic activity in Ames tests compared with that induced by chlorination was also demonstrated by Kool et al (1985). However, this effect was only observed at low doses of chlorine dioxide (<1 mgCl₂/l), and high doses (above those used in disinfection processes)
actually resulted in raised levels of mutagenic activity over the non-treated samples. Similarly, treatment of stored water from the Rhine and Meuse Rivers with chlorine dioxide (5 mg/l) resulted in an increased concentration of direct acting mutagens in XAD-concentrated samples, comparable to that produced upon chlorination (Zoeteman et al 1982).

A study of the effect of chlorine dioxide treatment on the mutagenic activity of stored river water and drinking water samples from six cities was performed by Kool and Hrubec (1986). The samples were concentrated (7000X) on XAD resins at neutral and acidic pH, and Ames' tests carried out, using *S. typhimurium* strains TA98 and TA100. The results obtained were variable, demonstrating that chlorine dioxide treatment could result in an increase, a decrease, or have little effect on the mutagenic activity of water from different sources.

Drinking water samples prepared by disinfection with chlorine dioxide, and concentrated by reverse osmosis (400X) at pH 2 showed no mutagenic activity in Ames tests, using *S. typhimurium* strains TA98 or TA100 either with or without metabolic activation. However, XAD-concentrated samples (1000X at pH 2) showed that chlorine dioxide treatment resulted in an increase in direct acting mutagenic activity in TA98 compared with untreated samples, although this activity was less than that observed in parallel streams treated with chlorine or monochloramine (Miller et al 1986).

In a study of the mutagenicity resulting from pilot plant water disinfected with chlorine, chlorine dioxide, chloramines and ozone (Anderson et al 1987), chlorine dioxide was the only oxidant capable of removing any observed mutagenic activity in raw water samples. However, only one dose level was used in the Ames' tests performed in this study.
Cognet et al (1986) studied the effect of using chlorine or chlorine dioxide as the final stage of water treatment on mutagenicity. Addition of chlorine or chlorine dioxide after GAC filtration showed that chlorine dioxide resulted in less mutagenic activity in Ames tests than did chlorination.

Studies on the substitution of chlorine with chlorine dioxide in the treatment of sulphite and kraft pulp mill effluents have demonstrated a marked reduction in mutagenicity of the final effluent (Moller et al 1986, Rapson et al 1980). The toxicity of pulp bleaching filtrates to Daphnia may also be reduced by low levels (4% and 10%) of chlorine dioxide substitution (Donnini 1983, Belt et al 1982).

4.3 Carcinogenicity of chlorine dioxide-treated water samples

There have been few studies investigating the carcinogenic effect of water treated with chlorine dioxide. Bull et al (1982) attempted to use mouse skin initiation/promotion assays to determine the carcinogenicity of water treated in this way. Administration of the concentrated water sample (X16R) to the skin of 50 mice, of which 75 animals subsequently received administration of the promoter TPA, induced a total of 8 systemic tumours (4 lung adenomas, 3 mammary gland tumours and one stomach tumour). No systemic tumours were observed in animals treated with non-disinfected samples. The percentage of animals with papillomas was also investigated and in two further repeat experiments performed in different seasons, and was found to be 0, 18 and 15%. Therefore, with such variation in results, no firm conclusions may be drawn regarding the carcinogenic activity of these water samples.

Miller et al (1986) investigated the carcinogenicity of water samples treated with
different disinfectants, concentrated by XAD resin adsorption (2000X and 4000X), using three short-term carcinogenicity assays.

1. The mouse lung adenoma assay. Male and female mice were administered 0.25 ml of sample by oral gavage, 3 times per week for 8 weeks. After an additional 16 weeks the number of lung adenomas were counted following lung perfusion.

2. The Sencar mouse initiation-promotion assay. Oral administration of 0.5 ml of sample, 3 times per week for 2 weeks, was followed 2 weeks later by topical administration (1.0 μg) of the promoter 12-tetradecanlyphorbai-13-acetate (TPA), 3 times per week for 20 weeks. After a total of 30 experimental weeks, the skin tumour incidence was determined.

3. The rat liver foci assay. Partially hepatectomised rats were orally administered the test sample for one week, after which sodium phenobarbitol was added to their drinking water for a further 56 days. All animals were sacrificed at day 70, and the number of gamma-glutamyltranspeptidase(GGT)-positive foci quantified. Concentrates of samples treated with chlorine, monochloramine, chlorine dioxide and ozone (4000X) all failed to give positive results in any of these assays.

4.4 Use of chlorine dioxide in the control of trihalomethanes

An important determination in consideration of the use of any alternative disinfectant is a comparison of its ability to form trihalomethanes (THMs) with that of chlorine. It is generally accepted that disinfection with chlorine dioxide alone does not result in the formation of THMs (Miltner 1976, Mallevalle 1978, Masschelin and Rice 1979, Oehler et al 1986).
Pre-oxidation by chlorine mixed with increasing concentrations of chlorine dioxide have been shown to result in decreasing concentrations of THMs (Miltner 1976, Schalekamp 1986), although in waters with a high bromide content, greater efficiency of THM removal was observed when chlorine dioxide was allowed to react with the precursors before the addition of chlorine (Rav-Acha et al 1985).

Pre-disinfection of raw water with chlorine dioxide followed by post-disinfection with chlorine in a pilot plant has been shown to reduce the concentration of THMs to approximately 40% that existing in the effluent of a full scale plant using pre- and post-chlorination (Lykins and Gries 1986).

Lin et al (1984) demonstrated the production of chloroform following treatment of aqueous model components of humic substances (2,4- and 3,5-dihydroxybenzoic acids, 2,4,6-trihydroxybenzoic acid and syringic acid) with a mixture of chlorine dioxide and chlorine.

4.5
Toxicity of organic byproducts

Little information is available on other products of reactions between chlorine dioxide and organic constituents of water, under conditions similar to those experienced during water treatment. Many of the identified products are the same as those observed to result from disinfection of water by ozonation. These include aldehydes (Stevens 1982), ketones (Chen et al 1982, Snoeyink et al 1982), aliphatic mono- and di-carboxylic acids (Colclough et al 1983), hydroquinone (Amor et al 1984), Rav-Acha et al 1983), benzoquinone (Belevzev 1985) and epoxides (Rice and Gomez-Taylor 1986, Lindgren and Ericsson 1969). The toxicity of these compounds has been reviewed under ozonation byproducts.
The reaction of organics with chlorine dioxide may result in the formation of a range of chlorinated products due to the presence of chlorine as a contaminant. The toxicology of the halogenated compounds produced during water chlorination has already been reviewed, (Hunt 1985, 1986, 1987, Hunt and Fawell 1986) and will not be considered further.

The reaction of chlorine dioxide with naphthalene has been shown to result in the production of phthalic acid (Taymaz et al 1979), and similar treatment of methyl naphthalenes led to the production of naphthaldehyde isomers, naphthoic acid isomers, naphthaquinones and chlorinated methyl naphthalenes. Methyl naphthalenes are known to have tainting properties in water. For example, 20% of the population are able to detect 0.0021 ppm and 2.0 ppm of 1- and 2-methyl naphthalene, respectively (Lillard et al 1975). However, it is not certain whether chlorinated methyl naphthalenes also possess similar odour potentials. Little toxicological information is available on any of these products, but mammalian studies have indicated that phthalic acid, and isomers of naphthaldehyde and naphthoic acid are of low acute toxicity (Registry of Toxic Effects of Chemical Substances 1986). Lake et al (1975) demonstrated that administration of seven daily oral doses of phthalic acid (850 mg/kg) to rats resulted in no changes of liver weight, compared with controls. Neither was there any significant alteration in the activities of biochemical parameters, including succinate dehydrogenase, glucose-6-phosphatase, aniline-4-hydroxylase, biphenyl-4-hydroxylase and cytochrome P-450. The genetic effect of phthalic acid on cells in vitro was tested by Phillips et al (1986). No chromosome aberrations were observed in Chinese hamster ovary cells or rat liver cells exposed to the acid.
It has been demonstrated that chlorine dioxide treatment may react with a number of polycyclic aromatic hydrocarbons to form quinone compounds. For example, anthraquinone and a number of benzpyrene quinones were identified following chlorine dioxide oxidation of anthracene (Rav Acha and Blits 1985) and 3,4-benzpyrene (Thielemann 1972), respectively. Anthraquinone was found to be non-mutagenic in Ames' tests, using $S$. typhimurium strains TA97, TA98 TA100, TA1535, TA1538 and TA2637, either with or without metabolic activation (Sakai et al 1985, Anderson and Styles 1978, Brown 1980, Tikkanen et al 1983). Administration of anthraquinone to mice orally (464 mg/kg) or subcutaneously (1000 mg/kg) daily, for 18 months, induced no significant increase in tumours compared with control animals (NCI 1968, Innes et al 1969). No further information on the toxicity of these products was found to be available.

Lin and Carlson (1984) identified a number of oxidised nitrogen heterocycles and ring-ruptured products following the reaction of chlorine dioxide with various heterocyclic compounds. However, the relevance of these reactions and products to those likely to occur in water treatment practice is uncertain, and little information on the toxicity of these products could be found.

A number of aromatic ketones and alcohols are reported to result from the reaction between hydrocarbons with benzylic hydrogen atoms and chlorine dioxide (Chen et al 1982). The ketones identified include acetophenone, benzophenone, indanone, fluorenone and tetralone. Little information is available on the toxicity of these compounds. Acetophenone, benzophenone and tetralone appear to be of low acute toxicity (Jenner et al 1964, Caprino et al 1976, Smyth et al 1969) and negative mutagenic activity has been
demonstrated for acetophenone, indanone and fluorenone in Ames' tests (Florin et al 1980). Information on carcinogenicity was only found for benzophenone, which failed to induce skin tumours in mice following lifetime dermal application of a 50% solution in acetone (Stenback and Shubik 1974).

The alcohols identified by Chen et al (1982) included benzohydrol, indanol, fluoreno1 and tetralol. These compounds are also of low acute toxicity (RTECS 1986), but little other information is available.

There is insufficient data available for those ketones and alcohols identified as products of the reaction between chlorine dioxide and hydrocarbons on which to base an assessment of the hazard they represent to human health.

4.6 Discussion

It is evident from the available data that treatment of water with chlorine dioxide has the potential to reduce mutagenic activity compared with chlorination. Similarly, mixtures of chlorine and chlorine dioxide have also proved useful in reducing the mutagenicity and concentration of trihalomethanes compared with chlorine alone. However, this is dependent on the dose used, since higher doses of chlorine dioxide may actually elevate the mutagenic activity. In addition, Miller et al (1986), discussed above, demonstrated that the detection of mutagens produced in chlorine dioxide-treated water may depend on methods of sample concentration used, or the concentration factor.

At present, there is little information concerning the production of organics during treatment of water with chlorine dioxide, under typical treatment plant conditions. The majority of those
products which have been identified do not appear to represent any significant hazard to human health. However, there are a number of identified products for which the likelihood of their formation during water treatment is unknown, and information concerning their toxicity is scarce. It is therefore possible that such compounds may be a cause for concern. In addition, it is likely that other organics products have yet to be identified. Therefore, at this stage it is difficult to make a prediction of the hazard to human health which treatment of water with chlorine dioxide would exert.


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5. CRITICAL ASSESSMENT OF THE TOXICITY OF CHLORINE DIOXIDE, CHLORITE AND CHLORATE

5.1 Introduction

During the disinfection process chlorine dioxide in water is converted to chlorite and chlorate, particularly at alkaline pH (Gordon et al 1972, Granstrom and Loe 1957, Medir and Giralt 1982).

\[2\text{ClO}_2 + \text{H}_2\text{O} \rightarrow \text{ClO}_3^- + \text{ClO}_2^- + 2\text{H}^+\]

At acid pH, chloride is an additional product of decomposition of chlorine dioxide (Brown 1952). Any ingested chlorine dioxide is rapidly converted to its inorganic reaction products in the mouth and stomach.

There have been numerous studies of the toxicity of chlorine dioxide and its byproducts to laboratory animals. One of the earliest such studies showed increased mortality in rats given 100 mg/l in their drinking water for two years (Haag 1949). It was shown that the apparent no effect levels in this study were 10 mg/l chlorine dioxide and 8 mg/l chlorite. However, the techniques used at that time would be unlikely to detect sub-clinical effects.

Later studies by Musil et al (1964) and Simon et al (1964) showed that, at high doses, chlorite was a fairly potent inducer of methaemoglobin in rats. This caused concern that the chlorite residual could be capable of causing problems similar to nitrite but at lower equivalent doses. Much of the modern research into the toxicity of chlorine dioxide and its breakdown products has been directed at studying the effects on the erythrocytes of red blood cells and the possible long-term consequences of such effects.
5.2 Metabolism and pharmacokinetics

The metabolism of chlorine dioxide, chlorite and chlorate has been studied in the rat (Abdel-Rahman et al 1980a, 1980b, 1982). Using 36Cl labelled compounds, radioactivity was shown to be rapidly absorbed from the gastro-intestinal tract. Chlorine dioxide was converted to chloride, chlorite and chlorate. Chlorite was excreted unchanged and as chloride, and chlorate was excreted unchanged and as chloride and chlorite. In all cases chloride was the major metabolite.

The plasma half-life of 36Cl was shown to be 44 hours but only about 40% of ClO₂-derived chlorine was eliminated in 72 hours.

5.3 Toxicology

The ability of chlorite to cause oxidative damage to erythrocytes was confirmed in vitro with cells from man, rat and guinea pig and in vivo in rats and cats (Heffernan et al 1979a, 1979b). However, methaemoglobin formation did not occur to any appreciable extent until biochemical damage to the red cell was well advanced. This consisted of glutathione depletion and increased hydrogen peroxide concentrations. Damage to the cell membrane was also observed by scanning electron microscopy. These findings indicate that chlorite belongs to the class of oxidants which produce haemolytic anaemia and differs from nitrite which does not display this activity (Cohen et al 1964a, 1964b, Abdel-Rahman et al 1980b, Couri et al 1982, Singelmann et al 1984). It is, therefore, unlikely to directly produce methaemoglobinaemia.

Exposure of rats to chlorine dioxide (0, 1, 10, 100 and 1000 mg/l), chlorite and chlorate (10 and 100 mg/l) in their drinking water for nine months resulted in a decrease in osmotic fragility of erythrocytes in all treatment groups. At 2, 4 and
6 months no significant haematological changes were observed in treated rats compared to control. However, after 9 months erythrocyte counts, haematocrit and haemoglobin were decreased in all treatment groups. Decreased blood glutathione was only seen in the chlorite and chlorate groups (Abdel-Rahman et al 1984a, 1985). However, there was no consistent dose response and only small numbers of animals were used. In a study with similar dose levels in rat and chicken, decreases in blood glutathione were observed after 6 months. Glutathione reductase was increased in the chlorine dioxide and chlorite groups but not in the chlorate groups (Couri and Abdel-Rahman 1980). Following in vitro studies on the mechanism of action of chlorine dioxide, chlorite and chlorate, Abdel-Rahman et al (1984b) concluded that the formation of disulphide bonds between sulphydryl groups in erythrocyte membranes and haemoglobin caused precipitation of haemoglobin and an apparent resistance to haemolysis.

Studies in strains of mice with differing levels of glucose-6-phosphate dehydrogenase (G6PD) activity indicated little difference between the strains in the haematological parameters measured following exposure to 100 ppm chlorine dioxide in their drinking water. Chlorite exposure under similar conditions produced increases in osmotic fragility, mean corpuscular volume and G6PD activity in red cells of both strains. When the tails of the mice were cut to obtain blood samples, a haemolytic crisis developed in the C57L/J (low G6PD) strain treated with chlorite (Moore and Calabrese 1979, 1980, 1982). Further studies with these strains to study the interactive effects of chlorine dioxide, chlorite and nitrite showed that after exposure to 100 ppm chlorite in the drinking water for 30 days increases in erythrocyte G6PD activity, mean corpuscular volume, osmotic fragility,
acanthocytosis (deformed erythrocytes) and erythrocyte cell size. There were no strain differences or synergistic effects with nitrite (Moore and Calabrese 1981). The authors caution that the suggestion that chlorite and nitrite could interact and could cause effects in high risk groups such as those with G6PD deficiency and neonates (Moore et al 1978), cannot be discounted since human G6PD deficient individuals have even lower levels of this enzyme than C57L/J mice.

Further studies in Dorset sheep, a variety of sheep with low erythrocyte G6PD, and man indicated an increase in methaemoglobin in sheep while in contrast humans showed decreases in glutathione (Moore et al 1980).

In non-human primates exposed to rising doses of chlorine dioxide ranging from 30 to 200 mg/l in drinking water and 25 to 400 mg/l chlorite and chlorate the chlorine dioxide was rapidly reduced to chlorite and chlorate in the mouth and stomach. A dose-dependent increase in oxidative stress on haematopoiesis was observed in the chlorite group indicated by decreased haemoglobin and erythrocyte count and increased methaemoglobin content. In addition, the chlorine dioxide exposed animals showed a dose related suppression of thyroid metabolism characterised by decreased plasma thyroxine levels (Bercz et al 1982). These findings were confirmed in primates, rats and pigeons (Harrington et al 1986, Orme et al 1985, Revis et al 1986). Subsequent studies have shown that the probable mechanism for this is by the oxidation of dietary iodide to its elemental form and subsequent reaction with organic compounds in the gastro-intestinal tract reducing its bio-availability (Harrington et al 1985, Revis et al 1986a, 1986b). However, it has been postulated that a trace iodinated molecule formed
in situ in the gastro-intestinal tract acts as an extremely potent anti-thyroid agent (Bercz et al 1986). In view of the fact that chlorine dioxide is unlikely to be present at the tap it would appear that the significance of this finding for public health is extremely doubtful.

Chlorate has been shown to produce nephritis in man and test animals at high doses and chlorite has even greater oxidising potential than with chlorate.

Studies in which mice with low erythrocyte G6PD and rats were given chlorite in drinking water at concentrations of 4, 20 and 100 ppm and 31.2, 125 and 500 ppm respectively for 30, 90 and 180 days could find no effects on kidney (Moore et al 1984, Connor et al 1985).

In studies on pigeons 1.5 ppm chlorine or 2 ppm chlorine dioxide in drinking water raised the plasma cholesterol of birds fed normal diets. Chlorite did not significantly increase plasma cholesterol. In birds fed a high lipid diet the increase was not significant at 2 ppm chlorine dioxide but at 15 ppm the increase was highly significant.

5.4 Mutagenicity and carcinogenicity

Sodium chlorite and sodium chlorate gave negative results in short-term in vivo studies for chromosomal damage (micronucleus assay and bone-marrow cytogenetics) and sperm-based abnormalities in mice (Meier et al 1985). Sodium chlorite has been studied in long-term bioassays for carcinogenicity. Rats and mice were given sodium chlorite in their drinking water at doses of 300 or 600 ppm and 250 or 500 ppm respectively for 85 weeks. It was concluded that sodium chlorite was not carcinogenic (Kurokava et al 1986).
Chlorite has been tested for reproductive and teratogenic effects in rats and mice. No teratogenic effects were observed in litters from A/J strain mice given 100 ppm sodium chlorite in the drinking water from conception to weaning. However, there was a small decrease in survival to weaning of pups in the treated group and pups of treated dams did not gain weight as rapidly as controls (Moore et al 1980). In addition, fewer treated dams produced litters than control dams which was interpreted as a reduction in conception rate.

No teratogenic effects were observed in rats with high doses of sodium chlorite in the drinking water or by intraperitoneal injections. Some maternal toxicity characterised by haematological effects were observed in dams receiving a 2% solution of sodium chlorite as drinking water or given intra-peritoneal injections of 10 or 20 mg/kg sodium chlorite. Injection of 50 mg/kg caused 100% mortality. Decreased litter size, increased stillbirths and resorption sites were seen in the groups in which maternal toxicity occurred (Couri et al 1982). Post-natal growth of the pups was not affected.

In a reproductive toxicology study in Long-Evans rats, males were exposed for 56 days and females for 14 days prior to breeding and during the 10-day breeding period to 0, 1, 10 or 100 ppm sodium chlorite in the drinking water. Females were also exposed during pregnancy and lactation. Decreases in serum thyroid hormones (T₃ and T₄) were observed on post-natal days 21 and 40 in pups exposed to 100 ppm sodium chlorite and above. Additionally groups of adult males were exposed to 0, 10, 100 or 500 ppm sodium chlorite in the drinking water. A significant increase in the percentage of abnormal sperm morphology and a significant decrease in
sperm direct progressive movement were seen in animals exposed to 100 ppm and 500 ppm of chlorite (Carlton et al 1987, Carlton and Smith 1985).

Alcide gel and liquid, germicidal agents consisting of sodium chlorite and lactic acid which produce chlorine dioxide when mixed have been tested for teratogenicity and foetal development in rats and mice. There were no significant differences in the treated groups which were treated topically with the gel at doses of 1 or 2 g/kg body weight or by gavage with the liquid at doses of 0.1 or 1.0 ml (mice and rats respectively) Skowronski et al 1985, Gerges et al 1985).

Chlorine dioxide, chlorite and chlorate administered in drinking water to rats for 3 months at doses of 10 or 100 mg/l inhibited $^3$H-thymidine into nuclei of testis, liver and kidney but increased incorporation into the nuclei of the small intestine (Abdel-Rahman 1984, 1985). However the group size was small and the significance is unclear in view of reports by other workers on the toxicity and mutagenicity of these compounds.

Studies on the neurobehavioural effects of chlorine dioxide showed a correlation between chlorine dioxide dosing, suppression of plasma thyroid hormone levels and suppression of exploratory activity of rat pups (Taylor and Pfohl 1984, Orme et al 1985). The effects on behaviour support the view that depression in serum thyroid hormones can result in developmental delays.

5.6 Studies in man

There have been several studies of the effects of drinking water containing chlorine dioxide or its breakdown products in human volunteers. These studies used small numbers of health and G6PD deficient volunteers drinking 500 ml of water per day containing 5 mg/l sodium chlorite for up to 12
weeks. No significant effects were observed in a battery of tests designed to measure the biochemical and physiological response to chlorite ingestion (Bianchine et al 1981, Lubbers et al 1982, 1984a, 1984b). This dose level is equivalent to an adult drinking 2 litres of water per day containing 1.25 mg/l sodium chlorite.

Two epidemiological studies have been carried out in populations exposed to chlorine dioxide disinfected water. In the first, morbidity and mortality of newborns in two similar communities were compared. The only significant positive association was between the exposure of the mother to chlorine dioxide treated water during pregnancy and prematurity of the newborn as assessed by the attending physician (Tuthill et al 1982). However, this finding was not significant when the age of the mother was controlled (Condie 1986).

The second epidemiological study was a prospective study conducted in a rural village in the USA. Data on haematology and serum chemistry were collected before exposure and after 3 months of drinking water which contained approximately 5 mg/l chlorite, failed to identify any exposure-related effects. However, the one G6PD-deficient individual in the study showed a decline in red cell count, haemoglobin and haematocrit over the exposure period and this returned to normal 3 months following cessation of exposure (Michael et al 1981).

5.7 Discussion

The toxicology of chlorine dioxide, and its breakdown products chlorite and chlorate, in drinking water indicates that the most probable effect at the low doses used in drinking water disinfection is oxidant stress on the erythrocytes. The groups at most risk from such effects would be
neonates whose haemoglobin is more susceptible to oxidation and who have a high intake of water in relation to body-weight, and individuals suffering from G6PD deficiency. This genetic defect is most common in those of African origin and from the East Mediterranean but rare in caucasians. The major uncertainty relates to additive effects with other oxidants, eg bottle-fed infants who are also exposed to high nitrate/nitrite.

The effects on thyroid observed in a number of studies seem to be unlikely to be of significance to man if oxidation of iodine in the gastro-intestinal tract is first required. This appears to be the case and unless substantial new evidence is produced to change this view it would appear that these findings can be ignored.

The Russian standard for the use of chlorine dioxide for disinfection appears to be 0.5 ppm (Fridlyard and Kagan 1971) based on studies in rats in which the weight gain in the group given water containing 5 mg/l chlorine dioxide was reduced.

The Safe Drinking Water Committee of the United States National Academy of Sciences (USNAS) have calculated no adverse response levels for chlorine dioxide, chlorite and chlorate. They calculated a 24-hr suggested no adverse response level for chlorite and chlorate of 0.125 mg/l and a 24-hr SNARL for chlorine dioxide of 1.2 mg/l on the basis of human studies. They also calculated a 7-day SNARL of 0.125 mg/l but claimed there were insufficient data to calculate a SNARL for chronic exposure (USNAS 1982).

It is clear from the data available that chlorine dioxide disinfection requires very careful control since the uncertainty factor between the apparent no effect level in human studies and the
concentrations found in drinking water is less than 10. This is extremely unusual for any chemical to which the general public will be exposed for long periods of time. If the use of chlorine dioxide were to become much more widespread in the UK then epidemiological studies of high risk groups in long-term exposed populations would be desirable to improve confidence in the safety in use of chlorine dioxide. This would be particularly desirable if the allowable concentration of chlorine dioxide and its breakdown products in final water remains at 0.5 mg/l.


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6. CRITICAL ASSESSMENT OF THE TOXICITY OF CHLORAMINE AND THE ORGANIC BYPRODUCTS OF CHLORAMINE DISINFECTION

6.1 Introduction

The use of chloramine in water treatment may result in the presence of inorganic residuals in the distribution system. Therefore, determination of the risk to human health represented by use of chloramine must involve consideration of the toxicity of the disinfectant itself, in addition to the toxic effects of any reaction byproducts. The requirement for an evaluation of the former has been recognised by a number of workers, and as a result of this, most of the available information was found to be concerned with the toxicity of chloramine itself.

6.2 The toxicity of chloramine

Rats given a single oral dose of 3.3 mg/kg body-weight of \(^{36}\)Cl-labelled chloramine exhibited half-lives for absorption and elimination from plasma of 2.5 hours and 38.8 hours respectively. \(^{36}\)Cl-labelled chlorine dioxide was absorbed significantly quicker than chloramine or hypochlorous acid but all three had similar half-lives of elimination. By five days after dosing, rats treated with chloramine had excreted about 25% of the label in the urine and 2% in the faeces. \(^{36}\)Cl-label remaining in the body at this time was greatest in the blood followed by testes, kidney, lung, stomach and bone marrow (Abdel-Rahman 1985).

In a 30-day study (Moore et al 1980), groups of 12 male A/J mice received drinking water containing 0, 2.5, 25, 50, 100 or 200 mg/l of chloramine (in pH 8.9 carbonate-bicarbonate buffer). The parameters measured were mortality, body-weight, water consumption, complete blood counts, reticulocyte counts and erythrocyte osmotic
fragility, glutathione and G6PD activity. The only statistically significant effects were slight weight loss and an increase in haematocrit at the 50, 100 and 200 mg/l dose levels and decreased water consumption with all concentrations above 2.5 mg/l.

Rats exposed for 45 days to drinking water containing 0, 10, 50 or 100 mg/l of chloramine (made up in a 0.05M boric acid buffer) exhibited no effects on body-weight gain, haemoglobin, packed cell volume and glutathione, while methaemoglobin levels were in fact slightly lowered at the highest dose level (Bull 1980).

African green monkeys receiving drinking water containing 100 mg/l of chloramine (dose equivalent to 10 mg/kg/day) exhibited little change in serum thyroxine levels after 42 days (Condie and Bercz 1985).

Revis et al (1986) investigated the effect of chloramine in drinking water on plasma thyroxine levels in pigeons, and also measured plasma cholesterol as a means of determining effects on the cardiovascular system. Exposure of the pigeons to 2 mg/l chloramine resulted in reduction of plasma thyroid hormone (T4) concentrations and an elevation of plasma cholesterol levels.

In a subchronic study (US National Toxicology Program 1981), groups of 10 male and 10 female F-344 rats and B6C3F1 mice were given drinking water containing either 0, 25, 50, 100, 200 or 400 mg/l of chloramine for 13 weeks. Compared to control animals, a depression in body-weight was observed for male rats at the two highest dose levels, for female rats at the highest dose and for both male and female mice at the two highest dose levels. The depression in body-weight gain may
have been related to the palatability of the drinking solutions. Male rats at the two highest dose levels also exhibited lowered absolute liver weights but there were no changes in liver-weight to body-weight ratios. However, both absolute liver weights and liver-weight to body-weight ratios were lowered in male mice at the 400 mg/l dose level and in females at 100, 200 and 400 mg/l. No pathological changes were observed in the rats but there was elevated urinary excretion of protein in males at the two highest dose levels. Female mice exhibited necrotic changes in the liver at low doses and an inflammatory response in the liver at the 100, 200 and 400 mg/l dose levels. Proliferative changes were observed in the livers of male mice at 100, 200 and 400 mg/l and to a lesser extent in female mice at 200 mg/l. The results of these experiments were used to recommend the dose levels for a chronic study. This study is now in progress.

Maziarka et al (1976) reported no effects on growth, behaviour, haemoglobin or histopathology of Wistar rats receiving drinking water containing up to 9 mg/l of chloramine for 12 months.

In another study (Abdel-Rahman et al 1984), male Sprague-Dawley rats received drinking water containing 0, 1, 10 or 100 mg/l of chloramine for up to 12 months. Depression of body-weight gain was noted at the highest dose level. Consistently lowered blood glutathione was evident in all treated groups. There was also evidence of increased blood osmotic fragility and although other changes in blood parameters were observed there was no consistent pattern over the treatment period. At three months, measurement of [3H]-thymidine uptake into cell nuclei of several organs revealed evidence of elevated DNA synthesis.
There is little information on the mutagenicity of chloramine in bacterial systems. Shih and Lederberg (1976) reported chloramine to be weakly mutagenic for reversion of trpC to trp+ in *Bacillus subtilis*. Thomas *et al* (1987) found little or no mutagenic activity for chloramine in the Ames test with *S. typhimurium* TA97a, TA100 or TA102 but some organic dichloramines were mutagenic, particularly to TA100.

Fetner (1962) reported evidence of chromosomal damage in *Vicia faba*. Seeds were soaked for 60 minutes in a 0.1 mM solution of chloramine (in pH 9 sodium borate buffer). They were then washed in dilute sodium thiosulphate solution and allowed to germinate. The number of abnormal anaphases (containing bridges or fragments) were elevated compared to a buffer control (28% incidence in chloramine treated seeds compared to 1% incidence in controls).

Chloramine gave negative results for genotoxicity in three in vivo tests in mice (Meier *et al* 1985). Chloramine solutions were prepared by addition of sodium hypochlorite to 1.5 M ammonium hydroxide in a molar ratio of 1:1. The tests were carried out at three dose levels; 40, 100 and 200 mg/l chlorine equivalents. In a micronucleus assay, groups of five male and five female CD-1 were given oral doses on five consecutive days. Six hours after the last dose the animals were killed and bone marrow erythrocytes examined for micronuclei. In a bone marrow chromosome aberration assay, groups of four male and four female CD-1 mice were treated in precisely the same way but in addition, some groups of animals were given only single doses and were then sacrificed at 6, 24 and 48 hours after exposure. Bone marrow samples were then prepared and metaphase cells examined for chromosome aberrations. For a sperm-head abnormality assay,
groups of 10 male B6C3F1 mice were given oral doses on five consecutive days. The animals were then killed at 1, 3 and 5 weeks after the last dose and sperm samples examined for sperm-head shape abnormalities.

The ability of drinking water disinfectants to induce epidermal hyperplasia in Sencar mice has been examined through measurement of skin thickness and cell numbers (Robinson et al 1986). Animals were treated by whole body exposure (except head) to 1, 10, 100, 300 and 1000 mg/l of either chlorine, chlorine dioxide or chloramine for a 10 minute period on each of four days. Although chlorine and chlorine dioxide did induce hyperplastic responses, treatment with chloramine had little effect on skin thickness or cell counts. In an earlier study (Pfeiffer 1978), repeated application of a 1% solution of chloramine to the skin of NMRI mice had no significant effect on the development of tumours when either applied with, before or after benzo(a)pyrene.

Chloramine gave negative results for induction of gamma-glutamyltranspeptidase-positive foci in rat liver. The rats were given a single dose of 14.75 mg/kg of chloramine, 24 hours after a 2/3 partial heptectomy. Seven days later the animals began receiving drinking water containing 500 mg/l of a promoter, phenobarbital. After 10 weeks of exposure to the promoter, the rats were sacrificed and liver sections were stained for the presence of gamma-glutamyl transpeptidase (Herren-Freund and Pereira 1986).

In a reproductive study (Abdel-Rahman et al 1982), female Sprague-Dawley rats were given drinking water containing either 0, 1, 10 or 100 mg/l of chloramine (in pH 9 bicarbonate buffer) for 2½ months. After mating, exposure to chloramine was
continued until the termination of the experiment on Day 20 of gestation. There was no evidence of teratogenic potential and very little evidence of embryotoxic potential for chloramine. In another study, Carlton et al (1986) dosed Long-Evans rats by gavage with either 0, 2.5, 5 or 10 mg/kg/day of chloramine. The males were treated for 56 days prior to breeding and throughout the 10-day breeding period while females were treated for 14 days prior to breeding and throughout breeding, gestation and lactation. No clinical signs of toxicity, haematological changes, effects on body-weight gain, or histopathological lesions in the reproductive tracts were evident in either the male or female rats. Sperm number, morphology, motility or progressive movement were also unaffected. No treatment-related effects were observed in the offspring though detailed examination for skeletal abnormalities was not carried out.

Lubbers and Bianchine (1984) studied the effects of rising dose of chloramine in human volunteers. A group of 10 healthy adult males were given 1 litre of drinking water containing 0.01 mg/l on Day 1, 1 mg/l on Day 4, 8 mg/l on Day 7, 18 mg/l on Day 10 and 24 mg/l on Day 13. During this period the subjects were allowed access to unlimited quantities of untreated water and no attempt was made to control daily activity or diet. An extensive battery of biochemical and physiological tests failed to reveal any significant changes compared to a control group. In a further study (Lubbers et al 1984), 10 adult male volunteers were given 500 ml/day of 5 mg/l chloramine for 12 weeks. Unlimited quantities of untreated water were again supplied to the participants for the duration of the study. No significant biochemical or physiological changes were found.
Studies in the US (Eaton et al 1973, Kjellstrand et al 1974, Jacoh et al 1975) have shown that chloraminated water can cause problems with dialysis. The adverse effect, development of acute haemolytic anaemia, is caused through both oxidation of haemoglobin to methaemoglobin and inhibition of erythrocyte reductive metabolism (by effects on hexose monophosphate shunt pathway). Hence, chloramine not only represents oxidative stress to red blood cells, but also inhibits reductive metabolism, the way in which the cell protects itself against oxidative stressors. As a consequence of the development of anaemia in renal dialysis patients, much of the experimental animal work on chloramine has been concerned with possible effects on the blood.

6.3 Studies on the byproducts produced by chloramine and their health effects

No chlorination or oxidation products were identified in diethyl ether extracts of chloraminated humic and fulvic acids. However, the colour of the fulvic material was bleached by chloramine (Seeger et al 1984). Similarly, Jensen et al (1985) found no evidence of ether extractable products of chloramine-fulvic acid reaction. Chloramine was also less reactive to aquatic fulvic acid compared to chlorine in terms of demand values and total organic halide.

Treatment of aldehydes and ketones with chloramine produces amides or chlorimines (Crochet and Kovacic 1973, Hauser and Hauser 1930). Chlorimines may be further transformed to nitriles (Hauser and Hauser 1930). Chloramine may also react with alkenes to produce other organic chloramines by addition of –Cl and –NH₂ across the double bond (Neale 1964) while the chlorine from chloramine is readily transferred to the nitrogen atoms of amines, amino acids and peptides producing other organic N-chloramines (Isaac and Morris 1983, 1985). Kanno
et al (1982a, 1982b) found that in the presence of excess ammonia, chloramine reacted with simple aromatic compounds to produce cyanogen chloride. However, Lin et al (1984) reported no substantial loss of biphenyl or naphthalene with chloramine at pH >6 either in the presence or absence of bromide. Carlson and Lin (1985) investigated the reaction between chloramine and several phenolic acids over a contact time of 24 hours. The major byproducts following chloramination of solutions of 4-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid, 2,4,6-trihydroxybenzoic acid and 4-hydroxy-3-methoxybenzoic acid were generally the monochloro- and dichloro-addition products. For 4-hydroxy-3-methoxycinnamic acid, the monochloro-cinnamic acid and 1-(4-hydroxy-3-methoxyphenol)-2-chloroethylene were the major byproducts. Chloramine was generally unreactive to heterocyclic compounds except at low pH (Lin and Carlson 1984). At pH 7, chloramine acts on both amino acids and nucleic acids though it tends to react more readily with the former, particularly cysteine, cystine, methionine and tryptophan (Jacangelo and Olivieri 1985).

Chloramine and organic chloramines can also react slowly with bromide to produce bromo- and bromochloroamines (Trofe et al 1980, Haag 1985).

Arber et al (1985) studied the effects of chloramine formed under four separate conditions at pilot plant scale. Chloramine was formed:

(i) far to the left of breakpoint where monochloramines predominate;

(ii) just to the left of breakpoint where dichloramines predominate;
(iii) following breakpoint, with subsequent addition of ammonia; and

(iv) preformed prior to addition to process stream.

At identical total residual chlorine concentrations, water treated under each of the above conditions showed differences with respect to trihalomethanes, total organic halide, organic chloramines, inorganic chloramines and bacteriological quality. Preformed chloramine resulted in the lowest trihalomethane and total organic halide levels but had a lower bactericidal effectiveness.

Chloramine disinfection and byproduct formation were investigated in another pilot plant study in the US (Lykins et al 1986, Lykins and Koffsky 1986, Miller et al 1986). Raw river water was pumped to the plant, clarified with dialkyldimethylammonium chloride or dimethylamine-type cationic polymers, fluoridated and then passed through high pressure sand filters. The water was then split into separate streams for disinfection with either chloramine, chlorine, ozone or chlorine dioxide. Contact times were about 30 minutes and residuals were 2.1 mg/l for chloramine (with 0.4 mg/l dichloramine), 1.0 mg/l for chlorine (with 0.2 mg/l chloramine and 0.3 mg/l dichloramine), 0.5 mg/l for both ozone and chlorine dioxide. Analysis of treated water samples for total organic halide (TOX), trihalomethanes and alkylbenzenes and the gas chromatographic profiles from flame ionisation detection and electron capture detection revealed that chloramination did result in the formation of organic byproducts but not to the extent of that of chlorination. Treated water samples were concentrated by either reverse osmosis at pH 2 (400-fold concentration) or XAD
resin extraction at pH 2 (4000-fold concentration) and the concentrates tested in five assays: the Ames Salmonella mutagenicity test, a 30-day toxicity test in mice, lung adenoma test in strain A mice, Sencar mouse initiation-promotion assay and rat liver foci assay. No samples gave significant results in the lung adenoma, Sencar mouse or rat liver foci assays and although some effects were noted in the 30-day test a consistent pattern of toxicity was not observed. In the Ames test, all reverse osmosis extracts gave negative results but XAD resin extracts of chloramine (3/4 samples) and chlorine (4/4 samples) disinfected samples were mutagenic in both the TA98 and TA100 strains. Extracts from chlorine dioxide treated water were mutagenic in strain TA98 only.

Unpublished studies by WRc have also demonstrated that concentrated extracts of chloraminated water are mutagenic in the Ames' test to S. typhimurium TA100 and TA98. Raw water samples were taken from lowland river sites and chloraminated in the laboratory at 2 mg/l for a contact time of 4 hours. The samples were concentrated by freeze-drying and the solids extracted with acetone.

In an abstract, Meier et al (1986) reported the effects of different disinfectants on the mutagenicity of clarified and sand filtered Mississippi river water. XAD resin extracts of samples disinfected with either chlorine, chloramine or chlorine dioxide were mutagenic in both the Ames and mouse lymphoma L5178Y TK+- assays, though chlorinated samples were the most potent. Ozone-treated or undisinfected samples had no activity in these assays. In an in vivo test, all samples proved negative for induction of micronuclei in mouse bone marrow.
Bull et al (1982) reported the results of three experiments on the ability of concentrated water samples to initiate tumours in mouse skin. Settled, coagulated and filtered Ohio river water was disinfected with either chlorine, chloramine, chlorine dioxide or ozone. In the first experiment, samples were concentrated 100-180 fold by reverse osmosis. Chlorinated, chloraminated and ozonated samples all induced a significant increase in skin papillomas compared to a non-disinfected sample. In two subsequent experiments, water samples were concentrated 400-fold by reverse osmosis supplemented with freeze-drying. In these cases, the disinfected water samples failed to increase the number of animals with skin tumours compared to non-disinfected samples.

A preliminary epidemiological study of 51,645 deaths in Massachusetts, USA, found no significant differences for death from different tumour types between communities drinking water that had been disinfected with either chlorine or chloramine (Zierler et al 1986). In another study, Marienfeld et al (1986) investigated cancer mortality rates following a change of disinfection practice in St Louis County. Until 1955 the plant added both chlorine and ammonia as the first stage of treatment, but after this time a higher pre-chlorine dose was given and ammonia was added only prior to distribution. The cancer rates for this area were compared with those for the population of St Louis City. In this case, water treatment was identical to the original method of St Louis County but St Louis City continued using this method after 1955. Trend analysis for the periods 1960-67 and 1972-76 revealed an increase in mortality rates from large bowel, liver and bladder cancer in St Louis County which was higher than that for St Louis City. However, total cancer mortality rate and most organ specific cancer
mortality rates were consistently higher for St Louis City than St Louis County.

6.4 Discussion

Few studies identifying the organic byproducts of chloramination of water have been performed and therefore an assessment of the hazard such compounds impose upon human health cannot be evaluated at present. However, it has been demonstrated that chloraminated water may cause anaemia in renal dialysis patients or in individuals showing glucose-6-phosphate dehydrogenase deficiency, through its ability to induce oxidant stress to red blood cells.

There is limited evidence for chloraminated water showing positive mutagenic activity in the Ames' test. However, other workers have demonstrated negative results in both mutagenicity assays in mice, and short-term carcinogenicity studies in rodents.


KANNO S, NOJIMA K and OHYA T (1982a) Formation of cyanide ion or cyanogen chloride through the cleavage of aromatic rings by nitrous acid or chlorine. IV. On the reaction of aromatic hydrocarbons with hypochlorous acid in the presence of ammonium ion. Chemosphere 11, 663-667.
KANNO S, NOJIMA K and OHYA T (1982b) Formation of cyanide ion or cyanogen chloride through the cleavage of aromatic rings by nitrous acid or chlorine. V. On the reaction of aromatic amines or phenolic compounds with hypochlorous acid in the presence of ammonium ion. *Chemosphere* 11, 669–673.


7. ULTRAVIOLET IRRADIATION

7.1 Introduction

Ultraviolet irradiation has been most commonly used for disinfection of small volume and remote rural supplies. Useful reviews of this treatment process have been published by Grocock (1984) and Collentro (1986).

Evaluation of the data available on ultraviolet irradiation is difficult as full operational details are not always given and hence dose is not easy to calculate.

Dose of ultraviolet irradiation is a function of intensity and time.

\[
\text{Dose} = [\text{intensity in microwatts/cm}^2] \times \text{time} \\
= \text{microwatt seconds/cm}^2
\]

However, if there is marked attenuation within the system due to large distances from the ultraviolet source or dissipation and absorption then minimum and maximum doses can be calculated from the equation:

\[
[S] = \frac{I}{4R}
\]

\[I = \text{intensity}\]
\[S = \text{intensity from UV source}\]
\[R = \text{distance from UV source}\]
\[= \text{absorbance coefficient}\]

7.2 Organic byproducts of UV irradiation

Very few investigations have been carried out on byproduct formation during drinking water disinfection with ultraviolet irradiation. In a pilot plant study (Zoeteman et al 1982, Kool et al...
1985), irradiation at 120 microwatt seconds/cm² was found to have little effect on the mutagenicity of stored Rhine water. Analysis of the treated water by GC-MS also revealed little effects on the volatile organic content though there were small rises in the concentration of some compounds including benzene and toluene.

It has been reported (Armstrong et al 1966) that ultraviolet radiation of wavelengths below 240 nm resulted in conversion of some organic nitrogen and nitrate in sea water to nitrite. In freshwater with a low nitrate content, Grocock (1984) observed about 1% conversion of nitrate to nitrite with two ultraviolet units used by Severn Trent Water.

In a laboratory experiment, Sierka and Amy (1985) found that ultraviolet irradiation of an aqueous solution of humic acid (4.3 mg/l) for 15 minutes had only a minor effect on both non-volatile total organic carbon and trihalomethane-forming potential.

The effects of ultraviolet irradiation of aromatic compounds in aqueous solution, in the presence of either nitrate or nitrite, have been investigated in a series of laboratory experiments by Japanese workers (Suzuki et al 1982a, 1982b, 1983, 1985, Ohta et al 1982). They found that irradiation of aromatic compounds in the presence of nitrate (16.5 mg/l NO₃/Nitrogen) or nitrite (16.2 mg/l NO₂/Nitrogen) produced mutagenic activity. Irradiation of the aromatic compounds in the absence of nitrate or nitrite did not result in mutagenic byproducts.

Some work has been carried out on the effects of ultraviolet irradiation of wastewater effluents. In laboratory experiments, Lee et al (1982) found
that irradiation of effluent samples at 27 000-29 000 microwatt seconds/cm² had very little effect on chemical oxygen demand, total Kjeldahl nitrogen and total organic carbon. Irradiation at 2000 and 5000 microwatt seconds/cm² also had minimal effects on six volatile constituents; chloroform, bromodichloromethane, trichloroethylene, tetrachloroethylene, ortho- and para-dichlorobenzene. Jolley et al (1983) irradiated effluent samples with ultraviolet light and assessed changes in the non-volatile organic content through the profile produced from HPLC separation. Ultraviolet irradiation at 25 000-30 000 microwatt seconds/cm² produced relatively few changes. However, at 60 000 microwatt seconds/cm² there was some destruction of a few of the original peaks while some new small peaks were produced.

Hein et al (1982) studied the effects of ultraviolet irradiation on coal conversion wastewaters (containing aromatic amines, oxygen and sulphur heterocyclics, and polycyclic aromatic hydrocarbons) and compared the biodegradation of irradiated and untreated samples in bench-top activated sludge units. Physical observation and gas chromatography analysis indicated that there were changes in composition, particularly in the base neutral fraction, following ultraviolet irradiation. Only minimal changes in total organic carbon and chemical oxygen demand were found. However, biodegradation of the irradiated sample proceeded more rapidly than with the untreated effluent.

In April 1978, samples were taken from the Northwest Bergen County wastewater treatment plant in the US and were concentrated (3000-fold) by freeze-drying. Both undisinfected and ultraviolet irradiated samples gave negative results for
mutagenicity in the Ames test. However, further samples were taken in August of the same year and following concentration by freeze-drying (1000-fold) the undisinfected sample gave negative results but the ultraviolet irradiated sample was weakly mutagenic in strain TA1535. The concentrated extracts from this second series of samples were also fractionated by HPLC. For the undisinfected sample, 3/18 fractions proved mutagenic and toxic to TA1535 and one other was weakly mutagenic in TA1538. It would appear that there were mutagens present in the concentrate from which these fractions were derived but the activity may have been masked by high toxicity. All the HPLC fractions from the ultraviolet irradiated sample gave negative results for mutagenicity. The mutagens present in the irradiated sample may have been lost or destroyed during the separation process (Cumming et al 1983).

Several studies have examined the effects of combined ozonation and ultraviolet irradiation on the degradation of organics (Kuo et al 1977, Peyton et al 1982, Glaze et al 1982, Prengle 1983, Sierka and Amy 1985, Benoit-Guyod et al 1986). In general, ozone in combination with ultraviolet irradiation degrades organic compounds more quickly than ozone alone. However, increasing the dose of irradiation tends to have little effect on the rate of degradation whereas the dose of ozone appears to be more critical.

7.3 Discussion

There are very little data on the by-products of UV disinfection. However, the data that does exist suggests that UV will have less effect on the organic matter present in the water than other disinfectants.
7.4 References


8. CONCLUSIONS

8.1 Production of organic by-products

From the data presented, it is evident that:

i) few studies of the reactivity of organics with disinfectants were conducted under realistic conditions;

ii) the studies which were conducted under realistic conditions made no attempt to relate by-products to starting material or vice versa;

iii) the remaining studies, whilst generally supplying data on the reactivity and products resulting from reactions between disinfectants and specific organic compounds were, for a variety of reasons (concentration, pH, contact time or temperature), atypical of normal water treatment practice.

While it has been possible to identify a number of organic by-products of disinfection by ozone, chlorine dioxide, chloramine and UV, the relevance of these compounds to disinfection under the conditions of water treatment practice is not clear. The range and quantity of compounds formed under water treatment conditions are likely to be very different.

In addition the problem encountered with the study of the organic by-products of chlorination; namely the difficulty of studying compounds not amenable to GC-MS also applies to the study of organic by-products from other disinfectants.

8.2 Organic by-products of ozonation

There are only limited data available on the likely organic by-products of ozonation. However, from
the data which are available there is no indication of the production of compounds which are likely to be of concern to health to the same extent as the by-products of chlorination. There are some possible by-products such as epoxides which would be of potential concern but these seem unlikely to survive to any significant extent in distribution.

The data on mutagenicity produced by ozonation is mixed. In comparison with chlorination it is difficult to predict whether mutagenicity will be produced under a given set of circumstances. However, ozone does appear to produce less mutagenicity than chlorination.

8.3 Organic by-products of chlorine dioxide

The production of organic by-products of chlorine dioxide has been relatively poorly studied and therefore it is difficult to draw any substantial conclusions. However, from the limited information available it would appear that many of the compounds produced are similar to those produced by ozonation.

The major implication with chlorine dioxide is that it is usually contaminated with chlorine and therefore some chlorinated compounds will be observed.

The information on mutagenicity indicates a potential to produce less mutagenicity than chlorination and to reduce mutagenicity when used in combination with chlorine. Only at high doses does it appear to give much higher levels of mutagenicity in contrast to ozone.

8.4 Inorganic by-products of chlorine dioxide

The effects most likely to be observed as a consequence of exposure to chlorite and chlorate is oxidant stress on the erythrocytes. The groups
most at risk would be those with a high water intake in relation to body weight and those more susceptible to the effects of oxidants on the red cells. Such groups would include dialysis patients, babies, particularly in areas of high nitrate, and individuals suffering from G-6-PD deficiency. There must remain some doubt about the safety in use of chlorine dioxide until further studies are carried out or until chlorite and chlorate levels entering supply are further restricted.

8.5
Organic by-products of chloramine and chloramine per se

There are very little data on the organic by-products of chloramination although there is only limited evidence for the formation of mutagens by chloramine. Chloramine may, however, act as an oxidant stressor on erthrocytes and has been demonstrated to cause anaemia in dialysis patients.

8.6
Organic by-products of UV irradiation

There are too few data available on the by-products of UV irradiation to draw any firm conclusions although there is some indication that UV will have less impact on organic matter than other disinfectants.

8.7
Overall conclusions

The data available on the organic by-products of alternative disinfectants are only limited. The major deficiency relates to studies of disinfection under the conditions of water treatment practice and of natural waters. Until such data are obtained no proper comparison with chlorination can be made. Chlorine dioxide, chloramine and UV have been very poorly studied and there are very little data of any sort.

One of the major concerns with chlorination has been the formation of mutagens. The alternative
disinfectants seem better in this respect but again there is a shortage of adequate data to make a fair comparison. In addition it is possible that lower mutagenic activity does not indicate a lower hazard if the mutagens formed are potent carcinogens. With regard to chlorine dioxide and, to a lesser extent, chloramine there are still gaps in the data on the effects of inorganic by-products. For chlorine dioxide the margin of safety between the concentrations found in water supply practice and possible health effects in consumers are relatively small. It is not possible to rule out health effects in high risk groups at these concentrations. More widespread use of chlorine dioxide would make reassurance in this regard even more desirable.

9. RECOMMENDATIONS

1. There is a need for analytical studies of the organic by-products of alternative disinfectants to chlorine under the conditions of water treatment practice and with natural waters. Survey work carried out with chlorination proved extremely successful by giving a clear indication of the incidence and concentrations of chlorination by-products. This would be less easy to carry out with the alternative disinfectants since fewer installations exist. However, consideration should be given to the use of pilot plants and obtaining samples from other European countries who use these disinfectants more frequently.

2. There is a need to carry out definitive studies of the mutagenicity formed by the alternative disinfectants so that a clear comparison can be made with chlorination. The same problems in studying these disinfectants apply as outlined above, however, initial laboratory work would
enable samples to be taken in the field with some confidence and use could be made of the appropriate water treatment plants in other European countries.

3. There is a need to provide reassurance that the inorganic by-products of chlorine dioxide and, to a lesser extent, chloramine do not pose a risk to vulnerable groups. It is difficult to see how such data could be obtained except by epidemiological studies since the animal models are of limited value in these circumstances due to the difficulties in making a quantitative extrapolation in the absence of appropriate data on Man.
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