CHLORINATED DRINKING-WATER

1. Description of the Process

1.1 History of chlorination of drinking-water

Chlorine in one form or another is by far the most commonly used chemical for the disinfection of water supplies. It is also active for other purposes associated with water treatment and supply, such as prevention of algal, bacterial and general slime growths in treatment plants and pipeworks, control of tastes and odours, and removal of iron, manganese and colour (White, 1986).

The history and use of chlorine in the treatment of water has been reviewed in detail (White, 1986), and the following summary is based largely on that work.

Chlorine was discovered in 1774 by Karl W. Scheele and identified as an element in 1810 by Humphrey Davy. Javel water (a solution of potassium hypochlorite) was introduced in 1785 by Berthollet, and the commercially important development of a cheap, stable bleaching powder, calcium hypochlorite, was achieved by Tennant in 1798.

One of the first reported uses of chlorination for the disinfection of water supplies was in 1897, when bleach solution was used to disinfect a water main in Maidstone, Kent, UK, following an outbreak of typhoid. Regular use in water treatment began around the beginning of the twentieth century. Probably, the first continuous application was in 1902 at Middelkerke, Belgium, where ferric chloride, used for 'coagulation' (see p. 47) was mixed with calcium hypochlorite, producing hypochlorous acid; in 1903, at Ostende, Belgium, chlorine was generated from potassium chlorate and oxalic acid. In the UK, the first known regular use (of sodium hypochlorite) was in 1905 in Lincoln after a typhoid epidemic. In 1908 in Chicago, IL, USA, George A. Johnson instituted chlorination by adding 'chloride of lime' to contaminated river water. Chlorination of a river water supply to Jersey City, USA, at the turn of the century was significant in that, in the litigation that developed, objections regarding the ineffectiveness, potential hazards and general undesirability of the addition of chlorine to water supplies were overcome. These developments were quickly followed by similar examples in most industrialized

countries. As a result, most large-scale public water supplies are now disinfected chemically by chlorine (White, 1986), although there are many small local supplies (small wells, private springs) that are not disinfected by any means.

Prior to the successful widespread introduction of chlorination, water treatment techniques existed that included filtration, followed by chemical precipitation and sedimentation techniques. These methods alone, however, could not guarantee a bacteriologically safe water supply.

The main diseases can be controlled (to varying extents) by good physical/chemical water treatment and chemical disinfection include typhoid fever, cholera, amœbic dysentry, bacterial gastroenteritis, shigellosis, salmonellosis, *Campylobacter* enteritis, *Yersina* enteritis, *Pseudomonas* infections, schistosomiasis, giardiasis and various viral diseases, such as hepatitis A (National Research Council, 1980; Hoff & Akin, 1986; White, 1986).

The early use of chlorine to disinfect drinking-water involved hypochlorite solutions. In 1910-20, it became possible to store and transport liquid chlorine, and the development of suitable chlorinator installations led to increased use of chlorine itself for this purpose, providing easier control and monitoring and better disinfection than the various hypochlorite solutions. Notable in these and subsequent developments in the field of water treatment chlorinators were Wallace and Tiernan, who patented a variety of control and safety devices (White, 1986). The introduction of chlorine-resistant plastics in the 1950s and increased understanding of the chemistry of chlorination hastened the process. Further major developments were the use of ammonia-chlorine reactions and the breakpoint phenomenon (see p. 51) to minimize the taste and odour of chlorine, precise control of chlorine residues by dechlorination with sulfur dioxide and, more recently, concern over organic chemical by-products and the possible need for their control. These developments are discussed in the following sections.

1.2 Overview of the addition of chlorine during drinking-water treatment

Before discussing the addition of chlorine during water treatment, it is useful to review the important stages of water treatment and the chemistry of chlorination.

(*a*) *Drinking-water treatment*

The fundamental purpose of water treatment is to protect the consumer from impurities that may be offensive or injurious to human health. A secondary purpose is to deal with impurities which, although not directly harmful to health, may cause problems such as corrosion and discoloration. These purposes are achieved by setting up barriers such as coagulation and filtration, which remove impurities by precipitation and particle capture. The final barrier is disinfection.

The main purpose of treatment prior to disinfection is to prepare the water for effective and reliable disinfection, for example by removing suspended solids which can impair disinfection efficiency.

Surface water sources, i.e., those exposed to air on the surface of the Earth, comprise waters of widely varying quality, from high quality waters containing little known contamination (such as treated or untreated wastewater) to lowland rivers that contain appreciable contamination from a variety of sources. Deep groundwaters, i.e., the water that is naturally contained in and saturates the subsoil, are normally of high quality. However, some groundwaters, particularly those that are shallow and those in highly permeable strata, are vulnerable to specific localized contamination by a variety of substances—especially volatile chlorinated hydrocarbons such as trichloroethylene (see IARC, 1987). Springs constitute a water source in which the groundwater meets an impermeable rock stratum and is 'forced' out of the ground; they are usually of high purity.

Surface waters are more prone to contamination than groundwaters and so more often need pretreatment. Some pretreatment may be afforded by storing the water in a reservoir, which can result in sedimentation of suspended solids and a significant reduction in the numbers of any pathogenic organisms present. Various additional pretreatment methods are used, generally to remove suspended solids and naturally occurring coloured impurities. The principles involved in these processes are discussed below. Apart from disinfection, high quality groundwaters need no or minimal physical or chemical treatment.

(i) *Coagulation, sedimentation and filtration*

Coagulation: Some impurities in natural waters cannot be removed by settling alone, either because they are dissolved or because they occur in a very finely divided ('colloidal') state. The addition of a chemical coagulant is needed to create large particles that can settle, called 'flocs'. The coagulants most commonly used are aluminium and ferric salts. When these chemicals are added, a precipitate of the metal hydroxide forms which removes suspended solids, algae and colour by a number of mechanisms, including adsorption and trapping. Mechanical or hydraulic mixing causes the hydroxide precipitate, together with impurities, to agglomerate into flocs a few millimetres in diameter. Other chemicals, called polyelectrolytes, can be used in addition to, or in place of, aluminium or iron coagulants to produce stronger or larger flocs. Once formed, the flocs are removed from the water by filtration, generally preceded by sedimentation.

Sedimentation and flotation: Sedimentation is used to remove the bulk of the flocs, so as to reduce the load on downstream filters. Sedimentation may take place in rectangular or circular, horizontal basins in which discrete settling of flocs occurs or, commonly in some European designs, in 'floc blanket' clarifiers, in which the

water flows upwards through a fluidized bed of flocs and treated water is taken from the top of the clarifier. Flocs have a density only marginally greater than water, so treatment rates must be low. Typical tank loadings are < 1-5 m/h.

An alternative process to sedimentation is dissolved air flotation. In this process, water saturated with air under pressure is released into the water containing flocs, and tiny air bubbles become attached to the flocs and float them to the surface of the water. This is a faster process than sedimentation; typical loadings being 5-12 m/h. Dissolved air flotation may be particularly suitable for the treatment of coloured, low-turbidity waters and algal-laden waters.

Filtration: Deep-bed filtration through sand is employed to remove the remaining particulate matter. Water is passed through a bed of sand, typically composed of grains 0.5-1.0 mm in diameter, one-metre deep. Particles are trapped within the bed by a variety of mechanisms including straining, sedimentation, interception and electrostatic adhesion. Filtration rates are typically 4-10 m/h. As particles are trapped within the bed, the resistance to flow increases, necessitating a greater head of water (pressure) to maintain a constant rate of flow. Once a limiting head loss is reached, or solids start to be released from the filter, the filter is cleaned by backflushing with clean water.

In the treatment of turbid waters, filtration is almost always preceded by sedimentation, and filters are of the open 'gravity filter' type. With some low turbidity waters, including coloured moorland waters, the sedimentation stage may be omitted and direct filtration employed. With direct filtration, pressure filtration can be used to conserve a hydrostatic head.

(ii) *Slow sand filtration*

Slow sand filtration, which is a well-established process, is an alternative to the coagulation process for waters with little colour and a moderately low concentration of suspended solids. A slow sand filter consists of a 0.5-1.5-m-deep bed of fine (0.15-0.35 mm) sand, supported on a layer of gravel by a system of underdrains. At the low flow rates used (0.1-0.3 m/h), solids settle onto the surface of the sand. The layer formed, known as the 'Schmutzdecke', contains mud, organic waste, bacterial matter and algae and is biologically active. The mechanisms involved in slow sand filtration are: removal of colloidal material by straining, adsorption and bacterial action; destruction of pathogenic organisms by bacterial action; and purification of the water above the filter by bacterial action, flocculation and pathogen death.

As filtration progresses, the head loss through the bed increases to the point at which the required flow rate cannot be maintained. The filter is then taken out of action and the top layer is skimmed off manually or mechanically. The sand is washed for re-use. Eventually the depth of sand in the filter becomes insufficient for effective filtration, and more sand is added.

(iii) *Other processes*

A number of other processes may be employed prior to disinfection of water; these processes are applicable to groundwaters as well as surface waters.

Aeration may be employed for a variety of reasons, including removal of volatile taste- and odour-producing compounds, precipitation of iron and manganese and removal of carbon dioxide.

Oxidation may be used for purposes other than disinfection; these include precipitation of iron and manganese, taste and odour control, colour removal and oxidation of trace organic compounds. The principal oxidizing agents employed in water treatment are chlorine, chloramine, ozone and chlorine dioxide (White, 1986).

pH Adjustment, usually to more alkaline levels, is used to achieve optimal values for other processes, including coagulation and disinfection, as well as to reduce the corrosiveness of the water supply. pH can be increased by adding chemicals such as lime, caustic soda or soda ash or by placing the water in contact with a bed of sparingly soluble material, such as marble.

The pH of drinking-water is typically in the range 6.5-8.5, but levels up to 9.5 can occur.

Softening: Hardness in water results from the presence of calcium and magnesium compounds. When hardness is excessive, it can be reduced by precipitation softening or ion exchange. In precipitation softening, lime (and sometimes soda ash) is added to precipitate calcium as calcium carbonate, which is removed in a sedimentation tank. Ion-exchange softening is used only for groundwaters; the water is passed through a bed of cationic resin which exchanges sodium for calcium and magnesium. When the resin is fully loaded with calcium and magnesium, it is regenerated using a strong brine solution.

Activated carbon may be employed to remove natural and synthetic organic chemicals. It is produced by the controlled combustion of wood, coal and other material to produce a porous material with a large surface area and a high affinity for organic compounds. A slurry of powder can be added to the water and then removed by subsequent treatment processes, such as coagulation. Alternatively, granular-activated carbon can be employed in purpose-built adsorbers, or as a replacement for some of the sand in a rapid gravity filter.

(*b*) *General chemistry of the addition of chlorine*

The basic chemistry of water chlorination has been studied by a large number of workers and has been reviewed (National Academy of Sciences, 1979; National Research Council, 1980; White, 1986). The main features are as follows.

Chlorine dissolves rapidly in water to establish an equilibrium with hypochlorous acid (HOCl) and hydrochloric acid (HCl):

$$Cl_2 + H_2O \leftrightarrows HOCl + H^+ + Cl^- \ (pK_a = 9.5).$$

In dilute solutions and at pH levels above 4.0, the equilibrium is displaced to the right and very little molecular chlorine exists in solution. Between pH 6.0 and 8.5, hypochlorous acid dissociates almost completely to form the hypochlorite ion (OCl^-):

$$HOCl \leftrightarrows OCl^- + H^+.$$

At pH levels above 9.0, hypochlorite ions are the dominant species.

Alternative sources of hypochlorite ions are calcium hypochlorite and sodium hypochlorite. Essentially the same active species and equilibria are established whether the source of chlorine is liquid or gaseous or a hypochlorite compound.

The total concentration of molecular chlorine, hypochlorous acid and hypochlorite ion is defined as 'free available chlorine'. Total available chlorine may be defined as the mass equivalent of chlorine contained in all chemical species that contain chlorine in an oxidized state. Combined available chlorine can be defined as the difference between total available chlorine and free available chlorine and represents the amount of chlorine that is in chemical association with various compounds (usually amino- or ammoniacal nitrogen) but that is also capable of disinfecting. Free chlorine species are generally more effective disinfectants than combined chlorine species.

Raw (untreated) water may contain a large number of compounds that can react with chlorine species, including inorganic reducing agents (H_2S, SO_3^{2-}, NO_2^-, Fe^{2+} and Mn^{2+}, which are oxidized to, for example, SO_4^{2-}, NO_3^-, Fe^{3+} and MnO_2); ammonia and amino-nitrogen groups; and organic substances.

The principal effects of these side-reactions are the formation of by-products and a loss of disinfection efficiency as active chlorine species are reduced to less active combined species, particularly the non-bactericidal chloride. The most significant side-reactions, in terms of chlorine demand, are those involving ammonia or amino-nitrogen groups.

The reaction between hypochlorous acid and ammonia in dilute aqueous solution yields, successively, monochloramine (NH_2Cl), dichloramine ($NHCl_2$) and trichloramine (more commonly known as nitrogen trichloride, NCl_3):

$$NH_3 + HOCl \leftrightarrows NH_2Cl + H_2O$$

$$NH_2Cl + HOCl \leftrightarrows NHCl_2 + H_2O$$

$$NH_2Cl + HOCl \leftrightarrows NCl_3 + H_2O.$$

Hypochlorous acid and ammonia may also react to yield nitrogen:

$$2NH_3 + 3HOCl \leftrightarrows N_2 + 3HCl + 3H_2O.$$

These reactions are dependent on pH, temperature and the initial ratio of chlorine to ammoniacal nitrogen.

An important reaction that often occurs in the chlorination of water is the formation of hypobromous acid from bromide:

$$HOCl + Br^- \rightarrow HOBr + Cl^-.$$

Even at low bromide concentrations, this reaction leads to readily detectable levels of brominated organic by-products, such as brominated trihalomethanes, due to the reactivity of hypobromous acid. Bromide concentrations in untreated water vary widely: for example, in nine rivers in various regions of the USA, bromide levels ranged from 10 to 245 µg/l (Amy *et al.*, 1985). The occasional detection of iodinated halomethanes is probably due to a similar mechanism involving iodides.

Organic chloramines are formed when chlorine reacts with amines, amino acids, proteinaceous material and other forms of organic nitrogen involving amino groups or linkages. Organic chloramines are usually formed at slower rates than inorganic chloramines and are not considered to be effective disinfectants. While some organic chloramines are stable, others are not and degrade to many other by-products.

Addition of chlorine to waters containing dissolved organic compounds can result in three possible reactions, which are classified as:

(i) addition,
(ii) ionic substitution and
(iii) oxidation.

While all of these reactions result in an increase in the oxidation state of the substrate, (iii) results only in unchlorinated products (Pierce, 1978). The amount of organic matter in untreated water varies considerably. Typically, high quality groundwater contains up to 1 mg/l (as organic carbon), river water contains 1-10 mg/l (as organic carbon), while upland water may contain up to 20 mg/l (as organic carbon) which is almost entirely of natural origin (in humic substances). The total organic matter present would be roughly double these concentrations.

The use of ammonia with chlorine in water treatment, often called the 'chloramination' or 'chloramine' process, is designed to convert fully or partially the free chlorine to chloramine. The chloramine produced has a disinfectant action. Although it is less effective than chlorine, it has a lesser tendency to react with organic matter to form by-products: it generates less chlorophenolic taste from phenol and, of more recent interest, fewer by-products such as trihalomethanes. Chloramines are also more persistent in the drinking-water distribution system. The development of the chloramination process has been reviewed (White, 1986).

Chloramination was popular until the discovery and understanding of the 'breakpoint phenomenon'. In breakpoint chlorination, the aim is to maintain an optimal free residue of chlorine; to achieve this, any ammonia in the water is destroyed by addition of sufficient chlorine. As described above, chlorine reacts

rapidly with ammonia in water to form monochloramine, dichloramine and trichloramine, depending on the ratio of chlorine to ammonia and other factors, such as pH. In practice, as the molar ratio of chlorine to ammonia increases towards 1:1, the combined chlorine residue in the water increases steadily. Beyond this ratio, i.e., with more added chlorine, the combined residue decreases quickly to a point beyond which further addition of chlorine produces a steady increase in free chlorine residue. This point (theoretically at around 1.5 mol chlorine to 1.0 mol ammonia) is the so-called breakpoint. For many waters, addition of sufficient chlorine to exceed the breakpoint, thus achieving a combined residue (free chlorine plus chloramines) containing about 85% free chlorine, produces the most satisfactory palatability. It was found recently that these levels of free chlorine often enhance levels of organic chemical by-products such as trihalomethanes; consequently, breakpoint chlorination has been replaced at some treatment works by other processes (White, 1986).

The concentration of chlorine entering the distribution system is often reduced slightly, to conform to operational requirements, by the addition of a small quantity of a reducing agent; typically, sulfur dioxide is used.

(c) *Addition of chlorine during water treatment*

Current drinking-water treatments reflect other objectives of chlorination, in addition to killing pathogenic organisms. These objectives include the destruction of substances and organisms that confer taste and odour on the supply and foul equipment, such as filters and pipelines, and the oxidation of undesirable chemical substances such as Fe^{2+} and Mn^{2+} in raw water.

Additions of chlorine during the treatment and distribution of drinking-water can be summarized as follows:

—prechlorination of raw water (i.e., prior to any treatment),
—addition at various points in the treatment process,
—addition after treatment but before distribution (i.e., final works disinfection),
—addition during distribution, and
—miscellaneous use during maintenance activities.

Prechlorination has been used extensively for the treatment of lower quality surface water. The amount of chlorine added is usually in the range of 1-10 mg/l—typically around 5 mg/l, although much higher levels have been used. Such additions of relatively large amounts of chlorine directly to raw water can produce high levels of by-products such as trihalomethanes; consequently, efforts have been made to reduce the level of prechlorination or to abandon it completely.

Chlorine (typically less than 5 mg/l) may also be added after coagulation/before sedimentation or after sedimentation/before filtration,

generally to maintain improved flow by preventing build-up of slimes and bacterial growth. At some works, chlorine is added (at 2-5 mg/l) to oxidize ferrous sulfate to ferric sulfate, which is then used as a coagulant.

The quantity of chlorine added for disinfection after treatment depends on the actual treatment process, but generally sufficient chlorine is added to provide the desired chlorine residue (free chlorine and chloramine), usually in the range of 0.5-1 mg/l. Higher levels have been used (e.g., up to 5 mg/l; White, 1986) when difficulties in maintaining a residue in distribution are experienced, for example, with long pipelines.

Within large distribution systems, further chlorine may be added to maintain a desired residue at consumer taps. The quantity of chlorine added, usually at a covered water storage reservoir, varies but is typically in the range of 0.5-2 mg/l.

High doses of chlorine (about 50 mg/l) are used for disinfecting new or repaired equipment such as distribution pipes; however, such highly chlorinated water is usually flushed to waste.

In Europe, the USA and in other industrialized countries, where most water supplies are disinfected, usually with chlorine, high-quality groundwater sources usually receive minimal treatment and relatively low doses of chlorine (up to around 1 mg/l) for disinfection. Surface waters generally receive more chlorine, depending on the quality of the source water, as discussed above.

1.3 Impurities in chlorine gas and liquid

Various processes have been used for the commercial production of chlorine gas and liquid; the relative popularity of each has often been governed by economic aspects—particularly the cost and availability of starting chemicals from other industrial processes. Most of the current production of chlorine is accomplished electrolytically from brine using diaphragm, mercury or membrane cells. To a lesser extent, hydrochloric acid is used instead of brine. Some chlorine is also produced by the catalytic oxidation of hydrochloric acid and the action of nitric acid on sodium chloride, known as the salt process.

The main impurities in chlorine that are of possible relevance to the quality of drinking-water are carbon tetrachloride (see IARC, 1987) and bromine. Generally, the level of carbon tetrachloride is such that the residual concentrations in drinking-water, if any, are very low. A detectable level (1 mg/l) that was reported appeared to be due to unsuitable chlorine manufacture (carbon tetrachloride was used in this particular process). Consequent to this incident, the American Water Works Association set a maximum level for carbon tetrachloride in chlorine at 150 mg/l (White, 1986).

Bromine in chlorine gas or liquid could result in brominated by-products. The levels of bromine in commercial chlorine available in the UK for drinking-water

treatment are, however, low (maxima, 850 and 2500 ppm (w/w) in two sources) (ICI Chemicals and Polymers Ltd, 1988), and typical levels in the USA are 50-125 ppm (maximum, 200 ppm) (The Chlorine Institute, USA, 1990).

1.4 Alternative disinfectants for drinking-water

Although chlorine is by far the most commonly used disinfectant (and oxidant) in drinking-water treatment, other chemicals, particularly ozone and chlorine dioxide, have been used for many years. Concern over possible risks to health due to the by-products of chlorination has led to a wider interest in alternatives.

Ozone is a powerful oxidant and an excellent disinfectant. It is used for treating drinking-water at many waterworks throughout the world, particularly in certain countries, for example France. It must be generated on site, and consequently it is less suited than chlorine to application at small treatment works. It does not leave a residue in the distribution system, since it decays quickly in water; therefore, if a residue is required, ozone must be used in conjunction with a disinfectant that gives such a residue (White, 1986).

Ozone produces a range of by-products, particularly aldehydes and organic acids (White, 1986), and it can generate low levels of bromoform (see monograph, p. 213) (Jacangelo *et al.*, 1989) by oxidation of bromide to hypobromous acid (Amy *et al.*, 1985). Evidence concerning the bacterial mutagenicity of ozonation by-products is conflicting; in general, ozone generates less mutagenicity than chlorine, but different mutagens are likely to be produced (National Academy of Sciences, 1979; National Research Council, 1980; Fielding & Horth, 1988).

Chlorine dioxide is used at a number of waterworks, particularly for water sources in which chlorophenolic tastes result from the use of chlorine (due to chlorination of phenol). It does not form trihalomethanes and it persists in drinking-water, which means that it provides a residue in the distributed supply. In use, however, it produces chlorite and chlorate, which must be carefully controlled, as they are relatively toxic species. The by-products of chlorine dioxide are not well characterized. In general, chlorine dioxide produces low levels of bacterial mutagenicity, but, as in the case of ozone, the mutagens involved are probably different from those produced by chlorine (National Academy of Sciences, 1979; National Research Council, 1980; Fielding & Horth, 1988).

Monochloramine is a less powerful disinfectant than chlorine, ozone or chlorine dioxide, but it is more persistent in drinking-water and has been used to maintain a low residual level in a distribution system over many years. Recently, interest in its use on a more substantial scale has been raised because it does not lead to high levels of trihalomethanes (Jacangelo *et al.*, 1989). Little information is available on the by-products of chloramine; it generates bacterial mutagenicity, but less consistently and at a lower level than does chlorine.

2. Occurrence and Analysis of Compounds Formed by the Chlorination of Drinking-water

2.1 Occurrence

The composition of chlorinated drinking-water to which the consumer is exposed varies according to location. The variables of established importance in the production of potentially toxic compounds are total organic carbon concentrations, pH, ammonium and bromide ion concentrations and the qualitative composition of the organic matter. Minor constituents are other inorganic ions such as nitrate, additives to drinking-water and other treatment processes.

The pK_a of HOCl (one of the forms that chlorine assumes in aqueous solution) is 9.5 (see equation on p. 50). At pH < pK_a, chlorination reactions are more prominent (White, 1986). Many by-products produced at low pH (2-7) are unstable at neutral to alkaline pH. This is particularly true of mutagenic chemicals formed on chlorination (Meier *et al.*, 1983, 1985). The concentrations of other by-products (e.g., dichloroacetic acid) appear to be more or less independent of the pH (Krasner *et al.*, 1989), while others decrease markedly at high pH (e.g., trichloroacetic acid and chloral). Conversely, the amount of trihalomethanes increases markedly as the pH becomes more alkaline. The pH of drinking-water is sometimes altered during the course of treatment (e.g., lime softening).

The relationship between chlorine dose and the amount of organic carbon that is present greatly affects the by-products formed. This becomes a critical issue in assessing whether the chlorine residue commonly maintained in chlorinated waters or the by-products of chlorination that are formed are responsible for any effects observed epidemiologically. The chlorine:carbon molar ratios normally found during the chlorination reaction in drinking-water treatment are very different from those found in ingested water in the gastrointestinal tract. In drinking-water, the ratio is typically in the range of 1.0-1.5, and that in the gastrointestinal tract is much lower. As a consequence, data gathered in the USA, where fairly high residual levels of chlorine remain in treated water as it is consumed at the tap (0.5-2 mg/l), may not be applicable to practice in other parts of the world where residues are deliberately maintained at low levels (< 0.1 mg/l). Finally, it is important to recognize that the actual practice in many locations is to maintain residues as 'combined residuals' (e.g., by adding ammonia to form chloramine) after using chlorine or other chemicals for primary disinfection.

As a consequence, chlorinated water in different locations cannot be considered to be the same entity. This fact has added a complex dimension to

evaluation of the carcinogenic hazard for humans of chlorinated water that is not ordinarily encountered in these *Monographs*. Nevertheless, it was the view of the Working Group that this issue was of great importance to public health. Consequently, it endeavoured to make as objective an evaluation as is possible, given the vagaries of the data. The Working Group considered it important that the appropriate public health and regulatory authorities recognize the need to clarify the *broad* issue of drinking-water disinfection with appropriate research efforts in the near future. This issue must be resolved in a way that first protects against the waterborne infectious diseases observed in past centuries and then provides for minimizing or even eliminating any carcinogenic hazards that are secondary to this primary goal.

The addition of chlorine to waters containing dissolved organic compounds results in complex reactions that lead to chlorination by-products. The nature and extent of reaction of organic substrates in natural waters with chlorine is controlled by several factors, particularly pH and the chlorine:substrate ratio. An additional factor of importance is the presence of bromide in the untreated water (see p. 54), which can lead to brominated compounds.

Improvements in analytical techniques over recent years have revealed a complex range of organic substances in water supplies (Commission of the European Communities, 1989), and it has become apparent that many of these are generated during water chlorination. The probable organic precursors of these substances occur commonly and are of natural origin; they include humic substances and organic nitrogen compounds, such as amino acids (White, 1986).

The following sections summarize the available information on groups of halogenated by-products, selected mainly on the basis of the frequency of their occurrence in chlorinated water.

(*a*) *Trihalomethanes*

The production of chloroform (see IARC, 1987) during chlorination of natural waters was first observed by Bellar *et al.* (1974) and Rook (1974); this finding initiated many investigations into the identity, source and significance of chlorination by-products. Subsequently, a variety of additional trihalomethanes was detected (for example, Fawell *et al.*, 1986; Fielding & Horth, 1986), which include bromodichloromethane (see monograph, p. 179), chlorodibromomethane (see monograph, p. 243), tribromomethane (bromoform) (see monograph, p. 213), iododichloromethane, iododibromomethane, bromochloroiodomethane and chlorodiiodomethane. Total trihalomethane levels in treated drinking-water were reported in one survey in the UK (Water Research Centre, 1980): Chlorinated water derived from a lowland river contained a mean level of 89.2 μg/l (SD, 0.9-3.9), and that from an upland reservoir, 18.7 μg/l (SD, 0.2-1.3). The study also showed that

chlorinated groundwater was contaminated by trihalomethanes to a significantly lesser extent than chlorinated surface waters. Chloroform was the predominant trihalomethane.

The occurrence of chloroform in drinking-water was reviewed in an earlier monograph (see IARC, 1979a), which indicated that unchlorinated waters contain low concentrations (typically < 1 μg/l), but chlorinated waters in several countries invariably contain chloroform at levels up to 311 μg/l (Symons *et al.*, 1975). Similar findings were reported in later surveys (for example, Brass *et al.*, 1977; Water Research Centre, 1980).

Chloroform was measured in a range of surface, reservoir, lake and groundwaters in the USA (Krasner *et al.*, 1989). The median values (according to season) ranged from 9.6 to 15 μg/l for chloroform, 4.1-10 μg/l for bromodichloromethane, 2.6-4.5 μg/l for chlorodibromomethane and 0.33-0.88 μg/l for bromoform. Concentrations of chloroform in 100 US surface waters were 0.1-1 μg/l (39%), 1-10 μg/l (49%), 10-100 μg/l (12%) and 100-1000 μg/l (< 1%) (Perwak *et al.*, 1980). Quaghebeur and De Wulf (1980) found mean total concentrations of trihalomethanes in Belgium of 7.7 μg/l in groundwater and 78 μg/l in surface water; chloroform was the predominant trihalomethane in treated surface waters. In the USA, three of 13 groundwater supplies had levels of < 0.2, 2.6 and 83 μg/l chloroform, while in the other 10 surface water supplies the levels ranged from 1.3 to 130 μg/l (Reding *et al.*, 1989).

Nicholson *et al.* (1984) reported chloroform concentrations in drinking-water from 17 countries at levels ranging from not detected to 823 μg/l; levels of bromodichloromethane ranged from not detected to 228 μg/l; those of chlorodibromomethane ranged from not detected to 288 μg/l; and those of bromoform from not detected to 289 μg/l.

Bromodichloromethane levels have been reported in many studies. In treated drinking-water, concentrations typically range from 1 to 50 μg/l, with higher or lower values at some locations compared with those in untreated water samples, which are typically less than 1 μg/l (see monograph, p. 179). Surface and groundwater samples showed a similar range; however, in certain groundwaters, the concentrations were higher than those in surface waters. An analysis of 19 550 water samples in the USA revealed a mean bromodichloromethane concentration of 11 μg/l (range, 0-10 133 μg/l) (US Environmental Protection Agency, 1985). [The Working Group noted that the very high concentrations seen may be misleading, since no information was available on possible contamination by wastewater or on measures of quality control.] Concentrations of bromodichloromethane in 118

surface waters in the USA ranged from 0.1 to 1 μg/l in 66% of the samples, 1-10 μg/l in 31% and 10-100 μg/l in 3% (Perwak *et al.*, 1980).

Chlorodibromomethane levels have also been reported in many studies. In treated drinking-water, concentrations typically ranged from 1 to 20 μg/l, with higher or lower values at some locations compared with those in untreated waters, which are typically less than 1 μg/l. Treated groundwater samples showed, in general, higher chlorodibromomethane concentrations than treated surface water (see monograph, p. 243). An analysis of 18 616 water samples in the USA revealed a mean chlorodibromomethane concentration of 10 μg/l (range, 0-10 133 μg/l) (US Environmental Protection Agency, 1985). [The Working Group noted that the very high concentrations may be misleading, since no information was available on possible contamination by wastewater or on measures of analytical quality control.] Concentrations of chlorodibromomethane in 115 surface waters in the USA ranged from 0.1 to 1 μg/l in 80% of samples and from 1 to 10 μg/l in 20% (Perwak *et al.*, 1980).

Bromoform has been determined in many chlorinated drinking-water samples (see monograph, p. 213). It was not usually found (< 1 μg/l) in untreated water sources in the USA (Symons *et al.*, 1975). Concentrations in surface water in the USA typically ranged from 1 to 10 μg/l, with a median of about 4 μg/l (Brass *et al.*, 1977; Perwak *et al.*, 1980). Maximal levels in chlorinated groundwaters tend to be higher (up to 240 μg/l) (Glaze & Rawley, 1979; Page, 1981). Levels of bromoform in chlorinated groundwater vary widely, probably because of variations in the natural bromide content; at high bromide levels, the median value for bromoform was 72 μg/l (Krasner *et al.*, 1989).

Heating or boiling drinking-water containing trihalomethanes causes the concentrations to decrease significantly (Table 1) (Lahl *et al.*, 1982).

Table 1. Effect of heating and boiling water on trihalomethane content[a]

Compound	Level (μg/l)				
	Original tap water	80°C: 1 min	100°C: 0 min	Boiling: 1 min	Boiling: 5 min
Chloroform	45.6	23.2	12.3	9.4	4.1
Bromodichloromethane	44.6	24.1	13.5	10.8	4.6
Chlorodibromomethane	42.3	24.1	14.4	12.3	5.5
Bromoform	35.9	21.3	13.9	13.5	6.8

[a]From Lahl *et al.* (1982)

Since the late 1970s, many countries have endeavoured to control the levels of trihalomethanes in water supplies to meet national standards, which range from 25 to 250 µg/l (World Health Organization, 1988). The World Health Organization (1984) set a guideline value for chloroform in drinking-water of 30 µg/l.

(*b*) *Halogenated acetic acids*

Halogenated acetic acids, although not investigated as thoroughly as trihalomethanes, are probably major chlorination by-products in drinking-water. Table 2 summarizes the levels reported.

Table 2. Halogenated acetic acids in chlorinated drinking-water

Water type (location)	Compound	Concentration range (µg/l)	Reference
Two chlorinated surface waters (USA)	Monochloroacetic acid Dichloroacetic acid Trichloroacetic acid Monobromoacetic acid Dibromoacetic acid	1 and 4 9.4 and 23 7.4 and 22 < 0.5 and 3.8 0.7 and 11	Jacangelo *et al.* (1989)
Range of surface, reservoir, lake, and groundwaters (USA)	Monochloroacetic acid Dichloroacetic acid Trichloroacetic acid Monobromoacetic acid Dibromoacetic acid	< 1–1.2[a] 5.0–7.3[a] 4.0–6.0[a] < 0.5–1.6[b] 0.9–19[b]	Krasner *et al.* (1989)
Tap water (reservoir) (USA)	Dichloroacetic acid Trichloroacetic acid	63.1–133 33.6–161	Uden & Miller (1983)
Tap water (Germany)	Trichloroacetic acid	Not detected–3	Lahl *et al.* (1984)
Surface waters (USA)	Trichloroacetic acid	4.23–53.8	Norwood *et al.* (1986)
Treated water (Australia)	Trichloroacetic acid Dichloroacetic acid	200 max (similar max)	Nicholson *et al.* (1984)

[a]Median value
[b]High bromide level

(*c*) *Halogenated acetonitriles*

A variety of halogenated acetonitriles (see monograph, p. 269) have been detected in chlorinated drinking-water samples, formed by the action of chlorine on natural organic matter in water (Oliver, 1983; Jacangelo *et al.*, 1989; Krasner *et al.*, 1989; Peters *et al.*, 1989). The levels found vary; the highest total concentration found was 42 µg/l in a survey in Florida (Trehy & Bieber, 1981). Halogenated acetonitriles were not detected in raw water (Oliver, 1983).

The most abundant of the acetonitriles is dichloroacetonitrile. In surveys in the USA, this compound was found in most chlorinated water supplies at concentrations of up to 24 μg/l, with a median of 1.2 μg/l. Bromochloroacetonitrile was found at concentrations up to 10 μg/l, with a median of 0.5 μg/l. Dibromoacetonitrile was found in some water supplies at maximum concentrations of 11 μg/l, with a median of 0.5 μg/l (Krasner *et al.*, 1989; Reding *et al.*, 1989).

(*d*) *Chlorinated ketones*

A range of chlorinated ketones are produced during chlorination (Table 3). Other chlorinated ketones that have been detected in drinking-water but have not been quantified, include 1,1,3,3-tetrachloropropanone, 3,3-dichloro-2-butanone, 1,1-dichloro-2-butanone, 1,1,1-trichloro-2-butanone and 2,2-dichloro-3-pentanone (Coleman *et al.*, 1984).

Table 3. Chlorinated ketones in chlorinated drinking-water

Water type (location)	Compound	Concentration range (μg/l)	Reference
Range of surface, reservoir, lake and groundwaters (USA)	1,1-Dichloropropanone	0.46–0.55[a] 2.2 (max)	Krasner *et al.* (1989)
	1,1,1-Trichloropropanone	0.35–0.80[a] 2.4 (max)	
Two chlorinated surface waters (USA)	1,1-Dichloropropanone 1,1,1-Trichloropropanone	0.16–0.24 1.1–1.8	Jacangelo *et al.* (1989)
Drinking-water (Australia)	1,1,1-Trichloropropanone	20 (max)	Nicholson *et al.* (1984)

[a]Median

(*e*) *Halogenated phenols*

Chloro-, chlorobromo- and bromophenols can be formed from phenol during chlorination. They add objectionable tastes or odours to drinking-water when present at levels over a few micrograms per litre. Although high concentrations may occur during phenol pollution of untreated water, typical levels in drinking-water are kept low to avoid consumer complaints. A recent investigation of drinking-water gave the following levels (μg/l): 2-chlorophenol, < 0.004-0.065; 4-chlorophenol, < 0.004-0.127; 2,4-dichlorophenol (see IARC, 1986), < 0.002-0.072; 2,6-dichlorophenol, < 0.002-0.033; 2,4,6-trichlorophenol (see IARC, 1987), < 0.008-0.719; pentachlorophenol (see IARC, 1987), < 0.004-0.034; bromodichlorophenol, < 0.002-0.78; chlorodibromophenol, < 0.004-0.022; 2,4-dibromophenol, < 0.002-0.084; and 2,4,6-tribromophenol, < 0.004-0.022 (Sithole & Williams, 1986).

(f) Other halogenated hydrocarbons

Other halogenated hydrocarbons have been detected in chlorinated drinking-water; although accurate quantitative data are not available, levels are typically < 1 µg/l (McKinney *et al.*, 1976; Suffet *et al.*, 1980; Anon., 1983; Coleman *et al.*, 1984; Kopfler *et al.*, 1985; Fielding & Horth, 1986; Fawell *et al.*, 1987; Horth *et al.*, 1989). These compounds include bromoethane (see monograph, p. 299), bromobutane, bromochloromethane, bromochloropropanes, bromopentachloroethane, bromopropane, bromopentane, bromotrichloroethylene, chlorobutane, chloroethane (see monograph, p. 315), dibromomethane, dichloromethane (see IARC, 1987), 1,1-dichloroethane, 1,2-dichloroethane (see IARC, 1979b), dichloropropene (see IARC, 1987), hexachloroethane (see IARC, 1979c), hexachlorocyclopentadiene, iodoethane, tetrachloromethane (carbon tetrachloride) (see IARC, 1987) and pentachloropropene. It is not clear, however, to what extent, if any, these compounds result from chlorination of water.

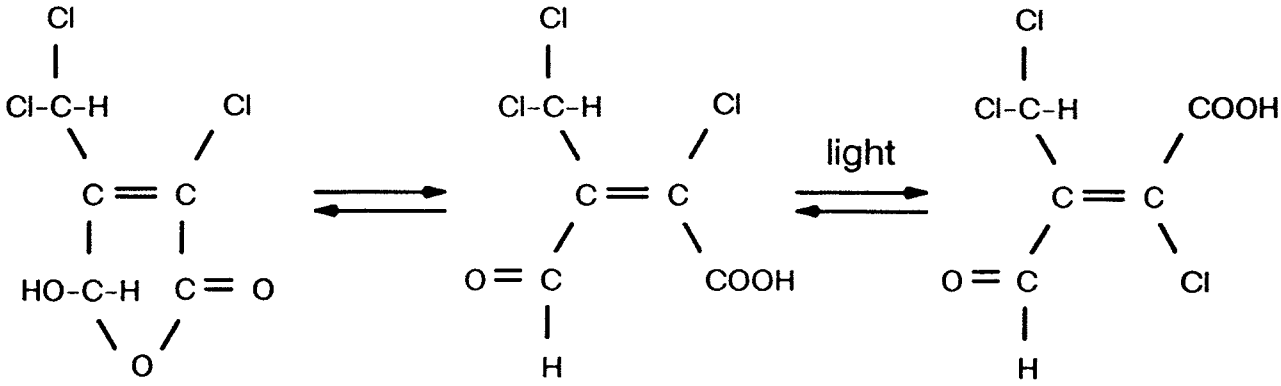
(g) Chlorinated furanones and related compounds

Studies on the possible identity of chemical mutagens formed during chlorination (see p. 71) have led to the detection in drinking-water (Kronberg & Vartiainen, 1988; Horth *et al.*, 1989; Fawell & Horth, 1990) of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone, referred to as MX, and E-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid, referred to as E-MX (see also the section on genetic and related effects, p. 66). Levels of MX and E-MX that have been detected are given in Table 4.

Table 4. Concentrations of MX and E–MX in chlorinated drinking-water

Water type (location)	Compound	Concentration range (µg/l)	Reference
Surface treated and chlorinated waters (Finland)	MX E-MX	< 0.004–0.067 0.002–0.059	Kronberg & Vertiainen (1988)
Treated and chlorinated lowland rivers (UK)	MX	Not detected–0.006	Fawell & Horth (1990)
Treated and chlorinated upland waters (UK)	MX	Not detected–0.041	Fawell & Horth (1990)

MX and E-MX are though to be related in the following manner:



(*h*) *Miscellaneous compounds found in chlorinated water*

Other compounds that have been reported to be present in chlorinated drinking-water are listed in Table 5.

Table 5. Concentrations of miscellaneous chlorination products in chlorinated drinking-water

Water type (location)	Compound	Concentration range (μg/l)	Reference
Eight treated waters (UK)	5-Chlorouracil 5-Chlorouridine 4-Chlororesorcinol 5-Chlorosalicylic acid	0.1–14.1 0.7–26.7 1.6–4.7 2.3–12.5	Crathorne *et al.* (1979)
Six treated waters (USA)	Chloral (hydrate)	7.2–18.2	Uden & Miller (1983)
Two utilities (USA)	Chloral (hydrate)	6.3–19	Jacangelo *et al.* (1989)
Range of surface, reservoirs, lake and groundwaters (USA)	Chloral (hydrate)	1.7–3	Krasner *et al.* (1989)
Range of surface, reservoir, lake and groundwaters (France, UK, USA)	Chloropicrin	0.07–1	Duguet *et al.* (1985); Fawell *et al.* (1986, 1987); Jacangelo *et al.*, 1989)
		0.10–0.16	Krasner *et al.* (1989)

Table 5 (contd)

Water type (location)	Compound	Concentration range (µg/l)	Reference
Range of surface and groundwaters (UK)	Bromodichloronitro-methane	Not quantified	Fawell *et al.* (1986)
	Bromochloronitromethane	Not quantified	
Not stated (USA)	Trichloropropenenitrile	Not quantified	Coleman *et al.* (1984)
Range of surface and groundwaters (UK)	Benzyl cyanide	Not quantified	Fawell *et al.* (1986, 1987)
	Chlorohydroxybenzyl cyanide	Not quantified	
Range of surface, reservoir, lake and groundwaters (USA)	Formaldehyde	2.1–17[a]	Krasner *et al.* (1989)
	Acetaldehyde	2.1–7.1[a]	

[a]Due to presence in untreated water and increase during chlorination

Formaldehyde and acetaldehyde were found in untreated water and were found at higher levels in water treated with various disinfectants, including chlorine. Ozone produced the highest levels (Krasner *et al.*, 1989).

(*i*) *Adsorbable organic halide*

The total halogenated matter generated by chlorination has been estimated by measuring adsorbable organic halide (halogenated organic compounds that can be adsorbed onto activated carbon; see p. 49). A recent survey of drinking-water (Krasner *et al.*, 1989) found median levels in the range of 150-250 µg/l. The relationships among the individual chlorination by-products covered by this measurement and between individual products and halogenated organic compounds vary substantially.

(*j*) *Sources of chlorination by-products*

At present, it is not possible to analyse all of the by-products of chlorination or other disinfectants/oxidants. In order to understand the production of by-products and to identify unknown by-products, many workers have studied substances occurring in raw water that could react with chlorine. Such studies have revealed by-products that have been found in water supplies and others that could be present but have not, as yet, been detected.

Rook (1977) suggested that humic substances are involved as precursors. These naturally occurring substances are an ill-defined mixture of chemically and

microbiologically degraded plant residues, bound together by chemical and physical processes, and are characterized as refractory, yellow-to-black materials. They are complex, high-molecular-weight, ubiquitous constituents of natural waters, where they consist mainly of humic and fulvic acids, the latter normally predominating. They vary in character to some extent from site to site and according to season; the organic matter in upland, coloured, natural water is mostly humic substances. They are extracted from water in several ways but usually by adsorption onto resins. Humic acids are defined operationally as becoming insoluble at pH < 2. Fulvic acids, however, are soluble in water at all pHs.

Several research groups have studied the chlorination of humic substances extracted from soil and water and confirmed that chloroform and dichloro- and trichloroacetic acids are produced as major reaction products. A variety of other products and intermediates have also been characterized. Christman *et al.* (1983) studied the chlorination of humic and fulvic substances extracted from water and found a wide variety of chlorinated saturated and unsaturated aliphatic acids. In a recent review, Christman *et al.* (1989) gave the significant products detected as: chloroform ($CHCl_3$), bromodichloromethane ($CHBrCl_2$), chloral (CCl_3–CHO), chloroacetic acid (H_2CCl–COOH), dichloroacetic acid (H_2CCl–COOH) and trichloroacetic acid (CCl_3–COOH), which are found in chlorinated drinking-water. Others produced in the laboratory are 2,2-dichloropropanoic acid (CH_3–CCl_2–COOH), 3,3-dichloropropenoic acid (CCl_2 = CH–COOH), 2,3,3-trichloropropenoic acid (CCl_2 = CCl–COOH), dichloropropanedioic acid (HOOC–CCl_2–COOH), butanedioic acid (HOOC–$(CH_2)_2$–COOH), chlorobutanedioic acid (HOOC–CH_2–CHCl–COOH), 2,2-dichlorobutanedioic acid (HOOC–CCl_2–CH_2–COOH), *cis*-chlorobutenedioic acid (HOOC–CH = CCl–COOH), *cis*-dichlorobutenedioic acid (HOOC–CCl = CCl–COOH) and *trans*-dichlorobutenedioic acid (HOOC–CCl = CCl–COOH).

Nonchlorinated products—for example, benzene carboxylic acids, carboxyphenylglyoxylic acids and mono- and dibasic alkanoic acids—were also reported. Depending on reaction conditions, chloroform, dichloro- and trichloroacetic acids accounted for over 50% of the adsorbable organic halides (see p. 63) produced during chlorination of humic substances. de Leer (1987) identified over 100 products of the chlorination of humic acids. These were mainly those found by previous workers, but, in addition, he described various other chlorinated carboxylic acids, cyano-alkanoic acids and trichloromethyl precursors of chloroform. Examples of the many precursors detected are: 3,3,3-trichloro-2-hydroxypropanoic acid (Cl_3C–CH(OH)–COOH), 4,4,4-trichloro-3-hydroxybutanoic acid (Cl_3C–CH(OH)–CH_2–COOH) and 2-chloro-3-(trichloroacetyl)butenedioic acid (COOH–(CCl_3–CO)C = CCl–COOH). These by-products have not been detected

in drinking-water but are probably reaction intermediates. The chloroform precursors may form chloroform in the following manner:

$$Cl_3C\text{–}CO\text{–}CCl=CCl\text{–}CCl_2\text{–}COOH \xrightarrow{H_2O} CHCl_3 + HOOC\text{–}CCl=CCl\text{–}CCl_2\text{–}COOH.$$

The cyanoalkanoic acids (which are presumably derived from nitrogen-containing elements of humic substances) were examined further by de Leer (1987). Cyanopropanoic acid and cyanoacetic acid (the latter was not detected as a chlorination by-product but its presence was postulated) reacted readily with chlorine. The following chlorination products were identified after reaction of cyanoacetic acid at pH 10: dichloroacetic acid ($CHCl_2$–COOH) and trichloroacetic acid (CCl_3–COOH), which are found in chlorinated drinking-water; and 2,2-dichloroacetamide ($CHCl_2$–$CONH_2$), 2,2-dichloro-*N*-hydroxyethaneimidoyl chloride ($CHCl_2$–CCl = NOH), 2,2-dichloro-2-carboxyacetamide (HOOC–CCl_2–$CONH_2$), 2,2-dichloropropanedioic acid (HOOC–CCl_2–COOH), 2,2,2-trichloro-*N*-hydroxyethaneimidoyl chloride (CCl_3–CCl = NOH) and 2,2-dichloro-2-carboxy-*N*-hydroxyethaneimidoyl chloride (HOOC–CCl_2–CCl = NOH). At lower pH, conversion to dichloroacetonitrile, dichloroacetic acid and dichlorosuccinic acid was favoured.

Several workers have concluded that most chlorination products are formed by a reaction involving 1,3-dihydroxybenzene (resorcinol) structures within the humic structure (Rook, 1980; Boyce & Hornig, 1983; de Leer, 1987; Christman *et al.*, 1989).

Unsaturated organic compounds, alkenes and unsaturated fatty acids such as oleic and linoleic acids, can react with chlorine in the laboratory under conditions similar to those of water treatment to form chlorohydrins (Gibson *et al.*, 1986); however, their presence in chlorinated drinking-water has not been investigated.

Amino acids are common constituents of raw water. Although they normally occur at low concentrations (typically up to 100 µg/l), bound amino acids, such as proteins and peptides, may predominate (Le Cloirec & Martin, 1985; Thurman, 1985).

The general reaction of amino acids with chlorine in aqueous solution has been known for many years, and reviews have been published (for example, Glaze *et al.*, 1982). It is now known that most, if not all, amino acids of the type R–CH_2–CH(COOH)NH_2 react readily with chlorine and initially form monochloramines (R–CH_2–CH(COOH)NHCl) and, depending on the conditions, dichloramines (R–CH_2–CH(COOH)NCl_2). Further reaction leads to nitriles (R–CH_2CN) and/or aldehydes (R–CH_2CHO). Le Cloirec and Martin (1985) postulated the mechanism involved. Amino acids have been shown to generate a wide range of other by-products during chlorination (Horth, 1989).

(*k*) *Mutagenic by-products*

Mutagenicity assays have been used in many countries to study the mutagenic potential of drinking-water samples (see p. 70). A number of the substances found in chlorinated drinking-water have been shown to be bacterial mutagens (Table 6). Only the chlorinated furanones and related compounds (see p. 61) are sufficiently potent and occur in sufficiently high concentrations to account for a significant proportion of the mutagenicity measured in some extracts of chlorinated drinking-water (Kronberg & Vartiainen, 1988; Horth, 1989). Many mutagens are generated during laboratory chlorination of humic substances and amino acids and during chlorination of wood pulp in experiments designed to indicate those substances that may be formed in the chlorination of natural waters; however, not all of these have been detected in drinking-water.

Table 6. Studies in which bacterial (*Salmonella typhimurium* TA100 without an exogenous metabolic system) mutagens were identified in chlorinated drinking-water, chlorinated solutions of humic substances and amino acids and in chlorinated wood pulp effluent

Mutagen	Reference[a]			
	Drinking-water	Humic substances	Amino acids	Wood pulp
Halo-alkanes				
Bromoform	1	ND	ND	ND
Bromochloromethane	1	ND	ND	ND
Bromodichloromethane	1	1,7	ND	2
Dibromomethane	1	ND	ND	2
Chlorodibromomethane	1	ND	ND	2
Dichloromethane	15	ND	ND	3
Bromoethane	1	ND	ND	ND
1-Bromopropane	1	ND	ND	ND
1-Bromobutane	1	ND	ND	ND
1,2-Dichloroethane	1	ND	ND	3
1,1,1-Trichloroethane	ND	ND	ND	3
1,1,2,2-Tetrachloroethane	ND	ND	ND	3
Iodoethane	1	ND	ND	ND
Chloro-alkenes				
Trichloroethylene	ND	ND	ND	2
Tetrachloroethylene	ND	ND	ND	2
Dichloropropene	1	ND	ND	ND
Tetrachloropropene	ND	ND	ND	2
Pentachloropropene	ND	4	ND	2

Table 6 (contd)

Mutagen	Reference[a]			
	Drinking-water	Humic substances	Amino acids	Wood pulp
Chloro–ketones				
1,1-Dichloropropanone	5	4,5	ND	ND
1,3-Dichloropropanone	ND	4,5	ND	2
1,1,1-Trichloropropanone	5	4,5	ND	ND
1,1,3-Trichloropropanone	ND	4,5	ND	ND
3,5,5-Trichloropent-4-ene-2-one	ND	ND	ND	2
1,1,3,3-Tetrachloropropanone	5	4,5	ND	2,3
Pentachloropropanone	ND	4,5	ND	3
Hexachloropropanone	ND	6	ND	2,3
Chloro-aldehydes/furanones				
Chloral (trichloroethanal)	1	16	7	ND
Chloroacetaldehyde	ND	ND	ND	2
2-Chloropropenal	ND	4	ND	2
Dichloropropanal	ND	4	ND	ND
2,3-Dichloropropenal	ND	4	ND	ND
3,3-Dichloropropenal	ND	4,5	ND	ND
Trichloropropanal	ND	4	ND	ND
2,3,3-Trichloropropenal	ND	4,5	ND	ND
2-Phenyl-2,2-dichloroethanal	ND	ND	7	ND
E-2-Chloro-3-[dichloromethyl]-4-oxo-butenoic acid (E–MX)	8	9	7	10
3-Chloro-4-[dichloromethyl]-5-hydroxy-2(5*H*)-furanone (MX)	8,11	9,10	7	2,12
3,4-Dichloro-5-[dichloromethyl]-5-hydroxy-2-furanone	ND	ND	ND	13
3-Chloro-4-[bromochloromethyl]-5-hydroxy-2(5*H*)-furanone (BMX-1)	ND	ND	14	ND
3-Chloro-4-[dibromomethyl]-5-hydroxy-2(5*H*)-furanone (BMX-2)	ND	ND	14	ND
3-Bromo-4-[dibromomethyl]-5-hydroxy-2(5*H*)-furanone (BMX-3)	ND	ND	14	ND
Halo–nitriles				
Bromochloroacetonitrile	1	ND	ND	ND
Dichloroacetonitrile	1	5,7	7	ND

Table 6 (contd)

Mutagen	Reference[a]			
	Drinking-water	Humic substances	Amino acids	Wood pulp
Miscellaneous				
Chloropicrin[b]	1	ND	ND	ND
Trichloro-1,2,3-trihydroxybenzene	ND	ND	ND	2
Benzyl chloride[c]	ND	ND	7	3
Benzoyl chloride[c]	ND	ND	7	ND
Bromo-*para*-cymene	ND	ND	ND	2
Dichloro-*para*-cymene	ND	ND	ND	2

[a]References: 1, Fielding & Horth (1986); 2, Rapson *et al.* (1985); 3, McKague *et al.* (1981); 4, Kopfler *et al.* (1985); 5, Meier *et al.* (1985); 6, de Leer (1987); 7, Horth (1989); 8, Kronberg & Vartiainen (1988); 9, Kronberg *et al.* (1990); 10, Holmbom *et al.* (1990); 11, Hemming *et al.* (1986); 12, Holmbom *et al.* (1984); 13, Strömberg *et al.* (1987); 14, Fawell & Horth (1990); 15, Anon. (1983); 16, Coleman *et al.* (1984)
[b]With S9 activation
[c]Tentative identification
ND, not detected

2.2 Analytical methods

Methods for the analysis of chlorinated compounds produced during the chlorination of drinking-water can be divided into three basic types: techniques for identifying unknown or suspected substances—not necessarily specific for halogenated compounds; specific techniques for the analysis of known or suspected halogenated compounds; and techniques designed for a gross estimate of halogenated organic matter in chlorinated water.

(*a*) *Analysis of unknown chlorination by-products*

In the 1970s, concern over the presence of organic micropollutants in drinking-water together with the emergence of powerful, sensitive analytical techniques for separating and identifying these substances, such as capillary column gas chromatography-mass spectrometry (GC-MS) led to the identification of a large number of organic compounds in drinking-water at low concentrations (Commission of the European Communities, 1989). Techniques involving GC-MS have been used extensively to analyse drinking-water for unknown and known chlorination by-products in addition to contaminants in general. With the exception of grossly contaminated drinking-water, concentrations of organic chemicals are such that direct application of identification techniques is usually

impossible and, consequently, some form of isolation/concentration process is required. The mixture of organic chemicals isolated is so complex that considerable separation (invariably by some form of chromatography) is also needed prior to application of instrumental techniques capable of providing structural information. Thus, the overall analytical technique deployed usually consists of:

(i) isolation/concentration (not necessarily as one step),
(ii) separation (of the components in the complex mixtures isolated) and
(iii) detection and structural analysis.

Various methods for isolating and concentrating organic chemicals, such as chlorination by-products, from drinking-water exist, and a number of validated methods have emerged that are based upon solvent extraction, adsorption (usually by XAD resin), followed by solvent elution of the adsorbent, headspace analysis and related methods. Application of these techniques is virtually routine, and examples abound in the literature (Keith, 1976; Coleman *et al.*, 1980; Keith, 1981; Fawell *et al.*, 1986).

(*b*) *Analysis of known or suspected chlorination by-products*

Analytical methods have been developed for a range of identified chlorination by-products. The following is a summary of those used for the substances discussed above.

(i) *Trihalomethanes*

Trihalomethanes were shown to be present in drinking-water as a result of chlorination (Bellar *et al.*, 1974; Rook, 1974) using purge and trap and solvent extraction methods of concentration, followed by GC with electron capture detection (ECD; Croll *et al.*, 1986). Subsequently, a number of analytical methods for the determination of trihalomethanes in drinking-water have been published (for review, see Croll *et al.*, 1986); they include direct aqueous injection (Nicholson *et al.*, 1977; Peters, 1980; Grob & Habich, 1983), liquid-liquid extraction (Dressman *et al.*, 1979; US Environmental Protection Agency, 1979a; Standing Committee of Analysts, 1980), purge and trap (Bellar *et al.*, 1974; Dressman *et al.*, 1979; US Environmental Protection Agency, 1979b) and headspace analysis (Rook, 1974; Otson *et al.*, 1979; Croll *et al.*, 1986) with separation and detection by GC-ECD. More information is given in the respective monographs about the analysis of bromodichloromethane, chlorodibromomethane and bromoform.

(ii) *Halogenated acetic acids*

Halogenated acetic acids are common by-products of water chlorination. Most of the analytical methods involve extraction into a solvent at low pH (0.5-2) with addition of sodium chloride to salt out the substances, derivatization and then

detection by GC-ECD (Krasner *et al.*, 1989; Uden & Miller, 1983; Lahl *et al.*, 1984), GC with microwave plasma detection (Miller *et al.*, 1982) or isotope dilution MS (Norwood *et al.*, 1986).

(iii) *Halogenated acetonitriles*

Dichloroacetonitrile and other halogenated analogues have been determined in drinking-water by solvent extraction with salting out using sodium chloride or sodium sulfate followed by GC-ECD (Oliver, 1983; Italia & Uden, 1988; Krasner *et al.*, 1989). More information on analytical methods for halogenated acetonitriles is given in the monograph.

(iv) *Chlorophenols*

Chlorophenols are well-known chlorination by-products, since they can confer objectionable tastes and odours in the supply. A variety of techniques have been developed for their analysis, which usually involve derivatization to methyl, acetyl or pentafluorobenzoyl derivatives, followed by GC-ECD (Renberg, 1981; Abrahamsson & Xie, 1983; Standing Committee of Analysts, 1985, 1988) or, in some cases, GC-MS with specific-ion monitoring (Sithole & Williams, 1986).

(v) *Chlorouracil, chlorouridine, chlororesorcinol and chlorosalicylic acid*

These unchlorinated substances occur in natural waters and can become chlorinated during water treatment. They have been determined after freeze-drying or vacuum evaporation, extraction with methanol and examination by high-performance liquid chromatography with confirmation by GC-MS (Crathorne *et al.*, 1979).

(vi) *Organic chloramines*

Chlorine can react extensively with organic amines, amino acids and related substances in water to produce chloramines. Specific analysis of organic chloramines in drinking-water is difficult, and, consequently, there is little detailed information on their presence and concentrations. In recent years, some specific methods have appeared which are based on derivatization followed by high-performance liquid chromatography with fluorescence detection (Scully *et al.*, 1984) or ultraviolet/electrochemical detection (Lukasewycz *et al.*, 1989).

(*c*) *Mutagens and mutagenicity in chlorinated drinking-water*

The presence of mutagenic chemicals in concentrated extracts of drinking-water is inferred from the positive results obtained in bacterial mutagenicity assays such as the *Salmonella*/microsome mutagenicity assay (Ames & Yanofsky, 1971; Ames *et al.*, 1975).

Organic compounds present at low concentrations in drinking-water must be extracted and concentrated prior to assays for mutagenicity. No single technique is capable of extracting all organic material from water, and therefore several methods have been used in combination with bacterial mutagenicity assays, including reverse osmosis or freeze drying, followed by extraction of the solids with organic solvent, or adsorption on resins followed by elution with solvents (for review, see Wilcox *et al.*, 1986). The most widely used technique involves adsorption on XAD macroreticular resin. Although a small proportion of the organic matter in drinking-water is recovered, the level of mutagenic activity of the extracts is high (Fielding & Horth, 1986; Ringhand *et al.*, 1987). Different compounds may be recovered by altering the pH of the water prior to XAD adsorption. Some groups have reported considerably higher levels of mutagenic activity in low pH/XAD extracts than in extracts obtained at sample pH (near neutral) (Kronberg *et al.*, 1985a; Ringhand *et al.*, 1987; Horth *et al.*, 1989). With all the methods, it is essential to check that mutagens are not generated as artefacts by the process itself, as impurities in solvents and other materials used or even their reaction products with free chlorine or chloramine in the water samples being processed.

Studies of the compounds responsible for the mutagenicity detected have led to the identification of strong bacterial mutagens, especially MX. The levels of this chlorination by-product have been determined in drinking-water by a method based on adsorption on resin at low pH followed by desorption with solvent, methylation and GC-MS with selected-ion monitoring (Hemming *et al.*, 1986; Horth *et al.*, 1989).

(*d*) *Measurement of total halogenated organic matter in drinking-water*

Methods have been used to estimate total (as near as possible) organically bound halogen in chlorinated drinking-water (for review, see Oake & Anderson, 1984). The basis of the most commonly used technique, which involves measuring adsorbable organic halogen, includes extraction of organic chlorine (or bromine) compounds from water by adsorption onto activated carbon, removal of inorganic halide by washing the carbon with a nitrate solution, conversion of organically bound halogen to inorganic halide (usually by combustion, although other approaches exist) and, finally, measurement of the halide (usually by microcoulometry). The terms 'total organic halide' and 'adsorbable organic halide' tend to be used in practice (Krasner *et al.*, 1989); however, the latter is preferable, since very polar and very volatile halogenated compounds would not be recovered quantitatively by the usual methods.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

Most of the studies reported here were designed to investigate the effects of organic extracts of drinking-water. These studies did not address the potential effects of by-products of disinfection, since that variable was not controlled for, i.e., generally, no control group of animals treated with extracts of raw water was included. Furthermore, the methods used to extract organic material from water were somewhat selective and would not result in equal concentration of all components (see p. 69); in particular, volatile substances may be lost. The extracts studied, therefore, may not be completely representative of the substances found in chlorinated water. Finally, the potential for introducing impurities into organic extracts by the interaction of free chlorine and chloramine in drinking-water with a solvent or resin may also be a confounding factor. Notwithstanding the difficulties in designing studies that control for this variable, it must be considered in their interpretation. Although of limited relevance to evaluating the carcinogenicity of chlorinated drinking-water, the studies of organic extracts are included for completeness.

(*a*) *Oral administration*

Mouse: Groups of 25 male and 25 female CFLP Han mice [age unspecified] were administered a chloroform (triple distillate) extract of disinfected river water from France (treatment procedure: flocculation, filtration, prechlorination, ozonization and postchlorination), dissolved in agar at a weight ratio of 1:20, prepared every two weeks and added to the diet for 104 weeks. The river water was collected over a two-year period. The treatment doses of organic material [1.2 and 2.4 mg/kg bw per day, respectively] corresponded to 100 and 200 times the calculated human dose, based on an assumed human consumption of 3 l/60 kg bw per day. The average yield of organic extract was 0.24 mg/l (mean of 10 samples). A group of 50 males and 50 females served as controls [control diet not specified]. No control receiving unchlorinated water was included. Increased mortality was observed in animals of each sex in the treated group [details not given]. The frequency of malignant tumours in males (predominantly thyroid gland tumours and lymphosarcomas) was: control, 4.9%; low-dose, 11.1%; and high-dose groups, 11.1%. The frequency of malignant tumours among females (predominantly mammary gland and ovarian adenocarcinomas and lymphosarcomas) was: control, 14.3%; low-dose, 43.8%; and high-dose, 45.0% (Truhaut *et al.*, 1979). [The Working Group noted the lack of an adequate control group to test for the extraction

procedure, and that the incidence of individual tumour types and the incidence of benign tumours were not given.]

Groups of 50 male and 50 female B6C3F$_1$ mice, six to eight weeks of age, received solutions of either chlorinated humic acids (carbon:chlorine ratio, 1:1 or 1:0.3), produced by the addition of sodium hypochlorite to a commercial preparation of humic acid, or unchlorinated humic acids in the drinking-water, prepared freshly once a week, for two years. The average daily intake of total organic carbon was 2.8-2.9 mg/mouse for males and 2.1-2.2 mg/mouse for females. Similar numbers of male and female mice received sodium chloride (daily intake, 26.4 mg/male mouse and 22 mg/female mouse) in the drinking-water. As a positive control, equal numbers of mice of each sex were given dibromoethane at doses of 1.4 mg/male and 1.2 mg/female. A group of 100 male and 100 female mice received no treatment. Surviving animals were killed at 24 months, with the exception of the dibromoethane-treated groups, which were killed at 18 months. At two years, more than 78% of treated and control animals were still alive, except among males given dibromoethane. There was no difference in the percentage or the number of tumour-bearing animals in the treated groups. Several types of tumours occurred at higher incidence in the groups treated with 1:1 chlorinated humic acids, 1:0.3 chlorinated humic acids or unchlorinated humic acids, when compared to the untreated group, but the incidences were not increased when compared to the sodium chloride-treated control group (Van Duuren *et al.*, 1986).

Rat: Groups of 25 male and 25 female Sprague-Dawley rats [age unspecified] were administered the same extract of disinfected river water described for CFLP Han mice, above, at the same treatment doses. A group of 25 males and 25 females served as controls [control diet not specified]. No control receiving unchlorinated water was included. A dose-dependent increase in mortaliity was observed in animals of each sex [details not given]. The frequency of malignant tumours in males (thyroid gland tumours and lymphosarcomas) was significantly increased: 0 in controls, 33.3% with the low dose and 50% with the high dose. The frequency of malignant tumours in females (mammary gland and ovarian adenocarcinomas and lymphosarcomas) was 4.5% in controls, 40% in low-dose animals and 57.1% in high-dose groups (Truhaut *et al.*, 1979). [The Working Group noted the lack of a control group to test for the extraction procedure, that exact incidences of individual tumours types were not given, that tumours of different origins were combined for analysis, and that the incidences of benign tumours were not given.]

Groups of 50 male and 50 female Wistar rats (RIV:Tox(M)), weighing 165 g and 130 g, respectively, were administered organic extracts of surface tap water from the Netherlands [disinfection procedure unspecified] in nonmutagenic drinking-water for 106 weeks. Water consumption was measured weekly. Extraction and concentration were carried out on XAD-4/8 resin, and elution with

dimethylsulfoxide, such that a 0.11-ml sample of concentrate contained 115 µg organic material, which corresponded to 1 l tap water. Daily dose levels were calculated as multiples of the expected human exposure based upon a daily consumption of 2 l water per 70 kg bw: 0, 4.5 times (11 mg/kg bw organic matter), 14 times (34 mg/kg bw), 40 times (97 mg/kg bw) for males and 0, 7 times (17 mg/kg bw), 22 times (53 mg/kg bw) and 68 times (165 mg/kg bw) for females. A slight increase in mortality was observed in the exposed groups. The numbers of animals with tumours (benign and malignant combined) were: males—control, 29/50; low-dose, 23/47; mid-dose, 27/50; and high-dose, 34/50; and females—control, 36/49; low-dose, 30/47; mid-dose, 33/47; and high-dose, 35/50. The frequency and types of tumours were similar in the treated and control groups (Kool *et al.*, 1985a). [The Working Group noted that no control group to test for the extraction procedure was used and that several contaminants are unstable in dimethylsulfoxide (see p. 82; Meier *et al.*, 1987; Kronberg & Vartiainen, 1988; Fielding & Horth, 1988).]

(*b*) *Skin application*

Mouse: Two groups of 40 male C57Bl mice, eight to ten weeks old, received skin applications of one drop of a tap water (collected over a period of one year) extract (preparation: US river water was treated by breakpoint chlorination, coagulation, filtration, concentration of organic compounds by passing through activated carbon, extraction of adsorbed organic matter with diethyl ether, removal of the ether by evaporation), either undiluted or diluted with methyl ethyl ketone (1:1) (Braus *et al.*, 1951). The original tap water contained 0.1-1 mg/l organic material. The extracts were painted on a 1-cm^2 area of shaved shoulder twice a week for five months. The two groups of mice were then combined, and the animals received one drop of undiluted sample twice a week for a further a 12-13 months, when survivors were killed. A vehicle control group of 25 male mice received one drop of methyl ethyl ketone on shaved skin twice a week for four months. One skin papilloma developed among the treated mice, whereas no skin tumour was observed among vehicle controls. Amyloidosis of the spleen, liver and kidneys was observed in several animals (Hueper & Ruchhoft, 1954). [The Working Group noted the lack of information on the quantity of tap water used for extraction, the quantity of organic material in the extracts and the lack of an adequate control group].

As part of a larger experiment, groups of 36 male and 36 female C57Bl mice, two months of age, received skin applications on the shaved neck region of either one drop of undiluted chloroform extract condensate prepared by passing chlorinated US tap water through activated carbon and elution with chloroform (yield, 1 g/620 gallons [2347 litres] water) once every two weeks (total of 28 applications), or one drop of undiluted ethanol-extract condensate prepared by passing chlorinated tap water through activated carbon (yield, 1 g/890 gallons [3369 litres] water) once every two weeks (total of 20 applications). Forty male and female

mice were untreated. The 12 surviving animals in the chloroform-extract group and the three in the ethanol-extract group were killed at 18 months. No tumour developed among the treated mice either locally or in distant organs (Hueper & Payne, 1963). [The Working Group noted the infrequent application of the material and the lack of an adequate control group.]

(*c*) *Subcutaneous administration*

Mouse: Groups of 36 male and 36 female C57Bl mice, two months of age, were injected subcutaneously in the neck region at two-week intervals with 4 mg condensate prepared by passing chlorinated tap-water from the USA through activated carbon and extraction with chloroform (yield, 1 g/620 gallons [2347 litres] water) in 0.05 ml tricaprylin (total of 28 injections [total dose, 112 mg/mouse]). Additional groups of 36 male and 36 female C57Bl mice were similarly treated with 4 mg of tap water condensate prepared by passing chlorinated drinking-water through activated carbon and extraction with ethanol (yield, 1 g/890 gallons [3369 litres] water) in 0.5 ml ethanol (total of 20 injections [total dose, 80 mg/mouse]). Three animals in the chloroform-extract group survived to 18 months, and ten animals in the ethanol-extract group survived to 15 months. One skin papilloma at the site of injection and one leukaemia/lymphoma developed in the chloroform extract-treated animals [sex unspecified]. One leukaemia/lymphoma was observed among the ethanol extract-treated mice (Hueper & Payne, 1963). [The Working Group noted the infrequent application of the test material and the lack of adequate control groups.]

Six groups of 50-72 non-inbred albino mice received a subcutaneous injection of an extract of drinking-water collected every two weeks from three water-treatment plants in the USA, based on surface water sources (5000 gallons [18 927 litres]; treatment procedure: coagulation, sedimentation, filtration and chlorination with free chlorine). The water was passed through an activated carbon filter, and the adsorbate was eluted either with chloroform or with ethanol; eluates were pooled to obtain a one-year representative sample for each type of eluate. Median organic yields from the three sources were 45-78 µg/l for chlorofom and 98-122 µg/l for ethanol extracts. The mice received three injections of one of the extracts in 0.025 ml diluted propylene glycol (1:1 with isotonic saline) on the following dosing schedule: shortly after birth (4-18 h), 0.5 mg; at 10 days of age, 1.0 mg; and at 20 days of age, 3.5 mg/mouse (total dose, 5 mg). Control mice received either diluted propylene glycol or saline by a similar injection schedule. High mortality was observed in the neonatal period in each of the six treated and two control groups; however, more animals died in the chloroform extract-treated groups than in the ethanol extract-treated and vehicle control groups. The total number of surviving animals at week 4 ranged between 43 and 53. Survivors were observed for 78 weeks and were killed at 1.5 years of age. No tumour had developed

at the injection sites. The types and numbers of other tumours were similar in the experimental and control groups (Dunham *et al.*, 1967). [The Working Group noted the infrequent injection schedule, the high early mortality, the short duration of the experiment and the lack of an adequate control group.]

(*d*) *Administration with known carcinogens*

Groups of 50 Sencar mice [sex unspecified], aged six to nine weeks, were given six subcutaneous injections over two weeks (to give a total dose of 1.5 ml) of water from a US river, disinfected, after settling, coagulation and filtration, by either chlorine (2.0-2.5 mg/l), chloramine (2.0-3.0 mg/l), chlorine dioxide (2.0-3.0 mg/l) or ozone (1.0-3.0 mg/l), then concentrated 100-180 fold using reverse osmosis. Treated but not disinfected water, similarly concentrated [organic material not quantified] served as control water. Equal numbers of mice received isotonic saline by the same treatment schedule. As a positive control, 7.5 μg 7,12-dimethylbenz[*a*]anthracene (DMBA) in 10% Emulphor were administered subcutaneously to 50 mice. Two weeks after the last initiating dose, 25 mice in each group received topical applications of 2.5 μg 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in 0.2 ml acetone three times a week for 18 weeks, and the remaining 25 received 0.2 ml acetone without TPA; all mice were then observed for an additional 28 weeks. The numbers of animals injected with concentrate followed by topical application of TPA that had macroscopic skin tumours at one year were: non-disinfected water condensate, 0/25; chlorine disinfected water condensate, 4/25; chloramine disinfected water condensate, 5/25; chlorine dioxide disinfected water concentrate, 0/25; ozone disinfected water concentrate, 7/25; saline control, 1/25; DMBA positive control, 16/25. Histologically verified skin tumours (papillomas and carcinomas) were observed at the end of the study in 1/25, 4/25, 3/25, 0/25, 4/25, 1/25 and 9/25 in these groups, respectively (Bull *et al.*, 1982). [The Working Group noted that no information was provided on skin tumour frequency in the acetone-treated control groups.]

In two experiments, groups of 60 Sencar mice [sex unspecified], aged six to nine weeks, were given six subcutaneous injections over two weeks (to give a total dose of 1.5 ml) of the same samples described above but which were concentrated 400 fold using reverse osmosis followed by freeze-drying. Equal numbers of animals received isotonic saline by the same treatment schedule. As a positive control, groups of mice [numbers unspecified] received 7.5 or 25 μg/mouse DMBA or 9 mg/mouse urethane. Two weeks after the last initiating dose, 40 mice in each group received topical applications of 1 μg/mouse TPA in 0.2 ml acetone three times a week for 20 weeks; the remaining 20 animals in each group received applications of 0.2 ml acetone without TPA and were observed for an additional 28 weeks. The incidence of skin papillomas observed macroscopically at one year was similar in

the treated and saline control group in both experiments (Bull *et al.*, 1982). [The Working Group noted that data were not provided on tumour incidence in the positive control groups or in the acetone-treated groups.]

Groups of 60 male Sencar mice, 8-10 weeks of age, received six subcutaneous injections over two weeks of two types of drinking-water concentrates obtained from five water-treatment plants with different water sources [method of disinfection unspecified]. One sample (ROE) was obtained by reverse osmosis followed by extraction with pentane and dichloromethane. The other sample (XAD) was obtained by passing the aqueous residue of the reverse osmosis extraction through XAD-2 resin and eluting with ethanol. The extracts were administered in 0.1 ml Emulphor to give a total dose of 150 mg/kg bw. A vehicle control group received 0.1 ml Emulphor alone. As a positive control, a total dose of 25 μg/mouse DMBA in 0.1 ml Emulphor was injected in six subcutaneous injections over a two-week period. Two weeks after the last initiating dose, 40 mice from each group received topical applications of 0.1 μg/mouse TPA in 0.1 ml acetone three times a week for 20 weeks; the remaining 20 animals in each group received 0.1 ml acetone only. Surviving animals were sacrified one year after completion of promotion. Skin tumours that persisted for three weeks or more were included in a cumulative count. There was a statistically significant increase in the number of skin papillomas per mouse in one group treated with the ROE sample from one source plus TPA, and in one group treated with the XAD sample from another source plus TPA, as compared with the vehicle control and the TPA control (Robinson *et al.*, 1981).

3.2 Other relevant data

As chlorine dissolves in water to produce hypochlorous acid and hypochlorite, the Working Group summarized experiments that utilized high concentrations of chlorine, hypochlorous acid and hypochlorite in the monograph on hypochlorite. Only studies that were directed specifically to by-products isolated from chlorinated drinking-water (in comparison to non-chlorinated water from the same source) or sought to model processes that are known to occur in chlorinated drinking-water are discussed here.

(*a*) *Experimental systems*

(i) *Toxic effects*

Organic material recovered from chlorinated water by reverse osmosis (reduced in volume by 100 and 400 times) and given to 10 male and 10 female CD-1 mice in each experimental group as drinking-water was compared in a 30-day study with non-disinfected water and water treated with other disinfectants. A significant increase in liver weights was reported in female but not male mice given the high dose of chlorinated water concentrate in comparison with the concentrate from

non-chlorinated control water. Male mice had reduced lung weights at both doses and decreased testicular weights at the high dose. No histological examination was performed (Miller *et al.*, 1986).

Organic chemicals from the same waters, recovered on XAD resin, were administered as 0.3 ml of a 1000- or 4000-times concentrate by gavage three times per week for four weeks to 10 CD-1 mice of each sex per group. Treatment had no effect on organ weights in animals of either sex; however, water that had been chlorinated, filtered through granular activated carbon and then rechlorinated increased liver weight in male mice at both doses and decreased lung weight at the high dose. Both doses reduced ovary weight in female mice. No histological finding was reported (Miller *et al.*, 1986).

A combined acid and neutral fraction of organic chemicals recovered on XAD resin, dissolved in dimethyl sulfoxide (corresponding to 100 l of chlorinated drinking-water), was administered intraperitoneally on two consecutive days to 10-day-old Wistar rats and once to 20-day-old rats. This treatment resulted in 50% mortality at 48 h in 10-day-old rats and in 30% mortality in 20-day-old rats; it also induced various alterations in drug metabolizing enzyme activities in liver fractions obtained from surviving animals. The most consistent effect was an increase in the level of hepatic 7-ethoxyresorufin-*O*-deethylase compared to solvent-treated controls (Liimatainen *et al.*, 1988). [The Working Group noted that no non-chlorinated water control was available.]

In a 90-day study in groups of 15 rats, humic acids dissolved at concentrations of 0.1, 0.5 and 1 g/l in distilled water and chlorinated with a 1:1 ratio of chlorine equivalents to organic carbon were given as drinking-water (pH 3). Renal weights were increased relative to body weight at 0.5 and 1 g/l, and there was a small increase in blood urea nitrogen. Haematuria was seen with the 1.0 g/l dose, which appeared to be related to the deposition of crystals [composition unspecified] in the renal pelvis (Condie *et al.*, 1985).

Chlorine reacts very rapidly with purified DNA and RNA (Hayatsu *et al.*, 1971). It chlorinates uracil to produce 5-chlorouracil at low chlorine:carbon ratios and dichlorouracil and ring cleavage at higher ratios (Gould *et al.*, 1984). Relatively stable organic chloramines are formed with cytosine. Purines (modelled by caffeine) are chlorinated to a very small extent, with ring cleavage to a complex array of products (Gould & Hay, 1982). A peroxide of adenosine 5′-monophosphate (AMP) has been shown to form at physiological pH, and this reaction is dependent on the NaOCl:AMP ratio, reaching a plateau when this reatio is less than 1 (Bernofsky *et al.*, 1987).

(ii) *Effects on reproduction and prenatal toxicity*

McKinney *et al.* (1976) reported an increased incidence of dead implantations and of litters with malformed fetuses among Swiss CD-1 mice given chlorinated tap

water from Durham, NC, USA. The effect was reported to be seasonal [data not shown]. The control group in this study was given the same water purified by filtration (to reduce organic material and remove microparticulates), demineralization and distillation.

These observations stimulated a series of teratogenicity studies in which Durham city tap water was compared with water purified by the same method as described above. Using much larger group sizes than McKinney *et al.* (1976), Staples *et al.* (1979) found no significant overall difference in the reproductive status of pregnant mice given tap water or purified water. Month-by-month comparisons over a nine-month period (including the critical winter months suspected of being important by McKinney *et al.*) indicated occasionally improved reproductive performance only in the tap water group. Chernoff *et al.* (1979) also found no significant effect on any fetal parameter in CD-1 mice, except for an increased incidence of supernumerary ribs, which the authors considered to be spurious, in the groups given Durham tap water. They considered the possibility that drinking-water quality had changed during the intervening years since the study by McKinney *et al.*

Kavlock *et al.* (1979) evaluated the effects of concentrates of organic materials from the drinking-water of five US cities representative of major sources of raw water. Because the reverse osmosis method used for concentrating these materials does not retain organohalides with a molecular weight of less than 200, an artificially constituted organohalide mixture was also prepared and evaluated. Groups of Swiss mice were given 300, 1000 or 3000 times the anticipated human dose of these materials by gavage on gestation days 7-14. No adverse effect on embryonal or fetal development was observed.

[The Working Group noted that these studies were designed to study the effects of the drinking-water of individual cities but not to investigate the developmental toxicity of chlorinated drinking-water, nor did they include a non-chlorinated water control.]

(iv) *Genetic and related effects*

The results obtained in a variety of short-term tests for samples of chlorinated water have been reviewed (Kraybill, 1980; Loper, 1980; Alink, 1982; Kool *et al.*, 1982a; Nestmann, 1983; Bull, 1985; Degraeve, 1986; Fielding & Horth, 1986; Meier, 1988). Many of the studies were concerned with the mutagenicity of drinking-water and not with the influence of chlorination. As the source of mutagenicity in drinking-water may also be polluted raw water, the role of water chlorination cannot be evaluated unless a comparison is made with an unchlorinated sample. Papers lacking this aspect and those in which no reference is made to the disinfection agent used are not summarized here. In many papers, data were available to allow

comparison of unchlorinated and chlorinated waters, and when the authors did not do this, the Working Group drew their own conclusions.

The Working Group also limited themselves to studies of water samples disinfected with chlorine or hypochlorite; studies on water samples disinfected with chlorine dioxide, monochloramine or ozone alone were not considered.

By far the majority of studies were with *Salmonella typhimurium* strains TA98 and TA100.

(1) *Chlorinated water* (Table 7)

Chlorination did not increase the mutagenicity of drinking-water prepared from surface or spring water, as studied in fluctuation tests with *S. typhimurium* strains TA98 and TA100. Mutations were not induced in *S. typhimurium* TA100 when samples of chlorinated water were used to prepare bottom agar for the test plates. [The Working Group noted that volatile substances would be lost if the water were autoclaved.] Chlorination of a tap water sample derived from surface water did not increase the number of micronuclei in *Tradescantia* pollen mother cells. Chromosomal aberrations were induced in *Allium cepa*, however, by a river water sample chlorinated in the laboratory. Cell transformation was not induced by chlorinated tap water in cultured Syrian hamster embryo cells or by finished drinking-water from a surface water source in mouse embryo cells.

(2) *Concentrates of chlorinated water* (Tables 8-10)

The most widely used method for isolating organic material from water samples is adsorption to macroreticular resin (various types of Amberlite XAD) followed by elution with an organic solvent. Liquid-liquid extraction with an organic solvent is also commonly used. The different concentration methods used for mutagenicity studies have been discussed (Forster & Wilson, 1981; Harrington *et al.*, 1983; Maruoka & Yamanaka, 1983; Monarca *et al.*, 1985a,b; Wigilius *et al.*, 1985; Vartiainen *et al.*, 1987a). The methods are more or less selective and do not concentrate all organic materials, e.g., XAD adsorption and liquid-liquid extraction techniques may result in the loss of highly polar compounds. Concentration of an extract invariably means that volatile substances are removed with the solvent. The extent of loss depends upon the solvent used: use of low-boiling-point solvents, e.g., ether and acetone, leads to smaller losses (compounds with boiling-points of about 120°C should be retained unless evaporation is to dryness), while use of high-boiling-point solvents, e.g., ethyl acetate and dimethyl sulfoxide, leads to greater losses.

Chlorination of surface water usually resulted in increased mutagenicity of concentrated samples towards *S. typhimurium*, particularly strains TA100 and TA98 (Tables 8 and 10). In the few studies in which these strains were not used, negative responses were obtained, perhaps because the most sensitive organism

Table 7. Summary of the influence of chlorine disinfection on the genetic and related effects of unconcentrated drinking-water samples in comparison with unchlorinated water

Source of water; disinfection method[a]	Test system	Result		Dose or dose range	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
	PROKARYOTES				
Italy; lake; NaOCl, flocculation, RSF, NaOCl	Mutation, *S. typhimurium* TA100 fluctuation test	-	0	5–100%	Monarca *et al.* (1985b)
Italy; river; NaOCl, flocculation, SSF, NaOCl	Mutation, *S. typhimurium* TA100 fluctuation test	-	0	5–100%	Monarca *et al.* (1985b)
Italy; spring water; NaOCl	Mutation, *S. typhimurium* TA100 fluctuation test	-	0	5–100%	Monarca *et al.* (1985b)
USA; chlorinated drinking-water from two supply systems	Mutation, *S. typhimurium* TA100	-	-	1–20 ml/plate	Schwartz *et al.* (1979)
	PLANTS				
Macomb (IL, USA); chlorinated tap water from city reservoir	Micronuclei, *Tradescantia* clone 03, pollen mother cells	-	0	Cuttings placed in sample	Ma *et al.* (1985)
Sava river (Zagreb, Yugoslavia); NaOCl 1–10 mg Cl/l in laboratory	Chromosomal aberations, *Allium cepa*	(+)	0	Roots suspended in sample	Al-Sabti & Kurelec (1985)
	MAMMALIAN CELLS *IN VITRO*				
Pretoria (South Africa); reclaimed tap water; activated sludge, clarification, Cl_2, clarification, alum, sand filtration, Cl_2, active carbon, Cl_2	Transformation, golden hamster embryo cells, colony morphology	-	0	Media made up from sample	Kfir & Prozesky (1982)
Mississippi river (USA); 115 finished water samples	Transformation, mouse embryo R846–DP–6 cells, growth pattern	-	0	72% of sample in medium	Pelon *et al.* (1980)

[a]RSF, rapid sand filtration; SSF, slow sand filtration

was not used. Inclusion of a metabolic activation system usually resulted in a reduced response or totally abolished it. Mutagenic effects were consistently found in samples of surface water that had a high content of natural organic compounds at the time of the chlorination. Water samples in which the organic content had been reduced before chlorination by water treatment procedures tended to show reduced or no mutagenicity.

Chlorinated ground- and spring water samples (Table 9) were less frequently mutagenic than chlorinated surface water samples (Table 10).

Much of the bacterial mutagenicity of concentrated chlorinated surface water samples is probably due to chlorination of natural constituents, such as humic and fulvic acids. Chlorination of aqueous solutions of fulvic and humic acids resulted in the formation of mutagenic compounds (Meier *et al.*, 1983; Kowbel *et al.*, 1984; Kopfler *et al.*, 1985; Kronberg *et al.*, 1985a,b; Meier *et al.*, 1985; Kowbel *et al.*, 1986; Maruoka, 1986; Meier *et al.*, 1986; Van Duuren *et al.*, 1986; Agarwal & Neton, 1989; Horth, 1989; Pommery *et al.*, 1989). The mutagenicity of chlorinated water samples is not due to the volatile trihalomethanes known to be formed at chlorination; much of the mutagenicity is due to nonvolatile acidic and polar substances. Such compounds require acidic conditions for efficient extraction by non-polar solvents. In several studies, the greatest mutagenic activity was seen when concentration was performed at low pH (e.g., pH 2) (Kool *et al.*, 1981; Van Der Gaag *et al.*, 1982; Kronberg *et al.*, 1985a,b; Vartiainen & Liimatainen, 1986; Ringhand *et al.*, 1987; Fawell & Horth, 1990). A single organic compound, MX, has been shown to be responsible for a significant portion of the bacterial mutagenicity of some concentrated chlorinated surface water samples. This compound is unstable at high pH and in dimethyl sulfoxide (Meier *et al.*, 1987; Kronberg & Vartiainen, 1988; Fielding & Horth, 1988).

Some concentrates of chlorinated tap water prepared from surface waters, groundwater or their mixture induced more sister chromatid exchange in Chinese hamster ovary cells than concentrates of the respective raw waters. In the only study of its kind, concentrates of chlorinated river water that had undergone extensive water treatment procedures did not increase the incidence of *hprt* locus mutations in Chinese hamster V79 cells. Chlorination was associated with an increase in the frequency of micronuclei in Chinese hamster ovary cells exposed to some samples of concentrated chlorinated tap water prepared from surface water and mixed ground- and surface water but not in those exposed to concentrated chlorinated groundwater. Concentrates prepared from chlorinated water from a river and a reservoir induced chromosomal aberrations in Chinese hamster ovary cells.

No studies in mammals *in vivo* were available.

Table 8. Summary of the influence of chlorine disinfection on the genetic and related effects of surface water concentrates in comparison with concentrates of unchlorinated water

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
		PROKARYOTES				
Belgium; rechlorination (0.5 mg/l) of contact water dechlorinated totally with sulfur dioxide	Freeze-drying, methanol	Mutation, *S. typhimurium*, fluctuation test				Wilcox & Denny (1985)
		TA100	+	0	0.02–0.1 l/ml	
		TA98	-	0	0.02–0.1 l/ml	
UK; chlorinated water water from lowland rivers[d]	Freeze-drying	Mutation, *S. typhimurium*, fluctuation test			0.00	Fielding & Horth (1988)
		TA100	+	(+)*		
		TA98	+ 4/5	+* 2/5		
UK; chlorinated water water from upland reservoirs[d]	Freeze-drying	Mutation, *S. typhimurium*, fluctuation test			0.00	Fielding & Horth (1988)
		TA100	+	(-)		
		TA98	+ 2/3	(+)		
Savojärovi (Finland); humic lake water; Cl_2 21 mg/l	XAD 4/8, ethyl acetate	Mutation, *S. typhimurium*				Backlund *et al.* (1985)
		TA100	+	0	10–200 ml/pl	
		TA98	+	0	10–200 ml/pl	
		TA97	+	0	10–200 ml/pl	
Mississippi River (USA); lime and alum, CO_2, activated carbon powder, Cl_2 4–8 ppm, alum[d]	XAD-4, acetone, dichloromethane	Mutation, *S. typhimurium*				Cheh *et al.* (1980)
		TA100	+	0	0.1–0.6 l/pl	
Oise River (France); O_3, storage, coagulation, flocculation, decantation, filtration, O_3, GAC, O_3, Cl_2 0.9 mg/l	XAD-4 and XAD-8, DMSO (700–1000x)	Mutation, *S. typhimurium*[e]				Bourbigot *et al.* (1983)
		TA100	-	-	0.00	
		TA98	-	-	0.00	

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Mississipi River (USA); lime and alum, CO_2, activated carbon powder, Cl_2 4–8 ppm, Na_2SO_3, alum[d]	XAD-4, acetone, dichloromethane	Mutation, *S. typhimurium* TA100	+	0	0.1–0.6 l/pl	Cheh *et al.* (1980)
Seine River (France); pulsation, RSF, GAC, Cl_2 residual 0.2 mg/l	XAD-2 and XAD-8, CH_2Cl_2 or CH_3OH	Mutation, *S. typhimurium*[e] TA98	–	–	1 ml/pl	Cognet *et al.* (1986, 1987)
Seine River (France); pulsation, RSF, O_3, GAC, Cl_2 residual 0.2 mg/l	XAD-2 and XAD-8, CH_2Cl_2 or CH_3OH	Mutation, *S. typhimurium*[e] TA98	–	–	1 ml/p	Cognet *et al.* (1986, 1987)
Houlle River (France); Cl_2 5 ppm, coagulation, flotation, GAC	XAD-2 and XAD-8, CH_2Cl_2 or CH_3OH	Mutation, *S. typhimurium* TA98	+	0	1–5 l/pl	Cognet *et al.* (1986)
Arno River (Florence, Italy); NaOCl 2.5–7.5 g Cl/m^3, activated carbon, coagulation, flocculation	XAD-2, CH_2Cl_2 and $CHCl_3$	Mutation, *S. typhimurium* TA100 TA1538	+ +	+* +	0.375–10 l/pl 10 l/pl	Dolara *et al.* (1981)
Ottawa (Canada); chlorinated tap water from Ottawa River	XAD-2, hexane:acetone (200 000x stock)	Mutation, *S. typhimurium* TA100 TA98	+} Toxicity observed +}	+ +	0.3–2 mg/pl 0.3–2 mg/pl	Nestmann *et al.* (1979)
Ontario (Canada); chlorinated tap water from a river	XAD-2, hexane:acetone	Mutation, *S. typhimurium* TA100	+	–	92–756 µg/pl DD: (2.3 l eq/ml)	Douglas *et al.* (1986)
Ontario (Canada); chlorinated tap water from a river	XAD-2, hexane:acetone	Mutation, *S. typhimurium* TA98	+	0	DD: (1.8 l/ml)	Douglas *et al.* (1986)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Ontario (Canada); chlorinated tap water from mixed ground- and surface water	XAD-2, hexane:acetone	Mutation, *S. typhimurium* TA98	+	0	DD: (1.5 l/ml)	Douglas *et al.* (1986)
Ontario (Canada); chlorinated tap water from two lakes	XAD-2, hexane:acetone	Mutation, *S. typhimurium* TA100	+	0	DD: (2.2–4.1 l/ml)	Douglas *et al.* (1986)
Calumet River (Indiana, USA); Cl_2 10 mg/l 2 h, 0.2–1 mg/l residual Cl_2	XAD-2, diethyl ether, CH_3OH	Mutation, *S. typhimurium* TA1538	+	*	0.06–0.5 l/pl	Flanagan & Allen (1981)
Calumet River (Indiana, USA); Cl_2 10 mg/l 2 h, alum and polymer addition, flocculation, sedimentation	XAD-2, diethyl ether, CH_3OH	Mutation, *S. typhimurium* TA1538	+	+	0.06–0.5 l/pl	Flanagan & Allen (1981)
Calumet River (Indiana, USA); alum and polymer addition, flocculation, sedimentation, Cl_2 10 mg/l 2 h	XAD-2, diethyl ether, CH_3OH	Mutation, *S. typhimurium* TA1538	+	*	0.06–0.5 l/pl	Flanagan & Allen (1981)
Fox River (Illinois, USA); Cl_2 10 mg/l 2 h, residual Cl 0.2–1 mg/l	XAD-2, diethyl ether, CH_3OH	Mutation, *S. typhimurium* TA1538	–	0	0.00	Flanagan & Allen (1981)
Fox River (Illinois, USA); Cl_2 10 mg/l 2 h, alum and polymer addition, flocculation, sedimentation	XAD-2, diethyl ether, CH_3OH	Mutation, *S. typhimurium* TA1538	–	0	0.00	Flanagan & Allen (1981)
Fox River (Illinois, USA); alum and polymer addition, flocculation, sedimentation (?), Cl_2 10 mg/l 2 h	XAD-2, diethyl ether, CH_3OH	Mutation, *S. typhimurium* TA1538	–	0	0.00	Flanagan & Allen (1981)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Parys (South Africa); water from Vaal River; alum flocculation, sedimentation, sand filtration, chlorination, residual Cl_2 0.4–0.5 mg/l	Liquid-liquid extraction, Cl_2CH_2 (10 000x), neutral	Mutation, *S. typhimurium* TA100 TA98 TA1535	 + + –	 – (+)* –	 112–190 µg/pl 112–190 µg/pl 112-190 µg/pl (doses equal to 1 l/pl used in comparison)	Grabow *et al.* (1981)
Parys (South Africa); water from Vaal River; alum flocculation, sedimentation, sand filtration, chlorination, residual Cl_2 0.4–0.5 mg/l	Liquid-liquid extraction, Cl_2CH_2 (10 000x), acidic	Mutation, *S. typhimurium* TA100 TA98 TA1535	 – (+) –	 – – –	 36–125 µg/pl 36–125 µg/pl 36–125 µg/pl (doses equal to 1 l/pl used in comparison)	Grabow *et al.* (1981)
Parys (South Africa); water from Vaal River; alum flocculation, sedimentation, sand filtration, chlorination, residual Cl_2 0.4–0.5 mg/l	Liquid-liquid extraction, Cl_2CH_2 (10 000x), basic	Mutation, *S. typhimurium* TA100 TA98 TA1535	 – – –	 – – –	 < 1–25 µg/pl < 1–25 µg/pl < 1–25 µg/pl (doses equal to 1 l/pl used in comparison)	Grabow *et al.* (1981)
Des Moines (Iowa, USA); chlorinated and fluoridated water from a river and an infiltration gallery	XAD-4, diethyl ether (> 200 000x)	Mutation, *S. typhimurium* TA100 TA98	 + +	 +* (+)	 0.00 0.00	Grimm-Kibalo *et al.* (1981)
Des Moines (Iowa, USA); chlorinated and fluoridated water from a river and an infiltration gallery	XAD-4, ethanol after diethyl ether (> 200 000x)	Mutation, *S. typhimurium* TA100 TA98	 + (+)	 +* (+)	 0.00 0.00	Grimm-Kibalo *et al.* (1981)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Como (Italy) outlet; mixed from ground water and Lake Como; conventional treatment and NaOCl	XAD-2 and XAD-7, acetone	Mutation, *S. typhimurium* TA100 TA98	 + +	 + +	 0.5–5.0 l/pl 0.5–5.0 l/pl	Galassi *et al.* (1989)
Netherlands; Rhine River; filtration, ferric chloride, filtration, pH adjustment, Cl_2 (NaOCl) 5–15.7 mg/l, pH 6.2	XAD-4/8, DMSO (1500x)	Mutation, *S. typhimurium* TA100 TA98	 (+) (+)	 0 0	 0.25–0.5 ml/pl 0.25–0.5 ml/pl	de Greef *et al.* (1980)
Netherlands; dune infiltrated river water after transport chlorination	XAD-4/8, DMSO (8000x)	Mutation, *S. typhimurium* TA98	 +	 –	 1.5 l/pl	Kool *et al.* (1981)
Netherlands; river water; transport chlorination; end of transport system	XAD-4/8, DMSO (8000x)	Mutation, *S. typhimurium* TA98	 +	 +	 1.5 l/pl	Kool *et al.* (1981)
Netherlands; river water; transport chlorination, RSF, filtration	XAD-4/8, DMSO (8000x)	Mutation, *S. typhimurium* TA98	 +	 +	 1.5 l/pl	Kool *et al.* (1981)
Netherlands; dune infiltrated river water; transport chlorination, RSF	XAD-4/8, DMSO (8000x)	Mutation, *S. typhimurium* TA98	 –	 –	 1.5 l/pl	Kool *et al.* (1981)
Netherlands; Rhine River; 5–15 mg Cl_2/l	XAD-4/8, DMSO (1800x)	Mutation, *S. typhimurium* TA100 TA98	 – +	 0 0	 0.25–0.5 ml/pl 0.25–0.5 ml/pl	Kool *et al.* (1981)
Netherlands; Meuse River; breakpoint chlorination	XAD-4/8, DMSO (7000x)	Mutation, *S. typhimurium* TA100 TA98	 + +	 – +	 1.5 l/pl 1.5 l/pl	Kool *et al.* (1982b)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Netherlands; Meuse River; breakpoint chlorination and activated carbon	XAD-4/8, DMSO (7000x)	Mutation, *S. typhimurium* TA100 TA98	 + -	 - -	 1.5 l/pl 1.5 l/pl	Kool *et al.* (1982b)
Netherlands; Meuse River; post-chlorination	XAD-4/8, DMSO (7000x)	Mutation, *S. typhimurium*[e] TA100 TA98	 + +	 - -	 1.5 l/pl 1.5 l/pl	Kool *et al.* (1982b)
Netherlands; Meuse River; transport chlorination	XAD-4/8, DMSO (8000x)	Mutation, *S. typhimurium*[e] TA98	 +	 +	 2 l/pl	Kool *et al.* (1982b)
Netherlands; Meuse River; transport chlorination, dune infiltration or activated carbon with RSF and SSF	XAD-4/8, DMSO (8000x)	Mutation, *S. typhimurium*[e] TA98	 -	 -	 2 l/pl	Kool *et al.* (1982b)
Netherlands; Rhine River; chlorination	XAD-4/8, acetone, XAD-4/8 (45 000x), TLC fraction	Mutation, *S. typhimurium* TA98	 +	 -	 0.00	Kool *et al.* (1982b)
Netherlands; Meuse River; breakpoint chlorination, O_3, activated carbon, post-chlorination	XAD-4/8, DMSO (7000x)	Mutation, *S. typhimurium*[e] TA100 TA98	 + -	 - -	 3 l/p 3 l/p	Kool *et al.* (1982b)
Netherlands; Meuse or Rhine River; prechlorination 5 mg Cl_2/l	XAD-4/8, DMSO (2000–4000x)	Mutation, *S. typhimurium* TA98	 +	 +*	 0.25–0.5 ml/pl	Zoeteman *et al.* (1982)
Nieuwegein (Netherlands); Rhine River; dune recharge, Cl_2 0.2 mg/l after 20 min	XAD-4, ethanol, cyclohexane/ethanol, pH 7 (4000x)	Mutation, *S. typhimurium* TA100	 +	 +*	 1–3 l/pl	Van Der Gaag *et al.* (1982)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Nieuwegein (Netherlands); Rhine River; dune recharge, Cl_2 0.2 mg/l after 20 min	XAD-4, ethanol, cyclohexane/ethanol, pH 2 (4000x)	Mutation, *S. typhimurium* TA100	+	+*	1–3 l/pl	Van Der Gaag *et al.* (1982)
Nieuwegein (Netherlands); Rhine River; active carbon filtration, Cl_2 0.2 mg/l after 20 min	XAD-4, ethanol, cyclohexane/ethanol, pH 7 (4000x)	Mutation, *S. typhimurium* TA100	–	–	1–4 l/pl	Van Der Gaag *et al.* (1982)
Nieuwegein (Netherlands); Rhine River; active carbon filtration, Cl_2 0.2 mg/l after 20 min	XAD-4, ethanol, cyclohexane/ethanol, pH 2 (4000x)	Mutation, *S. typhimurium* TA100	+	–	1.4 l/pl	Van Der Gaag *et al.* (1982)
Netherlands; Meuse River; Cl_2 1.5 mg/l	XAD-4/8, DMSO or acetone (7000x)	Mutation, *S. typhimurium*				Kool *et al.* (1985b)
		TA100	+	(-)	0.1–0.2 ml	
		TA98	+	+	0.1–0.2 ml	
		TA100NR$^-$	–	–	0.1–0.2 ml	
		TA98NR$^-$	+	–	0.1–0.2 ml	
Netherlands; Meuse River; Cl_2 5–15 mg/l	XAD-4/8, DMSO (4000x)	Mutation, *S. typhimurium*				Kool *et al.* (1985c)
		TA100	–	–	2 l/pl	
		TA98	+	+*	2 l/pl	
Netherlands; Meuse River; transport chlorination Cl_2 1–2 mg/l	XAD-4/8, DMSO	Mutation, *S. typhimurium* TA98	+	–	1.5 l/pl	Kool & Hrubec (1986); Kool *et al.* (1985b)
Netherlands; Meuse River; prechlorination Cl_2 1.8 mg/l	XAD-4/8, DMSO (7000x)	Mutation, *S. typhimurium*[e]				Kool & Hrubec (1986); Kool & van Kreijl (1984); Kool *et al.* (1985b)
		TA100	+	+*	3.5 l/pl	
		TA98	+	+*	3.5 l/pl	

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Netherlands; Meuse or Rhine River; postchlorination 0.15 mg Cl_2 after 20 min	XAD-4/8, DMSO (7000x)	Mutation, *S. typhimurium*[e] TA100 TA98	 + +	 + +*	 3.5 l/pl 3.5 l/pl	Kool & Hrubec (1986); Kool & van Kreijl (1984); Kool *et al.* (1985b)
Netherlands; Meuse River; Cl_2 1–5 mg/l	XAD-4/8, DMSO (3500x)	Mutation, *S. typhimurium* TA100 TA98	 + +	 0 +*	 1.7 l/pl 1.7 l/pl	Kool & Hrubec (1986)
Netherlands; Rhine River; Cl_2 1–5 mg/l	XAD-4/8, DMSO (3500x)	Mutation, *S. typhimurium* TA100 TA98	 – (+)	 0 (+)	 0.9 l/pl 0.9 l/pl	Kool & Hrubec (1986)
Netherlands; surface water from one city; Cl_2 1 mg/l	XAD-4/8, DMSO (7000x), neutral fraction	Mutation, *S. typhimurium* TA100 TA98	 – +	 – +	 3.5 l/pl 3.5 l/pl	Kool & Hrubec (1986)
Netherlands; surface water from one city; Cl_2 1 mg/l	XAD-4/8, DMSO (7000x), acidic fraction	Mutation, *S. typhimurium* TA100 TA98	 – –	 – +	 3.5 l/pl 3.5 l/pl	Kool & Hrubec (1986)
Netherlands; surface water from one city; Cl_2 1 mg/l	XAD-4/8, DMSO (7000x), neutral fraction	Mutation, *S. typhimurium* TA100 TA98	 + +	 – +	 3.5 l/pl 3.5 l/pl	Kool *et al.* (1985c)
Netherlands; surface water from one city; Cl_2 1 mg/l	XAD-4/8, DMSO (7000x), acidic fraction	Mutation, *S. typhimurium* TA100 TA98	 – +	 – –	 3.5 l/pl 3.5 l/pl	Kool *et al.* (1985c)
Cincinnati (Ohio, USA); Ohio River; presettling with aluminium sulfate, Cl_2, lime, F, ferric sulfate, coagulation, flocculation, sedimentation, RSF	XAD-2, hexane-acetone (10 000x)	Mutation, *S. typhimurium* TA100 TA98	 + +	 +* +*	 0.25–1.5 l/pl 0.25–1.5 l/pl	Loper *et al.* (1985)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Cincinnati (Ohio, USA); tap water; presettling with aluminium sulfate, Cl_2, lime, F, ferric sulfate, coagulation, flocculation, sedimentation, RSF	XAD-2, hexane-acetone (10 000x)	Mutation, *S. typhimurium* TA100 TA98	 + +	 (+)* (+)*	 0.25–1.5 l/pl 0.25–1.5 l/pl	Loper *et al.* (1985)
Cincinnati (Ohio, USA); Ohio River; presettling with aluminium sulfate, Cl_2, lime, F, ferric sulfate, coagulation, flocculation, sedimentation, RSF, GAC	XAD-2, hexane-acetone (10 000x)	Mutation, *S. typhimurium* TA100 TA98	 - -	 0 0	 0.25–1.5 l/pl 0.25–1.5 l/pl	Loper *et al.* (1985)
Cincinnati (Ohio, USA); Ohio River; presettling with aluminium sulfate, Cl_2, lime, F, ferric sulfate, coagulation, flocculation, sedimentation, RSF, GAC, Cl_2 2.6 mg/l	XAD-2, hexane-acetone (1000x)	Mutation, *S. typhimurium* TA100 TA98	 - -	 - -	 0.25–3 l/pl 0.25–2 l/pl	Loper *et al.* (1985)
Jefferson Parish (Louisiana, USA); pilot plant; Cl_2	XAD-2 and XAD-8, acetone (4000x), pH 2	Mutation, *S. typhimurium* TA100 TA98	 + +	 0 0	 0.00 0.00	Miller *et al.* (1986)
Jefferson Parish (Louisiana, USA); pilot plant; Cl_2, fresh GAC	XAD-2 and XAD-8, acetone (4000x)	Mutation, *S. typhimurium* TA100 TA98	 - -	 0 0	 0.00 0.00	Miller *et al.* (1986)
Jefferson Parish (Louisiana, USA); pilot plant, Cl_2, GAC after 14 months	XAD-2 and XAD-8, acetone (4000x)	Mutation, *S. typhimurium* TA100 TA98	 - +	 0 0	 0.00 0.00	Miller *et al.* (1986)
Jefferson Parish (Louisiana, USA); pilot plant; Cl_2, fresh GAC, Cl_2	XAD-2 and XAD-8, acetone (4000x)	Mutation, *S. typhimurium* TA100 TA98	 - -	 0 0	 0.00 0.00	Miller *et al.* (1986)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Jefferson Parish (Louisiana, USA); pilot plant; Cl_2, GAC after 6 months, Cl_2	XAD-2 and XAD-8, acetone (4000x)	Mutation, *S. typhimurium* TA100 TA98	 + +	 0 0	 0.00 0.00	Miller *et al.* (1986)
Jefferson Parish (Lopuisiana, USA); pilot plant; Cl_2	Reverse osmosis (400x)	Mutation, *S. typhimurium* TA100 TA98	 - -	 - -	 0.025–1 ml/pl 0.025–1 ml/pl	Miller *et al.* (1986)
Jefferson Parish (Louisiana, USA); pilot plant, Mississippi River; clarification, settling, F, sand filtration, Cl_2 0.2–7.5 ppm	XAD-2 and XAD-4, acetone pH 2	Mutation, *S. typhimurium* TA100	+	0	0.1–1.6 l/pl	Ringhand *et al.* (1987)
Jefferson Parish (Louisiana, USA); pilot plant; Mississipi River; clarification, settling, F, sand filtration, Cl_2, 0.2–7.5 ppm	XAD-2 and XAD-4, acetone pH 8	Mutation, *S. typhimurium* TA100	(+)	0	0.1–1.6 l/pl	Ringhand *et al.* (1987)
Cincinnati (Ohio, USA); pilot plant, Ohio River; clarification, coagulation, flocculation, sedimentation, RSF, Cl_2 0.2–7.5 ppm	XAD-2 and XAD-4, acetone pH 2	Mutation, *S. typhimurium* TA100	+	0	0.1–1.6 l/pl	Ringhand *et al.* (1987)
Cincinnati (Ohio, USA); pilot plant, Ohio River; clarification, sedimentation, coagulation, flocculation, sedimentation, RSF, Cl_2 0.2–7.5 ppm	XAD-2 and XAD-4, acetone pH 8	Mutation, *S. typhimurium* TA100	(+)	0	0.1–1.6 l/pl	Ringhand *et al.* (1987)
USA; chlorinated drinking-water	Polyurethane foam column, acetone, benzene (30 000x)	Mutation, *S. typhimurium* TA98	-	-	0.1– 1 l/pl	Schwartz *et al.* (1979)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Italy; lake; Cl_2, flocculation, RSF, Cl_2	Liquid-liquid extraction, CH_2Cl_2, neutral	Mutation, *S. typhimurium* TA100 TA98	 - -	 - -	 0.00 0.00	Monarca *et al.* (1985a)
Italy; lake; Cl_2, flocculation, RSF, Cl_2	Liquid-liquid extraction, CH_2Cl_2, acidic	Mutation, *S. typhimurium* TA100 TA98	 + -	 - -	 0.00 0.00	Monarca *et al.* (1985a)
Italy; lake; Cl_2, flocculation, RSF, Cl_2	Liquid-liquid extraction, CH_2Cl_2, basic	Mutation, *S. typhimurium* TA100 TA98	 + -	 - -	 0.00 0.00	Monarca *et al.* (1985a)
Italy; river, Cl_2, flocculation, SSF, Cl_2	Liquid-liquid extraction, CH_2Cl_2, neutral	Mutation, *S. typhimurium* TA100 TA98	 - +	 - -	 0.00 0.00	Monarca *et al.* (1985a)
Italy; river; Cl_2, flocculation, SSF, Cl_2	Liquid-liquid extraction, CH_2Cl_2, acidic	Mutation, *S. typhimurium* TA100 TA98	 + +	 - -	 0.00 0.00	Monarca *et al.* (1985a)
Italy; river; Cl_2, flocculation, SSF, Cl_2	Liquid-liquid extraction, CH_2Cl_2, basic	Mutation, *S. typhimurium* TA100 TA98	 - -	 - -	 0.00 0.00	Monarca *et al.* (1985a)
Italy; lake; Cl_2, flocculation, RSF, Cl_2	XAD-2, acetone	Mutation, *S. typhimurium* TA100 TA98	 - -	 - -	 0.00 0.00	Monarca *et al.* (1985a)
Italy; river; Cl_2, flocculation, SSF, Cl_2	XAD-2, acetone	Mutation, *S. typhimurium* TA100 TA98	 - -	 - -	 0.00 0.00	Monarca *et al.* (1985a)
Italy; lake; NaOCl, flocculation, RSF, NaOCl	XAD-2, acetone	Mutation, *S. typhimurium*, fluctuation test TA100	 -	 0	 0.1–1 l/test	Monarca *et al.* (1985b)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Italy; river; NaOCl, flocculation, SSF, NaOCl	XAD–2, acetone	Mutation, *S. typhimurium*[e] fluctuation test TA100	–	0	0.1–1 l/test	Monarca *et al.* (1985b)
Italy; lake; NaOCl, flocculation, RSF, NaOCl	Liquid-liquid extraction, CH_2Cl_2, neutral	Mutation, *S. typhimurium* fluctuation test TA100	–	0	0.1–1 l/test	Monarca *et al.* (1985b)
Italy; lake; NaOCl, flocculation, RSF, NaOCl	Liquid-liquid extraction, CH_2Cl_2, acidic	Mutation, *S. typhimurium* fluctuation test TA100	+	0	0.1–1 l/test	Monarca *et al.* (1985b)
Italy; lake; NaOCl, flocculation, RSF, NaOCl	Liquid-liquid extraction, CH_2Cl_2, basic	Mutation, *S. typhimurium* fluctuation test TA100	–	0	0.1–1 l/test	Monarca *et al.* (1985b)
Italy; river; NaOCl, flocculation, SSF, NaOCl	Liquid-liquid extraction, CH_2Cl_2, neutral	Mutation, *S. typhimurium* fluctuation test TA100	–	0	0.1–1 l/test	Monarca *et al.* (1985b)
Italy; river; NaOCl, flocculation, SSF, NaOCl	Liquid-liquid extraction, CH_2Cl_2, acidic	Mutation, *S. typhimurium* fluctuation test TA100	+	0	0.1–1 l/test	Monarca *et al.* (1985b)
Italy; river; NaOCl, flocculation, SSF, NaOCl	Liquid-liquid extraction, CH_2Cl_2, basic	Mutation, *S. typhimurium* fluctuation test TA100	–	0	0.1–1 l/test	Monarca *et al.* (1985b)
Italy; surface water; NaOCl, flocculation, sand filtration, NaOCl	Sep–Pak, methanol	Mutation, *S. typhimurium* fluctuation test TA100	+	0	from 0.1 l/test	Monarca *et al.* (1985b)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Kuopio, Finland; Kallavesi Lake; $Ca(OH)_2$, $Al_2(SO)_4)_3$, Cl_2 1 mg/l, CO_2, flotation, sand filtration, $Ca(OH)_2$, Cl_2 1 mg/l, F	Liquid-liquid extraction, diethyl ether	Mutation, *S. typhimurium* TA100 TA98	 + +	 + * + *	 3–33 ml/pl 4–35 ml/pl	Vartiainen & Liimatainen (1986)
Kuopio, Finland; Kallavesi Lake; $Ca(OH)_2$, $Al_2(SO)_4)_3$, Cl_2 1 mg/l, CO_2, flotation, sand filtration, $Ca(OH)_2$, Cl_2 1 mg/l, F	Liquid-liquid extraction, CH_2Cl_2	Mutation, *S. typhimurium* TA100 TA98	 + +	 0 0	 100–300 ml/pl 100–300 ml/pl	Vartiainen & Liimatainen (1986)
Kuopio, Finland; Kallavesi Lake; $Ca(OH)_2$, $Al_2(SO)_4)_3$, Cl_2 1 mg/l, CO_2, flotation, sand filtration, $Ca(OH)_2$, Cl_2 1 mg/l, F	XAD 8, ethyl acetate	Mutation, *S. typhimurium* TA100 TA98	 + +	 0 0	 4–40 ml/pl 4–40 ml/pl	Vartiainen & Liimatainen (1986)
Varkaus, Finland; lake; chlorinated drinking-water	Liquid-liquid extraction, CH_2Cl_2	Mutation, *S. typhimurium* TA100 TA98	 + +	 0 0	 0.00 0.00	Vartiainen & Liimatainen (1986)
Kuopio, Finland; Kallavesi Lake; $Ca(OH)_2$, Cl_2 0.7 g/m^3, $Al_2(SO)_4)_3$, CO_2, mixing, flotation or sedimentation, sand filtration, Cl_2 1.2 g/m^3, F, $Ca(OH)_2$	XAD 8, ethyl acetate, acidic/ neutral	Mutation, *S. typhimurium* TA100 TA98 TA97	 + + +	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1987b)
Kuopio, Finland; Kallavesi Lake; $Ca(OH)_2$, Cl_2 0.7 g/m^3, $Al_2(SO)_4)_3$, CO_2, mixing, flotation or sedimentation, sand filtration, Cl_2 1.2 g/m^3, F, $Ca(OH)_2$	XAD 8, ethyl acetate, basic	Mutation, *S. typhimurium* TA100 TA98 TA97	 – – –	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1987b)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Kuopio, Finland; artificially recharged water from Kallavesi Lake; aeration, $Ca(OH)_2$, ClO^- 1 mg/l, mixing, $Al_2(SO_4)_3$, flocculation, sedimentation, sand filtration, postchlorination 0.5 mg/l	XAD 8, ethyl acetate, acidic/neutral	Mutation, *S. typhimurium* TA100 TA98 TA97	 + + +	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1987b)
Kuopio, Finland; artificially recharged water from Kallavesi Lake; aeration, $Ca(OH)_2$, ClO^- 1 mg/l, mixing, $Al_2(SO_4)_3$, flocculation, sedimentation, sand filtration, postchlorination 0.5 mg/l	XAD 8, ethyl acetate, basic	Mutation, *S. typhimurium* TA100 TA98 TA97	 - + +	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1987b)
Kuopio, Finland; artificially recharged water from Kallavesi Lake; aeration, $Ca(OH)_2$, ClO^- 1 mg/l, mixing, $Al_2(SO_4)_3$, flocculation, sedimentation, sand filtration	XAD 8, ethyl acetate, acidic/neutral	Mutation, *S. typhimurium* TA100 TA98 TA97	 + + +	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1987b)
Kuopio, Finland; artificially recharged water from Kallavesi Lake; aeration, $Ca(OH)_2$, ClO^- 1 mg/l, mixing, $Al_2(SO_4)_3$, flocculation, sedimentation, sand filtration	XAD 8, ethyl acetate, basic	Mutation, *S. typhimurium* TA100 TA98 TA97	 - + +	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1987b)
Kuopio, Finland; artificially recharged water from Kallavesi Lake; aeration, $Ca(OH)_2$, ClO^- 1 mg/l, $KMnO_4$, mixing, $Al_2(SO_4)_3$, flocculation, sedimentation, sand filtration	XAD 8, ethyl acetate, acidic/neutral	Mutation, *S. typhimurium* TA100 TA98 TA97	 + + -	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1987b)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Kuopio, Finland; artificially recharged water from Kallavesi Lake; aeration, $Ca(OH)_2$, ClO^- 1 mg/l, $KMnO_4$, mixing, $Al_2(SO_4)_3$, flocculation, sedimentation, sand filtration	XAD 8, ethyl acetate, basic	Mutation, *S. typhimurium* TA100 TA98 TA97	 – – –	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1987b)
Kuopio, Finland; artificially recharged water from Kallavesi Lake; aeration, ClO^- 2 mg/l	XAD–8, ethyl acetate, acidic/ neutral	Mutation, *S. typhimurium* TA100 TA98 TA97	 + + +	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1987b)
Kuopio, Finland; artificially recharged water from Kallavesi Lake; aeration, ClO^- 2 mg/l	XAD 8, ethyl acetate, basic	Mutation, *S. typhimurium* TA100 TA98 TA97	 – + –	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1987b)
Finland; nine artificially recharged waters; alum coagulation, clarification, sand filtration, pH adjustment, Cl_2 1 $\pm$ 0.9 mg/l	XAD 8 at pH 2, ethyl acetate	Mutation, *S. typhimurium*[e] TA100 TA98 TA97	 + (+) –	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1988)
Finland; 14 surface waters; filtration, Cl_2 0.6 $\pm$ 0.6 mg/l	XAD 8 at pH 2, ethyl acetate	Mutation, *S. typhimurium*[e] TA100 TA98 TA97	 + + +	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1988)
Finland; 22 surface waters; alum coagulation with or without $Fe_2(SO_4)_3$, clarification, sand filtration, pH adjustment, Cl_2 1.3 $\pm$ 0.9 mg/l	XAD 8 at pH 2, ethyl acetate	Mutation, *S. typhimurium*[e] TA100 TA98 TA97	 + + +	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1988)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Finland; 22 surface waters; Cl_2 1.7 ± 1.2 mg/l, alum coagulation with or without $Fe_2(SO_4)_3$, clarification, sand filtration, pH adjustment, Cl_2 1 ± 0.5 mg/l	XAD 8 at pH 2, ethyl acetate	Mutation, *S. typhimurium*[e] TA100 TA98 TA97	 + + -	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1988)
Finland; three surface waters; $KMnO_4$, alum coagulation, clarification, sand filtration, pH adjustment, Cl_2 1.4 ± 0.3 mg/l	XAD 8 at pH 2, ethyl acetate	Mutation, *S. typhimurium*[e] TA100 TA98 TA97	 + (+) +	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1988)
Taipei (Taiwan); chlorinated river water; total Cl_2 before XAD 1.2–13.4 ppm	XAD-2, acetone, pH 7, 6.9 or 6	Mutation, *S. typhimurium* TA100 TA98	 + -	 - -	 0.00 0.00	Wei *et al.* (1984)
Taipei (Taiwan); chlorinated eiver water; total Cl_2 before XAD 36 ppm	XAD-2, acetone, pH 5.2	Mutation, *S. typhimurium* TA100 TA98	 + -	 +* -	 0.25–1 l/pl 0.00	Wei *et al.* (1984)
Taipei (Taiwan); chlorinated river water; total Cl_2 0.1–13.3 ppm, boiling before or after Cl_2	XAD-2, acetone, pH 6.5–8.5	Mutation, *S. typhimurium* TA100 TA98	 - -	 - -	 0.00 0.00	Wei *et al.* (1984)
Como (Italy) outlet; mixed from groundwater and Lake Como; conventional treatment, NaOCl	XAD-2 and XAD-7, acetone	Gene conversion, *Saccharomyces cerevisiae* 6117 cyh2 locus	+	0	0.2–5.0 l/ml	Galassi *et al.* (1989)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
		MAMMALIAN CELLS *IN VITRO*				
Oise River (France), O_3, storage, coagulation, flocculation, decantation, filtration, O_3, GAC, O_3, Cl_2 0.9 mg/l	XAD-4 and XAD-8, DMSO	Mutation, Chinese hamster V79 cells[f], *hprt* resistance including 'initiator and promoter activity'	-	0	0.00	Bourbigot *et al.* (1983)
Ontario (Canada); river; chlorinated tap water	XAD-2, hexane:acetone	Sister chromatid exchange, Chinese hamster CHO cells	+	(-)	DD: 1.2 l/ml	Douglas *et al.* (1986)
Ontario (Canada); river; chlorinated tap water	XAD-2, hexane:acetone	Sister chromatid exchange, Chinese hamster CHO cells	-	0	DD: 0.8 l/ml	Douglas *et al.* (1986)
Ontario (Canada); lake; chlorinated tap water	XAD-2, hexane:acetone	Sister chromatid exchange, Chinese hamster CHO cells	-	0	DD: 2.0 l/ml	Douglas *et al.* (1986)
Ontario (Canada); lake; chlorinated tap water	XAD-2, hexane:acetone	Sister chromatid exchange, Chinese hamster CHO cells	(+)	0	DD: 0.9 l/ml	Douglas *et al.* (1986)
Ontario (Canada); mixed surface and groundwater; chlorinated tap water	XAD-2, hexane:acetone	Sister chromatid exchange, Chinese hamster CHO cells	+	0	DD: 1 l/ml	Douglas *et al.* (1986)
UK; lowland river; chlorinated	XAD-2, acetone (10 000x)	Chromosomal aberrations, Chinese hamster CHO cells	+	0	0.5–2 l/ml	Wilcox & Williamson (1986)
UK; upland reservoir; chlorinated	XAD-2, acetone (10 000x)	Chromosomal aberrations, Chinese hamster CHO cells	+	0	1–4 l/ml	Wilcox & Williamson (1986)

Table 8 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Ontario (Canada); river; chlorinated tap water	XAD-2 hexane: acetone	Micronuclei, Chinese hamster CHO cells	(+)	-	4–27 μg/ml	Douglas *et al.* (1986)
Ontario (Canada); river; chlorinated tap water	XAD-2 hexane: acetone	Micronuclei, Chinese hamster CHO cells	-	0	0.00	Douglas *et al.* (1986)
Ontario (Canada); lake; chlorinated tap water	XAD-2 hexane: acetone	Micronuclei, Chinese hamster CHO cells	-	0	0.00	Douglas *et al.* (1986)
Ontario (Canada); lake; chlorinated tap water	XAD-2 hexane: acetone	Micronuclei, Chinese hamster CHO cells	+	0	DD: 2.8 l/ml	Douglas *et al.* (1986)
Ontario (Canada); mixed ground- and surface waters; chlorinated tap water	XAD-2 hexane:acetone	Micronuclei, Chinese hamster CHO cells	+	0	DD: 0.9 l/ml	Douglas *et al.* (1986)

[a]GAC, granular activated carbon; RSF, rapid sand filtration; SSF, slow sand filtration

[b]DMSO, dimethyl sulfoxide; TLC, thin-layer chromatography

[c]pl, plate; DD, doubling dose. Doses given in litres per unit are litre equivalents of the original water sample per that unit. Other units refer to the amount of concentrate added per plate, millilitre, etc.

[d]Treatment performed in laboratory instead of water treatment plant

[e]Mean of net revertants/litre compared to mean of net revertants/litre in raw waters

[f]Comparison to previous stage in the treatment process

*Lower effect than without metabolic activation

**Data not given but lower effect than without metabolic activation

Table 9. Summary of the influence of chlorination upon the genetic and related effects of groundwater and spring water concentrates in comparison with concentrates of unchlorinated water

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Netherlands; groundwater from five cities; Cl_2 1 mg/l	XAD-4/8, DMSO (7000x), neutral	Mutation, *S. typhimurium* TA100 TA98	(+) (1/5)[d] + (3/5)	- + (2/5)	3.5 l/pl 3.5 l/pl	Kool & Hrubec (1986)
Netherlands; groundwater from five cities; Cl_2 1 mg/l	XAD-4/8, DMSO (7000x), acidic	Mutation, *S. typhimurium* TA100 TA98	+ (2/5) + (2/5)	- (+) (1/5)	3.5 l/pl 3.5 l/pl	Kool & Hrubec (1986)
Netherlands; groundwater from five cities; Cl_2 1 mg/l	XAD-4/8, DMSO (7000x), neutral	Mutation, *S. typhimurium* TA100 TA98	+ (2/5) + (2/5)	- (+) (1/5)	3.5 l/pl 3.5 l/pl	Kool *et al.* (1985c)
Netherlands; groundwater from five cities; Cl_2 1 mg/l	XAD-4/8, DMSO (7000x), acidic	Mutation, *S. typhimurium* TA100 TA98	(+) (1/5) + (3/5)	- (+) (3/5)	3.5 l/pl 3.5 l/pl	Kool *et al.* (1985c)
Italy; spring water; Cl_2	Liquid-liquid extraction, CH_2Cl_2, neutral, acidic, basic	Mutation, *S. typhimurium* TA100 TA98	- -	- -	0.00 0.00	Monarca *et al.* (1985a)
Italy; spring water; Cl_2	XAD-2, acetone	Mutation, *S. typhimurium* TA100 TA98	- -	- -	0.00 0.00	Monarca *et al.* (1985a)
Italy; spring water; NaOCl	XAD-2, acetone	Mutation, *S. typhimurium*, fluctuation test TA100	-	0	0.1-1 l/test	Monarca *et al.* (1985b)

Table 9 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Italy; spring water; NaOCl	Liquid-liquid extraction, CH_2Cl_2, neutral, acidic, basic	Mutation, *S. typhimurium*, fluctuation test TA100	-	0	0.1–1 l/test	Monarca *et al.* (1985b)
Italy; spring water; NaOCl	Sep–Pak, methanol	Mutation, *S. typhimurium*, fluctuation test TA100	-	0.00	0	Monarca *et al.* (1985b)
Ontario (Canada); groundwater; chlorinated tap water	XAD-2, hexane:acetone	Mutation, *S. typhimurium* TA100	+	0	DD: (15.5 l/ml)	Douglas *et al.* (1986)
Siilinjärvi, Finland; groundwater; chlorinated drinking-water	Liquid-liquid extraction, CH_2Cl_2	Mutation, *S. typhimurium* TA100 TA98	- -	0 0	0.00 0.00	Vartiainen & Liimatainen (1986)
Siilinjärvi, Finland; groundwater; chlorinated Cl_2 2 or 20 mg/l[g]	Liquid-liquid extraction, CH_2Cl_2	Mutation, *S. typhimurium*[e] TA100 TA98	+[f] -	0 0	0.00 0.00	Vartiainen & Liimatainen (1986)
UK; groundwater; chlorinated	Freeze-drying	Mutation, *S. typhimurium* fluctuation test TA100 TA98	- +	- +		Fielding & Horth (1988)

Table 9 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[c]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Ontario (Canada); groundwater; chlorinated tap water	XAD-2, hexane:acetone	Sister chromatic exchange, Chinese hamster CHO cells	(+)	0	DD: 1.9 l/ml	Douglas *et al.* (1986)
Ontario (Canada); groundwater; chlorinated tap water	XAD-2, hexane:acetone	Micronuclei, Chinese hamster CHO cells	-	0	0.00	Douglas *et al.* (1986)

[a]GAC, granular activated carbon; RSF, rapid sand filtration; SSF, slow sand filtration.

[b]DMSO, dimethyl sulfoxide

[c]pl, plate; DD, doubling dose. Doses given in l per unit are litre equivalents of the original water sample per that unit. Other units refer to the amount of concentrate added per plate, millilitre, etc.

[d]In parentheses, number of cities

[e]Comparison to previous stage in the treatment process

[f]Only 20 mg Cl_2/l positive

[g]Treatment performed in laboratory and not in water treatment plant

Table 10. Summary of the influence of chlorination in combination with either chlorine dioxide or ozone treatment upon the genetic activity of surface water concentrates in comparison with concentrates of unchlorinated water

Source of water; disinfection method	Concentration and extraction method (concentration factor)	Test system	Result		Dose or dose range[a]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Savojärvi, Finland; humic lake water; O_3 5.7–33.2 mg/l, Cl_2 21 mg/l	XAD 4/8, ethyl acetate	Mutation, *S. typhimurium* TA100 TA98 TA97	 + + +	 0 0 0	 10–50 ml/pl 10–50 ml/pl 10–50 ml/pl	Backlund *et al.* (1985)
Savojärvi, Finland; humic lake water; Cl_2 10.5 mg/l, ClO_2 10.5 mg/l	XAD 4/8, ethyl acetate	Mutation, *S. typhimurium* TA100 TA98 TA97	 + + –	 0 0 0	 10–50 ml/pl 10–50 ml/pl 10–50 ml/pl	Backlund *et al.* (1985)
Savojärvi, Finland; humic lake water; alum flocculation, Cl_2 6.5 mg/l or O_3 2.9 mg/l, Cl_2 6.5 mg/l	XAD 4/8, ethyl acetate	Mutation, *S. typhimurium* TA100 TA98 TA97	 + + +	 0 0 0	 10–200 ml/pl 10–200 ml/pl 10–200 ml/pl	Backlund *et al.* (1985)
Savojärvi, Finland; humic lake water; alum flocculation, Cl_2 3.25 mg/l, ClO_2 3.25 mg/l	XAD 4/8, ethyl acetate	Mutation, *S. typhimurium* TA100 TA98 TA97	 (+) – –	 0 0 0	 10–200 ml/pl 10–200 ml/pl 10–200 ml/pl	Backlund *et al.* (1985)
Arno River (Florence, Italy); NaOCl 2.5–7.5 g, Cl/m^3, activated carbon, coagulation, flocculation, decantation, O_3	XAD-2, CH_2Cl_2, $CHCl_3$	Mutation, *S. typhimurium* TA100 TA1538	 (+) +	 + +	 0.375–10 l/pl 10 l/pl	Dolara *et al.* (1981)
Finland, seven surface waters; ClO_2 1 ± 0.5 mg Cl_2/l, alum coagulation, clarification, sand filtration, pH adjustment, Cl_2 0.7 ± 0.2 mg/l	XAD 8, pH 2, ethyl acetate	Mutation, *S. typhimurium* TA100 TA98 TA97	 + – +	 0 0 0	 0.00 0.00 0.00	Vartiainen *et al.* (1988)

Table 10 (contd)

Source of water; disinfection method[a]	Concentration and extraction method (concentration factor)[b]	Test system	Result		Dose or dose range[a]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
Finland, seven surface waters; O_3, alum coagulation, clarification, sand filtration, pH adjustment, Cl_2 0.7 $\pm$ 0.3 mg/l	XAD 8, pH 2, ethyl acetate	Mutation, *S. typhimurium*				Vartiainen *et al.* (1988)
		TA100	+	0	0.00	
		TA98	(+)	0	0.00	
		TA97	+	0	0.00	

[a]pl, plate

(*b*) *Humans*

(i) *Toxic effects*

The possible effect of drinking-water on serum lipids was examined in a cross-sectional study of about 1500 healthy persons who had resided for at least 10 years in 46 different communities in Wisconsin, USA. Alcohol consumption, smoking habits, dietary fat intake, dietary calcium intake and body mass were considered in the analyses. The public water supply varied in magnesium and calcium content (water hardness), whether it had been chlorinated or not. The prevalence of women whose serum cholesterol level exceeded 270 mg/dl was greater in communities served by chlorinated drinking-water (odds ratio, 2.0); the mean serum cholesterol concentrations in women on chlorinated and on nonchlorinated drinking-water supplies were 247.9 mg/dl and 239.8 mg/dl, respectively (a significant difference). A smaller difference in men was not significant. Water hardness did not influence the serum cholesterol levels in either women or men (Zeighami *et al.*, 1990).

(ii) *Effects on reproduction and prenatal toxicity*

Rausch (1980) evaluated pregnancy outcomes during 1968-77 in several villages in New York State, USA, served by nonchlorinated groundwater, chlorinated groundwater or chlorinated surface water supplies. A significantly greater incidence of late fetal deaths and neonatal deaths was observed in villages on nonchlorinated groundwater; and a significantly higher prevalence of anencephaly was seen in villages using surface water as compared to villages using groundwater. There was, however, no difference in the prevalence of anencephaly between villages using chlorinated groundwater and those using nonchlorinated groundwater. The confounders analysed were season and year of birth, sex and cause of death of the fetus or newborn, maternal age, education, previous reproductive history and prenatal care and hospital where delivery occurred.

In a retrospective study described in detail in the monograph on sodium chlorite, Tuthill *et al.* (1982) compared neonatal morbidity and mortality in two similar communities in the USA, one of which used chlorination and the other of which used chlorine dioxide for disinfecting potable water. The number of infants that were judged by the attending physician to be premature or to have greater weight loss after birth was significantly greater in the community with chlorine dioxide-treated water. [The Working Group noted the difficulties associated with establishing prematurity and poor weight gain after birth, especially in a retrospective study, and that confounding factors were not controlled for.]

In a case-control study of spontaneous abortions in relation to tap-water consumption in northern California, USA, Hertz-Picciotto *et al.* (1989) observed a crude odds ratio of 1.7 (95% confidence interval (CI), 1.2-2.3) for drinkers of tap

water as compared with drinkers of bottled water. After controlling for a large number of confounders, including demographic, reproductive and life-style variables, the results were still significant.

(iv) *Genetic and related effects*

No data were available to the Working Group.

3.3 Epidemiological studies of carcinogenicity in humans

The epidemiological investigation of the relation between exposure to chlorinated drinking-water and cancer occurrence is problematic because any increase in relative risk over that in people drinking unchlorinated water is likely to be small and therefore difficult to detect in epidemiological studies. It is particularly important to obtain valid assessment of disease status, of confounding factors (see also Preamble, p. 26) and, most relevantly, of the level of exposure to chlorinated water.

Relevant exposure to chlorinated water is particularly difficult to measure. A number of surrogates, such as use of surface water, depth of wells and residence in a community with a chlorinated water supply, have been used. To the extent that they do not reflect exposure to chlorinated water during the possibly relevant time periods for the etiology of the cancers in question, they will result in misclassification of subjects by exposure and will introduce bias. In some studies, concentrations of particular chlorination by-products have been modelled retrospectively; the assumptions underlying such models are, however, unproven.

Correlation studies are generally of uncertain validity, because exposure variables assessed for whole communities do not necessarily reflect the exposure of individuals. Such studies have been used extensively in relation to chlorinated drinking-water, however, as exposure may vary less within geographical units (such as towns) than between them.

Case-control studies are generally considered to provide greater opportunity for valid inference than correlation studies, because in these studies exposure and outcome are correlated at the individual level. Some of the case-control studies available for review were, however, based on exposure measured at the community level, because of the difficulties in assessing exposure of individuals.

Moreover, in many of these correlation and case-control studies, information on the nature of the water source and its chlorination status was obtained subsequently to or contemporaneously with the period over which cancer occurrence was measured. Because there are usually long latent periods between exposure and disease, cancer rates should be correlated with the characteristics of water supplies that were current before the cancers occurred. Most of these studies

also did not address the problem of migration in and out of communities over time: the degree of exposure misclassification consequent on population mobility can vary between geographical areas and thus lead to unpredictable bias.

In a small number of case-control studies of cancer incidence, detailed information was collected about the residential histories of subjects and their exposure to chlorinated water over long periods, estimated by reference to historical data on water supplies. The accuracy of such exposure measurements depends on the accuracy of recall by study subjects and the availability of relevant water supply records. Moreover, water consumed outside the home and the daily quantity of water consumed have rarely been taken into account. Thus, even in the best studies, errors in exposure measurement may still be a problem.

An additional problem encountered in assessing the effects of chlorinated water is that the profile of chemical exposures resulting from chlorination depends on local conditions and may vary from place to place and from time to time. It is possible therefore that one criterion for assessing causality—consistency of findings among epidemiological studies—may not be entirely appropriate.

Comparisons of populations living in communities served by chlorinated water supplies and populations living in towns served by unchlorinated sources could be confounded by many factors, including the constituents of water supplies other than chlorination by-products; socioeconomic, industrial and cultural (e.g., smoking, diet, use of medications) characteristics of the populations; and the medical facilities available for diagnosing cancer. In most of the studies, very few, if any, of these factors were mentioned. Virtually all of the studies reviewed are therefore susceptible to bias from confounding, to some degree.

(*a*) *Correlation studies*

(i) *Surface* versus *groundwater*

In the studies described below, the surrogate measure of exposure to chlorinated water was exposure to surface water, although the status of neither the surface nor the groundwater was known.

Page *et al.* (1976) studied 64 parishes in Louisiana, USA, in which 32% of the population were supplied by the Mississippi River, 56% by groundwater and 12% by other surface supplies. Age-adjusted 20-year mortality rates (Mason & McKay, 1974) for cancers of the gastrointestinal tract, urinary tract (these two groupings were necessary owing to small numbers of deaths from cancers at related individual sites in some counties), breast and prostate and for cancers at all sites were analysed by multiple regression, including as independent variables the percentage of the parish population drinking Mississippi River water, rurality, income, and occupation in the petroleum and coal, chemical and mining industries, in four subgroups: white men, white women, non-white men and non-white women. The

proportion of the parish population using Mississippi River water was positively ($p < 0.05$) associated with cancer of the gastrointestinal tract in all four groups, for urinary tract cancer in white men and non-white women and for cancers at all sites in white men, non-white men and non-white women. [The Working Group noted that the parishes using Mississippi River water were all located in the southern part of Louisiana, and the possible effects of water supply type and social and cultural differences cannot be separated.]

A further analysis of these and other data (DeRouen & Diem, 1977) also took account of region, i.e., northern or southern Louisiana, to allow for cultural and occupational differences. In a multiple regression analysis, percentage use of Mississippi River water was significantly associated with mortality from cancers at all sites for white men, non-white men and non-white women, with gastrointestinal tract cancer mortality for non-white men and non-white women, with urinary tract cancer for non-white women, and with cancer of the lung for non-white men. Southern Louisiana parishes in which part of the population was supplied with Mississippi River water showed significantly higher mortality from all cancers for non-white women, cancer of the stomach for non-white women, cancer of the colon for both white and non-white women, cancer of the rectum for white men, cancer of the urinary bladder for white men and cancer of the lung for non-white men. In these parishes, there was significantly lower mortality from cancer of the lung in white women and from cancer of the liver in white men.

Kuzma *et al.* (1977) classified 88 Ohio (USA) counties by ground- or surface water source on the basis of a survey of water supplies conducted in 1960. A substantial proportion of the population was served by sources not included in the survey: in only 39 counties was more than 50% of the population on a water source that was covered. Average annual age-adjusted cancer mortality rates among whites from 1950 to 1969 (Mason & McKay, 1974) were obtained for cancers of the stomach, large intestine, rectum, biliary passages and liver, pancreas and urinary bladder; for all cancers at all sites; for lung cancer in men; and for breast cancer in women. Analysis of covariance included the water classification variable as a factor and percentage urbanization, median income, population size and percentage of the male population in manufacturing activity and agriculture-forestry-fishery activity as covariables. Adjusted mean mortality in counties classified as supplied by surface water significantly ($p < 0.05$) exceeded that in those supplied by groundwater for cancers at all sites combined in men, for stomach cancer in both men and women and for bladder cancer in men. When the 39 counties in which more than 50% of the population was on a water source covered by the survey were analysed, similar results were obtained. [The Working Group noted that in the absence of information on county population size and water source, it was

impossible to confirm the adequacy of covariance analysis to control for population size.]

Bean *et al.* (1982) compared cancer incidence rates in Iowa (USA) municipalities served by water from surface sources with those from groundwater sources. They omitted municipalities with populations of fewer than 1000 and those in which less than 90% of the water used was from the classified source. Only municipalities receiving water from a single source type in 1965-79 were included. Cancer incidence data were obtained for 1969-71 and 1973-78, from the Third National Cancer Survey and from the Surveillance, Epidemiology and End Results Program, respectively. Age-standardized, sex-specific incidence rates were calculated for cancers of the urinary bladder, breast, colon, lung, rectum, prostate and stomach. Details of socioeconomic status and occupation were obtained from the 1970 census and from the Directory of Iowa Manufacturers. Using a previously conducted population-based case-control study of urinary bladder cancer which included subjects in Iowa (Hoover & Strasser, 1980), the authors derived information on several variables, including education, income, manufacturing, labour force, change in population between 1960 and 1970 and smoking habits, for residents of towns on groundwater and on surface water. The case-control study had shown that 63% of the controls over 55 years of age had been on the same water supply for at least 20 years before onset of their cancer, and 77% had been on the same supply for at least 10 years before onset. Analyses were based on log-linear models. After adjustment for population size, the incidences of lung cancer and rectal cancer were significantly greater [details not given] for men and women served by surface water than for those drinking groundwater. Trends of risk over three categories of well depth were not significant.

Kool *et al.* (1981) studied 19 cities in the Netherlands, representing approximately one-third of the population of that country. Directly standardized, sex-specific mortality rates for cancers of the bladder, lung, oesophagus, stomach, colon, rectum and liver were calculated for 1964-76. Organic constituents of tap water were determined in 1976. Correlation coefficients between source type (surface/groundwater) were calculated, and a transformation of the rates showed that mortality from liver and urinary bladder cancer in men and lung cancer in both men and women was significantly greater ($p < 0.05$) in cities supplied with surface water. [The Working Group noted that no potential confounding factor was taken into account, and the statistical methods were not adequately described.]

(ii) *Chlorination and chlorination by-products*

Cantor *et al.* (1978) calculated directly age-standardized, sex-specific, cancer mortality rates by site for whites for 1968-71 in 923 US counties with more than 50% urbanization in 1970. Chloroform and total trihalomethane (THM) levels were

obtained from two drinking-water surveys carried out in 1975 by the US Environmental Protection Agency, and levels of bromine-containing trihalomethanes (BTHM) were calculated by subtraction. The proportion of each county served in 1960 by the sampled municipal water supplies was estimated. The correlation between chloroform and BTHM levels was 0.54. Weighted linear regression using all 923 counties was used to predict sex- and site-specific cancer rates in 1970 by including the following variables in the model: urbanization (%; 1970), education (1970), population size (1970), ratio of 1970:1950 population, workforce in manufacturing (%; 1970), population in each of 10 ethnic groups (%) and region. The differences between the observed and predicted values (residuals) were correlated with log-THM in the 76 counties where 50% or more of the population was served by a water supply included in either of the two surveys and in the 25 counties where 85% or more of the population was so served. All cancer sites for which the sex-specific mortality rate was more than $1.5/10^5$ per year were studied. In the analysis of the 76 counties, the only significant correlation found was between the residual mortality rates for lung cancer in females and level of total THM (correlation coefficient, $r = 0.22; p = 0.05$). In the analysis of the subset of 25 counties, there were significant correlations between kidney cancer in men and chloroform level ($r = 0.42, p = 0.04$) and between urinary bladder cancer in women and BTHM level ($r = 0.45, p = 0.02$); whereas the correlations for kidney cancer in women and lung cancer in men were very low or negative, the correlation coefficient for male urinary bladder cancer and BTHM level was 0.38 ($p = 0.06$). Partial correlations controlling for high-risk occupation were calculated for cancers of the urinary bladder and lung. After allowance for lung cancer mortality [presumably a proxy for cigarette smoking], the partial correlations of urinary bladder cancer with log-BTHM level in counties in which 85-100% of the population was served by sampled supplies were 0.33 and 0.42 for men and women, respectively. Adjustment for occupational exposures left the correlations unchanged.

Hogan *et al.* (1979) used cancer mortality rates in US counties for 1950-69 (Mason & McKay, 1974) for white men and women in a multiple regression analysis. The cancers considered were of the tongue, oesophagus, stomach, large intestine, rectum, biliary passages and liver, pancreas, breast, ovary, kidney, urinary bladder and other urinary, thyroid and bone and cancers at all sites. Data on exposure to chloroform were taken from the two surveys carried out by the US Environmental Protection Agency in 1975 and referred to by Cantor *et al.* (1978). Weighted and unweighted analyses were carried out, which included the following independent variables: population density, percentage of urbanization, percentage of non-white, percentage of foreign born, median income, education, percentage in manufacturing industry, population size (all in 1960), region and chloroform in finished water. There were substantial differences in the results of three analyses

based on different methods of weighting the units of observation. Consistent positive associations were found with chloroform exposure level in men and women in all analyses (with at least one significant result, $p < 0.05$) for cancers of the urinary bladder, breast, rectum and large intestine using data for the counties covered in the first survey and for cancers of the liver and tongue using data from the second survey. A significant negative association was obtained for pancreatic cancer using data from the second survey. Finally, in an analysis restricted to counties in which 50% or more of the population was on a sampled supply, significant associations were found for cancer of the large intestine in both men and women and for urinary bladder cancer in women. [The Working Group noted that the geographical areas and exposure measures were similar to those used by Cantor *et al.* (1978), and these results, therefore, do not provide independent evidence.]

Carlo and Mettlin (1980) obtained age-adjusted cancer incidence rates from the New York State Tumor Registry for 218 census tracts in Erie County, NY, USA, between 1973 and 1976. Nine census tracts with rates greater than three standard deviations from the mean or large institutions were excluded; cases with incomplete residence data were excluded. The cancer sites studied were oesophagus, stomach, colon, rectum, urinary bladder and pancreas; socioeconomic factors, mobility, percentage of non-white, urbanicity and occupation (only for bladder cancer) were controlled for. Total THM, derived from State records, and type of water source were included in the analysis. Use of surface water was significantly ($p < 0.05$) associated with the incidence of oesophageal and pancreatic cancer; total THM was not significantly related to any cancer site studied. [The Working Group noted that the quality of data on THM levels could not be assessed; it is probable that a number of neighbouring census tracts shared the same water supply; and the statistical procedure used was unclear.]

Tuthill and Moore (1980) studied communities in Massachusetts (USA) served by surface water in 1949, excluding those with a population of fewer than 10 000 persons in 1970 or with a growth rate exceeding 25% between 1950 and 1970. They calculated sex-specific standardized mortality ratios (SMRs) for 1969-76 for nine digestive and urinary tract cancers and ten other cancer sites thought unlikely to be related to water quality. Correlations were made between SMRs and three measures of water quality: average past (1949-51) chlorine dose, recent chlorine dose and recent total THM level. Data analysis included correlation and stepwise multiple regression. Potential confounding variables included were ethnic group, income, education, percentage of foreign-born, occupation in the textile, printing and chemical industries and population growth betweeen 1950 and 1970. There was no significant correlation between sex-specific SMRs and average chlorine dose in 1949-51. For recent chlorine dose and recent total THM level, there was a significant ($p < 0.05$) positive association with stomach cancer for women and for rectal cancer

for men. For recent total THM level, there was a significant negative association with stomach cancer for men. After multiple regression analysis allowing for sociodemographic factors, the significant associations disappeared. [The Working Group noted that the number of communities studied was not explicitly stated and the methods of analysis were not fully presented.]

In the study by Kool *et al.* (1981) (described on p. 110), no significant relationship was found between levels of THM and cancer mortality in 19 cities in the Netherlands.

Isacson *et al.* (1983) extended the analysis of their earlier study (Bean *et al.*, 1982; see p. 110) to include the chlorination status of the water supply (chlorinated prior to 1966 or never chlorinated). Directly standardized incidence rates for rectal cancer in men were significantly lower in municipalities with chlorinated water in both categories of well depth (< 150 feet and ≥ 150 feet [$<$ or ≥ 45.7 m]), but this difference was no longer significant after adjustment for potential confounding by other methods of water treatment (aeration, filtration, coagulation and sedimentation). When water sources were classified by chloroform content (0-96, 100-230 and 260-900 µg/l), nonsignificant increases were observed across these levels for cancers of the colon, rectum and urinary bladder in men and cancers of colon and rectum in women. [The Working Group noted that no test for trend with increasing chloroform level was presented.]

Zierler *et al.* (1986) compared mortality rates from cancers of the stomach, colon, rectum, urinary bladder, breast, lung, pancreas, kidney and lymphatic system in 23 Massachusetts (USA) communities provided with chlorinated water with mortality from these cancers in Massachusetts as a whole. There were higher mortality rates in communities served by a chlorinated water source for cancer of the stomach among both males and females and for cancer of the lung among males. [The Working Group noted that the exposed group provided a substantial proportion of the reference population.]

(iii) *Time-trend study*

Cech *et al.* (1987) used the introduction of a new water supply in Houston, TX, USA, as a natural experiment. Lake Houston was constructed in 1954, and much of the population of Houston, previously supplied with water from lightly chlorinated underground sources, thereafter received heavily chlorinated surface water. Fifty-six census tracts were studied: group A (138 697 residents) had used groundwater over the whole period of the study; group B (46 394) changed from ground- to surface water in 1954; and groups C (84 159) and D (163 466) changed from ground- to surface water after 1954. THM levels were measured in 1978-79, and average concentrations in source areas A, B, C and D were 4, 111, 129 and 50 µg/l, respectively. The outcome measure was age-adjusted five-year average

mortality from urinary tract cancer in 1940-74; control causes of death were respiratory cancer, bronchitis-emphysema and homicide. Trends in death rates over time showed little variation that could be related to the change in water supply. The slopes of the regression lines for urinary cancer rates for 1940-59 and for 1960-74 in area B showed a significant ($p < 0.05$) decrease for white men and a significant increase for white women; no such difference was found in other areas. Adjustment of mortality rates for education, population density, percentage population employed in high-risk industries, percentage population foreign-born, presence of metal or petroleum industries, and presence of hospitals with oncological units had no effect on the results. A cohort analysis was also carried out: there was some evidence of a birth cohort effect for urinary cancer in white women in area B. The authors concluded that there was little evidence of an effect of chlorination.

(*b*) *Case-control studies*

(i) *Community exposure data*

Alavanja *et al.* (1978) carried out a death certificate case-control study in seven counties of New York State (USA), chosen because the water supplies were diverse (including chlorinated surface and chlorinated and nonchlorinated groundwater); individual supplies had been stable for at least 15 years before the date of the study, and immigration had been low during the same period. In all, 3446 deaths occurring in 1968-70 from cancers of the gastrointestinal tract (oesophagus, stomach, small intestine, large intestine, rectum, liver, intrahepatic bile ducts, gall-bladder and bile ducts, pancreas and peritoneum and retroperitoneal tissue) and urinary tract (bladder, kidney, renal pelvis, ureter and other unspecified urinary organs) and 1416 lung cancer deaths were individually matched to noncancer deaths [not further defined] by year of death, race, sex, birthplace and county of residence. The 'usual place of residence' on the death certificate was taken as the place of residence. Water distribution maps [from an unspecified period] were used to locate the water supply for each case and control individually. The odds ratios associated with chlorination for gastrointestinal and urinary tract cancers combined were 1.44 for women and 2.09 for men (both $p < 0.005$). For lung cancer in all urban and rural areas combined, the odds ratios were 1.55 (not significant) for women and 1.83 ($p < 0.005$) for men. For individual cancer sites among men, all odds ratios were significantly greater than 1; only the odds ratio for stomach cancer was significantly raised for women. Random samples of cases and controls were taken in order to compare possible occupational exposures; male cases were more likely to have had occupational exposure to carcinogens than male controls (odds ratio, 1.25; not statistically significant). [The Working Group noted that no allowance for potential confounders (such as occupational exposure and smoking) was made in the

analysis, the statistical analysis of the data is inadequately described, and it is likely that the matching was not dealt with appropriately.]

Brenniman *et al.* (1980) carried out a death certificate case-control study incorporating 3208 deaths from gastrointestinal and urinary tract cancer occurring in Illinois, USA, between 1973 and 1976 and 43 666 non-cancer deaths as controls, excluding deaths from complications of pregnancy, congenital anomalies, perinatal disorders, mental disorders, senility and infectious diseases. The study was restricted to whites and to communities served by groundwater—272 chlorinated and 270 nonchlorinated. Data on water supply were obtained from an inventory of municipal water facilities published in 1963 and verified, where possible, by a questionnaire sent to the water supply source. Allowance was made in the analysis for age, sex, urbanicity and residence in a standard metropolitan statistical area. Cancers of the oesophagus, stomach, large intestine, rectum, liver, gall-bladder and bile ducts, pancreas, bladder and other urinary organs were studied for men and women separately. No significantly elevated odds ratio was found for any individual site.

Results from a study based on southern Louisiana (USA) parishes were presented in three articles (Gottlieb *et al.*, 1981, 1982; Gottlieb & Carr, 1982). Gottlieb *et al.* (1982) carried out a case-control study of 10 205 cancer deaths in 13 parishes in southern Louisiana. Deaths from the following cancers formed the case series: urinary bladder, colon, kidney, liver, non-Hodgkin's lymphoma, rectum, stomach, breast, brain, oesophagus, pancreas, Hodgkin's disease, leukaemia, lung, malignant melanoma, multiple myeloma and prostate. For each case, a control matched on sex, age, race and year of death was selected from among deaths from causes other than cancer, excluding causes related to each cancer. Analyses of surface *versus* groundwater were carried out (i) according to water source at death and (ii) restricted to subjects on the same source type at birth and at death (lifetime exposure). The former analysis revealed only three significant odds ratios: 1.79 for rectal cancer, 1.21 for breast cancer and 0.70 for multiple myeloma. The analysis of lifetime water use gave significant odds ratios of 2.50 for rectal cancer and 1.30 for breast cancer. [Confidence intervals were not given.] A dose-related response was seen for each of these cancers in the categories lifetime surface water, some surface water (only birth or death in a parish served by surface water) and lifetime groundwater use. Odds ratios were elevated for rectal cancer among men according to water use at death (2.21; 95% CI, 1.57-3.12) and for men and women according to lifetime water use (3.18; 1.96-5.19 in men and 1.73; 0.97-3.10 in women). There were also elevated risks for lung cancer among men on surface water at death (1.30; 1.05-1.62) and for breast cancer among women both on surface water at death (1.21; 1.00-1.46) and with lifetime surface water use (1.30; 1.00-1.69). An additional analysis based on 11 349 case-control pairs from 20 parishes (Gottlieb & Carr, 1982)

did not provide different results. [The Working Group noted some inconsistencies in the number of parishes studied; the analysis is inappropriate since matching was broken, and the results are not presented in an understandable format.] A further analysis of these data (Gottlieb *et al.*, 1981) was restricted to a sample of 692 deaths from rectal cancer and 1167 from colon cancer; 1859 controls were selected from strata based on age at death (± five years), race, sex, year of death and parish. Four categories of estimated lifetime surface water use were derived as in the earlier work: mostly surface (birth and death in a surface water parish), some surface (either birth or death in a surface water parish), possible surface (death in a groundwater parish, birth outside the study area), least surface (birth and death in a groundwater parish). Of the total population, 99.2% could be classified into one of the four surface water exposure levels. For rectal cancer, relative risks of 1.61 (95% CI, 0.91-2.85) and 2.11 (1.17-3.84) were found for a residence served by surface water for 10-19 and > 30 years, respectively, as compared with residence served by groundwater. A significantly increasing trend with a relative risk of 2.07 (95% CI, 1.49-2.88) was also found for lifetime consumption of 'mostly surface water' relative to the 'least surface water' category. No elevation in odds ratios for any of the variables of interest was found for colon cancer. [The Working Group noted some internal inconsistencies in these papers in the numbers of cases and controls, and the method of selection of controls and whether they were individually matched is unclear.]

A study of cancer mortality in Wisconsin, USA, in relation to water chlorination was reported by Young *et al.* (1981), Kanarek and Young (1982) and Young and Kanarek (1983). Young *et al.* (1981) carried out a matched case-control study based on the death certificates of white females who had died of cancer in Wisconsin during 1972-77, restricted to the 28 counties in which the population was relatively stable and in which there were both chlorinated and unchlorinated water supplies. A total of 8029 deaths due to cancers of the gastrointestinal and urinary tracts, lung, breast and brain were matched to white female noncancer deaths on county of residence, year of death and birth date. The water supply serving the usual place of residence as recorded on each subject's death certificate was obtained from a 1970 survey of the 202 water sources serving the study areas, gathered from a postal questionnaire to the water utilities. These data included type of water source (surface or ground), presence or absence of environmental factors that might influence organic content (e.g., rural run-off) and mean daily chlorination doses over the previous 20 years in four levels: none, low (< 1.00 ppm), medium (1.00-1.70 ppm) and high (1.71-7.00 ppm). In an unmatched analysis, adjusted for marital status, urban residence and high-risk occupations specific for certain cancer sites, significantly high odds ratios were found for colon cancer: 1.53 (95% CI, 1.11-2.11), 1.53 (1.08-2.00) and 1.51 (1.06-2.14) for the low, medium and

high categories, respectively. No significant increase in risk was found for other cancers. In areas with a rural run-off into the water supply, the odds ratios for colon cancer were higher, and these increased slightly after adjustment for depth of groundwater source and purification. In an additional analysis using matched data, similar results were obtained (Young & Kanarek, 1983). In a further analysis of these data (Kanarek & Young, 1982), in which organic contamination, source depth and purification were taken into account, the odds ratio for colon cancer among persons using chlorinated in relation to that for people using unchlorinated water sources (1.43; $p < 0.02$) increased to 1.81 ($p = 0.03$) for chlorinated sources contaminated by organic compounds and to 2.81 ($p = 0.01$) for chlorinated surface water.

In a case-control study of multiple cancer sites based on death certificates, Zierler *et al.* (1986) (see p. 113) compared communities in Massachusetts (USA) in which surface water was disinfected by chloramine treatment (see p. 51) (20 communities) or chlorine treatment (23 communities). More than 50 000 deaths from cancers of the urinary bladder, colon, kidney, pancreas, rectum, stomach, lung and breast occurring between 1969 and 1983 in persons aged 45 years or more were identified. Over 200 000 deaths from lymphatic cancer, cardiovascular disease, cerebrovascular disease, pulmonary disease and pneumonia/influenza were used as controls. Exposure was defined as residence in a community supplied with chlorinated water at the time of death; nonexposure, in a community supplied with chloraminated water. No elevated risk for cancer at any site was observed.

(ii) *Individual exposure data*

Lawrence *et al.* (1984) carried out a case-control study based on death certificates of white women who had been members of the New York State (USA) Teachers' Retirement System and had died from cancers of the colon and rectum. After geographical restrictions and other exclusions, 395 deaths occurring between 1962 and 1978 were included. Controls (395) were selected randomly from deaths due to any cause except malignant tumours and matched to the cases by age and year of death (within two years). Information was obtained on residence and employment 20 years prior to death, and water records were abstracted for both home and work addresses over the 20-year period. A model-based estimate of exposure to THM was derived from a study of New York State surface water systems. Potential confounding factors included in the matched and unmatched logistic regression analyses were population density, marital status, age and year of death. Only analyses for grouped colon and rectal cancers were reported. Results from the matched and unmatched analyses were identical. There was no significant finding in relation either to source type (odds ratio, 1.07; 90% CI, 0.79-1.43), to 20

years' cumulative chloroform dose or to five other measures of exposure to chlorine or THM.

Cantor *et al.* (1985, 1987) conducted a population-based case-control interview study of urinary bladder cancer in 10 areas of the USA comprising 2982 cases aged 21-84 who had been newly diagnosed in 1978 (73% of the eligible pool). A total of 5782 controls were selected by random-digit dialling for those age 21-64 and by Health Care Financing Agency listings for those 65 and older. Interviews in the homes of the subjects gathered information on residential history, fluid consumption and potential confounders (smoking, occupation, lower urinary tract infection, artificial sweetener use, use of hair dyes). Data on water source and treatment since 1900 was obtained from an independent survey of water utilities. Year-by-year profiles of water source (surface and ground) and water treatment (chlorinated and not) were derived for the lifetime of each respondent by merging individual residential and water utility information; 76% of all person-years could be related to a known water source. Reported consumption of drinking-water was added to the intake of other home beverages containing tap water to estimate total daily ingestion of tap water. Since one goal of the study was to estimate the risk associated with consumption of chlorinated surface water in comparison with nonchlorinated groundwater, the primary analyses were restricted to a subset of respondents who had lived at least 50% of their lifetime prior to interview at residences served by one or both of these two types of water source [59% of all cases and controls]. Analysis was by logistic regression. In initial analyses (Cantor *et al.*, 1985) that did not consider tap-water consumption levels, an association was found between duration of residence with a chlorinated surface source and risk of urinary bladder cancer. Only among nonsmokers was there a significant odds ratio for those exposed for more than 60 years (odds ratio, 2.3; 95% CI, 1.3-4.2); there was a nonsignificant inverse trend for current smokers. For all groups combined (controlling for smoking), odds ratios for the duration measure were close to one. In subsequent analyses (Cantor *et al.*, 1987), current total fluid and tap-water consumption were considered in conjunction with duration of exposure to chlorinated surface water. Total fluid consumption was related to urinary bladder cancer risk, and tap water was the main risk factor (test for trend: males, $\chi^2 = 22.6$, $p < 0.0001$; females, $\chi^2 = 3.15$, $p = 0.08$). These findings were not modified by extent of disease. When respondents were grouped by duration of chlorinated surface water use, significant trends with tap-water intake were restricted to persons who had consumed chlorinated water for 40-59 years and ≥ 60 years. The odds ratios for the highest (≥ 1.96 l/day) *versus* the lowest (≤ 0.80 l/day) quintiles of intake in these two duration strata were 1.7 and 2.0, respectively, with significant trends ($p = 0.006$ and $p = 0.014$, respectively). The trends in odds ratios with tap-water intake were nonsignificant for up to 39 years' duration. There was a

significant trend with duration of residence with a chlorinated surface water supply, but only among women whose tap-water consumption was above the median ($p = 0.02$). The overall increase in the odds ratio with duration seen among nonsmokers in the previous analysis (Cantor *et al.*, 1985) was more exaggerated among respondents whose tap-water consumption was above the median ($p = 0.01$ for trend) than in those whose consumption was below the median ($p = 0.40$ for trend).

Lynch *et al.* (1989) conducted an analysis of the Iowa respondents in the study of Cantor *et al.* (1987), comprising 354 cases of urinary bladder cancer and 752 controls. Chlorination was quantified in four ways, with increasing levels of specificity: (i) assuming that the respondent's lifetime was spent consuming the type of water provided by the community of his or her most recent place of residence; (ii) assuming that the person's most recently used water supply (whether or not his or her community's supply) was used for life; (iii) applying the most recent water supply to the number of years of actual residence at this place; and (iv) using the entire lifetime residential/water supply history. For methods (ii), (iii) and (iv), there were significant trends with exposure to chlorination, the highest odds ratio being found for method (iv) (test for trend: $\chi^2 = 10.90$, $p = 0.001$). The odds ratios for 1-25, 26-50 and > 50 years of exposure to chlorination relative to no exposure using method (iv) were 1.42, 1.70 ($p < 0.01$) and 2.14 ($p < 0.01$). After adjustment for age and smoking, the odds ratio for history of any exposure to chlorinated water was 1.47. The highest unadjusted odds ratio [no adjusted odds ratio reported] was found for exposure only to prechlorinated or prefiltered surface or shallow groundwater (odds ratio, 2.95; 95% CI, 1.52-5.75). In this study subset of Iowa respondents, cigarette smokers (more than 25 pack-years) who had had exposure to chlorinated drinking-water had a higher odds ratio (4.48; 95% CI, 2.47-8.13) than smokers never so exposed (2.89; 1.41-5.89), relative to nonsmokers never exposed to chlorinated drinking-water. This result contrasts with the findings from the overall study in which smokers who had used chlorinated surface water were not at excess risk (Cantor *et al.*, 1985).

Cragle *et al.* (1985) identified 200 cases of colon cancer newly diagnosed between 1978 and 1980 at seven hospitals in North Carolina (USA) who had had at least 10 years' residence in the state. At least two hospital controls were matched to each case by age, race, sex, vital status, date of diagnosis and hospital. Information on residential history and a variety of potential confounding factors was collected from the respondents by either a personal interview or by mail questionnaire. Each subject's residence history for 1953-78 was related to data from the water company to derive estimates of the duration of residence on chlorinated and nonchlorinated supplies. A logistic regression analysis was carried out which included a chlorination variable and several potential confounders. The authors concluded

that there was an association between chlorination and colon cancer in people over the age of 60. [The Working Group noted that a number of details of the study design are not adequately described: it is not stated how many deceased cases and controls were selected and what procedure was used for obtaining data on these subjects; it is not clear how the chlorination variable was treated in the analysis; and, in spite of the matched nature of the design, an unmatched analysis was apparently carried out.]

A study in Wisconsin (USA), reported by Young *et al.* (1987) was designed to estimate the risk for colon cancer associated with chronic ingestion of THMs occurring as by-products of water chlorination. White men and women aged 50-90 were included. Cases were identified from a state-wide hospital tumour registry over a two-year period; 347 cases (45% of those sampled) were included in the analysis. Two sets of controls were used: 639 cancer controls identified from the same source as the cases, and 611 population controls identified from driver's license records, representing 48% of controls sampled. Self-completed questionnaires, supplemented with medical records, were used to obtain lifetime histories of residence, water use and medical and occupational histories. Water company records and contemporary measurements of THM were used to estimate the THM content of all types of water source in the past and then to construct estimates of lifetime ingestion of THM for each subject. Odds ratios for colon cancer relative to population controls, adjusted for age, sex and population size were 1.10 (95% CI, 0.68-1.78) for estimated cumulative exposure to 100-300 mg THM and 0.73 (0.44-1.21) for 300 mg or more, relative to the baseline group (less than 100 mg lifetime ingestion of THM). Analyses comparing surface with groundwater sources and chlorinated with nonchlorinated sources also showed no association with colon cancer risk. [The Working Group considered that the response rate in this study was too low to permit reliable inferences to be made.]

Zierler *et al.* (1988) carried out a case-control study of urinary bladder cancer based on death certificates of residents of 43 Massachusetts (USA) communities served by surface water disinfected by chlorine or chloramine. A total of 1057 deaths from urinary bladder cancer in people aged 45 or more occurring between 1978 and 1984 were identified. Controls were obtained from an age-stratified sample of deaths from the following causes: lung cancer, lymphoma, cardiovascular disease, cerebrovascular disease and chronic obstructive pulmonary disease (total, 2144). A large number of the cases and controls included in this study were also included in a previous case-control study carried out by the same authors (Zierler *et al.*, 1986; see p. 117). Informants were found for 614 (58%) of the cases and 1074 (50%) of controls and were interviewed about the decedents' residential and smoking history. Each subject's residential history was linked to historical data on water source obtained from the US Environmental Protection Agency and State

water authorities. Four categories of lifetime exposure to chlorinated water were defined, and each individual was placed into one of these. Information on socioeconomic status and high-risk occupations was obtained indirectly at the level of the community. Odds ratios for usual and lifetime exposure to chlorinated water with respect to lifetime exposure to chloramine were 1.4 (95% CI, 1.1-1.8) and 1.6 (1.2-2.1). When analysis was restricted to 30 communities each supplied by a single authority, the odds ratio for lifetime exposure with respect to lifetime nonexposure was 1.6 (1.1.-2.4). [The Working Group noted that the response rate was very low. It was unclear whether information on water supplies was obtained when individuals resided outside the 43 communities. The choice of controls may not have been appropriate. Confounding by city size was not addressed. Differences between the results of this study and those of Zierler *et al.* (1986) may be due to the fact that the exposure information in this study was more precise or to selection biases due to low response rates.]

(*c*) *Cohort study*

Wilkins and Comstock (1981) conducted a cohort study in Washington County, Maryland (USA) on a population of 14 553 white men and 16 227 white women over 25 years of age, who were resident in 1963. Follow-up over a 12-year period to mid-1975 was through death certificate records, the cancer registry and medical records at Washington County Hospital. [No information was given on completeness of follow-up.] Data on personal and socioeconomic variables in 1963 (age, marital status, education, smoking history, length of residence, frequency of church attendance, adequacy of housing and persons per room, source of drinking-water) were available. Sex- and site-specific incidence rates were calculated for cancers of the biliary passages and liver, kidney and urinary bladder. Mortality rates were calculated for the same sites and also for cancers of the oesophagus, stomach, colon, rectum, pancreas, lung, breast, cervix, ovary, prostate and brain, and leukaemia, and non-cancer causes of death (cirrhosis of the liver, bronchitis and emphysema, pneumonia, aortic aneurysm, road accident, fall, suicide, arteriosclerotic heart disease, hypertension, stroke and all causes.) Water sources were classified into three groups according to the subjects' residence in 1963: high exposure (23 727 urban residents served by chlorinated surface water systems; average chloroform concentration, 107 μg/l), low exposure (2231 users of unchlorinated, deep wells), and an intermediate group of 4842 residents of four small towns served by combined chlorinated surface and groundwater. In the incidence study, the only consistent results for men and women were adjusted relative risks (high *versus* low exposure) of 1.80 and 1.60 for urinary bladder cancer based on five and two cases in the low-exposure category (both $p > 0.05$). Only for urinary bladder cancer in men was there a relationship with duration of exposure

(relative risk, 6.46; 95% CI, 1.00- > 100 for 12 or more years at one address). In the mortality study, a significant result was obtained only for breast cancer (2.27; 1.16-4.89); however, when the relative risks were ranked, three of the four highest were for sites for which there was an a-priori suspicion of an association with organic contamination of drinking-water (liver: 2.98, 0.92-14.84; kidney: 2.76, 0.67-23.06; and urinary bladder: 2.20, 0.71-9.39). Relative risks were 0.89 (0.57-1.43) for cancer of the colon and 1.42 (0.70-3.16) for cancer of the rectum. [The Working Group noted that the large number of liver cancer deaths may indicate the inclusion of secondary liver cancers.]

Studies relevant to the evalution

Table 11 gives a summary of the results from those studies on which the final evaluation was based. Some studies were excluded because of the methodological limitations described on pp. 107-108 and in the square brackets following the descriptions of some studies; some were excluded because they largely overlapped with other studies included in the Table. For correlation studies, only an indication of the direction of the results is given; odds ratios or relative risks (with 95% CI when available) are given for case-control studies and for the cohort study.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

Water supplies were first chlorinated at the turn of the century, and over the following two decades chlorination was introduced for disinfection of drinking-water in most industrialized countries. In the chlorination process, chlorine reacts mainly with natural water constituents to produce a complex mixture of by-products, including a wide variety of halogenated compounds, the actual levels of which depend on the amount of chlorine added and the type of water source. In general, groundwaters produce lower levels, while surface waters often tend to produce higher levels of chlorination by-products; however, there is some evidence that groundwaters can give higher levels of brominated substances, probably due to higher levels of bromide in the untreated water. Estimates of the total halogenated organic matter generated during chlorination suggest typical levels in the range < 10-250 µg/l as chlorine. The main chlorination by-products are trihalomethanes and chlorinated acetic acids, which usually occur in the range 1-100 µg/l (although higher levels have been reported). Many products occur in the range 1-10 µg/l, while a large number can be detected at levels of < 1 µg/l. The

Table 11. Summary of results of selected epidemiological studies[a]

Author, year	Exposure variable	Bladder		Colon		Rectum		Stomach		Lung	
		M	F	M	F	M	F	M	F	M	F
Correlation studies											
DeRouen & Diem (1977)	Surface *vs* groundwater										
	Whites	(+)[b]	(+)[b]	(+)[c]	(+)[c]						
	Nonwhites	(+)[b]	+[b]	+[c]	+[c]						
	River *vs* non-river										
	Whites	+	(-)	(+)	+	+	(+)	(-)	(-)	(+)	-
	Nonwhites	(+)	(+)	(+)	+	(+)	(+)	(+)	+	+	(-)
Kuzma *et al.* (1977)	Surface *vs* groundwater	+	(+)	(+)	+/-	(+)	+/-	+	+	(+)	
Bean *et al.* (1982)	Surface *vs* groundwater	+/-	(-)	+/-	+/-	+	+	+/-	+/-	+	+
Cantor *et al.* (1978)	Chlorinated surface *vs* unchlorinated groundwater	(+)	+	+/-	+/-	+/-	+/-	+/-	(-)	(+)	(+)
Tuthill & Moore (1980)	Trihalomethanes in 1978	+/-	(+)	+/-	+/-	+	(+)	-	+	+/-	+/-
	Chlorine dose in 1950	+/-	+/-	+/-	+/-	(-)	+/-	(-)	(-)	+/-	(-)
Isacson *et al.* (1983)	Chlorinated *vs* unchlorinated	(+)	(-)	(+)	(-)	-	(+)	(-)	(-)	(+)	(+)
Time-trend studies											
Cech *et al.* (1987)	Chlorinated *vs* unchlorinated	(-)[b]	(+)[b]							(-)	(+)
Case-control studies, community-based exposure definition											
Brenniman *et al.* (1980)	Chlorinated *vs* unchlorinated	0.99	0.95	1.04	1.17	1.14	1.35	0.91	1.07		
Gottlieb *et al.* (1982)	Lifetime use of surface *vs* groundwater.	1.32 (0.88–1.97)	1.02 (0.57–1.82)	0.90 (0.60–1.37)	1.05 (0.73–1.51)	3.18*[d] (1.96–5.19)	1.73*[d] (0.97–3.10)	1.25 (0.85–1.84)	1.01 (0.61–1.66)	1.05 (0.77–1.43)	1.39 (0.63–3.11)
Young & Kanarek (1983)	Chlorinated *vs* unchlorinated[e]		1.08		1.41*		1.19		0.72		0.86
Zierler *et al.* (1986)	Chlorinated *vs* chloraminated	1.04 (0.94–1.16)	1.05 (0.92–1.21)	0.85 (0.80–0.90)	0.92 (0.87–0.97)	0.98 (0.88–1.09)	0.94 (0.84–1.05)	0.95 (0.87–1.03)	1.01 (0.92–1.10)	0.91 (0.86–0.96)	0.95 (0.91–0.98)

Table 11 (contd)

Author, year	Exposure variable	Bladder		Colon		Rectum		Stomach		Lung	
		M	F	M	F	M	F	M	F	M	F
Case–control studies, individual exposure definition											
Lawrence *et al.* (1984)	Surface *vs* groundwater[a]					1.07 (0.79–1.43)[f]					
Cantor *et al.* (1987)	60 years or more on chlorinated water, water consumption > median	1.2 (0.7–2.1)	3.2*[g] (1.2–8.7)								
Zierler *et al.* (1988)	Chlorinated *vs* chloraminated, lifetime exposure	1.6* (1.2–2.1)									
Cohort study											
Wilkins & Comstock (1981)	Chlorinated *vs* unchlorinated	1.80 (0.80–4.75)	1.60 (0.54–6.32)								

[a](+), positive association; +, positive association, $p < 0.05$; (-) negative association; -, negative association, $p < 0.05$; +/-, no association
[b]Urinary tract
[c]Gastrointestinal tract
[d]Significant trend (both sexes combined) across two levels of exposure (source at death, lifetime source)
[e]Women only
[f]Colorectal cancer; 90%confidence interval
[g]Women only
[g]Significant trend across five levels of duration of residence with a chlorinated surface drinking-water source (0, 1–19, 20–39, 40–59 and ≥ 60 years)
*$p < 0.05$

by-products responsible for most of the bacterial mutagenicity found in chlorinated drinking-water, 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5*H*]-furanone (MX) and associated substances, are present at very low concentrations (< 0.1 µg/l).

4.2 Experimental carcinogenicity data

Two series of studies were considered to provide evidence that could support an evaluation of the potential carcinogenicity of chlorinated drinking-water.

Samples of material concentrated from treated and undisinfected or treated and chlorinated water samples were tested in mice in three initiation-promotion experiments (by subcutaneous injection followed by topical application of 12-*O*-tetradecanoylphorbol 13-acetate). None of the concentrates derived from the chlorinated water induced a significantly increased incidence of skin tumours when compared with concentrates derived from undisinfected water samples or with saline.

In one experiment in mice, oral administration of chlorinated humic acids in the drinking-water did not increase the incidence of tumours over that in animals receiving unchlorinated humic acids or in saline-treated controls.

4.3 Human carcinogenicity data

Seven case-control studies conducted in the USA were considered to provide evidence that could support an evaluation. Four of these had community exposure data, and three had individually derived exposure data. The four studies with community exposure data each included several cancer sites. One study showed a significant increase in risk for colon cancer only; another showed a significant increase only for rectal cancer; the other two studies showed no excess risk for cancer.

Of the three case-control studies with individual exposure data, one was a population-based study of urinary bladder cancer carried out by interview in 10 areas of the USA. Many potential confounding factors, including smoking, were taken into account in the analyses. An early analysis of the study showed a significant association between long-term use at home of a chlorinated surface water source (as compared to an unchlorinated groundwater source) and urinary bladder cancer in nonsmokers only. In a subsequent analysis, tap-water intake was considered in addition to home water source, and consumption level of tap water was significantly associated with urinary bladder cancer; this effect was substantially confined to those who had lived for 40 years or more in a house with a chlorinated surface water source. There were significant and increasing trends in urinary bladder cancer risk with duration of residence in a house with a chlorinated surface water source for both women and nonsmokers whose tap-water

consumption was above the median. In a further report based only on Iowa participants in this study, risk for urinary bladder cancer was associated with duration of use of a chlorinated water source, and the association became stronger with increasing accuracy of the exposure measure.

In the second of these case-control studies, carried out in Massachusetts (USA), the authors reported an excess risk for mortality from urinary bladder cancer among people who had lived in areas with chlorinated water supplies as compared with chloraminated supplies. Some confounding factors, including smoking, were taken into account; however, the proportion of eligible subjects for whom exposure could be ascertained was low.

In a third case-control study, based on deaths among members of the New York State Teachers' Retirement System, no association was found between deaths from cancers of the colon and rectum combined and estimated use of surface water or intake of chloroform from domestic and workplace water supplies over the 20 years prior to death. Few confounding variables were taken into acount.

A cohort of the general population in a county in Maryland (USA) was enrolled and surveyed in 1963 and followed up to 12 years. Urinary bladder cancer incidence was found to be higher in both men and women residents supplied mainly by a chlorinated surface water source compared with county residents who obtained their drinking-water from unchlorinated deep wells; but the effects of chlorinated drinking-water could not be distinguished from factors related to urbanicity, and the numbers were too small to rule out a chance effect.

Six correlation studies and one time-trend study were considered by the Working Group to provide some useful data. These studies showed moderately consistent patterns of a positive correlation between use of surface water or of chlorinated water and cancers of the stomach, colon, rectum, urinary bladder and lung, with the most consistent patterns for cancers of the urinary bladder and rectum.

The studies that were considered informative, and therefore included in this summary, were nevertheless difficult to interpret in an evaluation of the carcinogenicity of chlorinated drinking-water. The water variables studied—whether surface or groundwater and others—were generally imperfect surrogates for the subject of this monograph. There is cause for some scepticism about the estimates of exposure to chlorinated drinking-water in all of these studies. Furthermore, very few attempted to document exposure over long periods of the subjects' lives. Chlorination by-products differ according to local conditions and practices of chlorination, and the health effects found in one place may not be found elsewhere. Many variables, such as smoking habits, dietary practices and environmental conditions, influence the risks for cancer, and they may differ between populations served by chlorinated and unchlorinated water supplies. Such

factors should ideally be taken into account in an epidemiological study; however, in most of the studies evaluated, there was little if any information available about them. When the data are examined on the basis of individual cancer sites, the evidence of elevated risk is strongest for cancer of the urinary bladder. The strongest study of cancer at this site supports the hypothesis of an elevated risk due to drinking chlorinated surface water compared with unchlorinated groundwater. However, the sum of the evidence from other studies, although showing some degree of consistency, is severely compromised by the weaknesses outlined above.

4.4 Other relevant data

Elevated serum cholesterol levels were reported in women but not in men living in communities served by chlorinated *versus* nonchlorinated water supplies in one study. No difference in the prevalence of ancephaly was observed between villages served by chlorinated and nonchlorinated groundwater in another study.

In regard to studies of genetic and related effects, only those reports were included in which the role of chlorination could be evaluated. Samples of unconcentrated chlorinated drinking-water were not genotoxic in bacteria or in a micronucleus assay in plants and did not induce morphological transformation in cultured mammalian cells. Samples of organic material concentrated from chlorinated surface waters were usually genotoxic in bacteria and induced sister chromatid exchange, micronuclei and chromosomal aberrations in single studies with cultured mammalian cells. In a single study, no activity was observed in a mammalian cell assay for mutation.

Samples of organic material concentrated from chlorinated groundwaters were less frequently mutagenic in bacteria than those from chlorinated surface waters; in a single study, they induced sister chromatid exchange but not micronuclei in cultured mammalian cells.

Samples of organic material concentrated from surface water treated with either chlorine dioxide or ozone followed by chlorination induced mutation in bacteria in some studies.

4.5 Evaluation[1]

There is *inadequate evidence* for the carcinogenicity of chlorinated drinking-water in humans.

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[1]For definition of the italicized terms, see Preamble, pp. 30-33.

There is *inadequate evidence* for the carcinogenicity of chlorinated drinking-water in experimental animals.

Overall evaluation

Chlorinated drinking-water *is not classifiable as to its carcinogenicity to humans (Group 3).*

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