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Trihalomethanes in Drinking-water

Background document for development of WHO *Guidelines for Drinking-water Quality*

"Brominated trihalomethanes" and "Total trihalomethanes" originally published in *Guidelines for drinking-water quality*, 2nd ed. Vol. 2. *Health criteria and other supporting information.* Geneva, World Health Organization, 1996; "Chloroform" originally published in *Guidelines for drinking-water quality*, 2nd ed. Addendum to Vol. 2. *Health criteria and other supporting information.* Geneva, World Health Organization, 1998.

Preface

One of the primary goals of WHO and its member states is that "all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water." A major WHO function to achieve such goals is the responsibility "to propose ... regulations, and to make recommendations with respect to international health matters ...."

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects reviewing selected microorganisms was published in 2002.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared/updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants examined in drinking-water.

For each chemical contaminant or substance considered, a lead institution prepared a health criteria document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom and United States of America prepared the requested health criteria documents.

Under the responsibility of the coordinators for a group of chemicals considered in the guidelines, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors before the documents were submitted for final evaluation by the experts meetings. A "final task force" meeting reviewed the health risk assessments and public and peer review comments and, where appropriate, decided upon guideline values. During preparation of the third edition of the GDWQ, it was decided to include a public review via the world wide web in the process of development of the health criteria documents.

During the preparation of health criteria documents and at experts meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the joint FAO/WHO Meetings on Pesticide Residues and the joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO internet site and in the current edition of the GDWQ.

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The first draft of the chloroform section of this background document was prepared by Ms M.E. Meek, Health Canada, to whom special thanks are due.

Ms Marla Sheffer of Ottawa, Canada, was responsible for the scientific editing of the entire document.

The efforts of all who helped in the preparation and finalization of this document, including those who drafted and peer reviewed drafts, are gratefully acknowledged.

Acronyms and abbreviations used in the text

BDCM	bromodichloromethane
CAS	Chemical Abstracts Service
CHO	Chinese hamster ovary
DBCM	dibromochloromethane
DNA	deoxyribonucleic acid
IARC	International Agency for Research on Cancer
IUPAC	International Union of Pure and Applied Chemistry
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
NCI	National Cancer Institute (USA)
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NTP	National Toxicology Program (USA)
SGPT	serum glutamate–pyruvate transaminase
TDI	tolerable daily intake
THM	trihalomethane
USA	United States of America
USP	US Pharmacopeia

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A. CHLOROFORM

1. GENERAL DESCRIPTION

1.1 Identity

CAS No:	67-66-3
Molecular formula:	$CHCl_3$

Chloroform is the most commonly occurring trihalomethane (THM); THMs are halogen-substituted single-carbon compounds with the general formula CHX_3.

1.2 Physicochemical properties[1]

Chloroform is degraded photochemically, is not flammable and is soluble in most organic solvents. However, its solubility in water is limited. Phosgene and hydrochloric acid may be formed by chemical degradation. The most important physical properties of chloroform are presented below (IARC, 1979; Budavari et al., 1989):

Property	*Value*
Physical state	Clear, colourless liquid
Boiling point at 101.3 kPa	61.3 °C
Melting point	-63.2 °C
Relative density (20 °C)	1.484
Auto-ignition temperature	>1000 °C
Water solubility (25 °C)	7.5–9.3 g/litre
Vapour density (101.3 kPa, 0 °C)	4.36 kg/m^3
Vapour pressure	8.13 kPa at 0 °C; 21.28 kPa at 20 °C
Stability	Air- and light-sensitive; breaks down to phosgene, hydrogen chloride and chlorine
Log octanol–water partition coefficient	1.97

1.3 Organoleptic properties

Chloroform has a characteristic odour and a burning, sweet taste. Its odour threshold values are 2.4 mg/litre in water and 420 mg/m^3 in air (Budavari et al., 1989; ATSDR, 1993).

1.4 Major uses

Commercial production of chloroform was 440 000 t in 1987. Chloroform has been used primarily in the production of chlorodifluoromethane, although smaller amounts have also been used as solvents, as cleaning agents and in fumigants. Although

[1] Conversion factor in air: 1 mg/m^3 = 0.20 ppm.

chloroform was used in the past as an anaesthetic and in proprietary medicines, these applications have been prohibited in many countries.

1.5 Environmental fate

It is assumed that most chloroform present in water is ultimately transferred to air as a result of its volatility. Chloroform has a residence time in the atmosphere of several months and is removed from the atmosphere through chemical transformation. It is resistant to biodegradation by aerobic microbial populations of soils and aquifers subsisting on endogenous substrates or supplemented with acetate. Biodegradation may occur under anaerobic conditions. Bioconcentration in freshwater fish is low. Depuration is rapid (IPCS, 1994a).

2. ANALYTICAL METHODS

There are several methods for the analysis of chloroform in air, water and biological materials. The majority of these methods are based on direct column injection, adsorption on activated adsorbent or condensation in a cool trap, then desorption by solvent extraction or evaporation by heating and subsequent gas chromatographic analysis. In water, detection limits range from 0.02 to 1 μg/litre (IPCS, 1994a; ISO, 1997).

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Outdoor air

Chloroform levels in ambient air in remote areas of the USA range from 0.1 to 0.25 $\mu g/m^3$. In urban and source-dominated areas, concentrations are 0.3–9.9 $\mu g/m^3$ and 4.1–110 $\mu g/m^3$, respectively (ATSDR, 1993). The population-weighted mean concentration of chloroform at 17 urban sites sampled across Canada in 1989 was 0.2 $\mu g/m^3$ (Environment Canada, 1992).

Hourly average concentrations of chloroform in the Netherlands, determined during 1979–1981, were generally 0.15 $\mu g/m^3$ (estimated detection limit) or less, the maximum value being 10 $\mu g/m^3$ (den Hartog, 1980, 1981). Average concentrations of chloroform during 1990 in four German cities (Berlin, Tübingen, Freudenstadt and Leipzig) ranged from 0.26 to 0.9 $\mu g/m^3$; the maximum value was 30 $\mu g/m^3$, detected in Tübingen (Toxicology and Environmental Health Institute of Munich Technical University, 1992).

3.2 Indoor air

Concentrations of chloroform in indoor air are generally higher than those in ambient outdoor air owing primarily to volatilization during water use. In a population survey in the USA, the observed increase was approximately 85%, which was consistent with assumptions concerning daily water use and likely release of chloroform from water into air (Wallace, 1987).

In a nationwide survey of 757 randomly selected one-family houses in Canada sampled over a 10-month period in 1991, the mean level of chloroform in indoor air was 4.1 μg/m^3; the maximum value was 69 μg/m^3 (Otson et al., 1992). Ullrich (1982) reported comparable concentrations in indoor air (1–3 μg/m^3) in Germany.

Chloroform levels in the air of enclosed swimming pools are also increased as a result of transfer from water. They vary as a function of several factors, such as the degree of ventilation, the level of chlorination, water temperature, the degree of mixing at the water surface and the quantity of organic precursors present (Lahl et al., 1981a). Over a period of 11 months, the levels of chloroform directly above the water surface in indoor public swimming pools in Bremen, Germany, ranged from 10 to 380 μg/m^3, with an average of about 100 μg/m^3 (Bätjer et al., 1980; Lahl et al., 1981a). Ullrich (1982) reported a similar mean value in four public swimming pools in Germany.

In an experimental study in which the mean concentration of chloroform in water in an indoor swimming pool was increased from 159 to 553 μg/litre, corresponding mean air chloroform levels ranged from 2.9 to 8.0 mg/m^3 (Levesque et al., 1994).

3.3 Water

Sources of chloroform in the aquatic environment include paper bleaching with chlorine, chlorination of recreational (pool) water, cooling water and wastewater. Chloroform is present in drinking-water through direct contamination of the source and through formation from naturally occurring organic compounds during chlorination. The rate and degree of formation of chloroform during chlorination are a function primarily of the concentrations of chlorine and humic acid, temperature and pH. Levels vary seasonally, with concentrations generally being greater in summer than in winter (IPCS, 1994a).

Concentrations of chloroform in groundwater vary widely, depending principally on proximity to hazardous waste sites (ATSDR, 1993). For example, chloroform was detected at levels ranging from 11 to 866 μg/litre in samples from five out of six monitoring wells drilled 64 m apart in a direction perpendicular to the northward flow of groundwater at a contaminated site in Pittman, Nevada, USA (the depth of unconfined groundwater was 2–4 m at this selected site) (Kerfoot, 1987).

Finished drinking-water collected in 1988–1989 from 35 US sources, 10 of which were located in California, in all four seasons contained median concentrations of chloroform ranging from 9.6 to 15 μg/litre. The overall median for all four seasons was 14 μg/litre (Krasner et al., 1989). In a 1987–1989 survey conducted in the USA, the mean chloroform concentration in finished water for surface water systems serving more than 10 000 people was 38.9 μg/litre (90th percentile, 74.4 μg/litre). The comparable mean value in the distribution system was 58.7 μg/litre (US EPA, 1992).

In a national survey of the water supplies of 70 communities in Canada conducted during the winter of 1976–1977, concentrations of chloroform in treated water of the

distribution system 0.8 km from the treatment plant averaged 22.7 µg/litre (Williams et al., 1980). Concentrations at 10 different locations in southern Ontario sampled in the early 1980s were 4.5–60 µg/litre in water leaving the treatment plant and 7.1–63 µg/litre 1.6 km from the plant (Oliver, 1983). In a more recent survey of samples collected during winter and summer of 1993 at 53 sites in nine provinces of Canada, chloroform was the major THM detected at all but three sites, these being groundwater sources that were treated with minimal chlorination. The contribution of chloroform to total THMs (ranging from 75.5 to 91.4%) was higher in summer than in winter and was slightly higher for chlorine–chlorine versus chlorine–chloramine or ozone–chloramine treatment. Although the majority of treatment facilities had relatively low total THM levels (<50 µg/litre), a small number had relatively high levels (>100 µg/litre), particularly in the summer (except for chlorine–chloramine disinfection) (Health Canada, 1995).

Chloroform levels in drinking-water in 100 German cities sampled in 1977 ranged from <0.1 to 14.2 µg/litre and averaged 1.3 µg/litre. Concentrations were similar in other surveys conducted in Germany in the late 1970s and early 1980s (Lahl et al., 1981a).

Mean levels of chloroform in drinking-water in the Netherlands in 1994 ranged up to 8.9 µg/litre (Versteegh et al., 1996).

3.4 Food

In an early study in Germany, chloroform was detected in several foodstuffs, in particular decaffeinated coffee (20 µg/kg), olive oil (28 µg/kg), pork (10 µg/kg) and sausages (17 µg/kg). Occasionally, concentrations were higher: up to 80 µg/kg in coffee and 90 µg/kg in sausages. Levels of 1–10 µg/kg were detected in flour products, potatoes, cod liver oil, margarine, lard, fish, mussels and milk; levels in most foodstuffs, however, were less than 1 µg/kg (Bauer, 1981).

Chloroform was detected in about 90 of 300 samples in a market basket survey of 231 "table-ready" foodstuffs (prepared and cooked as normally served) in the USA, most often in fat-containing samples (Daft, 1988). In a later account (Daft, 1989), it was reported that chloroform concentrations of 2–830 µg/kg of food were detected in 68% of 549 samples of foodstuffs obtained in a market basket survey (average of 71 µg/kg).

Chloroform was not detected in composite samples of meat/fish/poultry or in composite samples of oil/fat in 39 different foods in the USA, although it should be noted that the quantification limits were higher (18–28 µg/kg) than those in the studies described above. However, a chloroform concentration of 17 µg/litre was found in the composite of dairy foods (Entz et al., 1982).

Concentrations of chloroform in soft drinks range from 3 to 50 µg/litre, with levels for cola being at the upper end of the range (Abdel-Rahman, 1982; Entz et al., 1982; Wallace et al., 1984).

3.5 Estimated total exposure and relative contribution of drinking-water

Based on a daily inhalation volume for adults of 20 m^3, a body weight of 60 kg, the assumption that 20 out of 24 h are spent indoors and the mean levels of chloroform in indoor air presented above (1–4 μg/m^3), the mean intake of chloroform from indoor air for the general population is estimated to be 0.3–1.1 μg/kg of body weight per day.

Individuals may be exposed during showering to elevated concentrations of chloroform from chlorinated tap water (Jo et al., 1990a,b). Based on experimental studies with humans, these authors concluded that the contribution of dermal exposure was approximately equivalent to inhalation exposure during showering and that the average intake of chloroform (inhalation and dermal absorption) was 0.5 μg/kg of body weight per shower for a person weighing 70 kg.

Based on a review of relevant estimates, Maxwell et al. (1991) concluded that the ratio of the dose of chloroform received over a lifetime from inhalation to that received from ingestion of drinking-water is probably in the range of 0.6–1.5 but could be as high as 5.7. The ratio of the dose received dermally compared with that received orally over a lifetime from drinking-water was considered to be approximately 0.3 but could be as high as 1.8.

Based on daily consumption of 2 litres of drinking-water for adults, a body weight of 60 kg and the mean levels of chloroform presented above (generally <20 μg/litre), the estimated mean intake of chloroform from drinking-water for the general population is less than 0.7 μg/kg of body weight per day. Actual levels of exposure may be less than those estimated on the basis of mean levels in drinking-water, as most of the chloroform would be expelled from drinking-water that is heated before consumption (tea, coffee, soups, sauces). For example, approximately 96% of the total volatile halogenated hydrocarbon fraction was eliminated in water boiling for 5 min, whereas 50–90% was eliminated upon heating at 70–90 °C (Bauer, 1981). Owing to the wide variations in concentrations of chloroform in water supplies, intake from drinking-water could be considerably greater than estimated here for some segments of the general population.

Based on a daily ingestion of solid foodstuffs for adults of 1.5 kg (IPCS, 1994b), a mean body weight of 60 kg and the mean level and percent detection of chloroform in foodstuffs in the market basket survey reported by Daft (1989), the estimated intake of chloroform from foodstuffs is approximately 1 μg/kg of body weight per day.

Based on estimates of mean exposure from various media, therefore, the general population is exposed to chloroform principally in food, drinking-water and indoor air, in approximately equivalent amounts. The estimated intake from outdoor air is considerably less. The total estimated mean intake is approximately 2–3 μg/kg of body weight per day. For some individuals living in dwellings supplied with tap water containing relatively high concentrations of chloroform, estimated total intakes from

drinking-water through ingestion, inhalation and dermal contact are up to 10 µg/kg of body weight per day.

Pools are also an important source of exposure to chloroform for swimmers. Based on an experimentally determined relationship, Levesque et al. (1994) estimated that the daily dose of chloroform resulting from a 1-h swim (65 µg/kg of body weight per day) in conditions commonly found in public swimming pools is 141 times greater than that for a 10-min shower and 93 times greater than that for tap water ingestion.

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

The kinetics and metabolism of chloroform were reviewed in IPCS (1994a). Chloroform is well absorbed in animals and humans after oral administration, but the absorption kinetics are dependent upon the vehicle of delivery. After inhalation exposure in humans, 60–80% of the inhaled quantity is absorbed, with kinetics being dependent upon concentration and species-specific metabolic capacities. Chloroform is readily absorbed through the skin of humans and animals, and significant dermal absorption of chloroform from water while showering has been demonstrated (Jo et al., 1990a). Hydration of the skin appears to accelerate absorption of chloroform.

Chloroform distributes throughout the whole body, with levels being highest in the fat, blood, liver, kidneys, lungs and nervous system. Distribution is dependent on exposure route; extrahepatic tissues receive a higher dose from inhaled or dermally absorbed chloroform than from ingested chloroform. Placental transfer of chloroform has been demonstrated in several animal species and humans. Unmetabolized chloroform is retained longer in fat than in any other tissue.

The oxidative biotransformation of chloroform is catalysed by cytochrome P-450 to produce trichloromethanol. Loss of hydrogen chloride from trichloromethanol produces phosgene as a reactive intermediate. Phosgene may be detoxified by reaction with water to produce carbon dioxide or with thiols, including glutathione and cysteine, to produce adducts. The reaction of phosgene with tissue proteins is associated with cell damage and death. Little binding of chloroform metabolites to DNA is observed. Chloroform also undergoes cytochrome P-450-catalysed reductive biotransformation to produce the dichloromethyl radical, which becomes covalently bound to tissue lipids. A role for reductive biotransformation in the cytotoxicity of chloroform has not been established.

In animals and humans exposed to chloroform, carbon dioxide and unchanged chloroform are eliminated in the expired air. The fraction of the dose eliminated as carbon dioxide varies with the dose and the species. The rate of biotransformation to carbon dioxide is higher in rodent (hamster, mouse, rat) hepatic and renal microsomes than in human hepatic and renal microsomes. Also, chloroform is biotransformed more rapidly in mouse than in rat renal microsomes.

5. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

5.1 Acute exposure

The liver is the target organ for acute toxicity in rats and several strains of mice. Liver damage is characterized mainly by early fatty infiltration and balloon cells, progressing to centrilobular necrosis and then massive necrosis. The kidney is the target organ in male mice of other more sensitive strains. The kidney damage starts with hydropic degeneration and progresses to necrosis of the proximal tubules. Significant renal toxicity has not been observed in female mice of any strain.

Acute toxicity varies depending upon the strain, sex and vehicle. In mice, the oral LD_{50} values range from 36 to 1366 mg/kg of body weight; for rats, they range from 450 to 2000 mg/kg of body weight (IPCS, 1994a).

5.2 Short-term exposure

The most universally observed toxic effects of chloroform are liver and kidney damage. The severity of these effects per unit dose administered depends on the species, vehicle and method by which the chloroform is administered.

Many histological and biochemical parameters were examined in female and male CD1 mice (7–12 per sex per group) administered chloroform at 0, 50, 125 or 250 mg/kg of body weight per day in water by gavage for 14 and 90 days (Munson et al., 1982). After 90 days, a depression in the number of antibody-forming cells was found at the highest dose level in both sexes. In females at the highest dose level, a decrease in cell-mediated-type hypersensitivity was observed. Liver weight was increased after 90 days of exposure at all dose levels in females and at the highest dose level in males. After 90 days of exposure, the animals demonstrated tolerance against a challenging dose of 1000 mg/kg of body weight. Histological changes observed in the kidneys of all dosed animals included small intertubular collections of chronic inflammatory cells; in the liver, they included generalized hydropic degeneration of hepatocytes and occasional small focal collections of lymphocytes. In females, small amounts of extravasated bile were occasionally noted in the sinusoidal Kupffer cells.

Jorgenson & Rushbrook (1980) administered chloroform to female $B6C3F_1$ mice for 90 days at concentrations of 0, 200, 400, 600, 900, 1800 or 2700 mg/litre of drinking-water (equal to 0, 34, 66, 92, 132, 263 and 400 mg/kg of body weight). In the first week of the experiment, some mice in the highest dose group died of dehydration as a result of reduced water consumption. Concentration-related depression of the central nervous system occurred. The only treatment-related histopathological findings consisted of a mild adaptive and transitory fatty change in the livers of animals dosed with 66 mg/kg of body weight per day or more and a mild lymphoid atrophy of the spleen at 92 mg/kg of body weight per day and higher dose levels.

There is evidence that the vehicle in which chloroform is administered significantly affects its toxicity. Bull et al. (1986) reported that chloroform administered by gavage

in corn oil was significantly more hepatotoxic than equivalent doses administered in an aqueous emulsion (2% Emulphor, polyoxyethylated vegetable oil, GAF Corp.) to male and female $B6C3F_1$ mice (10 per sex per group) administered doses of 0, 60, 130 or 270 mg/kg of body weight per day for 90 days, based on examination of serum hepatic enzymes and hepatic histopathology.

In female $B6C3F_1$ mice exposed for 1 week to chloroform vapour at concentrations of 0, 4.9, 14.7, 49, 147, 490 or 1470 mg/m^3, there was increased cell proliferation in the nasal passages. This response was markedly less than that in F344 rats exposed to similar concentrations (Mery et al., 1994).

Palmer et al. (1979) exposed 10 male and 10 female SPF Sprague-Dawley rats to chloroform by intragastric gavage (in toothpaste) daily for 13 weeks. Dose levels were 0, 15, 30, 150 or 410 mg/kg of body weight per day. At 150 mg/kg of body weight per day, there was "distinct influence on relative liver and kidney weight" (significance not specified). At the highest dose, there was increased liver weight with fatty change and necrosis, gonadal atrophy in both sexes and increased cellular proliferation in bone marrow.

In a 90-day study by Chu et al. (1982), groups of 20 male and 20 female Sprague-Dawley rats were exposed to chloroform via drinking-water at dose levels of 0, 0.17, 1.3, 12 or 40 mg/day for males and 0, 0.12, 1.3, 9.5 or 29 mg/day for females; this was followed by 90 days of recovery. Water and food intake were reduced in the highest dose group. At the 40 mg/day level, mortality was increased. Upon histological examination, there were mild liver and thyroid lesions, especially in the highest dose group. In livers of both males and females, there was an increase in cytoplasmic homogeneity, density of the hepatocytes in the periportal area, mid-zonal and centrilobular increase in cytoplasmic volume, vacuolation due to fatty infiltration and occasional nucleic vesiculation and hyperplasia of biliary epithelial cells. Thyroid lesions consisted of a reduction in follicular size and colloid density, increase in epithelial cell height and occasional collapse of follicles. Liver and thyroid lesions diminished in severity during the 90-day recovery period.

Jorgenson & Rushbrook (1980) administered chloroform in drinking-water to male Osborne-Mendel rats for 90 days at concentrations of 0, 200, 400, 600, 900 or 1800 mg/litre (equal to 0, 20, 38, 57, 81 and 160 mg/kg of body weight per day). A concentration-related central nervous system depression was seen. Body weights in the highest dose group were reduced throughout the study. In biochemical investigations of serum, there were no important deviations from control values other than a dose-related increase in cholesterol at dose levels of 38 mg/kg of body weight per day or more after 60 days and a decrease in triglycerides in the highest dose group from 30 days onwards. After 90 days of administration, however, these parameters were affected in the two highest dose groups only. No dose-related histopathological changes were reported.

Exposure of male F-344 rats for 1 week to chloroform vapour at concentrations of 0, 4.9, 14.7, 49, 147, 490 or 1470 mg/m^3 resulted in concentration-dependent lesions in

the nasal passages. Changes included increased epithelial mucosubstances in the respiratory epithelium of the nasopharyngeal meatus and a complex set of responses in specific regions of the ethmoid turbinates. The NOEL for these responses ranged from 14.7 to 490 mg/m^3, with histological changes and induced cell proliferation being the most sensitive parameters (Mery et al., 1994).

5.3 Long-term exposure

Beagle dogs (eight per sex per dose) were given chloroform in a toothpaste base in gelatin capsules, 6 days per week for 7.5 years, at doses of 15 or 30 mg/kg of body weight per day (Heywood et al., 1979). After 6 weeks of treatment, there were significant increases in SGPT levels in dogs given the high dose. At the low dose level, significant increases were observed at 34 weeks and after. Similar effects were not observed in the vehicle control (16 dogs of each sex) or untreated control (8 dogs of each sex) groups. "Fatty cysts" of the liver were observed in both dose groups at the end of this study. The LOAEL in this study was 15 mg/kg of body weight per day.

5.4 Reproductive and developmental toxicity

Adverse effects on reproduction in ICR mice have been observed, but only at doses that are hepatotoxic (Borzelleca & Carchman, 1982). There are also some limited data to suggest that chloroform is toxic to the fetus in studies in Sprague-Dawley rats, but only at doses that are maternally toxic (>20 mg/kg of body weight per day) (Thompson et al., 1974; Ruddick et al., 1983).

5.5 Mutagenicity and related end-points

The results of genotoxicity assays for chloroform must be considered in light of two important aspects that could potentially compromise interpretation. First, there is the possibility that ethylcarbonate and diethylcarbonate, produced by reaction of phosgene with the ethanol that is routinely added to USP chloroform, could generate false-positive results. Secondly, owing to its volatility, chloroform must be tested in a sealed system to avoid false-negative results.

In three separate studies conducted under sealed conditions to ensure chloroform retention, results were negative in the Ames assay. In validated systems in *Salmonella typhimurium* and *Escherichia coli*, results have been negative both with and without metabolic activation. In only one uncommon test with *Photobacterium phosphorum* were positive results reported (Wecher & Scher, 1982).

Chloroform did not induce gene mutations in V79 Chinese hamster cells (Sturrock, 1977) or chromosomal aberrations in human lymphocytes *in vitro* (Kirkland et al., 1981).

In three of four micronucleus tests in mice, results were negative (Gocke et al., 1981; Salamone et al., 1981; Tsuchimoto & Matter, 1981). In the fourth micronucleus test, a weakly positive result was reported (San Augustin & Lim-Sylianco, 1978). The same

authors found a positive effect in the mouse host-mediated assay with *Salmonella typhimurium* TA1537 but not with TA1535.

In investigations of the potential of chloroform to induce unscheduled DNA synthesis *in vitro* and *in vivo* (single doses of 238 and 477 mg/kg of body weight by gavage in corn oil) at the most sensitive site for tumour formation, the female mouse liver, results have been uniformly negative and are consistent with the suggestion that neither chloroform nor its metabolites react directly with DNA *in vivo*. No increase in DNA repair was observed in freshly prepared primary cultures of human hepatocytes from discarded surgical material from four different individuals exposed to concentrations as high as 1 mmol of chloroform per litre (Butterworth et al., 1989).

Given the large number of sensitive assays to which chloroform has been submitted, it is noteworthy that the reported positive responses are so few. Furthermore, these few positive responses were randomly distributed among the various assays with no apparent pattern or clustering for any test system. Taken together, the weight of evidence indicates that neither chloroform nor its metabolites would appear to interact directly with DNA or possess genotoxic activity (IPCS, 1994a).

5.6 Carcinogenicity

Roe et al. (1979) administered 0, 17 or 60 mg of USP-grade chloroform per kg of body weight per day in a toothpaste base (vehicle) to ICI mice (control group 104 animals per sex, dose groups 52 animals per sex) by gavage, 6 days per week for 80 weeks, followed by a 16-week observation period. The control toothpaste did not contain eucalyptol and peppermint oil, whereas the toothpaste containing chloroform did contain these substances. Treatment with chloroform resulted in slightly increased survival, especially in the males. The most common cause of death was respiratory failure. A slightly increased incidence of fatty degeneration was observed among the chloroform-treated animals, and there was a slight increase in total tumours in male mice. Renal tumours (3 hypernephromas and 5 cortical adenomas) were reported in 8 out of 38 males in the high-dose group.

In a second experiment by Roe et al. (1979), the influence of peppermint oil, eucalyptol and chloroform was determined separately. In this study, male ICI mice received 60 mg of chloroform per kg of body weight daily, in a similar manner as in the study reported above. The vehicle control (toothpaste without chloroform, eucalyptol and peppermint oil) and dose groups consisted of 260 and 52 male animals, respectively (the groups receiving a dose of peppermint oil or eucalyptol also consisted of 52 animals). Again, the survival in the chloroform-dosed group was better than in the control group. However, administration of chloroform resulted in a kidney tumour frequency of 9/49 (2 hypernephromas and 7 adenomas), compared with 6/240 in controls.

In a third study by Roe et al. (1979), a chloroform dose of 60 mg/kg of body weight in toothpaste (containing eucalyptol and peppermint oil) was administered daily to male mice (52 per group) of the ICI, CBA, C57BL and CF1 strains for a period of 80

weeks. The chemical was also administered in arachis oil to male mice of the ICI strain. Each strain had its own control group. Terminal sacrifice was at 93, 97–99, 104 and 104 weeks for the CF1, ICI, C57BL and CBA strains, respectively. In this study, a treatment-related increase in survival was found in all strains tested, except for the CF1 strain. Treatment with chloroform resulted in a higher incidence of renal changes in the CBA and CF1 strains but not in the C57BL strain. The cause of death in all four strains was renal neoplasia in combination with respiratory and renal disease. In the C57BL, CBA and CF1 strains, no changes in tumour frequencies were observed. In the ICI mice, after treatment with chloroform in either the toothpaste vehicle or arachis oil, there was an increase in the incidence of malignant kidney tumours.

In an NCI carcinogenicity study, $B6C3F_1$ mice (20 animals per sex in the control group; 50 animals per sex in the dosed groups) received USP-grade chloroform stabilized with ethanol (0.5–1%) in corn oil 5 times a week by gavage (NCI, 1976a,b). Dosing was stopped after 78 weeks, and the animals were sacrificed after 92 weeks. The dose levels changed after 18 weeks, resulting in time-weighted average dose levels of 138 (low) and 277 (high) mg of chloroform per kg of body weight per day for male mice and 238 (low) and 477 (high) mg of chloroform per kg of body weight per day for female mice. Administration of the higher dose of chloroform reduced survival in the female mice. Causes of death were related to the observed liver tumours, pulmonary inflammation and cardiac thrombosis. This latter lesion was not observed in either the control or the low-dose group. There was a dose-related increased incidence of hepatocellular carcinomas in males and females. Mice presented clinical signs of illness (i.e., reduced food intake and an untidy appearance), but clear information on non-neoplastic lesions was not provided.

Increases in tumour incidence were not observed in a carcinogenicity bioassay in which female $B6C3F_1$ mice were exposed to 0, 200, 400, 900 or 1800 mg of chloroform per litre of drinking-water (number of animals: 430, 430, 150, 50 and 50, respectively) for a period of 2 years. These concentrations (monitored by analysis) correspond to time-weighted average daily chloroform doses of 0, 34, 65, 130 and 263 mg/kg of body weight (Jorgenson et al., 1985). Matched controls (50 females) received an amount of water without chloroform equal to that consumed by the 1800 mg/litre group. Initially, 25% of the animals in the two highest dose groups died, but later on the death rate was more or less equal to that in the control group. After 3 months, livers of animals exposed to chloroform concentrations of 65 mg/kg of body weight per day or more had a higher fat content than those of the controls (as examined by chemical techniques). After 6 months, liver fat content was increased in all exposed groups. Data on organ weights were not provided. No treatment-related effects on either liver or total tumour incidence were observed.

Palmer et al. (1979) exposed 50 male and 50 female SPF Sprague-Dawley rats to 60 mg of chloroform per kg of body weight per day for 80 weeks, then sacrificed them after 95 weeks. The same number of controls was gavaged with toothpaste containing essential oils. Respiratory disease was observed in both sexes. At week 95, survival was 32% for exposed males and 22% for controls. For females, survival was 26% for exposed animals and 14% for controls. There were no significant differences in any

tumour incidence. There was a significant decrease in plasma cholinesterase. Although there was a significant decrease in relative liver weight, there was no histological evidence of toxicity.

Osborne-Mendel rats (20 animals per sex in the control group; 50 animals per sex per dose group) received USP-grade chloroform stabilized with ethanol (0.5–1%) in corn oil 5 times a week by gavage (NCI, 1976a,b). Dosing was stopped after 78 weeks, and the animals were sacrificed after 111 weeks. The dose levels changed after 23 weeks, resulting in time-weighted average daily dose levels of 90 and 180 mg of chloroform per kg of body weight for males and 100 and 200 mg of chloroform per kg of body weight for females. Administration of chloroform reduced survival in male and female rats in all dose groups. A clear pathological reason for this effect in the rats was not given. In male rats, dose-related increased frequencies of kidney epithelial tumours were observed. In the females, a non-significant increase in the frequency of thyroid tumours was found. Rats presented clinical signs of illness (i.e., reduced food intake and an untidy appearance). However, clear information on non-neoplastic lesions was not provided.

Reuber (1979) re-evaluated the histological sections of the NCI (1976a,b) study and reported the same neoplastic lesions as the NCI. In addition, he noted that chloroform-dosed female rats developed liver lesions that were not seen in the control females.

Male Osborne-Mendel rats were exposed to 0, 200, 400, 900 or 1800 mg of chloroform per litre of drinking-water (number of animals: 330, 330, 150, 50 and 50, respectively) for a period of 2 years (Jorgenson et al., 1982). These concentrations (monitored by analysis) correspond to time-weighted average daily chloroform doses of 0, 19, 38, 81 and 160 mg/kg of body weight (Jorgenson et al., 1985). Matched controls received an amount of water without chloroform equal to that consumed by the 1800 mg/litre group. Additional rats were used for intermediate biochemical and histopathological examination. As a probable consequence of reduced drinking and reduced body weights, death rate was reduced with increasing chloroform dosage and in the matched control group. Biochemical examination of blood after 6, 12 and 18 months showed that chlorine, potassium, total iron and albumin levels and the albumin/globulin ratio tended to be increased after chloroform treatment, whereas levels of cholesterol, triglycerides and lactate dehydrogenase were decreased in all treated groups. These deviations were also observed in the matched controls, but the decreases in serum triglycerides and cholesterol levels were more severe at the two highest dose levels than in the matched control group. Data on organ weights were not provided. The only clear dose-related effect was an increase in renal tubular cell adenomas and adenocarcinomas. From 38 mg/kg of body weight per day upwards, the increase in the frequency of all kidney tumours was statistically significant. Although increased incidence of non-neoplastic histopathological effects was not reported, histological sections have been re-evaluated recently (ILSI, in preparation).

In a range of initiation/promotion assays in the liver, chloroform has not initiated tumour induction. Although chloroform had promotional activity in several studies, particularly when administered in corn oil (Capel et al., 1979; Deml & Oesterle, 1985,

1987), it inhibited the growth or formation of precancerous or cancerous cells in the majority of the assays in liver (Pereira et al., 1985; Herren-Freund & Pereira, 1986; Klaunig et al., 1986; Reddy et al., 1992) and in several in the gastrointestinal tract (Daniel et al., 1989, 1991).

5.6.1 Mechanism of carcinogenicity

The weight of the available evidence indicates that chloroform has little, if any, capability of inducing gene mutation or other types of direct damage to DNA. Moreover, chloroform does not appear capable of initiating hepatic tumours in mice or of inducing unscheduled DNA synthesis *in vivo* (IPCS, 1994a).

The pattern of chloroform-induced carcinogenicity in rodents in bioassays conducted to date can be summarized as follows. Chloroform induced hepatic tumours in $B6C3F_1$ mice (males and females) when administered by gavage in corn oil at doses in the range of 13–477 mg/kg of body weight per day (NCI, 1976a,b). However, when similar doses were administered to the same strain in drinking-water, hepatic tumours were not increased (Jorgenson et al., 1985). Liver tumours are observed, therefore, only in mice following administration by gavage in corn oil. This observation is consistent with those in initiation/promotion assays in which chloroform has promoted development of liver tumours, particularly when administered by gavage in a corn oil vehicle.

Chloroform also induces renal tumours, but at lower rates than liver tumours in mice. Chloroform induced kidney tumours in male Osborne-Mendel rats at doses of 90–200 mg/kg of body weight per day in corn oil by gavage (NCI, 1976a,b). However, in this strain, results were similar when the chemical was administered in drinking-water, indicating that the response is not entirely dependent on the vehicle used (Jorgenson et al., 1985). It should be noted, however, that at the higher doses in this study, there were significant reductions in body weight. In an early, more limited investigation, kidney tumours were increased in ICI mice but not in CBA, C57BL or CF1 mice administered chloroform by gavage in toothpaste (Roe et al., 1979). Therefore, the tumorigenic response in the kidney, although observed in both rats and mice (males), is highly strain-specific.

To investigate the possible role of replicative proliferative effects in the carcinogenicity of chloroform, a wide range of studies have been conducted in which replicative proliferative effects have been examined in similar strains of rats and mice exposed to similar doses or concentrations of chloroform, although for shorter periods, as in the principal carcinogenesis bioassays (Larson et al., 1993, 1994a,b,c, 1995a,b, 1996; Lipsky et al., 1993; Pereira, 1994; Templin et al., 1996a,b,c). Most of these studies involved evaluation of histopathological changes and cell proliferation in the kidney and liver, the latter determined as a BrdU labelling index in histological tissue sections.

The weight of evidence on genotoxicity, sex and strain specificity and concordance of cytotoxicity, regenerative proliferation and tumours is consistent with the hypothesis

that marked cytotoxicity concomitant with a period of sustained cell proliferation may underlie a secondary mechanism for the non-linear induction by a metabolite of chloroform-related tumours. The weight of evidence is strongest in this regard for hepatic and renal tumours in mice but more limited for renal tumours in rats.

Specifically, histopathological effects and regenerative proliferation were observed consistently following administration by gavage in corn oil but not following administration by gavage in drinking-water or continuous administration in drinking-water in the liver of $B6C3F_1$ mice (Larson et al., 1994a; Pereira, 1994) and kidney of F344 rats (Lipsky et al., 1993; Larson et al., 1994b). These data are consistent with the hypothesis that chloroform-induced cancer following gavage administration is secondary to events associated with induced cytolethality and cell proliferation. Results of available studies also indicate that the proliferative response is less when exposure is not continuous (e.g., inhalation for 5 days per week versus 7 days per week) (Larson et al., 1996; Templin et al., 1996c) and returns to baseline following a recovery period.

Correlations for renal tumours in the rat are less clear primarily due to the relative paucity of data on intermediate end-points and renal cancer in various strains and both sexes. There is some evidence of variations in sensitivity among strains, with those with most severe renal disease being most susceptible; however, available data are limited in this regard. Tumours occur at the site of renal injury following exposure via both gavage in corn oil and drinking-water. Based on studies conducted primarily in a strain of rats in which tumours have not been observed, a mode of action for carcinogenicity in the kidney based on cytotoxicity and tubular cell regeneration is plausible.

In a recent review article (Chiu et al., 1996), lack of observation of histopathological effects in test groups with tumours and lack of correlation between cytotoxicity and tumours in the kidneys of individual male rats at autopsy in the study of Jorgenson et al. (1982) are considered to be inconsistent with the above-mentioned seemingly plausible mechanism. Slides from this bioassay have recently been re-evaluated (ILSI, in preparation).

Based on studies conducted primarily in the F344 rat, available data are consistent with a mode of action for carcinogenicity in the kidney based on tubular cell regeneration. Studies in this strain indicate that chloroform causes damage and increases cell replication in the kidney at doses similar to those that induce tumours in Osborne-Mendel rats following gavage in corn oil for periods up to 3 weeks (Larson et al., 1995a,b). However, there has been no clear dose–response for renal damage or proliferation in F344 rats exposed to concentrations in drinking-water that were similar to those that induced tumours in Osborne-Mendel rats in the carcinogenesis bioassay of Jorgensen et al. (1985) (Larson et al., 1995b). In a single study in which the proliferative response was compared in F344 and Osborne-Mendel rats at 2 days following a single gavage administration, it was concluded that these strains were about equally susceptible to chloroform-induced renal injury, although a statistically significant increase in labelling index was observed at a much lower dose in the

Osborne-Mendel rat (10 mg/kg of body weight) than in the F344 rat (90 mg/kg of body weight); this latter observation may have been a function of the low value in controls for the Osborne-Mendel rats.

Data on the proliferative response in the strain in which renal tumours have been observed (Osborne-Mendel rats) are limited to examination at 2 days following a single administration by gavage in corn oil (Templin et al., 1996b); studies in which the proliferative response was examined in Osborne-Mendel rats following administration in drinking-water have not been identified. Although the results of this study are not inconsistent with a mode of action of induction of tumours based on tubular cell regeneration, they are considered inadequate in themselves to quantitatively characterize the dose–response relationship for an intermediate end-point for cancer induction.

5.7 Other special studies

Balster & Borzelleca (1982) administered chloroform in water to male ICR mice (8–12 per group) and examined their performance in a battery of neurobehavioural tests (several exposure periods and several dose levels). The only effect observed was a reduced achievement in an operant behaviour test after dosing with 100 or 400 mg of chloroform per kg of body weight in water for 60 days. At the chloroform level of 400 mg/kg of body weight, about half the treated animals died. No adverse effects on behaviour were observed after 90 days of dosing with 31 mg of chloroform per kg of body weight in water.

According to Burkhalter & Balster (1979), oral administration of chloroform to mice from 3 weeks before mating until the end of the lactating period (in both sexes the dose was 31 mg/kg of body weight) did not result in retardation of the development of responses to a battery of neurobehavioural tests in the pups.

5.8 Factors modifying toxicity

The *in vivo* toxicity of chloroform is modified by a range of factors. The rate of its biotransformation is a significant determinant of its toxicity. Hence, factors that increase or decrease chloroform biotransformation may alter the intensity of chloroform-induced toxicity. Moreover, the activities of the enzymes that metabolize chloroform may be increased or decreased by exposure to chemicals, and exposure to chloroform itself may alter chloroform metabolism.

In addition to differences in the rates of chloroform bioactivation, treatments that alter susceptibility are also important determinants of chloroform-induced toxicity. Cellular glutathione concentrations are an important determinant of susceptibility, and perturbations of glutathione homeostasis may markedly affect the toxicity of chloroform.

In experimental studies, the drinking-water contaminants monochloroacetic acid, dichloroacetic acid and trichloroacetic acid have potentiated the toxicity of

chloroform, although mechanisms were not elaborated (Davis, 1992; Davis & Berndt, 1992).

6. EFFECTS ON HUMANS

In general, chloroform elicits the same symptoms of toxicity in humans as in animals. In humans, anaesthesia may result in death as a result of respiratory and cardiac arrhythmias and failure. Renal tubular necrosis and renal dysfunction have also been observed in humans. The lowest levels at which liver toxicity due to occupational exposure to chloroform has been reported are in the range of 80–160 mg/m^3 (with an exposure period of less than 4 months) in one study and in the range of 10–1000 mg/m^3 (with exposure periods of 1–4 years) in another study (IPCS, 1994a). The mean lethal oral dose for an adult is estimated to be about 45 g, but large interindividual differences in susceptibility occur.

Analytical epidemiological studies of potential associations between ingestion of chlorinated drinking-water and colorectal cancer have been conducted in the USA in Wisconsin (Young et al., 1990), New York (Lawrence et al., 1984) and Iowa (Cantor et al., 1996). Similar investigations of bladder cancer have been conducted in Colorado, USA (McGeehin et al., 1993), Ontario, Canada (King & Marrett, 1996), and Iowa, USA (Cantor et al., 1996). Cumulative exposure to THMs was slightly higher in New York than in Wisconsin, but no increased colon cancer risk associated with THM exposure was observed in either study. Data reported thus far from a study in Iowa indicate that colon cancer was not associated with estimates of past exposure to chlorination by-products, but rectal cancer risk was associated with measures of exposure to chlorination by-products, duration of exposure to chlorinated water and increasing amounts of lifetime exposure to THMs. Levels of THMs were not reported. At this time, the evidence for an association between exposure to THMs in drinking-water and rectal cancer must be considered inconclusive. No evidence is available to suggest an increased risk of colon cancer, but studies have been conducted in areas where cumulative exposures are generally low.

Data reported thus far from a study in Iowa indicate that risk of bladder cancer is not associated with estimates of past exposure to chlorination by-products, except among men and smokers, for whom bladder cancer risk increased with duration of exposure after control for cigarette smoking. No increased risk of bladder cancer was associated with exposure to THMs in Colorado; cumulative exposure in this study was similar to that in New York and Wisconsin, where there was no increased risk for colon cancer. In Ontario, King & Marrett (1996) found an increased bladder cancer risk with increasing duration of exposure to THM levels; however, only after 35 or more years of exposure is the association statistically significant and of higher magnitude. The bladder cancer incidence was about 40% higher among persons exposed to greater than 1956 (µg/litre)-years THMs in water compared with those exposed to less than 584 (µg/litre)-years. Although it is not possible to conclude on the basis of available data that this association is causal, observation of associations in well conducted studies where exposures were greatest cannot be easily dismissed; however, the degree of evidence should be considered to be limited. In addition, it is not possible to

attribute these excesses to chloroform per se, although it is generally the disinfection by-product present at highest concentration in drinking-water.

There have also been a smaller number of epidemiological investigations of associations between consumption of chlorinated drinking-water and developmental/reproductive effects. In a recent review of relevant information, Reif et al. (1996) concluded that the existing database was inadequate to determine an association between exposure to disinfection by-products and adverse reproductive and developmental effects, drawing attention particularly to the variability in exposure assessments and examined end-points and potential for exposure misclassification and confounding.

7. GUIDELINE VALUE

The weight of evidence for genotoxicity is considered negative (IPCS, 1994a). The weight of evidence for liver tumours in mice is consistent with a threshold mechanism of induction. Although it is plausible that kidney tumours in rats may similarly be associated with a threshold mechanism, there are some limitations of the database in this regard.

A guideline value can therefore be developed on the basis of a TDI for threshold effects. The most universally observed toxic effect of chloroform is damage to the centrilobular region of the liver. The severity of these effects per unit dose administered depends on the species, vehicle and method by which the chloroform is administered. The lowest dose at which liver damage has been observed is 15 mg/kg of body weight per day administered to beagle dogs in a toothpaste base over a period of 7.5 years. Effects at lower doses were not examined. Somewhat higher doses are required to produce hepatotoxic effects in other species. Effects in the proximal tubules of the kidney cortex have been observed in male mice of sensitive strains and in both male and female rats of several strains. Levels inducing adverse histopathological effects in the range of 30 mg/kg of body weight per day have been reported in some studies in sensitive strains.

Based on the study by Heywood et al. (1979) in which slight hepatotoxicity (increases in hepatic serum enzymes and fatty cysts) was observed in beagle dogs ingesting 15 mg/kg of body weight per day in toothpaste for 7.5 years, and incorporating an uncertainty factor of 1000 (100 for inter- and intraspecies variation and 10 for use of a LOAEL rather than a NOAEL and a subchronic study), a TDI of 13 µg/kg of body weight (corrected for 6 days per week dosing) is derived. Allocation of 50% of total intake to drinking-water is a reasonable default based on estimates of mean exposure that indicate that the general population is exposed to chloroform principally in food, drinking-water and indoor air in approximately equivalent amounts and that most of the chloroform in indoor air is present as a result of volatilization from drinking-water. Moreover, the population is additionally exposed dermally to chloroform in drinking-water during showering. Based on an average body weight of 60 kg and daily ingestion of 2 litres of drinking-water, the guideline value is 200 µg/litre (rounded figure).

It is noted that a drinking-water concentration for a 10^{-5} excess lifetime cancer risk estimated on the basis of the default linearized multistage model for renal tumours in rats is similar to the value developed on the basis of non-neoplastic effects.

It is cautioned that where local circumstances require that a choice be made between meeting microbiological guidelines or guidelines for disinfection by-products such as chloroform, the microbiological quality must always take precedence. Efficient disinfection must *never* be compromised.

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B. BROMINATED TRIHALOMETHANES

1. GENERAL DESCRIPTION

1.1 Identity

Compound	*CAS No.*	*Molecular formula*
Bromoform	75-25-2	$CHBr_3$
Dibromochloromethane (DBCM)	124-48-1	$CHBr_2Cl$
Bromodichloromethane (BDCM)	75-27-4	$CHBrCl_2$

Trihalomethanes (THMs) are halogen-substituted single-carbon compounds with the general formula CHX_3, where X may be fluorine, chlorine, bromine or iodine, or a combination thereof. From the point of view of drinking-water contamination, only four members of the group are important — bromoform, DBCM, BDCM and chloroform (see Part A), the last of these being the one most commonly found. The IUPAC name of bromoform is tribromomethane.

1.2 Physicochemical properties (Verschueren, 1977; US EPA, 1980, 1985; Hawley, 1981; Hansch & Leo, 1985; Budavari et al., 1989; Montgomery & Welkom, 1990)

Property	*Bromoform*[2]	*DBCM*[3]	*BDCM*[4]
Boiling point (°C)	149–150	119	90
Melting point (°C)	8.3	–	-57.1
Density at 20 °C (g/cm^3)	2.90	2.38	1.98
Vapour pressure (kPa)	0.75 (25 °C)	2.0 (10 °C)	6.67 (20 °C)
Water solubility (mg/litre)	3190 (30 °C)	1050 (30 °C)	3320 (30 °C)
Log octanol–water partition coefficient	2.38	2.08	1.88

1.3 Organoleptic properties

The odour threshold for bromoform in water is 0.3 mg/litre (Hansch & Leo, 1985; Budavari et al., 1989).

1.4 Major uses

Brominated THMs have been used as laboratory reagents, as chemical intermediates for the synthesis of organic compounds and as fluids for mineral ore separation. They were formerly used as solvents for fats, waxes and resins and as flame retardants. Bromoform has been used as a sedative and cough suppressant (ATSDR, 1989a).

1.5 Environmental fate

In air, brominated THMs may be degraded by photo-oxidative interaction with atmospheric hydroxyl radicals; their typical atmospheric half-life is about 2 months (Radding et al., 1977; US EPA, 1980).

Volatilization is a major transport process for THMs. Estimated volatilization half-lives from rivers and streams are 1 h to 24 days for bromoform, 0.7 h to 16 days for DBCM and 0.5–24 h for BDCM (Mackay et al., 1982; Kaczmar et al., 1985).

Under anaerobic conditions, brominated THMs are readily biodegraded within days in the presence of methane-producing bacteria and under denitrifying and sulfate-reducing conditions (Bouwer et al., 1981; Bouwer & McCarty, 1983). Hydrolysis of brominated THMs in aqueous media is very slow; estimated half-lives are 1000, 274 and 686 years for BDCM, DBCM and bromoform, respectively (Mabey et al., 1982). Based on partition coefficients, bioaccumulation of THMs in aquatic organisms may occur, but only to a limited degree (ATSDR, 1989a).

Brominated THMs are expected to be mobile in soil, based on their partition coefficients and data from percolation studies. Studies in aqueous media suggest that

[2] Conversion factor in air: 1 ppm = 10.34 mg/m^3.
[3] Conversion factor in air: 1 ppm = 8.52 mg/m^3.
[4] Conversion factor in air: 1 ppm = 6.70 mg/m^3.

anaerobic biodegradation could be a major removal process in soil if volatilization is restricted (ATSDR, 1989a).

2. ANALYTICAL METHODS

The preferred technique for the determination of THMs is gas chromatography, with detection by flame ionization, electron capture or mass spectroscopy (Fishbein, 1985). The purge-and-trap gas chromatographic procedure is well suited to biological and environmental samples that are soluble in water; it has a detection limit of approximately 0.5 μg/litre (US EPA, 1979).

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Air

Ambient air concentrations at several urban locations in the USA averaged 37 ng/m^3 for bromoform, 32 ng/m^3 for DBCM and 7.4 ng/m^3 for BDCM (highest values reported were 0.73, 0.23 and 1.3 μg/m^3, respectively). The maximum concentration of bromoform in the air sampled in Toronto, Canada, was 0.1 μg/m^3 (54 samples) (Environment Canada, 1986; Department of National Health and Welfare, 1990).

3.2 Water

THMs are generated principally as by-products of the chlorination of drinking-water. Hypochlorous acid oxidizes bromide ion to form hypobromous acid, which reacts with endogenous organic materials (e.g., humic or fulvic acids) to form brominated THMs (US EPA, 1980). The amount of each THM formed depends on the temperature, pH and chlorine and bromide ion concentrations (Aizawa et al., 1989). THMs are rarely found in raw water but are often present in finished water (Rook, 1974; Boland, 1981).

In a Canadian survey of the water supplies of 70 communities, the average concentrations of bromoform, DBCM and BDCM were 0.1, 0.4 and 2.9 μg/litre, respectively (Department of National Health and Welfare, 1977). In a survey of 105 systems in the USA using surface water, bromoform was found in 14 supplies at <1.0–5.7 μg/litre (median 1.3 μg/litre), DBCM in 70 supplies at <0.5–45 μg/litre (median 3.2 μg/litre) and BDCM in 99 supplies at <0.5–62 μg/litre (median 8.2 μg/litre). In a survey of 315 systems using groundwater, bromoform was found in 81 supplies at <1.0–110 μg/litre (median 3.0 μg/litre), DBCM in 107 supplies at <0.5–32 μg/litre (median 4.1 μg/litre) and BDCM in 104 supplies at <0.5–51 μg/litre (median 3.5 μg/litre) (Brass et al., 1981).

THMs can be found in chlorinated swimming pools, total concentrations ranging from 120 to 660 μg/litre (Beech, 1980). BDCM concentrations were about the same in saltwater as in freshwater pools (13–34 μg/litre) (ATSDR, 1989b).

3.3 Food

BDCM is not a common food contaminant but has been found in trace amounts in some samples: 1.2 µg/kg in one dairy composite and 7 µg/kg in butter (Entz et al., 1982).

3.4 Estimated total exposure and relative contribution of drinking-water

The major routes of exposure to THMs are via drinking-water and inhalation. Indoor air contamination from such sources as volatilization from household uses of chlorinated water (e.g., showers, cleaning) probably contributes more to human exposure than outdoor air.

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Available studies indicate that gastrointestinal absorption is high for all THMs (Lehman & Hasegawa, 1910; Mink et al., 1986). Because of their high lipophilicity, accumulation is higher in tissues of high lipid content, including body fat, liver and kidneys (Mink et al., 1986).

In rats, THMs are oxidized by the hepatic cytochrome P-450 mixed-function oxidase system to trihalomethanols, which then decompose to yield highly reactive dihalocarbonyls (Gopinath & Ford, 1975; Ahmed et al., 1977). The amount metabolized depends on the species, being higher in mice than in rats (Mink et al., 1986). As the reactive metabolites of THMs may be responsible for their toxicity or carcinogenicity (US EPA, 1985), interspecies differences in metabolic patterns should be taken into account in the extrapolation of toxicity or carcinogenicity data from experimental animals to humans (Reitz et al., 1978).

Excretion of unchanged compounds and carbon dioxide occurs primarily in exhaled air, only small amounts being excreted in urine (Mink et al., 1986).

5. EFFECTS ON LABORATORY ANIMALS AND IN VITRO TEST SYSTEMS

5.1 Bromoform

5.1.1 Acute exposure

The oral LD_{50}s for bromoform administered in an aqueous vehicle to male and female mice were 1400 and 1550 mg/kg of body weight, respectively (Bowman et al., 1978). For male and female rats given bromoform in corn oil, LD_{50}s were 1388 and 1147 mg/kg of body weight, respectively (Chu et al., 1980).

5.1.2 Short-term exposure

Bromoform was administered in drinking-water at levels of 0, 5, 50, 500 or 2500 mg/litre (0, 0.6, 7, 52 or 250 mg/kg of body weight per day) to Sprague-Dawley rats (20 per sex per dose) for 90 days. Mild to moderate histological changes in the liver and thyroid and a significant increase in the severity of hepatic lesions were observed at the highest dose, and lactate dehydrogenase activity was significantly reduced. Based on the observed liver effect, the NOAEL was 52 mg/kg of body weight per day (Chu et al., 1982).

Young adult rats (10 per sex per dose) were given bromoform by gavage in corn oil at doses of 0, 12, 25, 50, 100 or 200 mg/kg of body weight per day, 5 days per week for 13 weeks. Male and female mice were given doses of 0, 25, 50, 100, 200 or 400 mg/kg of body weight per day. Growth was not affected except at the highest dose in male mice, in which it was slightly suppressed. Male mice at the two highest dose levels showed "minimal to moderate" hepatocellular vacuolation in a few cells. Male rats showed a dose-related increase in hepatocellular vacuolation, which became statistically significant at 50 mg/kg of body weight per day. The NOAELs for hepatocellular vacuolation were 25 and 100 mg/kg of body weight per day in male rats and male mice, respectively (NTP, 1989a).

5.1.3 Long-term exposure

The effect of feeding bromoform (microencapsulated and mixed in the diet) was evaluated in Wistar SPF rats (40 per sex) dosed for 2 years at 0.04%, 0.16% or 0.65% (18, 71 or 480 mg/kg of body weight per day for males and 30, 120 or 870 mg/kg of body weight per day for females). Animals given the highest dose exhibited body weight depression; decreases in serum triglycerides, non-esterified fatty acids, glucose and cholinesterase activity; elevated γ-glutamyl transpeptidase activity; and yellowing and roughening of the liver surface. Similar but less severe effects were seen in the mid-dose groups. Based on body weight depression and serum enzyme changes, the authors considered the NOAELs to be 18 and 30 mg/kg of body weight per day for male and female rats, respectively (Tobe et al., 1982).

Rats of both sexes and female mice (50 per dose) were given bromoform by gavage in corn oil at doses of 100 or 200 mg/kg of body weight per day, 5 days per week for 2 years. Male mice received 50 or 100 mg/kg of body weight per day. Survival was reduced relative to controls in male rats in the high-dose group. A dose-related suppression of growth was also noted in male rats, but female rats showed an adverse effect on growth only at the high dose level. Male mice at the lower dose showed no effect on growth, but female mice showed a slight suppression that was not clearly related to dose. Rats of both sexes and female mice showed a dose-related increased incidence of fatty change (or vacuolation) in the liver. An increased incidence of mild fatty changes was noted in both low-dose and high-dose female mice but not in male mice. The LOAEL was 100 mg/kg of body weight per day for hepatocellular vacuolation and suppression of weight gain in rats and female mice (NTP, 1989a).

5.1.4 Reproductive toxicity, embryotoxicity and teratogenicity

The effect of bromoform on fertility and reproduction was investigated in Swiss CD-1 mice (20 pairs per dose) dosed for 105 days at 0, 50, 100 or 200 mg/kg of body weight per day in corn oil by gavage. No apparent effect on fertility or reproduction (e.g., litters per pair, live pups per litter, pup body weights) was reported in either the parental or the F_1 generation, and a reproductive NOAEL of 200 mg/kg of body weight per day was identified (NTP, 1989b).

5.1.5 Mutagenicity and related end-points

Bromoform was positive in the Ames test in *Salmonella typhimurium* strain TA100 without activation (Simmon et al., 1977; Ishidate et al., 1982) and negative or equivocal in strains TA1535 or TA1937 with and without activation (NTP, 1989a). Bromoform gave positive results in the following assays: chromosomal aberration in CHO cells with activation (Ishidate et al., 1982) and in mouse bone marrow cells *in vivo* (NTP, 1989a), sister chromatid exchange in human lymphocytes (Morimoto & Koizumi, 1983), in CHO cells without activation (NTP, 1989a) and in mouse bone marrow cells *in vivo* (Morimoto & Koizumi, 1983; NTP, 1989a) and gene mutation in mouse lymphoma cells (NTP, 1989a). It was negative for sister chromatid exchange in CHO cells with activation (NTP, 1989a), and results were equivocal in the micronucleus assay (Ishidate et al., 1982; NTP, 1989a).

5.1.6 Carcinogenicity

When bromoform (4, 48 or 100 mg/kg of body weight) was administered intraperitoneally to male strain A mice (20 per dose) 3 times per week for 8 weeks, and the mice were kept under observation for 16 additional weeks, an increased incidence of lung tumours was seen at the intermediate dose (Theiss et al., 1977).

Groups of 50 male B6C3F_1 mice were given bromoform by gavage in corn oil at doses of 0, 50 or 100 mg/kg of body weight per day, 5 days per week for 105 weeks. Females received doses of 0, 100 or 200 mg/kg of body weight per day. No increase in tumours was reported in any tissue in any group. In a similar study, Fischer 344/N rats (50 per sex per dose) were also exposed to bromoform by gavage in corn oil at doses of 0, 100 or 200 mg/kg of body weight per day, 5 days per week for 105 weeks. Adenomatous polyps or adenocarcinoma (combined) of the large intestine (colon or rectum) was induced in three male rats given the highest dose and in eight female rats given the highest dose. The increase was considered to be significant, as these tumours are rare in control animals. On the basis of these data, it was concluded that there was "some evidence" of carcinogenic activity in male rats and "clear evidence" in female rats. There were no tumours in the control rats and one in a mid-dose female rat (NTP, 1989a).

5.2 Dibromochloromethane

5.2.1 Acute exposure

The oral LD_{50}s for DBCM administered in an aqueous vehicle to male and female mice were 800 and 1200 mg/kg of body weight, respectively (Bowman et al., 1978). LD_{50}s for male and female rats given the compound in corn oil were 1186 and 848 mg/kg of body weight, respectively (Chu et al., 1980).

5.2.2 Short-term exposure

DBCM was administered in drinking-water at levels of 0, 5, 50, 500 or 2500 mg/litre (0, 0.6, 7, 52 or 250 mg/kg of body weight per day) to Sprague-Dawley rats (20 per sex per dose) for 90 days. Mild to moderate histological changes in the liver and thyroid and a significant increase in the severity of hepatic lesions were observed at the highest dose. Based on the observed liver effect, the NOAEL was 52 mg/kg of body weight per day (Chu et al., 1982).

Fischer 344/N rats and $B6C3F_1$ mice (10 per sex per dose) were given DBCM by gavage in corn oil at dose levels of 0, 15, 30, 60, 125 or 250 mg/kg of body weight per day, 5 days per week for 13 weeks. The final body weights of rats that received 250 mg/kg of body weight were depressed. A dose-dependent increase in hepatic vacuolation was observed in male rats. Based on this hepatic effect, the NOAEL in rats was 30 mg/kg of body weight per day. Kidney and liver toxicity were observed in male and female rats and male mice at 250 mg/kg of body weight per day. Survival rates for treated animals and corresponding controls were comparable except in high-dose rats. Clinical signs in the treated animals and controls were comparable. Based on the renal and hepatic lesions, a NOAEL of 125 mg/kg of body weight per day was identified in mice (NTP, 1985).

5.2.3 Long-term exposure

The effect of feeding DBCM (microencapsulated and mixed in the diet) was evaluated in Wistar SPF rats (40 per sex) dosed for 2 years at 0.022%, 0.088% or 0.35% (10, 39 or 210 mg/kg of body weight per day for males and 17, 66 or 350 mg/kg of body weight per day for females). Animals receiving the highest dose exhibited depressed body weight; decreases in serum triglycerides, non-esterified fatty acids, glucose and cholinesterase activity; elevated γ-glutamyl transpeptidase activity; and yellowing and roughening of the liver surface. Similar but less severe findings were present in the mid-dose groups. Based on the body weight depression and serum enzyme changes, the authors considered the NOAELs to be 10 and 17 mg/kg of body weight per day for male and female rats, respectively (Tobe et al., 1982).

Rats (50 per sex per dose) were given DBCM by gavage in corn oil at 0, 40 or 80 mg/kg of body weight, 5 days per week for 104 weeks, and mice (50 per sex per dose) received 0, 50 or 100 mg/kg of body weight by gavage for 105 weeks. Survival in rats and female mice was comparable in all dose groups, whereas it was decreased in

high-dose male mice. An overdosing accident at week 58 killed 35 male mice in the low-dose group, so this group was not evaluated further. Mean body weights of high-dose male rats and high-dose mice of both sexes were lower than those of the vehicle controls. The incidence of fatty metamorphosis of the liver was increased in male and female rats and female mice at both low and high dose levels. Male mice showed liver effects at the high dose. There was an increased incidence of kidney nephrosis in female rats and in male mice but not in male rats or female mice. Follicular cell hyperplasia of the thyroid occurred at increased incidence in female mice but not in males. Based on the hepatic lesions, LOAELs of 50 and 40 mg/kg of body weight per day for mice and rats, respectively, were identified (NTP, 1985).

5.2.4 Reproductive toxicity, embryotoxicity and teratogenicity

In a multigeneration reproductive study, groups of 10 male and 30 female ICR mice were treated with DBCM in Emulphor at 0, 0.1, 1.0 or 4.0 g/litre (0, 17, 171 or 685 mg/kg of body weight per day) in drinking-water for 35 days, then mated; subsequent rematings occurred 2 weeks after weaning. The F_1 mice were treated with the same test solution for 11 weeks after weaning and then mated; remating occurred 2 weeks after weaning. At 17 mg/kg of body weight per day, there was only a slight depression in the body weight of the newborn pups in the F_{2b} generation. At 171 mg/kg of body weight per day, there was a significant decrease in female body weight and an increase in the occurrence of gross liver pathology of F_0 and F_{1b} mice; the lesions varied in severity from fat accumulation to distinct masses on the liver surface. Although not occurring in every generation, there were significant decreases in litter size, pup viability, postnatal body weight and lactation index. At 685 mg/kg of body weight per day, the effects were of the same types but more severe. Body weight gain was significantly reduced in both males and females at the highest dose (685 mg/kg of body weight per day) and in females at the middle dose (171 mg/kg of body weight per day). Animals in both these groups exhibited enlarged livers with gross morphological changes. In addition, the gestation index, fertility and survival of the F_1 generation were significantly reduced. Based on maternal toxicity and fetotoxicity, a NOAEL of 17 mg/kg of body weight per day was identified (Borzelleca & Carchman, 1982).

5.2.5 Mutagenicity and related end-points

DBCM was positive in the Ames test with *S. typhimurium* strain TA100 without activation (Simmon et al., 1977; Ishidate et al., 1982) but negative in strains TA98, TA1535 and TA1537 with or without activation (Borzelleca & Carchman, 1982). It gave positive results for chromosomal aberration in CHO cells with activation (Ishidate et al., 1982) and for sister chromatid exchange in human lymphocytes and mouse bone marrow cells *in vivo* (Morimoto & Koizumi, 1983); it was negative in the micronucleus assay (Ishidate et al., 1982).

5.2.6 Carcinogenicity

DBCM was administered to rats and mice (50 per sex per dose) in corn oil by gavage at doses of 0, 40 or 80 mg/kg of body weight per day for rats and 0, 50 or 100 mg/kg of body weight per day for mice, 5 days per week for 104–105 weeks. An overdose killed 35 of the 50 low-dose male mice, so that this group could not be used for the study of carcinogenicity. DBCM significantly increased the incidence of hepatocellular adenomas and the combined incidence of hepatocellular adenomas and carcinomas in high-dose female mice. The incidence of hepatocellular carcinomas was significantly increased in high-dose male mice; the combined incidence of hepatocellular adenomas and carcinomas was marginally significant by the lifetable test but not by the incidental tumour test. DBCM did not produce an increased incidence of tumours in treated rats. There was "no evidence" of carcinogenic activity in male or female rats, "equivocal evidence" of carcinogenicity in male mice and "some evidence" of carcinogenicity in female mice under the conditions of this study (NTP, 1985).

5.3 Bromodichloromethane

5.3.1 Acute exposure

Oral LD_{50}s for BDCM administered in an aqueous vehicle to mice were 450 and 900 mg/kg of body weight for males and females, respectively (Bowman et al., 1978). Male and female rats given the compound in corn oil had LD_{50}s of 916 and 969 mg/kg of body weight, respectively (Chu et al., 1980).

5.3.2 Short-term exposure

BDCM was administered in drinking-water at levels of 0, 5, 50, 500 or 2500 mg/litre (0, 0.6, 7, 52 or 250 mg/kg of body weight per day) to Sprague-Dawley rats (20 per sex per dose) for 90 days. Mild to moderate histological changes in the liver and thyroid and a significant increase in the severity of hepatic lesions were observed at the highest dose. Based on the observed liver effect, the NOAEL was 52 mg/kg of body weight per day (Chu et al., 1982).

Fischer 344/N rats and $B6C3F_1$ mice were given BDCM by gavage in corn oil 5 days per week for 13 weeks. Rats (10 per sex per dose) were given 0, 19, 38, 75, 150 or 300 mg/kg of body weight per day. Male mice (10 per dose) were given 0, 6.3, 12.5, 50 or 100 mg/kg of body weight per day, and female mice were given 0, 25, 50, 100, 200 or 400 mg/kg of body weight per day. Of the male and female rats that received the highest dose, 50% and 20%, respectively, died before the end of the study. None of the mice died. Body weights decreased significantly in male and female rats given BDCM at 150 and 300 mg/kg of body weight per day. Centrilobular degeneration of the liver was observed at 300 mg/kg of body weight per day in male and female rats and at 200 and 400 mg/kg of body weight per day in female mice. Degeneration and necrosis of the kidney were observed at 300 mg/kg of body weight per day in male rats and at 100 mg/kg of body weight per day in male mice. The NOAELs in rats were

75 and 150 mg/kg of body weight per day for body weight reduction and for hepatic and renal lesions, respectively. The NOAEL for renal lesions in mice was 50 mg/kg of body weight per day (NTP, 1987).

5.3.3 Long-term exposure

The effect of feeding BDCM (microencapsulated and mixed in the diet) was evaluated in Wistar SPF rats (40 per sex) dosed for 2 years at 0.014%, 0.055% or 0.22% (6, 24 or 130 mg/kg of body weight per day for males and 11, 41 or 220 mg/kg of body weight per day for females). Animals receiving the highest dose exhibited depressed body weight; decreases in serum triglycerides, non-esterified fatty acids, glucose and cholinesterase activity; elevated γ-glutamyl transpeptidase activity; and yellowing and roughening of the liver surface. Similar but less severe findings were present in the mid-dose groups. Based on the body weight depression and serum enzyme changes, the authors considered the NOAELs to be 6 and 11 mg/kg of body weight per day for male and female rats, respectively (Tobe et al., 1982).

Groups of Fischer 344/N rats (50 per sex per dose) were given 0, 50 or 100 mg of BDCM per kg of body weight per day in corn oil by gavage 5 days per week for 102 weeks. Male $B6C3F_1$ mice (50 per dose) were given 0, 25 or 50 mg/kg of body weight per day and female mice received 0, 75 or 150 mg/kg of body weight per day by gavage for 102 weeks. Renal cytomegaly was observed in male rats at 50 mg/kg of body weight per day and above and in male mice at 25 mg/kg of body weight per day and above. Fatty metamorphosis of the liver was observed in male and female rats at 50 mg/kg of body weight per day and above and in male mice at 25 mg/kg of body weight per day and above. Compound-related follicular cell hyperplasia of the thyroid was also observed in male and female mice. Survival was decreased in female mice only. Mean body weights were decreased at 100 mg/kg of body weight per day in rats and at 50 and 150 mg/kg of body weight per day in male and female mice, respectively. Based on the observed renal and liver effects, a LOAEL of 50 mg/kg of body weight per day was identified for rats. Based on the observed renal, liver and thyroid effects in male mice and thyroid effects in female mice, a LOAEL of 25 mg/kg of body weight per day was identified for mice (NTP, 1987).

5.3.4 Reproductive toxicity, embryotoxicity and teratogenicity

A dose-related increased incidence in sternebral anomalies was reported in fetuses from groups of 9–15 pregnant rats exposed to BDCM in corn oil by gavage at doses of 0, 50, 100 or 200 mg/kg of body weight per day on days 6–15 of gestation. The authors interpreted the sternebral anomalies as evidence of a fetotoxic (rather than a teratogenic) effect. The LOAEL based on this fetotoxic effect was 50 mg/kg of body weight per day (Ruddick et al., 1983).

5.3.5 Mutagenicity and related end-points

BDCM was positive in the Ames test with *S. typhimurium* strain TA100 without activation (Simmon et al., 1977; Ishidate et al., 1982) but negative in strains TA98,

TA1535 and TA1537 with or without activation (NTP, 1987). It induced gene mutations in mouse lymphoma cells with, but not without, activation (NTP, 1987). BDCM gave conflicting results for chromosomal aberration in CHO cells with and without activation (Ishidate et al., 1982; NTP, 1987), positive results for sister chromatid exchange in human lymphocytes and in mouse bone marrow cells *in vivo* (Morimoto & Koizumi, 1983) and negative results for the micronucleus assay (Ishidate et al., 1982) and sister chromatid exchange in CHO cells (NTP, 1987).

5.3.6 Carcinogenicity

When BDCM (20, 40 or 100 mg/kg of body weight) was administered intraperitoneally to male strain A mice (20 per dose) 3 times per week for 8 weeks, and the mice were kept under observation for 16 additional weeks, an increased incidence of lung tumours was seen at the highest dose (Theiss et al., 1977).

Fischer 344/N rats (50 per sex per dose) were given BDCM in corn oil by gavage at 0, 50 or 100 mg/kg of body weight, 5 days per week for 102 weeks. Male $B6C3F_1$ mice (50 per dose) were given 0, 25 or 50 mg/kg of body weight per day by gavage, and females received 0, 75 or 150 mg/kg of body weight per day. BDCM caused a significant increase in tumours of the kidney in male mice, the liver in female mice and the kidney and large intestine in male and female rats. In male mice, the incidences of tubular cell adenomas and the combined incidences of tubular cell adenomas and adenocarcinomas of the kidneys were significantly increased at 50 mg/kg of body weight per day. In female mice, significant increases in hepatocellular adenomas occurred at 75 and 150 mg/kg of body weight per day, whereas hepatocellular carcinomas were significantly increased at 150 mg/kg of body weight per day. In male and female rats, the incidence of tubular cell adenomas and adenocarcinomas and the combined incidence of adenomas and adenocarcinomas of the kidneys were significantly increased only at 100 mg/kg of body weight per day. Adenosarcomas of the large intestine were increased in male rats at both doses and in high-dose female rats. Adenomatous polyps were significantly increased in male rats in a dose-dependent manner but were present in females at the high dose only. Based on the data, it was concluded that, under the conditions of this study, there was "clear evidence" of carcinogenic activity for male and female mice and rats (NTP, 1987).

6. EFFECTS ON HUMANS

In the past, orally administered bromoform was used as a sedative for children with whooping cough. Typical doses were around 180 mg, given 3–6 times per day. Deaths as a result of accidental overdose were occasionally reported. The clinical signs in fatal cases were central nervous system depression followed by respiratory failure (Burton-Fanning, 1901; Dwelle, 1903). Based on these clinical observations, the estimated lethal dose for a child weighing 10–20 kg is probably about 300 mg/kg of body weight, and the LOAEL for mild sedation is around 54 mg/kg of body weight per day.

In several epidemiological studies (Brenniman et al., 1980; Cragle et al., 1985), associations between the ingestion of chlorinated drinking-water (which typically contains THMs) and increased cancer mortality rates have been reported. In one study (US EPA, 1975), there was an apparent association between bladder cancer and THMs, and a higher degree of correlation was noted with the brominated THMs than with chloroform. However, as chlorinated water contains many by-products, it is not possible from these epidemiological studies to conclude that brominated THMs are human carcinogens.

7. GUIDELINE VALUES

7.1 Bromoform

In a bioassay carried out by the NTP in the USA, bromoform induced a small increase in relatively rare tumours of the large intestine in rats of both sexes but did not induce tumours in mice. Data from a variety of assays on the genotoxicity of bromoform are equivocal. IARC has classified bromoform in Group 3.

A TDI was derived on the basis of a NOAEL of 25 mg/kg of body weight per day for the absence of histopathological lesions in the liver in a well conducted and well documented 90-day study in rats (NTP, 1989a). This NOAEL is supported by the results of two long-term studies. The TDI is 17.9 µg/kg of body weight, correcting for exposure on 5 days per week and using an uncertainty factor of 1000 (100 for intra- and interspecies variation and 10 for possible carcinogenicity and the short duration of the study). With an allocation of 20% of the TDI to drinking-water, the guideline value is 100 µg/litre (rounded figure).

7.2 Dibromochloromethane

In an NTP bioassay, DBCM induced hepatic tumours in female and possibly in male mice but not in rats. The genotoxicity of DBCM has been studied in a number of assays, but the available data are considered inconclusive. IARC has classified DBCM in Group 3.

A TDI was derived on the basis of a NOAEL of 30 mg/kg of body weight per day for the absence of histopathological effects in the liver in a well conducted and well documented 90-day study in rats (NTP, 1985). This NOAEL is supported by the results of long-term studies. The TDI is 21.4 µg/kg of body weight, correcting for exposure on 5 days per week and using an uncertainty factor of 1000 (100 for intra- and interspecies variation and 10 for the short duration of the study). An additional uncertainty factor for potential carcinogenicity was not applied because of the questions regarding mouse liver tumours from corn oil vehicles and inconclusive evidence of genotoxicity. With an allocation of 20% of the TDI to drinking-water, the guideline value is 100 µg/litre (rounded figure).

7.3 Bromodichloromethane

In an NTP bioassay, BDCM induced renal adenomas and adenocarcinomas in both sexes of rats and male mice, rare tumours of the large intestine (adenomatous polyps and adenocarcinomas) in both sexes of rats and hepatocellular adenomas and adenocarcinomas in female mice. BDCM has given both positive and negative results in a variety of *in vitro* and *in vivo* genotoxicity assays. IARC (1991) has classified bromodichloromethane in Group 2B.

Cancer risks have been estimated on the basis of increases in the incidence of kidney tumours in male mice observed in the NTP (1987) bioassay, as these tumours yield the most protective value. Hepatic tumours in female mice were not considered owing to the possible role of the corn oil vehicle in their induction, although the estimated risks are within the same range. Using the linearized multistage model, the range of concentrations of BDCM in drinking-water associated with excess lifetime cancer risks of 10^{-4}, 10^{-5} and 10^{-6} for kidney tumours are 600, 60 and 6 µg/litre, respectively. These values are supported by a recently published feeding study in rats that was not available for full evaluation.

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C. TOTAL TRIHALOMETHANES

1. GUIDELINE VALUE

The trihalomethanes (THMs) may act as an indicator for the presence of other chlorination by-products. Control of the four most commonly occurring THMs in drinking-water should help to reduce levels of other uncharacterized chlorination by-products.

Because these four compounds usually occur together, it has been the practice to consider total THMs as a group, and a number of countries have set guidelines or standards on this basis. In the first edition of the *Guidelines for Drinking-water Quality*, a guideline value was established for chloroform only; few data existed for the remaining THMs, and, for most water supplies, chloroform was the most commonly encountered member of the group. In this edition, no guideline value has been set for total THMs; however, guideline values have been established separately for all four THMs.

For authorities wishing to establish a total THM standard to account for additive toxicity, the following fractionation approach could be taken:

$$\frac{C_{bromoform}}{GV_{bromoform}} + \frac{C_{DBCM}}{GV_{DBCM}} + \frac{C_{BDCM}}{GV_{BDCM}} + \frac{C_{chloroform}}{GV_{chloroform}} \leq 1$$

where C = concentration and GV = guideline value.

Authorities wishing to use a guideline value for total THMs should not simply add up the guideline values for the individual compounds in order to arrive at a standard, because the four compounds are basically similar in toxicological action.

In controlling THMs, a multistep treatment system should be used to reduce organic THM precursors, and primary consideration should be given to ensuring that disinfection is never compromised.