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Trihalomethanes in Drinking-water

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

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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 on selected chemicals in 1998 and on microbial aspects in 2002. The third edition of the GDWQ was published in 2004, and the first addendum to the third edition was published in 2005.

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 and 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 of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a lead institution prepared a background 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 documents for the third edition and addenda.

Under the oversight of a group of coordinators, each of whom was responsible for a group of chemicals considered in the GDWQ, 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. The draft documents were also released to the public domain for comment and submitted for final evaluation by expert meetings.

During the preparation of background documents and at expert 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 draft text was discussed at the Working Group Meeting for the first addendum to the third edition of the GDWQ, held on 17–21 May 2004. The final version of the document takes into consideration comments from both peer reviewers and the public. The input of those who provided comments and of participants in the meeting is gratefully acknowledged.

The WHO coordinator was Dr J. Bartram, Coordinator, Water, Sanitation and Health Programme, WHO Headquarters. Ms C. Vickers provided a liaison with the International Programme on Chemical Safety, WHO Headquarters. Mr Robert Bos, Water, Sanitation and Health Programme, WHO Headquarters, provided input on pesticides added to drinking-water for public health purposes.

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Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comment are greatly appreciated.

Acronyms and abbreviations used in the text

BDCM	bromodichloromethane
BrdU	bromodeoxyuridine
CEPA	*Canadian Environmental Protection Act, 1999*
CYP	cytochrome P450
DBCM	dibromochloromethane
DNA	deoxyribonucleic acid
ECD	electron capture detector
EPA	Environmental Protection Agency (USA)
FAO	Food and Agriculture Organization of the United Nations
GAC	granular activated carbon
GC	gas chromatography
GDWQ	*Guidelines for Drinking-water Quality*
GSTT1-1	glutathione-*S*-transferase T1-1
Ieq	ingestion-equivalents
ITD	ion trap detector (mass spectrometry)
LD_{50}	median lethal dose
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
MQL	method quantification limit
NCI	National Cancer Institute (USA)
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program (USA)
PAC	powdered activated carbon
PBPK	physiologically based pharmacokinetic
ppm	part per million
SCE	sister chromatid exchange
TDI	tolerable daily intake
TGF	transforming growth factor
THM	trihalomethane
UDS	unscheduled DNA synthesis
USA	United States of America
VMRATEK	mean rate of metabolism per unit kidney cortex volume during each dose interval
VRAMCOR	maximum rate of metabolism per unit kidney cortex volume
WHO	World Health Organization

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1. GENERAL DESCRIPTION

1.1 Identity

Trihalomethanes (THMs) are halogen-substituted single-carbon compounds with the general formula CHX_3, where X represents a halogen, which may be fluorine, chlorine, bromine, or iodine, or combinations thereof. The THMs most commonly present in drinking-water are chloroform ($CHCl_3$), bromodichloromethane or dichlorobromomethane ($CHBrCl_2$) (BDCM), dibromochloromethane or chloro-dibromomethane ($CHClBr_2$) (DBCM), and bromoform ($CHBr_3$). Consideration of information relevant to the derivation of drinking-water guidelines for THMs is restricted to these compounds.

1.2 Physicochemical properties[1]

The four compounds considered here are liquids at room temperature. They are relatively to extremely volatile, with vapour pressures at 25 °C ranging from 0.80 kPa for bromoform to 23.33 kPa for chloroform. The THMs are only slightly soluble in water, with solubilities less than 1 mg/ml at 25 °C. Their log octanol–water partition coefficients range from 1.97 (chloroform) to 2.38 (bromoform).

1.3 Major uses and sources in drinking-water

THMs are formed in drinking-water primarily as a result of chlorination of organic matter present in raw water supplies. The rate and degree of THM formation increase as a function of the chlorine and humic acid concentration, temperature, pH, and bromide ion concentration (Stevens et al., 1976; Amy et al., 1987). As well as being the most common THM, chloroform is also the principal disinfection by-product in chlorinated drinking-water (LeBel & Williams, 1995). In the presence of bromides, brominated THMs are formed preferentially and chloroform concentrations decrease proportionally (Aizawa et al., 1989).

The use of chloroform as an anaesthetic, in dentifrices, in liniments, and in anti-tussives has been largely discontinued. Chloroform is used as a solvent and in the production of other chemicals (Environment Canada & Health Canada, 2001). BDCM is used in the synthesis of other chemicals and as a solvent, whereas DBCM is an intermediate in the manufacture of refrigerants, pesticides, propellents, and other organic chemicals (Keith & Walters, 1985). Bromoform is used in the synthesis of pharmaceuticals, as a solvent, and in the aircraft and shipbuilding industries as an ingredient in fire-resistant chemicals and gauge fluid. THMs are released into the environment from industrial sources.

[1] Conversion factors in air are as follows: chloroform, 1 ppm = 4.96 mg/m^3; BDCM, 1 ppm = 6.70 mg/m^3; DBCM, 1 ppm = 8.52 mg/m^3; and bromoform, 1 ppm = 10.34 mg/m^3.

2. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

2.1 Water

The chemistry of the reactions between chlorine and the organic materials present in water is complex and, although extensively studied, poorly understood; however, important factors include the type and concentrations of organic materials in the raw water, the chlorine reaction time, temperature, and chlorination pH.

Levels of chloroform, the most common THM, are generally higher in chlorinated water originating from surface water than in groundwater, because of higher organic matter in the former. The extent of formation of chloroform varies with different water treatment processes. Concentrations of chloroform in chlorinated water in treatment plants and distribution systems are approximately twice as high during warmer months as during colder months. This is a consequence of the higher concentrations of precursor organic materials and especially of the higher rates of formation of disinfection by-products in the raw water during the warmer period (LeBel et al., 1997). Levels can increase as the chlorinated water moves from the water treatment plant through the distribution system, because of the continued presence of a chlorine residual. Further increases in concentrations of chloroform in water can occur in domestic hot water tanks. However, storage in the hot water tank increases the level of chloroform twice as much in colder months, when more hot water is required to maintain the shower temperature, as in warmer months, so that concentrations of chloroform in the warm water used for showering are relatively constant for both periods (Williams et al., 1995; Benoit et al., 1997).

Concentrations of THMs have been determined in drinking-water supplies at a considerable number of locations across Canada (Water Quality Issues Sub-Group, 2003). Eight provinces provided 1994–2000 THM data for just over 1200 water systems serving a sampled population of over 15 million Canadians. The methods of sampling and analysis varied and were often not well described, but generally samples were taken from the midpoints and/or end-points of the water systems, and the typical methods of analysis were either liquid–liquid extraction or purge-and-trap gas chromatography (GC).

Based on the data received from these eight Canadian provinces, the mean THM level was about 66 µg/litre in drinking-water samples from all systems. Some systems had average values in the 400 µg/litre range, and some systems had maximum or peak values in the 800 µg/litre range. From the eight provinces, 282 water systems (23% of sampled systems), representing a sampled population of 523 186 (3.4% of the sampled population served), reported having mean THM levels greater than 100 µg/litre, whereas 506 water systems (41%), serving a sampled population of 2 509 000 (16%), reported at least one instance of THM levels being greater than 100 µg/litre (Water Quality Issues Sub-Group, 2003).

System mean chloroform levels for 1994–2000 were generally less than 50 µg/litre, with some single maximum or peak values in the 400 µg/litre range. From those

suppliers who reported chloroform data, 290 water systems (26%), serving a sampled population of 1 130 000 (8%), reported mean chloroform levels greater than 75 µg/litre, while 425 water systems (39%), serving 1 740 000 consumers (12%), had a peak concentration greater than 75 µg/litre in their drinking-water during this period (Water Quality Issues Sub-Group, 2003).

Mean concentrations of both BDCM and DBCM in systems were generally less than 10 µg/litre, although some averages were higher, and several locations reported one-time samples in excess of 200 µg/litre. From those suppliers who reported BDCM data, 87 water systems (8% of reporting systems), representing a sampled population of 285 000 (2% of the population served), reported having mean BDCM levels greater than 10 µg/litre, whereas 192 water systems (18%), serving a sampled population of 1 165 000 (8%), reported at least one instance of BDCM levels being greater than 10 µg/litre (Water Quality Issues Sub-Group, 2003).

Mean concentrations of bromoform were typically less than the detection limit, or approximately 0.5 µg/litre, and individual values were less than 10 µg/litre. In a few systems, however, average and maximum bromoform levels exceeded 30 µg/litre over this period (Water Quality Issues Sub-Group, 2003).

Generally speaking, the smaller centres with less sophisticated treatment systems had higher THM levels in their drinking-water. In this 1994–2000 national survey, it was found that where the population was unreported or less than 1000, 274 systems had average THM levels greater than 75 µg/litre, and 45 systems had average BDCM levels greater than 10 µg/litre. Conversely, where the population was greater than 50 000 (and where more sophisticated treatment plants would be expected), there were only four systems whose average THM levels were greater than 75 µg/litre, and only one system had an average BDCM level greater than 10 µg/litre. For population centres with greater than 10 000 people, the 118 systems serving 11 036 000 people had an average system THM level of 37 µg/litre — a value significantly lower than the average of 66 µg/litre reported for all systems, regardless of size. For population centres with greater than 50 000 people, the 41 systems serving 9 439 000 people had an average THM level of about 27 µg/litre (Water Quality Issues Sub-Group, 2003).

In the USA, monitoring data were collected over an 18-month period between July 1997 and December 1998 from approximately 300 water systems operating 501 plants and serving at least 100 000 people. Summary occurrence data stratified by raw water source (groundwater or surface water) are available for finished water, the distribution system average, and the distribution system high values. The mean, median, and 90th-percentile values for surface water distribution system average concentrations in the US survey are 8.6, 70.2, and 20.3 µg/litre, respectively, for BDCM (range of individual values 0–65.8 µg/litre); 2.4, 4.72, and 13.2 µg/litre, respectively, for DBCM (range 0–67.3 µg/litre); and 0, 1.18, and 3.10, respectively, for bromoform (range 0–3.43 µg/litre) (US EPA, 2001b).

2.2 Multiroute exposure through drinking-water

The importance of exposure to chloroform and BDCM via inhalation and dermal absorption from tap water during showering and bathing was evaluated. A modifying factor for each compound, in terms of ingestion-equivalents (Ieq) per day, was estimated by evaluating the relative contribution of inhalation and dermal exposures associated with showering and bathing.

Krishnan (2003) determined Ieq/day values for dermal and inhalation exposures of adults and children (6-, 10-, and 14-year-olds) during showering and bathing with tap water containing chloroform (5 µg/litre) and BDCM (5 µg/litre).[1] The Ieq/day values for a 10-min shower and a 30-min bath were calculated using the physiologically based pharmacokinetic (PBPK) model-generated data on the absorbed fraction (Corley et al., 1990, 2000; Haddad et al., 2001; Price et al., 2003). The "absorbed fraction" for the dermal and inhalation exposures took into consideration the dose that was absorbed following exposure as well as that portion that was exhaled in the following 24 h.

Calculations done for chloroform and BDCM accounted for inter-chemical differences in water-to-air factor (based on differences in Henry's law constants), fraction of dose absorbed during inhalation and dermal exposures, and skin permeability coefficient. Complete (100%) absorption of ingested chloroform and BDCM in drinking-water was assumed for all subpopulations; this was supported by the available information on the extent of hepatic extraction of these THMs (Corley et al., 1990; DaSilva et al., 1999).

Ieq/day values for the inhalation and dermal routes were higher for the 30-min bath scenario than for the 10-min shower for all subpopulations based on the longer exposure time. The highest total exposure values for drinking-water were for adults in the 30-min bath scenario: 4.61 Ieq/day (2 litres ingestion, 1.7 litres inhalation, 0.91 litre dermal) for chloroform and 4.05 Ieq/day (2 litres ingestion, 0.67 litre inhalation, 1.38 litres dermal) for BDCM. Both values are considered to be conservative, since most people do not take a 30-min bath daily. In the event that individuals spend more than 10 min in a shower or are exposed to chloroform or BDCM via other household activities or additional bathroom time, the above-calculated Ieq/day values (which account for inhalation and dermal exposures from a 30-min bath) are likely to be adequate for assessment.

2.3 Food and beverages

Data from the USA and Canada were sufficient to serve as a basis for estimating the minimum, midpoint, and maximum concentrations of chloroform in 131 of the 181 foods for which per capita daily intake rates (i.e., g/day) are available. The midpoint concentrations were greater than 100 µg/kg in 12 food items (i.e., butter, margarine, vegetable fats and oils, baby food cereal, pizza, marine fish, fresh fish, crackers,

[1] Ieq/day values are unlikely to change in the THM µg/litre range typically observed.

pancakes, veal, beef roast, and cheese). The highest concentrations of chloroform have frequently been measured in dairy products (Environment Canada & Health Canada, 2001).

Maximum concentrations of 2200 µg of chloroform per kg and 3 µg of BDCM per kg were detected in the fat of nine species of fish from six areas of the Norwegian coastline that were contaminated principally by discharges from pulp and paper plants, but also by agricultural runoff, chemical plants, and other industries. Bromoform and DBCM were detected in only one sample, at concentrations of 115 µg/kg and 9 µg/kg, respectively (Ofstad et al., 1981). Neither chloroform nor BDCM was detected in composite samples of meat/fish/poultry (quantification limits were 18 ng/g and 4.5 ng/g, respectively) or oil/fat (quantification limits were 28 ng/g and 8.3 ng/g, respectively) from 39 different foods in the USA (Entz et al., 1982). In the composite sample of dairy foods, concentrations of chloroform and BDCM were 17 µg/litre and 1.2 µg/litre, respectively.

THM concentrations in six different cola and non-cola beverages (five samples of each) in New Jersey, USA, ranged from 3.2 to 44.8 µg/litre (Abdel-Rahman, 1982). Concentrations of chloroform and BDCM in unspecified beverage composites from the USA averaged 32 µg/litre and 1.0 µg/litre, respectively (Wallace et al., 1984). Chloroform concentrations are approximately 10 times higher in cola soft drinks than in non-cola soft drinks, even for similar water sources (Abdel-Rahman, 1982; Entz et al., 1982; Wallace et al., 1984). This may be due to the method of extraction of the cola or the presence of caramel in these soft drinks. Chloroform was detected in 11 of 13 beverages sampled in Ottawa, Canada, at a maximum concentration of 14.8 µg/kg in a fruit drink (Environment Canada & Health Canada, 2001). Bottled water prepared from a chlorinated municipal water supply may also contain THMs.

2.4 Consumer products

In the USA, emissions from approximately 5000 materials were determined, with a small number of these products emitting chloroform, usually in trace amounts. Emissions of chloroform were detected from the following materials (with median emission levels reported in parentheses): ink and pen (10.0 µg/g), miscellaneous housewares (4.85 µg/g), photographic equipment (2.5 µg/g), rubber (0.9 µg/g), electrical equipment (0.23 µg/g), lubricant (0.2 µg/g), adhesives (0.15 µg/g), fabric (0.1 µg/g), photographic film (0.1 µg/g), tape (0.05 µg/g), and foam (0.04 µg/g) (Environment Canada & Health Canada, 2001).

2.5 Swimming pools and hot tubs

The use of swimming pools results in ingestion of, inhalation of, and dermal exposure to THMs due mainly to the reaction between chlorine and organic matter. In indoor pool environments, concentrations of chloroform in plasma increase with the level of exertion of swimmers and are closely correlated with the chloroform concentrations in air and time spent swimming (Aggazzotti et al., 1990). In general, competitive swimmers are potentially exposed to higher levels of chloroform than are leisure

swimmers due to higher breathing rates and longer durations of exposure (Health Canada, 1999).

The inhalation route appears to be significantly more important than the dermal route for swimmers (Strähle et al., 2000; Erdinger et al., 2004). Lévesque et al. (1994) determined that when swimmers (in indoor pools) are exposed to high concentrations of chloroform in the pool water and air, 78% and 22% of the body burden were due to inhalation and dermal uptake, respectively. Ingestion of swimming pool water is also a potential route of intake, although recent measured data indicate an extreme intake of about 100 ml (WHO, in revision).

Limited information suggests that users of hot tubs may have more significant dermal uptake than swimmers due to higher water temperatures (Wilson, 1995).

2.6 Estimates of total exposure to chloroform

For the general population, mean intake of chloroform from indoor air can be estimated to be 0.3–1.1 μg/kg of body weight per day. The average intake of chloroform (inhalation and dermal absorption) during showering is 0.5 μg/kg of body weight per shower. Preliminary results from a study by Benoit et al. (1998), based on four volunteers, suggested that showering for 10 min with warm water that has been treated with a chlorinated disinfectant is equivalent to drinking 2.7 litres of cold water per day from the same water supply, on an annual average. Dermal absorption accounts for an average of 30% of the total uptake. The estimated mean intake of chloroform from ingestion of drinking-water for the general population, based on an average concentration of <20 μg/litre, is less than 0.7 μg/kg of body weight per day. The estimated intake of chloroform from foodstuffs is approximately 1 μg/kg of body weight per day. Outdoor air exposure is estimated to be considerably less than exposure from other sources. 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, estimates of total intake are up to 10 μg/kg of body weight per day.

As described above, where chlorine is used as a disinfectant in swimming pools, disinfection by-products, including chloroform, will be produced. In some circumstances, this can be a significant source of exposure for swimmers. Lévesque 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.

The *Canadian Environmental Protection Act, 1999* (CEPA) Priority Substances List assessment report on chloroform (Environment Canada & Health Canada, 2001) developed deterministic estimates of chloroform exposure for six age groups based on data on concentrations of chloroform in outdoor and indoor air acquired in national surveys in Canada and on estimates of the concentrations of chloroform in foods in Canada and the USA. Estimates of intake in drinking-water were based on monitoring

data from the provinces and territories. Estimates of the average daily intake of chloroform by inhalation and dermal absorption during showering were also derived for teenagers, adults, and seniors.

The main pathways of exposure to chloroform for the general population are inhalation of indoor air and ingestion of tap water. The contributions of outdoor air and food are considerably less than the contributions from indoor air and tap water. Most of the chloroform in indoor air is present as a result of volatilization from drinking-water. A recent study using PBPK modelling (Krishnan, 2003) found the highest chloroform exposure values among adults taking a 30-min bath daily. Indoor air exposure to chloroform (and BDCM) is particularly important in countries with low rates of ventilation in houses and high rates of showering and bathing.

3. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

3.1 Absorption

THMs are generally well absorbed, metabolized, and rapidly eliminated by mammals after oral or inhalation exposure (IPCS, 2000).

3.1.1 Chloroform

The absorption kinetics of chloroform following intragastric intubation are dependent upon the vehicle of delivery. Based on the calculated area under blood concentration–time curves, uptake of chloroform following administration of 75 mg/kg of body weight by intragastric intubation in aqueous solution was 8.7 times greater than that for a similar dose administered in corn oil in paired Wistar rats (Withey et al., 1983).

Chloroform is readily absorbed through the skin of humans and animals, and significant dermal absorption of chloroform from water while showering has been demonstrated. Hydration of the skin appears to accelerate absorption of chloroform (Jo et al., 1990).

3.2 Distribution

3.2.1 Chloroform

Chloroform is distributed 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.

3.2.2 Brominated THMs

Brominated substitution would be expected to confer greater lipophilicity on the brominated THMs compared with chloroform, which would affect tissue solubility. Mink et al. (1986) found that the liver, stomach, and kidneys were the organs containing the highest BDCM levels. Mathews et al. (1990) found that repeated doses had no effect on the tissue distribution of BDCM in rats. Lilly et al. (1998) found slightly higher maximum concentrations of BDCM in the liver and kidneys after aqueous administration compared with corn oil delivery in male rats.

3.3 Metabolism

THMs are metabolized primarily to carbon dioxide and/or carbon monoxide.

3.3.1 Chloroform

Available data indicate that the toxicity of chloroform is attributable to its metabolites. Both oxidative and reductive pathways of chloroform metabolism have been identified, although *in vivo* data are limited. The metabolism of chloroform proceeds through a cytochrome P450-dependent activation step, regardless of whether oxidative or reductive reactions are occurring. The balance between oxidative and reductive pathways depends on species, tissue, dose, and oxygen tension. Tissues with chloroform-metabolizing ability include liver, kidney cortex, and tracheal, bronchial, olfactory, oesophageal, laryngeal, tongue, gingival, cheek, nasopharyngeal, pharyngeal, and soft palate mucosa. Of these, the liver is the most active, followed by the nose and kidney. 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. Strain- and sex-related differences in sensitivity of mice to nephrotoxicity are correlated with the ability of the kidney to metabolize chloroform. Chloroform is biotransformed more rapidly in mouse than in rat renal microsomes (Environment Canada & Health Canada, 2001).

The oxidative biotransformation of chloroform is catalysed by cytochrome P450 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 by reaction with thiols, including glutathione and cysteine, to produce adducts. Carbon dioxide is the major metabolite of chloroform generated by the oxidative pathway *in vivo*. Both products of oxidative activation, phosgene and hydrochloric acid, can cause tissue damage. Phosgene reacting with tissue proteins is associated with cell damage and death. Increased covalent binding of chloroform metabolites in the liver occurs when glutathione is depleted (Environment Canada & Health Canada, 2001). Phosgene can bind covalently to cellular nucleophiles, but little binding of chloroform metabolites to DNA is observed. Chloroform also undergoes cytochrome P450-catalysed reductive biotransformation to produce the dichloromethyl radical (with and without phenobarbital induction), which becomes covalently bound to tissue lipids.

Secondary metabolic pathways are reductive dehalogenation via CYP2B1/2/2E1 (leading to free radical generation) and glutathione conjugation via theta-class glutathione-*S*-transferase T1-1 (GSTT1-1), which generates mutagenic intermediates. Glutathione-*S*-transferase-mediated conjugation of chloroform to glutathione can occur only at extremely high chloroform concentrations or doses (IPCS, 2000). Reduced glutathione is capable of scavenging essentially all chloroform metabolites produced in incubations with mouse liver microsomes when chloroform concentrations are not too high (Environment Canada & Health Canada, 2001). Although the findings should be interpreted with caution, Delic et al. (2000) used PBPK modelling to estimate that humans would need to be exposed to chloroform at 645 mg/m^3 by inhalation in order to attain levels of active metabolites associated with a concentration of 50 mg/m^3 in mice. Based on comparison of the formation of reactive metabolites as measured by binding of radioactivity from [^{14}C]$CHCl_3$ (0–10 mmol) in rat and human liver microsomes, it was concluded that the metabolism in these species is similar, although less efficient in humans (Cresteil et al., 1979).

In eight human volunteers ingesting gelatin capsules containing chloroform (500 mg in olive oil), a maximum of 68.3% and 50.6% of the dose was found in the expired air as chloroform and carbon dioxide, respectively, 8 h post-administration (Fry et al., 1972; NAS, 1987). There was an inverse relationship between the adipose tissue content of the body and pulmonary elimination of chloroform (Fry et al., 1972).

3.3.2 Brominated THMs

BDCM is metabolized to phosgene, whereas DBCM and bromoform are metabolized to brominated analogues of phosgene. The rate of metabolism of these compounds to carbon monoxide both *in vivo* and *in vitro* generally follows the halide order: namely, bromoform >> DBCM > BDCM >> chloroform. IPCS (2000) postulated that the brominated THMs may be more rapidly and more extensively metabolized than their chlorinated counterparts. Although this may be true for BDCM, support for this statement, as it pertains to DBCM or bromoform, is difficult to determine from the limited literature that is currently available. The majority of the comparative metabolism studies conducted to date are limited to chloroform or BDCM. Nonetheless, it would appear that the toxicity of BDCM and likely other brominated THMs is mediated through a bioactivation pathway (IPCS, 2000).

Thornton-Manning et al. (1994) concluded that there were clear interspecies differences in metabolism of BDCM, which may explain the greater sensitivity of rats, relative to mice, to the hepatotoxicity of orally administered BDCM. Within 8 h following intragastric administration of 150 mg/kg of body weight (rats) or 100 mg/kg of body weight (mice) in corn oil, 4–18% and 40–81% of total radiolabelled THMs were eliminated as carbon dioxide through the lungs in expired air in rats and mice, respectively. In the same experiment, 41–67% and 5–26% of the parent compound were eliminated unchanged in rats and mice, respectively. Less than 10% of the total radiolabel for each of the chemicals was detected in the urine of both species 36–48 h post-exposure; the proportion excreted in the urine for both species was greatest for chloroform, followed by, in descending order, bromoform, BDCM, and DBCM. The

authors considered the metabolism of these compounds to be 4- to 9-fold greater in the mouse than in the rat; however, it should be noted that the administered doses were high and that metabolism in both species is more complete following administration of lower, more relevant doses.

Pegram et al. (1997) provided evidence that the mutagenic metabolic pathway for brominated THMs is mediated by GSTT1-1 conjugation and that the mutagenic pathway of chloroform is not. These findings suggest that chlorinated and brominated THMs may be activated by different mechanisms. DeMarini et al. (1997) examined the ability of GSTT1-1 to mediate the mutagenicity of various THMs, reported nucleotide transitions (GC → AT) mediated by glutathione-*S*-transferase in *Salmonella*, and ranked the THMs according to relative mutagenic potency as follows: bromoform = DBCM > BDCM. GSTT1-1 conjugation of BDCM was confirmed by Ross & Pegram (2003), who characterized the reaction kinetics of the conjugation of BDCM with glutathione in mouse, rat, and human hepatic cytosols. Reactive glutathione conjugates produced may result in the formation of DNA adducts. Furthermore, these reactive intermediates produced by glutathione conjugation of BDCM are more mutagenic/genotoxic than intermediates produced from dichloromethane.

Allis et al. (2001) and Lilly et al. (1997) investigated the metabolism of BDCM following inhalation exposure in male rats. The findings suggest that CYP2E1 is the dominant enzyme involved in the metabolism of inhaled BDCM in rats (GlobalTox, 2002). Lilly et al. (1998) also found that more of the parent BDCM compound was eliminated unmetabolized via exhaled breath after aqueous dosing than after corn oil gavage.

3.3.3 Mixtures of THMs

A PBPK model was developed by DaSilva et al. (2000), who found that exposures to binary mixtures of chloroform and BDCM, DBCM, or bromoform would likely result in significant increases in the levels of unmetabolized chloroform in the blood, relative to chloroform administered alone. This study also demonstrated that clearance of THMs may be impacted by toxicokinetic interactions between THMs. Bromoform and DBCM appear to persist in blood and tissues for longer periods of time when co-administered with chloroform than when given alone (GlobalTox, 2002).

3.4 Elimination

3.4.1 Chloroform

In humans and laboratory animals exposed to chloroform, carbon dioxide and unchanged chloroform are rapidly eliminated in the expired air. The fraction of the dose eliminated as carbon dioxide varies with the dose and the species (IPCS, 2000).

3.4.2 BDCM

Mink et al. (1986) estimated BDCM half-lives of 1.5 and 2.5 h in the rat and mouse, respectively. Mathews et al. (1990) found that urinary and faecal elimination were low at all dose levels in male rats. Elimination kinetics of BDCM have been studied in humans who had been swimming in chlorinated pools; BDCM half-lives of 0.45–0.63 min for blood were estimated using breath elimination data (Lindstrom et al., 1997; Pleil & Lindstrom, 1997).

4. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO *TEST SYSTEMS*

4.1 Acute exposure

At acutely toxic doses, chloroform causes central nervous system depression and cardiac effects. In rats, the clinical signs of acute toxicity for all of the THMs are similar and include piloerection, sedation, flaccid muscle tone, ataxia, and prostration. LD_{50}s for chloroform, BDCM, DBCM, and bromoform were 908, 916, 1186, and 1388 mg/kg of body weight, respectively, in male rats and 1117, 969, 848, and 1147 mg/kg of body weight, respectively, in female rats. In surviving animals, there were a variety of effects, including reduced food intake, growth retardation, increased liver and kidney weights, haematological and biochemical effects, and histological changes in the liver and kidney (Chu et al., 1980). Keegan et al. (1998) characterized the no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) for acute hepatotoxicity in F344 rats for both chloroform and BDCM delivered in an aqueous vehicle. For both chloroform and BDCM, the oral NOAEL was 0.25 mmol/kg of body weight, and a LOAEL of 0.5 mmol/kg of body weight was determined. Assessment at later time points indicated that liver damage caused by BDCM is more persistent than that caused by chloroform.

Based on data on chloroform and limited data on BDCM, DBCM, and bromoform, the literature suggests that rats are more sensitive than mice to acute effects of THMs. The critical effects associated with acute oral exposure in animals, irrespective of the target organ, are cellular degeneration, damage, and/or necrosis (GlobalTox, 2002).

4.2 Short-term exposure

4.2.1 THMs

The liver and thyroid, rather than the liver and kidney, were the organs most affected following administration of each of the THMs in a subchronic study (Chu et al., 1982a,b). Groups of 20 male and female SD rats ingested drinking-water containing chloroform, BDCM, DBCM, or bromoform at concentrations of 5, 50, 500, or 2500 mg/litre for 90 days; estimated doses were 0.11–0.17, 1.2–1.6, 8.9–14, and 29–55 mg/day per rat, respectively. Ten animals in each group were killed at the end of exposure, and the remaining animals were sacrificed 90 days later.

The growth rate was suppressed in animals administered chloroform and BDCM at 2500 mg/litre at the end of exposure but not following the 90-day recovery period. Food consumption was also depressed during both exposure and recovery periods in groups receiving chloroform, DBCM, or BDCM at 2500 mg/litre. Food consumption in males was depressed during exposure to 2500 mg of bromoform per litre but was normal at the end of the recovery period. Lymphocyte counts were decreased at the end of the recovery period in groups receiving 500 mg of chloroform per litre, 2500 mg of DBCM per litre, or 2500 mg of bromoform per litre. Mild, reversible histological changes in the liver and thyroid of exposed groups were reported, with the hepatotoxicity being greatest for bromoform, followed by, in descending order, BDCM, DBCM, and chloroform; however, the incidence of the lesions was not dose-related, although the frequency of more severe changes was greater in higher dose groups (statistical significance not reported). As the histological effects were mild and reversible and the haematological effects observed in chloroform-exposed animals were not dose-related, the NOAEL for all of the THMs in this study is considered to be 500 mg/litre; the LOAEL is considered to be 2500 mg/litre (Chu et al., 1982a,b).

4.2.2 Chloroform

In a 90-day study in which CD-1 male and female mice (7–12 animals of each sex per treatment group) received 50, 125, or 250 mg of chloroform per kg of body weight per day by intubation in Emulphor deionized water, there was a dose-related increase in liver weights and a decrease in hepatic microsomal activities in high-dose males and in females at all dose levels (Munson et al., 1982). Hexobarbital sleeping times were also increased in mid- and high-dose females. Blood glucose was increased in the high-dose groups of both sexes, and humoral immunity was decreased in high-dose males and mid- and high-dose females. Cellular immunity was decreased in high-dose females. The authors also reported slight histopathological changes in the kidney and liver of both sexes but did not provide information on the prevalence, severity, or dose–response relationship. The LOAEL for female mice in this study is considered to be 50 mg/kg of body weight; for males, the LOAEL is 250 mg/kg of body weight and the NOAEL is 125 mg/kg of body weight. The absence in this investigation of an increase in serum glutamic–pyruvic transaminase and serum glutamic–oxaloacetic transaminase observed in the high-dose groups in a 14-day study with a similar dosing regimen by the same investigators led the authors to conclude that some tolerance to the hepatotoxic action of chloroform may develop following long-term exposure.

The importance of the vehicle of administration in the toxicity of chloroform was demonstrated in a study in which groups of 80 male and female B6C3F1 mice were exposed to 60, 130, or 270 mg/kg of body weight per day by gavage in corn oil or a 2% Emulphor suspension for 90 days. Chloroform caused more marked hepatotoxic effects when administered in corn oil than in aqueous suspension, as determined by body and organ weights, serum chemistry, and histopathological examination (Bull et al., 1986).

Chloroform was administered by corn oil gavage to five male B6C3F1 mice per dose group at doses of 0, 34, 90, 138, or 277 mg/kg of body weight for 4 days or 3 weeks (5 days per week). Mild degenerative changes in centrilobular hepatocytes were noted in mice given 34 or 90 mg/kg of body weight per day after 4 days of treatment, but these effects were absent at 3 weeks. At 138 and 277 mg/kg of body weight per day, centrilobular necrosis was observed at 4 days and with increased severity at 3 weeks. Hepatic cell proliferation was increased in a dose-dependent manner at all chloroform doses after 4 days, but only in the 277 mg/kg of body weight group at 3 weeks. Renal tubular necrosis was observed in all treated groups after 4 days, while 3 weeks of exposure produced severe nephropathy at the highest dose and regenerating tubules at the lower doses. The nuclear labelling index was increased in the proximal tubules at all doses after 4 days of treatment, but was elevated only in the two highest dose groups after 3 weeks (Larson et al., 1994a).

In a similar study, five female B6C3F1 mice per dose group were administered chloroform dissolved in corn oil by gavage at doses of 0, 3, 10, 34, 238, or 477 mg/kg of body weight per day for 4 days or 3 weeks (5 days per week). Dose-dependent changes included centrilobular hepatic necrosis and markedly elevated labelling index in mice given 238 or 477 mg/kg of body weight per day. The NOAEL for histopathological changes (cytolethality and regenerative hyperplasia) was 10 mg/kg of body weight per day, and for induced cell proliferation, 34 mg/kg of body weight per day. In the same study, 14 female B6C3F1 mice per dose group were continuously exposed to chloroform in the drinking-water at concentrations of 0, 60, 200, 400, 900, or 1800 mg/litre for 4 days or 3 weeks. There was no increase in the hepatic labelling index after either 4 days or 3 weeks in any of the dose groups, nor were any microscopic alterations observed in the liver, even though the cumulative daily amount of chloroform ingested in the high-dose group was 329 mg/kg of body weight per day. The authors suggested that mice provided with chloroform in the drinking-water *ad libitum* received the dose over the entire day with much smaller peak tissue levels than when the compound was administered as a bolus dose (Larson et al., 1994b).

Five female F344 rats per dose group were given chloroform by corn oil gavage at doses of 0, 34, 100, 200, or 400 mg/kg of body weight per day for 4 consecutive days or 5 days per week for 3 weeks (Larson et al., 1995b). In the liver, mild degenerative centrilobular changes and dose-dependent increases in hepatocyte proliferation were noted at doses of 100, 200, and 400 mg/kg of body weight per day. At 200 and 400 mg/kg of body weight per day, degeneration and necrosis of the renal cortical proximal tubules were observed. Increased regenerative proliferation of epithelial cells lining proximal tubules was seen at doses of 100 mg/kg of body weight per day or more. Lesions of the olfactory mucosa lining the ethmoid region of the nose (new bone formation, periosteal hypercellularity, and increased cell replication) were seen at all doses, including the lowest dose of 34 mg/kg of body weight per day.

Larson et al. (1995a) also administered chloroform to 12 male F344 rats per dose group by corn oil gavage (0, 10, 34, 90, or 180 mg/kg of body weight per day) or in the drinking-water (0, 60, 200, 400, 900, or 1800 mg/litre) for 4 days or 3 weeks.

Gavage of 90 or 180 mg/kg of body weight per day for 4 days induced mild to moderate degeneration of renal proximal tubules and centrilobular hepatocyte changes that were no longer present after 3 weeks. Increased cell proliferation in the kidney was noted only at the highest gavage dose after 4 days. The labelling index was elevated in the livers of the high-dose group at both time points. With drinking-water administration, rats consuming the water containing 1800 mg/litre were dosed at a rate of 106 mg/kg of body weight per day, but no increase in renal or hepatic cell proliferation was observed at this or any lower dose.

The cardiotoxicity of chloroform was examined in male Wistar rats given daily doses of 37 mg/kg of body weight (0.31 mmol/kg) by gavage in olive oil for 4 weeks. Chloroform caused arrhythmogenic and negative chronotropic and dromotropic effects as well as extension of the atrioventricular conduction time and depressed myocardial contractility (Muller et al., 1997).

In an inhalation study, Templin et al. (1996b) exposed BDF1 mice to chloroform vapour at concentrations of 0, 149, or 446 mg/m^3 for 6 h per day for 4 days or 2 weeks (5 days per week). In the kidneys of male mice exposed to 149 or 446 mg/m^3, degenerative lesions and 7- to 10-fold increases in cell proliferation were observed. Liver damage and an increased hepatic labelling index were noted in male mice exposed to 149 and 446 mg/m^3 and in female mice exposed to 446 mg/m^3. Both doses were lethal in groups exposed for 2 weeks (40% and 80% mortality at 149 and 446 mg/m^3, respectively).

A 90-day chloroform inhalation study was conducted using male and female B6C3F1 mice and exposure concentrations of 0, 1.5, 10, 50, 149, and 446 mg/m^3 for 6 h per day, 7 days per week. Large, sustained increases in hepatocyte proliferation were seen in the 446 mg/m^3 groups at all time points (4 days and 3, 6, and 13 weeks). In the more sensitive female mice, a NOAEL of 50 mg/m^3 for this effect was established. Renal histopathology and regenerative hyperplasia were noted in male mice at 50, 149, and 446 mg/m^3 (Larson et al., 1996). In another 90-day inhalation study, F344 rats were exposed to chloroform at concentrations of 0, 10, 50, 149, 446, or 1490 mg/m^3 for 6 h per day, 7 days per week. The 1490 mg/m^3 level was extremely toxic and deemed by the authors to be inappropriate for chronic studies. Increases in renal epithelial cell proliferation in cortical proximal tubules were observed at concentrations of 149 mg/m^3 and above. Hepatic lesions and increased proliferation were noted only at the highest exposure level. In the ethmoid turbinates of the nose, enhanced bone growth and hypercellularity in the lamina propria were observed at concentrations of 50 mg/m^3 and above, and a generalized atrophy of the turbinates was seen at all exposure levels after 90 days (Templin et al., 1996c).

Jamison et al. (1996) reported that F344 rats exposed to a high concentration of chloroform vapour (1490 mg/m^3) for 90 days developed atypical glandular structures lined by intestinal-like epithelium and surrounded by dense connective tissue in their livers. These lesions appeared to arise from a population of cells remote from the bile ducts. The authors also observed a treatment-related increase in transforming growth factor-alpha (TGF-α) immunoreactivity in hepatocytes, bile duct epithelium, bile

canaliculi, and oval cells and an increase in transforming growth factor-beta (TGF-β) immunoreactivity in hepatocytes, bile duct epithelium, and intestinal crypt-like ducts. The lesions occurred only in conjunction with significant hepatocyte necrosis, regenerative cell proliferation, and increased growth factor expression or uptake.

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.

4.2.3 BDCM

Thornton-Manning et al. (1994) administered five consecutive daily BDCM doses to female F344 rats and female C57BL/6J mice by aqueous gavage and found that BDCM is both hepatotoxic and nepthrotoxic to female rats (150–300 mg/kg of body weight per day) but only hepatotoxic to female mice (75–150 mg/kg of body weight per day). Munson et al. (1982) administered BDCM (50, 125, or 250 mg/kg of body weight per day) to male and female CD-1 mice by aqueous gavage for 14 days and reported evidence for hepatic and renal toxicity as well as effects on the humoral immune system (decreases in both antibody-forming cells and haemagglutination titres). A subsequent study by French et al. (1999) found no effects of BDCM on immune function. Based on the degree of aspartate aminotransferase and alanine aminotransferase elevations in this study, BDCM was found to be a more potent hepatotoxicant than chloroform, DBCM, and bromoform.

F344/N rats and B6C3F1 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, 6.25, 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 or 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).

4.2.4 DBCM

DBCM-induced cardiotoxicity was reported in male Wistar rats after short-term exposure (4 weeks of daily dosing with 0.4 mmol/kg of body weight). Arrhythmogenic and negative chronotropic and dromotropic effects were observed, as well as extension of atrioventricular conduction times. Inhibitory actions of DBCM on calcium ion dynamics in isolated cardiac myocytes were also noted (IPCS, 2000).

F344/N rats and B6C3F1 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).

A 90-day corn oil gavage study was conducted using Sprague-Dawley rats and doses of 0, 50, 100, or 200 mg/kg of body weight per day. Body weight gain was significantly depressed in the high-dose groups to less than 50% and 70% of the controls in males and females, respectively. Observations of liver damage included elevated alanine aminotransferase in mid- and high-dose males, centrilobular lipidosis (vacuolization) in males at all doses and in high-dose females, and centrilobular hepatic necrosis in high-dose males and females. Kidney proximal tubule cell degeneration was induced by DBCM in all high-dose rats and to a lesser extent at 100 mg/kg of body weight per day in males and at both 50 and 100 mg/kg of body weight per day in females (Daniel et al., 1990).

4.2.5 Bromoform

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, 1989).

4.3 Reproductive and developmental toxicity

4.3.1 THMs

The teratogenicity of THMs was investigated in one study in which BDCM, DBCM, or bromoform at doses of 50, 100, or 200 mg/kg of body weight per day or chloroform at doses of 100, 200, or 400 mg/kg of body weight per day was administered to groups of 15 pregnant Sprague-Dawley rats by oral intubation in corn oil on gestation days 6–15. Maternal weight gain was depressed in the high-dose groups (200 mg/kg of body weight per day) receiving BDCM and DBCM, but to a lesser extent than in the high-dose group for chloroform (400 mg/kg of body weight per day). Maternal liver weight was also increased at the highest dose of BDCM (200 mg/kg of body weight per day). BDCM and bromoform were considered to be fetotoxic, based on the observation of interparietal anomalies, although the statistical significance of the observed increases was not reported. These compounds also appeared to increase the incidence of aberrations of the sternebrae. The LOAEL based on this fetotoxic effect was 50 mg/kg of body weight per day (Ruddick et al., 1983).

A survey of available toxicological literature on reproductive and developmental effects of disinfection by-products including chloroform and BDCM was conducted for the US Environmental Protection Agency (EPA) by Tyl (2000), who concluded that current published studies are not sufficient for quantitative assessment of reproductive or developmental risk but are sufficient for determination of hazard. The potential hazards identified for chloroform and BDCM were whole-litter resorption and fetotoxicity, and for BDCM, male reproductive toxicity (Tyl, 2000).

4.3.2 Chloroform

Available data on the teratogenicity of the THMs are confined principally to chloroform. In studies conducted to date, chloroform has not been teratogenic in rats, rabbits, or mice at doses up to 400 mg/kg of body weight following administration by gavage in corn oil or Emulphor:saline (Thompson et al., 1974; Burkhalter & Balster, 1979; Ruddick et al., 1983). Fetotoxic effects (e.g., decreased body weights and sternebral and interparietal malformations) were sometimes observed, but only at doses that were toxic to the mothers.

In a continuous-breeding study, male and female CD-1 mice were administered chloroform in corn oil by gavage at actual doses of 0, 6.6, 15.9, or 41.2 mg/kg of body weight per day for 7 days prior to and throughout the 98-day cohabitation period. Control and high-dose F_1 pups were administered chloroform after weaning at postnatal day 21 according to the same dosing schedule as their F_0 parents. There were no significant effects on fertility or reproduction in either gender over two generations. Histopathological changes indicative of hepatotoxicity were observed in the F_1 females at all treatment dose levels (Gulati et al., 1988).

Hoechst (1991) examined the embryotoxicity and developmental toxicity of inhaled chloroform. Female Wistar rats were mated, then exposed by whole-body inhalation to chloroform at 0, 15, 50, or 149 mg/m^3 for 7 h per day between gestation days 7 and 16. Slight reductions in food consumption and significant reductions in body weights were observed in dams exposed at 50 and 149 mg/m^3. These findings were hypothesized to result in the slight stunting of fetuses produced by these animals. A NOAEL of 15 mg/m^3 was established based on the lack of embryotoxicity or teratogenicity (GlobalTox, 2002).

4.3.3 BDCM

Narotsky et al. (1997) examined the effects of BDCM in F344 rats using doses of 0, 25, 50, or 75 mg/kg of body weight per day in aqueous or oil gavage vehicles. BDCM induced full-litter resorptions in the 50 and 75 mg/kg of body weight per day dose groups with either vehicle of administration. For dams receiving corn oil, full-litter resorptions were noted in 8% and 83% of the litters at 50 and 75 mg/kg of body weight per day, respectively. All vehicle control litters and litters from the group given 25 mg/kg of body weight per day survived the experimental period. BDCM had been shown to cause maternal toxicity at these doses in a previous study (Narotsky et al., 1992).

In a developmental study conducted by Christian et al. (2001), Sprague-Dawley rats and New Zealand White rabbits were dosed with BDCM continuously in drinking-water on gestation days 6–21 in rats and gestation days 6–29 in rabbits. Mean consumed doses were 0, 2.2, 18.4, 45.0, or 82.0 mg/kg of body weight per day for rats and 0, 1.4, 13.4, 35.6, or 55.3 mg/kg of body weight per day for rabbits. In rats, water consumption was reduced in all treatment doses, and body weight gain and feed consumption were reduced at ≥45.0 mg/kg of body weight per day. In rabbits, body weight gain and feed consumption were reduced at ≥35.6 mg/kg of body weight per day. The maternal NOAELs were 18.4 and 13.4 mg/kg of body weight per day for rats and rabbits, respectively. Minimal delays in the ossification of forepaw phalanges and hindpaw metatarsals and phalanges occurred in rat fetuses at 82.0 mg/kg of body weight per day and were considered marginal, reversible, and associated with severely reduced maternal weight gain. There were no treatment-related effects observed in rabbit fetuses. The developmental NOAELs were 45.0 and 55.3 mg/kg of body weight per day for rats and rabbits, respectively (Christian et al., 2001).

In a two-generation reproduction study conducted by Christian et al. (2002), Sprague-Dawley rats were treated with BDCM continuously via the drinking-water at concentrations of 0, 50, 150, or 450 mg/litre (equal to 0, 4.1–12.6, 11.6–40.2, or 29.5–109.0 mg/kg of body weight per day). In the top two dose groups, mortality and clinical signs associated with reduced water consumption, reduced body weights and weight gains, and reduced food consumption were observed. Reduced body weights were associated with reduced organ weights and increased organ weight ratios. Small delays in sexual maturation (preputial separation, vaginal patency) and more F_1 rats with prolonged diestrus were also attributed to severely reduced body weights. The NOAEL for general toxicity and the NOAELs for reproductive and developmental

toxicity were at least 4.1–12.6 mg/kg of body weight per day. If the delayed sexual maturation associated with severely reduced body weights is considered general toxicity, reproductive and developmental NOAELs for BDCM are greater than 29.5–109.0 mg/kg of body weight per day (Christian et al., 2002).

Bielmeier et al. (2001) investigated rat strain sensitivity between F344 and Sprague-Dawley rats as measured by full-litter resorption after dosing with BDCM. Following aqueous gavage with BDCM at 75 mg/kg of body weight per day on gestation days 6–10, F344 rats had a 62% incidence of full-litter resorption, whereas all SD rats maintained their litters. Additionally, rats treated with BDCM at 75 mg/kg of body weight per day on gestation days 6–10, the critical period encompassing the luteinizing hormone (LH)-dependent period of pregnancy, had a 75% incidence of full-litter resorption, but rats treated on gestation days 11–15 with BDCM at 75 or 100 mg/kg of body weight per day were unaffected. Twenty-four hours after a single dose, all dams with full-litter resorption had markedly reduced serum progesterone levels; however, LH levels were unaffected. The high full-litter resorption rate during the LH-dependent period, the lack of response thereafter, and the reduced progesterone levels without an associated reduction in LH levels suggest that BDCM disrupts luteal responsiveness to LH (GlobalTox, 2002).

Klinefelter et al. (1995) studied the potential of BDCM to alter male reproductive function in F344 rats. BDCM was consumed in the drinking-water for 52 weeks, resulting in average dose rates of 22 and 39 mg/kg of body weight per day. No gross lesions in the reproductive organs were revealed by histological examination, but exposure to the high BDCM dose significantly decreased the mean straight-line, average path, and curvilinear velocities of sperm recovered from the cauda epididymis (IPCS, 2000).

Chen et al. (2003) examined the effect of BDCM on chronic gonadotrophin secretion by human placental trophoblast cultures. A BDCM dose-dependent reduction in the secretion of bioactive and immunoreactive chorionic gonadotrophin from human placental trophoblasts was observed, suggesting that BDCM targets these cells. A reduction in chorionic gonadotrophin could have adverse effects on pregnancies, since this hormone plays a vital role in maintaining pregnancy.

4.3.4 DBCM

In a multigeneration reproduction 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 groups exhibited enlarged livers with gross morphological changes. In addition, the gestation index, fertility, and survival of the F_1 generation were significantly reduced. Only fertility was decreased (high dose) in the F_2 generation (IPCS, 2000). Based on maternal toxicity and fetotoxicity, a NOAEL of 17 mg/kg of body weight per day was identified (Borzelleca & Carchman, 1982).

4.3.5 Bromoform

Bromoform was found to induce full-litter resorptions in pregnant F344 rats when administered orally on gestation days 6–15, but at higher doses (150 and 200 mg/kg of body weight per day) than those required to produce the same effect for BDCM (Narotsky et al., 1993).

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, sex of live pups, 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, 1989).

4.4 Genotoxicity and related end-points

4.4.1 THMs

All four THMs have induced sister chromatid exchanges (SCE) in human lymphocytes *in vitro* (bromoform > DBCM > BDCM > chloroform) and in mouse bone marrow cells *in vivo* (Morimoto & Koizumi, 1983).

In contrast to the predominantly non-genotoxic and non-mutagenic finding for chloroform, the weight of evidence favours a finding of mutagenicity and genotoxicity for the brominated THMs. Pegram et al. (1997) provided evidence that the mutagenic metabolic pathway for brominated THMs is mediated by GSTT1-1 conjugation and that mutagenic effects were not nearly as common with chloroform as with brominated THMs. The ability of GSTT1-1 to mediate the mutagenicity of various brominated THMs and induce almost exclusively GC → AT transitions suggests that it is likely that these THMs are activated by similar pathways (DeMarini et al., 1997).

4.4.2 Chloroform

The current weight of evidence suggests that chloroform is only slightly mutagenic and unlikely to be genotoxic. Varma et al. (1988) reported that chloroform was

mutagenic in *Salmonella typhimurium* without metabolic activation, although a mixture of chloroform (85%) and bromoform (15%) was not mutagenic in the same assay with or without metabolic activation. LeCurieux et al. (1995) and Roldan-Arjona & Pueyo (1993) found that chloroform was not mutagenic with or without metabolic activation using several strains in an *S. typhimurium* assay. Shelby & Witt (1995) reported that chloroform was genotoxic in a mouse micronucleus assay in B6C3F1 mice but negative in an *in vivo* chromosomal aberration assay. Pegram et al. (1997) reported chloroform to be mutagenic in *S. typhimurium* TA1535, although not to the same extent as brominated THMs. Chloroform was not genotoxic in a number of unscheduled DNA synthesis (UDS) and/or repair, micronuclei, chromosomal aberration, and SCE assays (GlobalTox, 2002).

4.4.3 BDCM

Although BDCM has given mixed results in bacterial assays for genotoxicity, the results have tended to be positive in tests employing closed systems to overcome the problem of the compound's volatility (IARC, 1991, 1999a; Pegram et al., 1997). LeCurieux et al. (1995) found that BDCM was negative both with and without metabolic activation in the Ames assay. BDCM tested positive in several independent chromosomal aberration assays with and without metabolic activation but was negative in UDS and a mouse micronucleus assay. Fujie et al. (1993) reported that BDCM induced SCE. In addition, Pegram et al. (1997) provided evidence that a mutagenic metabolic pathway for brominated THMs is mediated by GSTT1-1 conjugation.

4.4.4 DBCM

DBCM is mostly positive in genotoxicity tests employing closed systems to overcome the problem of volatility (IARC, 1991, 1999a; Pegram et al., 1997). DBCM has given mostly positive results in eukaryotic test systems (Loveday et al., 1990; IARC, 1991, 1999a; McGregor et al., 1991; Fujie et al., 1993), although there is less consistency in results between the different assays when considered with or without an exogenous metabolic system. 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 aberrations in Chinese hamster ovary cells with activation (Ishidate et al., 1982) and for SCE in human lymphocytes and mouse bone marrow cells *in vivo* (Morimoto & Koizumi, 1983); it was negative in the micronucleus assay (Ishidate et al., 1982) and UDS in the liver of rats (IPCS, 2000).

4.4.5 Bromoform

There is some evidence to suggest that bromoform may be weakly mutagenic (GlobalTox, 2002). Bromoform, in common with the other brominated THMs, is largely positive in bacterial assays of mutagenicity conducted in closed systems (Zeiger, 1990; IARC, 1991, 1999a). Bromoform was positive in the Ames test in *S.*

typhimurium strain TA100 without activation (Simmon et al., 1977; Ishidate et al., 1982), positive with and without activation in TA98, and negative or equivocal in strains TA1535 or TA1937 with and without activation (NTP, 1989).

Bromoform gave increased SCE and chromosomal aberrations in mouse and rat bone marrow cells (Morimoto & Koizumi, 1983; Fujie et al., 1990). It gave negative results in mouse bone marrow (Hayashi et al., 1988; Stocker et al., 1997), in the rat liver UDS assay (Pereira et al., 1982; Stocker et al., 1997), and in the dominant lethal assay (Ishidate et al., 1982). In studies carried out by the NTP (1989), it was positive for micronuclei and SCE, but negative for chromosomal aberrations in mouse bone marrow. Potter et al. (1996) found that bromoform did not induce DNA strand breaks in the kidneys of male F344 rats following seven daily doses of 1.5 mmol/kg of body weight. As with bacterial assays, bromoform appeared more potent than the other brominated THMs (Morimoto & Koizumi, 1983; Banerji & Fernandes, 1996).

4.5 Carcinogenicity

4.5.1 Chloroform

Chloroform has been carcinogenic in two animal species in extensive bioassays. In an early study conducted by the US National Cancer Institute (NCI), chloroform was administered by gavage in corn oil to groups of 50 male and 50 female Osborne-Mendel rats and B6C3F1 mice. Male rats received 0, 90, or 180 mg/kg of body weight 5 times per week for 78 weeks; female rats received 0, 125, or 250 mg/kg of body weight 5 times per week for the first 22 weeks and the same doses as the males thereafter. In the first 18 weeks, doses of 0, 100, or 200 mg/kg of body weight were administered to male mice, and 0, 200, or 400 mg/kg of body weight were administered to female mice. After 18 weeks, the doses were changed to 0, 150, and 300 mg/kg of body weight for male mice and 0, 250, and 500 mg/kg of body weight for female mice for the remainder of the exposure period (NCI, 1976a).

In male rats, there was a statistically significant dose-related increase in the incidence of carcinomas of the kidney (0/99, 4/50, and 12/50 for control, low doses, and high doses, respectively). These tumours were not observed in female rats, although there was a non-significant increase in tumours of the thyroid (adenocarcinomas and carcinomas) in this sex. Highly significant increases in hepatocellular carcinomas were observed in both sexes of mice (males: 1/18, 18/50, 44/45; females: 0/20, 36/45, 39/41 for control, low doses, and high doses, respectively). Nodular hyperplasia was also observed in low-dose males. It should be noted, however, that the weight loss in exposed animals was greater than 10% (NCI, 1976a).

Upon re-examination of tissue samples from the NCI carcinogenesis bioassay, Reuber (1979) also reported increases in the incidence of several types of benign and malignant tumours of the liver in female rats and malignant lymphomas in both sexes of mice.

In a more recent and larger study, 0, 200, 400, 900, or 1800 mg of chloroform per litre was administered in drinking-water (a more appropriate vehicle than that used in the NCI bioassay described above) to male Osborne-Mendel rats (50–330 animals per group) and female B6C3F1 mice (50–430 animals per group) for 104 weeks; the time-weighted average doses on a body weight basis ranged from 19 to 160 mg/kg of body weight per day for the rats and from 34 to 263 mg/kg of body weight per day for the mice (Jorgenson et al., 1985). To increase the sensitivity for detecting low response rates, group sizes were larger for the lower doses; there were two control groups (n = 330 and n = 50), one of which (n = 50) was matched for water intake with the high-dose groups.

In rats, there were dose-related decreases in water consumption and body weight gain that persisted in the two highest dose groups; survival increased with dose, probably as a result of leaner body composition in the higher dose groups (e.g., after 104 weeks, only 12% of controls had survived, whereas 66% of the animals in the high-dose group were still alive; this is a common occurrence in such studies). Consistent with the results of the NCI bioassay described above, there was also a dose-related increase in the incidence of kidney tumours. The incidence of tubular cell adenomas and adenocarcinomas combined was slightly lower than that in the NCI bioassay: 1/50, 4/313, 4/148, 3/48, and 7/50 in the matched control and increasing dose groups, respectively. Although there were increases in other neoplastic lesions in rats, including neurofibromas, leukaemias, lymphomas, and circulatory system tumours, they were not considered to be treatment-related because of a lack of a clear dose–response relationship or statistical significance or because they appeared to be attributable to the longer survival of the chloroform-treated animals.

With respect to the non-neoplastic histopathological changes in the kidney in this study, the authors commented only that "nontumour pathology of the kidney was high in all animals regardless of treatment." As a result, "it was not possible to relate tumour pathology with other tissue damage on either an individual animal or across-group basis." The incidence of nephropathy was 91% in the control group, 90% in the matched control, and 95%, 95%, 100%, and 92% in the increasing dose groups, respectively. Kidney tissue from this investigation (Jorgenson et al., 1985) has recently been microscopically re-evaluated for evidence of cytotoxicity and regeneration. Toxic injury in proximal tubular epithelial cells was observed in all high-dose males (1800 mg/litre, the dose at which there was a statistically significant increase in tumour incidence) at all time points and approximately half of animals receiving the second highest dose (900 mg/litre) for 18 or 24 months. None of the other treatment groups or controls had these characteristic changes. Although a systematic evaluation was not possible due to degradation of the slides and frequent autolytic change, the authors confirmed that such changes were also present in males of the same strain in the 1976 NCI bioassay in which exposure was by corn oil gavage (Hard & Wolf, 1999).

In mice, drinking-water consumption was markedly depressed, leading to the death of about 25% of the two highest dose groups and 6% of the next highest dose group in the first week; after this initial period, survival did not differ significantly among

groups. In contrast to the NCI bioassay described above, in which hepatic tumours in both sexes of mice were observed, there were no treatment-related increases in the incidence of any tumours in female mice in this study. Jorgenson et al. (1985) suggested that the hepatic tumours in mice in the NCI study may have been attributable to the interaction of chloroform with the corn oil vehicle.

In different studies in which four strains of mice (C57Bl, CBA, CF/1, and ICI) were administered chloroform for 80 weeks by gavage in toothpaste (0, 17, or 60 mg/kg of body weight per day in ICI male and female mice) or in toothpaste or arachis oil (0 or 60 mg/kg of body weight per day in males of all four strains), there were no treatment-related effects on the incidence of any type of tumour in males of three of the four strains (C57Bl, CBA, and CF/1 mice). There was, however, an increase in the incidence of epithelial tumours of the kidney at 60 mg/kg of body weight per day in male ICI mice, which was greater when chloroform was administered in arachis oil than in toothpaste (Roe et al., 1979).

Several other studies on the potential carcinogenicity of chloroform have been conducted. In B6C3F1 male mice (35 animals per group) ingesting chloroform in drinking-water (0, 600, or 1800 mg/litre) for periods up to 52 weeks, there were no increases in tumour incidence (Klaunig et al., 1986). However, these results may have been a function of the short observation period or small group sizes. The potential of chloroform to promote tumours induced by known initiators was also investigated in this study. Mice of the same strain (35 animals per group) ingested drinking-water containing diethylnitrosamine at 10 mg/litre for 4 weeks followed by 600 or 1800 mg of chloroform per litre for up to 52 weeks. There were two control groups: after diethylnitrosamine treatment, the positive control group ingested drinking-water containing phenobarbital (500 mg/litre), while the vehicle control group received untreated drinking-water. The induction of liver tumours was enhanced by exposure to phenobarbital but not by exposure to chloroform after diethylnitrosamine treatment. In contrast, in a study conducted by Deml & Oesterle (1985), chloroform administered in corn oil (at 100, 200, or 400 mg/kg of body weight, twice weekly for 11 weeks, 1 week after administration of a single dose of 8 mg of diethylnitrosamine) promoted the development of diethylnitrosamine-initiated preneoplastic foci liver tumours in Sprague-Dawley rats.

In a study designed to assess the safety of chloroform in toothpaste, 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 0, 15, or 30 mg/kg of body weight per day (Heywood et al., 1979). After 6 weeks of treatment, there were significant increases in serum glutamate–pyruvate transaminase 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 (eight dogs of each sex) groups. “Fatty cysts” characterized by aggregations of vacuolated hepatocytes and minimal hepatic fibrosis were observed in animals within each group (including controls). These findings were more frequent and of greater magnitude in animals of either gender treated with chloroform at either

dose level than in controls. The LOAEL in this study was 15 mg/kg of body weight per day.

4.5.2 Mechanism of carcinogenicity for chloroform

Significant effort has been made in recent years to characterize the mechanism of carcinogenicity for chloroform and to understand the variability in effects from different routes and vehicles of administration. The current weight of evidence suggests that chloroform is a threshold carcinogen in rodents. There is strong evidence that the carcinogenic activity of chloroform in both rats and mice is mediated by a non-genotoxic mechanism of action that is secondary to cytotoxicity and cellular proliferation. There is strong evidence that the tumorigenicity of chloroform depends on the rate of its delivery to the target organ, and this suggests that detoxification mechanisms must be saturated before the full carcinogenic potential of chloroform is realized (GlobalTox, 2002). The weight of available evidence also indicates that chloroform has little, if any, capability of inducing gene mutation or other types of direct damage to DNA (IPCS, 2000).

IPCS (2000) summarized the pattern of chloroform-induced carcinogenicity in rodent bioassays conducted up to that time as follows: Chloroform induced hepatic tumours in B6C3F1 mice (males and females) when administered by gavage in corn oil at doses in the range of 138–477 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 bromodeoxyuridine (BrdU) labelling index in histological tissue sections. 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.

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 (IPCS, 2000).

Environment Canada & Health Canada (2001) also discussed the weight of evidence for the mechanism of carcinogenicity for chloroform. This report stated that for Osborne-Mendel rats, the results of reanalyses of the original renal tissues (Hard & Wolf, 1999; Hard et al., 2000), from both the drinking-water bioassay (Jorgenson et al., 1985) and the gavage study (NCI, 1976a), have been critical. They provide strong support for the argument that the mode of induction of these tumours is consistent with the hypothesis that sustained proximal tubular cell damage is a requisite precursor lesion for chloroform-induced tumours.

When comparing short-term studies in rats and mice using similar chloroform exposure regimens, the experimental conditions employed in studies that led to cellular proliferation and cytotoxicity led to tumour formation when employed in cancer bioassays. However, the converse is not always true.

The hypothesized mode of carcinogenesis for chloroform is in keeping with the growing body of evidence supporting the biological plausibility that prolonged regenerative cell proliferation can be a causal mechanism in chemical carcinogenesis. This has been addressed in numerous articles, including Ames & Gold (1990, 1996), Cohen & Ellwein (1990, 1991, 1996), Preston-Martin et al. (1990), Ames et al. (1993), Tomatis (1993), Cohen (1995), Cunningham & Matthews (1995), Butterworth (1996), Farber (1996), and Stemmermann et al. (1996).

In summary, chloroform has induced liver tumours in mice and renal tumours in mice and rats. The weight of evidence of genotoxicity, sex and strain specificity, and concordance of cytotoxicity, regenerative proliferation, and tumours is consistent with the hypothesis that cytotoxicity with a period of sustained cell proliferation likely represents a secondary mechanism for the induction of tumours following exposure to chloroform. This is consistent with a non-linear dose–response relationship for induction of tumours. This cytotoxicity is primarily related to rates of oxidation of chloroform to reactive intermediates, principally phosgene and hydrochloric acid. The weight of evidence for this mode of action is strongest for hepatic and renal tumours in mice and more limited for renal tumours in rats (Environment Canada & Health Canada, 2001).

There has been little evidence to support other mechanisms of carcinogenicity, especially at low doses where cytotoxicity and cellular proliferation are not expected. Chloroform toxicity is clearly enhanced in rodents when administered in corn oil, compared with when it is received in drinking-water, supporting the hypothesis that tumorigenicity of chloroform depends on the rate of its delivery to the target tissue and further suggesting that detoxification mechanisms must be saturated before the full carcinogenic potential of chloroform is realized (GlobalTox, 2002).

4.5.3 BDCM

In one carcinogenesis bioassay conducted for BDCM, groups of 50 male and 50 female F344/N rats and B6C3F1 mice were administered the compound by gavage in corn oil, 5 days per week for 102 weeks. Rats received 0, 50, or 100 mg/kg of body weight per day; male mice received 0, 25, or 50 mg/kg of body weight per day, while female mice received 0, 75, or 150 mg/kg of body weight per day (NTP, 1987).

In rats, there was some decrease in body weight gain in the high-dose groups of both sexes (statistical significance not specified), increased incidence of cytomegaly of the renal tubular epithelial cells in males (both doses), nephrosis in the high-dose group of females, and hepatic changes, including necrosis, clear cell change, eosinophilic cytoplasmic change, focal cellular change, and fatty metamorphosis, in both sexes, but predominantly in the high-dose group of females. There was clear evidence of carcinogenicity in male and female rats, with increases in the incidence of renal tubular cell adenomas and adenocarcinomas (combined incidence in control, low-dose, and high-dose groups: males, 0/50, 1/50, and 13/50; females, 0/50, 1/50, and 15/50) and rare tumours (adenomatous polyps and adenocarcinomas) of the large

intestine (combined incidence: males, 0/50, 13/50, and 45/50; females, 0/46, 0/50, and 12/47). Increased incidence of skin neoplasms in low- but not high-dose male rats was also observed but was not considered to be compound-related. The neoplasms of the kidney in rats in this bioassay were not similar to those observed for other compounds, such as 1,4-dichlorobenzene, for which tumours occurred principally in males and were associated with severe nephropathy and increased incidence of calcification and hyaline droplet formation, associated with reabsorption of α-2-microglobulin (Charbonneau et al., 1989).

There was a decrease in body weight gain of female mice, and survival was significantly lower than that of controls, due partly to ovarian abscesses not considered to be treatment-related. The incidences of renal cytomegaly and hepatic fatty metamorphosis in male mice were also increased. Pathological changes in the thyroid gland and testis were also observed but were not considered to be treatment-related. There was also clear evidence of carcinogenicity in male and female B6C3F1 mice, based on increased incidences of adenomas and adenocarcinomas (combined) of the kidney in males (incidence in control, low-dose, and high-dose groups, 1/49, 2/50, and 9/50, respectively) and of hepatocellular adenomas and carcinomas (combined) in female mice (incidence 3/50, 18/48, and 29/50).

Moore et al. (1994) administered BDCM in drinking-water (containing 0.25% Emulphor) to male F344 rats and B6C3F1 mice for 1 year and evaluated clinical indicators of kidney toxicity. Water containing BDCM concentrations of 0.08, 0.4, and 0.8 g/litre for rats and 0.06, 0.3, and 0.6 g/litre for mice resulted in average daily doses of 4.4, 21, and 39 mg/kg of body weight for rats and 5.6, 24, and 49 mg/kg of body weight for mice. A urinary marker for renal proximal tubule damage, *N*-acetyl-β-glucosaminidase, was elevated above controls in each dose group in rats and at the highest treatment level in mice. Significant increases in urinary protein, indicative of glomerular damage, were also noted in low- and mid-dose rats as well as high-dose mice.

While cytotoxic effects of BDCM may potentiate tumorigenicity in certain rodent tissues at high dose levels, direct induction of mutations by BDCM metabolites may also play a carcinogenic role. The extent to which each of these processes contributes to the induction of tumours observed in chronic animal studies is, however, questionable (IPCS, 2000).

DeAngelo et al. (2002) examined the ability of THMs administered in drinking-water to induce aberrant crypt foci in the colons of B6C3F1 mice and F344/N rats. Preneoplastic aberrant crypt foci were induced in the colon of rats following the administration of some brominated THMs. However, unlike DBCM and bromoform, colon neoplasms were not found upon chronic administration of BDCM to rats via drinking-water. BDCM did, however, induce colon cancer in male rats when administered in corn oil gavage.

In a recent study reported in draft form (NTP, 2004), male F344/N rats and female B6C3F1 mice were exposed to BDCM in drinking-water for 2 years. Groups of 50

male F344/N rats were exposed to target concentrations equivalent to average daily BDCM doses of 0, 6, 12, or 25 mg/kg of body weight. Survival and mean body weights of all exposed groups were generally similar to those of the controls throughout the study. There were no increased incidences of neoplasms that were attributed to BDCM. The incidences of chronic inflammation in the liver of the two higher dose groups were significantly greater than that in the controls; however, the biological significance of these increases is uncertain. Groups of 50 female B6C3F1 mice were exposed to target concentrations equivalent to average daily BDCM doses of 0, 9, 18, or 36 mg/kg of body weight. Survival of exposed groups was similar to that of the controls, but mean body weights of all exposed groups were generally less than those of the controls from week 4 through the end of the study. The incidence of hepatocellular adenoma or carcinoma decreased with increasing dose, and the incidence in the highest dose group was significantly decreased relative to that in the control group. The incidence of hemangiosarcoma in all organs was significantly decreased in the 18 mg/kg of body weight per day group.The authors of the study concluded that under the conditions of this 2-year drinking-water study, there was no evidence of carcinogenic activity of BDCM in male F344/N rats exposed to target concentrations of 6, 12, or 25 mg/kg of body weight per day and in female B6C3F1 mice exposed to target concentrations of 9, 18, or 36 mg/kg of body weight per day (NTP, 2004). However, this report has not yet been finalized.

4.5.4 DBCM

In a US National Toxicology Program (NTP) carcinogenesis bioassay, DBCM was administered in doses of 0, 40, or 80 mg/kg of body weight by gavage in corn oil 5 times per week for 104 weeks to groups of 50 male and female F344/N rats. In addition, 0, 50, or 100 mg/kg of body weight per day was administered in similar fashion to groups of 50 male and female B6C3F1 mice 5 days per week for 105 weeks. Body weight gain in the high-dose group of male rats was decreased, and there was a dose-related increase in lesions (fatty metamorphosis and ground-glass cytoplasmic changes) of the liver in both sexes and nephrosis of the kidney (dose-related) in females. There was, however, no evidence of carcinogenicity in rats (NTP, 1985).

In male mice, survival was significantly lower in both dose groups, and 35 animals in the low-dose group were accidentally killed during weeks 58–59. In both sexes, the incidences of hepatic lesions were increased, including fatty metamorphosis (both sexes), hepatocellular necrosis (dosed males), hepatocytomegaly (high-dose males), and calcification of the liver (high-dose females). Nephrosis (high dose) and renal calcification in males and follicular cell hyperplasia of the thyroid gland (possibly related to a bacterial infection) in females were also increased. There was equivocal evidence of carcinogenicity in male B6C3F1 mice based on an increased incidence of hepatocellular carcinomas, but only a marginal increase in hepatocellular adenomas or carcinomas (combined) (incidence of hepatocellular carcinomas in control and high-dose groups, 10/50 and 19/50, respectively; incidence of hepatocellular adenomas and carcinomas combined, 23/50 and 27/50, respectively). The number of surviving animals in the low-dose group of male mice, however, was inadequate for analysis of

tumour incidence, owing to a dosing error. There was also some evidence of carcinogenicity in female mice, based on an increased incidence of hepatocellular adenomas and hepatocellular adenomas and carcinomas (combined). The incidence of hepatic adenomas and carcinomas (combined) in the control, low-dose, and high-dose groups was 6/50, 10/49, and 19/50, respectively (NTP, 1985).

Mechanistic issues for DBCM are similar to those addressed for BDCM.

4.5.5 Bromoform

In an NTP carcinogenesis bioassay, 0, 100, or 200 mg of bromoform per kg of body weight per day was administered by gavage in corn oil 5 days per week for 103 weeks to groups of 50 F344/N rats of each sex and to female B6C3F1 mice (NTP, 1989). Male B6C3F1 mice were administered 0, 50, or 100 mg/kg of body weight on the same schedule. In rats, there was a reduction of body weight gain in low- and high-dose males and high-dose females; survival in the high-dose group of males was also significantly lower than that in controls. As well, dose-related, non-neoplastic effects in the salivary gland (squamous metaplasia and chronic active inflammation in both sexes), prostate (squamous metaplasia), forestomach (ulcers in the males), lung (chronic active inflammation — males only), and spleen (pigmentation — high-dose females) were also observed, although the lesions of the salivary gland and lung were characteristic of infection by rat corona virus, to which a positive serological reaction was observed early in the study. There was some evidence of carcinogenicity in male rats and clear evidence in female rats, based on increased incidences of uncommon neoplasms (adenomatous polyps and adenocarcinomas of the large intestine) in both sexes. The incidences of these tumours (combined) in the control, low-dose, and high-dose groups of females were 0/50, 1/50, and 8/50, respectively; in males, the comparable values were 0/50, 0/50, and 3/50. Although the incidence of these tumours in females was similar to that observed in the NTP bioassay for BDCM, the incidence in males was much less. Reduced survival in the high-dose group of male rats administered bromoform may, however, have lowered the sensitivity of the bioassay for detecting a carcinogenic response. The incidence of neoplastic nodules in low-dose female rats was also greater than that in controls, but it was not considered to be a chemically induced neoplastic effect, as the lesions did not fit the current NTP criteria for hepatocellular adenomas, nor was the incidence significantly increased in high-dose female rats or in dosed male rats.

In female mice, there was a decrease in body weight gain and survival (partially attributable to utero-ovarian infection) and increases in the incidence of follicular cell hyperplasia of the thyroid (high dose) and fatty change of the liver (both doses). There was no evidence of carcinogenicity in male or female mice (NTP, 1989).

Bromoform was administered in drinking-water (containing 0.25% Emulphor) to male F344 rats and B6C3F1 mice for 1 year, and clinical indicators of kidney toxicity were examined (Moore et al., 1994). Water containing bromoform concentrations of 0.12, 0.6, and 1.2 g/litre for rats and 0.08, 0.4, and 0.8 g/litre for mice resulted in average daily doses of 6.2, 29, or 57 mg/kg of body weight for rats and 8.3, 39, or 73 mg/kg of

body weight for mice. Several indicators of tubular and glomerular damage were elevated at each treatment level in mice, and mice appeared more susceptible to the nephrotoxic effects of bromoform than to those of BDCM. As in mice, urinary protein was increased in all rat dose groups, but little evidence of loss of tubule function was observed in rats.

Although bromoform seems to have a greater propensity for metabolism and is a more potent mutagen than BDCM, it appears to be a less potent toxicant and carcinogen based on the results of the NTP (1985, 1987) bioassays and numerous other *in vivo* studies of toxicity. As with DBCM, a possible explanation is less bioavailability resulting from the greater lipophilicity of this compound and the use of corn oil as the vehicle of administration. This concept may be supported by the occurrence of bromoform-induced tumours in the intestinal tract, but not in the liver or kidneys. Greater lipophilicity and reactivity of bromoform metabolites may also prevent it from reaching critical target sites. Moreover, when bromoform was injected intraperitoneally, its metabolism was greater than that of the other THMs (Anders et al., 1978; Tomasi et al., 1985). When administered by corn oil gavage, however, bromoform was the least metabolized THM (Mink et al., 1986).

4.6 Neurotoxicity

Neurotoxicological findings reported for the THMs are observations of anaesthesia associated with acute high-dose exposures to brominated THMs (bromoform, BDCM, DBCM) and results from a behavioural study conducted by Balster & Borzelleca (1982) in adult male mice dosed by aqueous gavage for up to 90 days. Treatment with 1.2 or 11.6 mg/kg of body weight per day was without effect in various behavioural tests, and dosing for 30 days with 100 mg/kg of body weight per day did not affect passive avoidance learning. Animals dosed with either 100 or 400 mg/kg of body weight per day for 60 days exhibited decreased response rates in an operant behaviour test. These effects were greatest early in the regimen, with no evidence of progressive deterioration (IPCS, 2000).

4.7 PBPK models

PBPK modelling is a technique that may inform and improve toxicological assessments, through a better assessment of the magnitude of the uncertainty factors applied in current risk assessment by informing on issues relating to extrapolation between and within species (Delic et al., 2000).

4.7.1 Chloroform

The 2001 CEPA assessment report on chloroform (Environment Canada & Health Canada, 2001) indicated that the exposure–response relationship for exposure to chloroform associated with cancer and rates of formation of reactive metabolites in the target tissue is upheld by evidence supporting the following assumptions inherent in the PBPK modelling:

- In both experimental animals and humans, metabolism of chloroform by CYP2E1 is responsible for production of the critical reactive metabolite, phosgene.
- The ability to generate phosgene and phosgene hydrolysis products determines which tissue regions in the liver and kidney are sensitive to the cytotoxicity of chloroform.
- This dose–effect relationship is consistent within a tissue, across gender, and across route of administration, and it may also be consistent across species.

The CEPA report presented a PBPK model that was a "hybrid" animal model of the International Life Sciences Institute Expert Panel, which was revised for their assessment and developed to permit its extension to humans (ILSI, 1997; ICF Kaiser, 1999). For this assessment, maximum rate of metabolism per unit kidney cortex volume (VRAMCOR) and mean rate of metabolism per unit kidney cortex volume during each dose interval (VMRATEK) were considered (Environment Canada & Health Canada, 2001).

4.7.1.1 Neoplastic assessment

The results of the exposure–response neoplastic assessment presented were for the combined incidence of renal adenomas and adenocarcinomas in Jorgenson et al. (1985). The VMRATEK associated with a 5% increase in tumour risk in humans estimated on the basis of the PBPK model is 3.9 mg/litre per hour (lower 95% confidence limit = 2.5, chi-square = 0.04, degrees of freedom = 1, *P*-value = 0.84). This dose would result from continuous lifetime exposure to chloroform at 3247 mg/litre in water or 149 mg/m^3 in air. Respective lower 95% confidence limits for these values are 2363 mg/litre and 74 mg/m^3.

Although the data on dose–response were less robust than those for the cancer bioassay, for comparison, a benchmark dose was developed for histological lesions in the kidney in the reanalysis of a subset of the slides from the Jorgenson et al. (1985) biassay. The VMRATEK in humans associated with a 5% increase in histological lesions characteristic of cytotoxicity is 1.7 mg/litre per hour (lower 95% confidence limit = 1.4, chi-square = 3.9, degrees of freedom = 2, *P*-value = 0.14). This dose rate would result from continuous lifetime exposure to 1477 mg/litre in water or 33.8 mg/m^3 in air (Environment Canada & Health Canada, 2001).

4.7.1.2 Non-neoplastic assessment

Short-term exposure by inhalation resulted in cellular proliferation in nasal passages in rats and mice at concentrations as low as 9.9 mg/m^3, with ossifications being observed at slightly higher concentrations following long-term exposure. Moderate hepatic changes were observed in short-term studies in mice at 50 mg/m^3; following both short- and long-term exposure to 124–149 mg/m^3, there were multiple adverse effects in the kidney and liver in both rats and mice in several studies. Following ingestion in drinking-water, regenerative proliferation after short-term exposure of mice to doses as low as 17 mg/kg of body weight has been observed. Following bolus dosing, increases in proliferation in the liver of rats have been observed after short-

term exposure of rats at 10 mg/kg of body weight per day and fatty cysts in the liver of dogs at 15 mg/kg of body weight per day. As one of the lowest oral dose levels at which effects on liver and kidney have been observed was in dogs in a study by Heywood et al. (1979), a PBPK model in dogs was developed, keeping in mind that effects on the liver of rodents have also been observed in a similar dose range. Two dose metrics were investigated in exposure–response: the mean rate of metabolism per unit centrilobular region of the liver and the average concentration of chloroform in the non-metabolizing centrilobular region of the liver. The two dose metrics were selected in order to evaluate the possibility of the fatty cyst formation in the dogs being the result of the solvent effects of chloroform or effects of a reactive metabolite. Results of a model fitting supported the assumption that a metabolite rather than chloroform itself was responsible for the observed effects. This hypothesis means that the effect of chloroform on the liver will vary depending on the rate of metabolism. The mean rate of metabolism per unit centrilobular region of the liver in humans associated with a 5% increase in fatty cysts estimated on the basis of the PBPK model is 3.8 mg/litre per hour (lower 95% confidence limit = 1.3, chi-square = 0.00, degrees of freedom = 1, *P*-value = 1.00). This dose rate would come from continuous lifetime exposure to 37 mg/litre in water or 9.8 mg/m^3 in air. Respective lower 95% confidence limits for these values were 12 mg/litre and 3.4 mg/m^3.

The 2001 CEPA assessment report concluded, based on the above PBPK models, that the exposure of the general population is considerably less than the level to which it is believed a person may be exposed daily over a lifetime without deleterious effect. Underestimates in exposure due to use of hot rather than cold water and increased chloroform levels in the distribution system compared with the water treatment plant were noted (Environment Canada & Health Canada, 2001).

4.7.2 BDCM

A PBPK model has been developed to describe the absorption, distribution, tissue uptake and dosimetry, metabolism, and elimination of BDCM in rats. The metabolism model, derived from inhalation exposure data, was subsequently linked to a multicompartment gastrointestinal tract submodel. This model accurately predicted tissue dosimetry and plasma bromide ion concentrations following oral exposure to BDCM and can be utilized in estimating rates of formation of reactive intermediates in target tissues (Lilly et al., 1997, 1998).

5. EFFECTS ON HUMANS

5.1 Cancer epidemiology

Epidemiological studies conducted prior to 1993 that explored associations between chlorinated disinfection by-products and adverse health outcomes often had limitations, particularly in the area of exposure measurement. In the case–control epidemiological studies conducted prior to 1993, associations were found between ingestion of chlorinated drinking-water and the incidences of colon cancer for those aged 60 years or more (Cragle et al., 1985) and bladder cancer among non-smokers

(Cantor et al., 1985, 1987). In the investigation by Cantor et al. (1985), which involved 1244 cases and 2500 control subjects who had never been exposed in high-risk occupations for bladder cancer and for whom detailed information on geographic mobility, water source (non-chlorinated ground source or chlorinated surface source for 50% of their lifetime), and potential confounders was collected, there was a positive association between bladder cancer risk, level of tap water ingestion, and duration of exposure, predominantly among study subjects with long-term residence in communities with chlorinated surface water (NAS, 1987). Among non-smokers, there was an association between water intake and relative risk, and the odds ratio for those over 60 years of age with more than median surface water intake compared with lifelong groundwater consumers was 2.3.

There has been an ongoing effort since 1993 to improve the design of these epidemiological studies in order to more clearly identify both the possible agents of concern in chlorinated drinking-water and the associated adverse health effects. More recent analytical epidemiological 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). 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 who had ever smoked, for whom bladder cancer risk increased with duration of exposure after control for cigarette smoking. No increased relative risk of bladder cancer was associated with exposure to chlorinated municipal surface water supplies or to chloroform or other THM species in a cohort of women, but the follow-up period of 8 years was very short, resulting in few cases for study. In Ontario, King & Marrett (1996) found an increased bladder cancer risk with increasing duration of exposure and THM levels. The association was statistically significant and of higher magnitude only after 35 or more years of exposure. The bladder cancer incidence was about 40% higher among persons exposed to greater than 1956 (µg of THMs per litre)-years[1] 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. 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 (IPCS, 2000). In 2002, an expert panel convened by Health Canada to identify critical end-points for assessment of health risks related to THMs in drinking-water also agreed that THMs are used in epidemiological studies as a surrogate for exposure to chlorinated disinfection by-products more generally, and the complexity of chlorinated disinfection by-product mixtures in drinking-water makes the assignment of causation to any single component or class of components extremely difficult (Health Canada, 2003).

In 2002, Health Canada commissioned a review of the non-bladder cancer epidemiology of THMs in drinking-water (SENES Consultants Ltd., 2002). The studies reviewed focused on colon, rectal, pancreatic, kidney, brain, and

[1] The unit (µg of THMs per litre)-years is a composite of THM levels and years of exposure.

haematological/lymphoreticular cancer sites. There were only a few studies with significant odds ratios for colon, rectal, brain, and pancreatic cancer; studies were not significant for kidney and the blood-related cancers.

For colon cancer, there were two studies showing a statistically increased risk of colon cancer with exposure to chlorinated drinking-water. King et al. (2000a) showed a significant association only for the male cohort, whereas Doyle et al. (1997) showed one only in females, as only females were considered. The results of the King et al. (2000a) study suggest that there may be different risk factor profiles for the different sexes insofar as there was no significant risk for females. However, the Iowa cohort (Doyle et al., 1997) indicates that this may not be the case.

Results from the studies involving rectal cancer were inconclusive. Of the studies examined, the only study showing significance was a population-based case–control study by Hildesheim et al. (1998). Hildesheim et al. (1998) and Doyle et al. (1997) both used the Iowa population and cancer registry for their studies. Their methodologies differed, in that Hildesheim et al. (1998) used a case–control design, examining rectal and colon cancers for both men and women, whereas Doyle et al. (1997) used a cohort design, examining only women in the population, prospectively, for colon and rectal cancers. Doyle et al. (1997) found an association only for colon cancer, whereas Hildesheim et al. (1998) found one for rectal cancer.

The only recent study involving the association between brain cancer and exposure to THMs indicated that such an association exists (Cantor et al., 1999). This study involved the same Iowa-based cohort used by Hildesheim et al. (1998) and Doyle et al. (1997).

In summary, even though recent studies suggest that some association exists between colon, rectal, and brain cancer and exposure to disinfection by-products in drinking-water, the data presented in the studies are not sufficient to reliably confirm a dose–response or causal relationship (SENES Consultants Ltd., 2002).

The only study that found any significant relationship between treated water and pancreatic cancer was an ecological study by Koivusalo et al. (1995) involving 56 communities in Finland in 1950. The inherent limitations and uncertainties associated with ecological studies make it difficult to acknowledge the outcome of this study and raise concerns about confidence in the results.

Several studies have attempted to estimate exposures to THMs or chloroform and the other THM species, but the studies did not consider exposures to other disinfection by-products or other water contaminants, which may differ between surface water and groundwater sources. Because inadequate attention has been paid to assessing exposure to water contaminants in epidemiological studies, it is not possible to properly evaluate the increased relative risks that have been reported. Specific risks may be due to other disinfection by-products, mixtures of by-products, or other water contaminants, or they may be due to other factors for which chlorinated drinking-water or THMs may serve as a surrogate (IPCS, 2000).

5.2 Reproductive epidemiology

Epidemiological studies have raised concerns regarding the potential effects of exposure to chlorinated disinfection by-products in drinking-water and reproductive and developmental outcomes, supported in part by the findings that some chlorinated disinfection by-products cause reproductive and developmental toxicity in laboratory animals, albeit at doses much higher than those encountered by humans. In 1997, both Health Canada and the US EPA held scientific panel workshops that concluded that the evidence at the time was insufficient to establish a causal relationship between chlorinated water or THMs and adverse pregnancy outcomes (Mills et al., 1998; IPCS, 2000).

Reif et al. (2000) conducted a critical review of the most recent epidemiological evidence. This review examined studies that used either 1) qualitative exposure assessment, which examined associations between source of water supply or method of disinfection and risk of adverse reproductive outcome, or 2) quantitative exposure assessment, relying predominantly on reported concentrations of THMs in drinking-water supplies. Reif et al.'s (2000) conclusions were as follows:

a) *Effects on fetal growth*: The epidemiological evidence for an association between THMs and effects on fetal growth is inconsistent. Weak but statistically significant associations (odds ratios: 1.2–2.6) with birth weight, low birth weight, and intrauterine growth retardation were described in epidemiological studies at concentrations of ≥61 µg of THMs per litre (Gallagher et al., 1998), >80 µg of THMs per litre (Bove et al., 1992), and >100 µg of THMs per litre (Bove et al., 1995). Increases in risk for intrauterine growth retardation were also reported at concentrations of chloroform and BDCM of ≥10 µg/litre, although the latter was not statistically significant (Kramer et al., 1992). Conversely, two studies (Savitz et al., 1995; Dodds et al., 1999) were unable to demonstrate a statistically significant association with any of these related outcomes. Among these studies, all adjusted for socioeconomic status and race or restricted the analysis to Caucasians. Smoking was controlled for in all but one (Bove et al., 1995) study. The two largest studies, each with good statistical power, reached different conclusions, despite relative similarity in exposure assessment and other methods (Bove et al., 1995; Dodds et al., 1999).

In a hospital-based study in Italy, Kanitz et al. (1996) reported lower mean birth weights among mothers older than 30 years of age consuming chlorinated water. Kallen & Robert (2000) also reported an effect of chlorine-treated systems on somatic parameters of body length and head circumference, as well as an association with low birth weight and preterm delivery. However, Jaakkola et al. (1999) reported no association between chlorinated water use and measures of fetal growth or prematurity. Yang et al. (2000) found no evidence of an association between low birth weight and chlorination in Taiwan, but municipalities using chlorination had a significantly higher rate of preterm delivery.

b) *Effects on fetal viability*: Epidemiological evidence is inconsistent in associating chlorinated disinfection by-products with an increased risk of spontaneous abortion and stillbirth. Although these end-points were grouped together in the Reif et al. (2000) report, their mechanisms of induction may differ. Increased rates of spontaneous abortion were reported in a cohort study by Waller et al. (1998) in California with heavy consumption of water (five or more glasses of cold tap water per day) containing ≥75 µg of THMs per litre. When specific THMs were considered, only heavy consumption of water containing BDCM (≥18 µg/litre) was associated with a risk of miscarriage (IPCS, 2000). An increased risk of spontaneous abortion associated with disinfection by-product formation is supported by findings from Aschengrau et al. (1989), who reported a doubling in risk for the consumption of surface water compared with groundwater and mixed water systems. Savitz et al. (1995) found a statistically significant relationship with increasing concentration of THMs and with the highest sextile of exposure, but there was no relationship with ingested dose or with water source.

An increased risk of stillbirth was reported for women from Nova Scotia, Canada, who were exposed to water containing more than 100 µg of THMs per litre (Dodds et al., 1999). In further analyses of these data, King et al. (2000b) found dose-dependent increases in adjusted risk for stillbirth with exposure to THMs, chloroform, and BDCM. Exposure to BDCM at levels of ≥20 µg/litre was associated with a doubling in risk. In New Jersey, USA, Bove et al. (1992, 1995) found little evidence for an association with THMs at 80 µg/litre, but did report a weak association between stillbirth and consumption of drinking-water from surface water systems. Aschengrau et al. (1993) found an association between stillbirth and the use of a chlorinated versus chloraminated surface water supply.

c) *Effects on risk for fetal malformations*: Relatively strong associations of several types of congenital anomalies with THMs were described by Bove et al. (1992, 1995). The highest risks were found for central nervous system, oral cleft, and major cardiac defects at THM concentrations above 80 or 100 µg/litre. Other studies of neural tube defects (Dodds et al., 1999; Klotz & Pyrch, 1999) and cardiac anomalies (Shaw et al., 1991; Dodds et al., 1999) found lower risks or no evidence of an association with THMs. The literature to date presents an inconsistent pattern of association with congenital anomalies collectively and a lack of consistency with specific anomalies across the relatively few studies that have explored these outcomes.

6. PRACTICAL ASPECTS

6.1 Analytical methods and analytical achievability

THMs can be determined by a number of different analytical techniques, including purge-and-trap, liquid–liquid extraction, and direct aqueous injection in combination with a chromatographic system. The chromatographic system will permit concurrent determination of all four THMs. The method quantification limit (MQL) by the purge-and-trap and liquid–liquid extraction methods is approximately 0.1–0.2 µg/litre.

Some of the techniques are known to give different values; for example, chloroform levels in water analysed by direct aqueous injection are usually higher than levels determined by the purge-and-trap technique. The variation is attributed to the formation of chloroform from the breakdown of chlorinated disinfection by-product precursors in the hot injection port of the gas chromatograph used in the direct aqueous injection technique.

During recent Health Canada studies on disinfection by-products in drinking-water, THMs were determined using a liquid–liquid extraction approach adapted from US EPA Method 551, with analysis of the extract by gas chromatography–electron capture detector (GC-ECD). Samples were also determined using the purge-and-trap technique followed by gas chromatography–mass spectrometry (ion trap) detector (GC-ITD).

The liquid–liquid extraction approach also allows for the concurrent determination of other disinfection by-products, including chloral hydrate, di- and trichloropropanones, haloacetonitriles, and chloropicrin. The method was later modified to include the concurrent determination of cyanogen chloride (LeBel & Williams, 1996, 1997; LeBel & Benoit, 2000) and other halogenated acetaldehydes (Koudjonou & LeBel, 2003). An essential requirement of the method was the pH adjustment (to pH 4.5) of the water samples at the time of field sampling to prevent further production of chloroform during storage of the sample between collection and analysis; the effect due to pH diminished with time (distance) in the distribution system (LeBel & Williams, 1995).

Data from recent Health Canada studies indicate that 1,1,1-trichloro-2-propanone (LeBel et al., 2002) and trihalogenated aldehydes (Koudjonou & LeBel, 2003) will degrade in water to their corresponding THMs at increased pH and temperature. However, they are stable in water at sampling/storage conditions (pH 4.5, 4 °C).

Both the purge-and-trap/GC-ITD and liquid–liquid extraction/GC-ECD techniques can be used for the determination of THMs in drinking-water samples. For similarly treated samples (same pH and preservative), the results using both techniques are comparable, but the purge-and-trap technique gives slightly higher values of chloroform due to breakdown of some chlorinated intermediates (LeBel & Williams, 1995). As well, the purge-and-trap technique is not generally amenable to the analysis of the more hydrophilic disinfection by-product analytes targeted by the liquid–liquid extraction approach. Therefore, the liquid–liquid extraction approach is preferred for its versatility and reliability.

6.2 Treatment and control methods and technical achievability

The preferred method of controlling disinfection by-products is precursor removal; however, any method of control employed *must not* compromise the effectiveness of water disinfection. It is also important to ensure that efforts to manage THM levels through adjustment of pH not increase the formation of haloacetic acids.

6.2.1 Chloroform

Chloroform removal using packed tower aeration depends on the air-to-water ratio, water loading rate, and packing depth; 78% removal from an inlet concentration of approximately 100 µg/litre was achieved in pilot-scale studies under the following conditions: air-to-water ratio = 38:1; water loading rate = 21 mm/s; and packing depth = 2.4 m (Umphres et al., 1983).

Pilot-scale packed tower aeration of a groundwater containing 258 µg of chloroform per litre, using a packed height of 2.7 m and an air-to-water ratio of 30:1, produced a removal of 98.6% (Bilello & Singley, 1986). Other experiments using packed tower aeration have achieved substantial removal of chloroform (Amy & Cooper, 1986).

A pilot plant study using air stripping in a tower with countercurrent air flow has been used for the treatment of groundwater contaminated with traces of volatile hydrocarbons including chloroform. The study reported that the total chlorinated hydrocarbon concentration was reduced to below 0.05 µg/litre (Reijnen, 1985).

Air stripping with an air-to-water ratio of 40:1, at pilot and full scale, has been reported to achieve chloroform removals of between 88% and 96% and between 50% and 90%, respectively (Bishop et al., 1985).

Cascade cross-flow air stripping was reported to achieve removals in excess of 90% for water contaminated with chloroform, methylene chloride, 1,2-dichloroethane, and carbon tetrachloride (Wood et al., 1990).

Membrane air stripping, in which water is passed through hollow fibre hydrophobic membranes and air is passed countercurrent on the outside of the fibres, is reported to offer better mass transfer rates for removal of volatile organic chemicals including chloroform (Castro & Zander, 1995).

Pilot plant trials have shown the combination of air stripping, precipitation, and activated carbon adsorption to be effective in the removal of chloroform from groundwater. Removals of 99.9% were achieved from an influent with a chloroform concentration of 1.1 mg/litre (Kelly et al., 1981).

An isotherm for chloroform on Filtrasorb 300 in distilled water gave the following loadings (Dobbs & Cohen, 1980):

Concentration (µg/litre)	Carbon load (mg/g)
1	0.02
10	0.09
100	0.48

Other isotherm data have been found for chloroform. Carbon (F400) capacities of 3.47 mg/g and 0.52 mg/g have been calculated for solutions containing 226 µg/litre and 13.2 µg/litre, respectively, at equilibrium (Speth & Miltner, 1990).

A powdered activated carbon (PAC) dose of 300 mg/litre is sufficient to reduce a chloroform concentration of 10 µg/litre to less than 1 µg/litre (Jacangelo et al., 1989).

A pilot-scale granular activated carbon (GAC) (F400) column, operating with an empty bed contact time of 6.2 min, treated 5800 bed volumes of groundwater containing 62 µg of chloroform per litre before the effluent concentration reached 30 µg/litre (Wood & DeMarco, 1979).

GAC was effective in removing chloroform by adsorption during the early operating period, which corresponded to 4000–5000 bed volumes throughput. However, it was noted that the chloroform concentration in the effluent increased with GAC operating time and throughput (Wang et al., 1985).

Pilot plant trials of ultrafiltration achieved removals of 14% from a river water with a chloroform concentration of 39 µg/litre and no measurable removals from an influent river water with a chloroform concentration of 3.2 µg/litre (Jacangelo et al., 1992).

6.2.2 BDCM

BDCM removal using packed tower aeration depends on the air-to-water ratio, water loading rate, and packing depth; 74% removal from an inlet concentration of approximately 100 µg/litre was achieved in pilot-scale studies under the following conditions: air-to-water ratio = 38:1; water loading rate = 21 mm/s; and packing depth = 2.4 m (Umphres et al., 1983).

Isotherm data have been found for adsorption of BDCM onto F400 GAC (Speth & Miltner, 1990). Carbon capacities of 1.2 mg/g and 10.8 mg/g have been calculated for solutions containing 12 µg/litre and 333 µg/litre, respectively, at equilibrium (i.e., BDCM is not well adsorbed).

6.2.3 DBCM

DBCM removal using packed tower aeration depends on the air-to-water ratio, water loading rate, and packing depth; 59% removal from an inlet concentration of approximately 100 µg/litre was achieved in pilot-scale studies under the following conditions: air-to-water ratio = 38:1; water loading rate = 21 mm/s; and packing depth = 2.4 m (Umphres et al., 1983).

Isotherm data have been found for adsorption of DBCM onto F400 GAC (Speth & Miltner, 1990). Carbon capacities of 0.1 mg/g and 3.1 mg/g have been calculated for solutions containing 1.5 µg/litre and 40 µg/litre, respectively, at equilibrium (i.e., DBCM is not well adsorbed).

6.2.4 Bromoform

Bromoform removal using packed tower aeration depends on the air-to-water ratio, water loading rate, and packing depth; 45% removal from an inlet concentration of approximately 100 µg/litre was achieved in pilot-scale studies under the following conditions: air-to-water ratio = 38:1; water loading rate = 21 mm/s; and packing depth = 2.4 m (Umphres et al., 1983). Removal efficiencies in excess of 95% from solutions containing 1.5–2.2 mg/litre using a co-current wetted butterfly valve scrubber system have been reported (Beg & Obaid-ur-Rehman, 1993).

Isotherm data have been found for adsorption of bromoform onto F400 (Speth & Miltner, 1990). Carbon capacities of 2.9 mg/g and 9.7 mg/g have been calculated for solutions containing 5.9 µg/litre and 100 µg/litre, respectively, at equilibrium. A pilot plant study (Paune et al., 1998) gave the following results for bromoform removal by virgin GAC:

	Average concentration (µg/litre)
Influent	662
Coal-based GAC	421
Coal-based GAC	284
Coconut-based GAC	240
Coal-based GAC	286

Bromoform reacts very slowly with ozone (Hoigne & Bader, 1983). Dibromomethane has been identified as a reaction product of ozonation (Kim et al., 1997).

Membrane stripping has been shown to remove 48% of bromoform from a water supply (Zander et al., 1989). Bromoform removal by reverse osmosis was monitored in a study of seawater desalination (Magara et al., 1996). Seawater was chlorinated, coagulated with ferric chloride, filtered, dechlorinated, and fed to the reverse osmosis plant. Operating at a recovery ratio of about 40% (99% salt rejection), bromoform was reduced from 68 µg/litre in the reverse osmosis influent to 1 µg/litre.

7. GUIDELINE VALUES

Although not complete, available epidemiological data are consistent with the hypothesis that ingestion of chlorinated drinking-water, if not THMs specifically, may be associated with cancers of the bladder and colon (Krasner et al., 1989). Additionally, epidemiological data available since 1993 have associated adverse reproductive outcomes with exposure to THMs, particularly the brominated THMs, although neither clear evidence of a threshold nor a dose–response pattern of increasing risk with increasing concentration of total THMs has been found (Reif et al., 2000). Nevertheless, in view of the potential link between such adverse health effects and THMs, particularly brominated THMs, it is recommended that THM levels in drinking-water be kept as low as practicable.

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

7.1 Chloroform

Chloroform is the THM present in greatest concentration in drinking-water and the THM for which there is most scientific data available. Chloroform has been classified as possibly carcinogenic to humans (Group 2B) based on limited evidence of carcinogenicity in humans but sufficient evidence of carcinogenicity in experimental animals (IARC, 1999b). There is compelling mechanistic evidence that both the hepatic and renal tumorigenic responses observed in previous carcinogenicity studies of chloroform (NCI, 1976a; Jorgenson et al., 1985) are mediated by a non-genotoxic mechanism (IPCS, 2000). One of the hypothesized modes of action for chloroform for tumour induction in rodents includes the following requisite precursor steps to cancer: 1) metabolism of chloroform by the target cell population; 2) induction of sustained cytotoxicity by metabolites; and 3) subsequent persistent regenerative cell proliferation (Environment Canada & Health Canada, 2001).

The nature of the vehicle appears to be an important factor in the toxicity and carcinogenicity of chloroform. More marked hepatotoxic effects and increased incidence of liver tumours in rats and mice are observed following administration of chloroform in corn oil compared with drinking-water, probably as a result of the major shift in the nature of the caloric intake associated with the former vehicle.

It is considered appropriate to use the tolerable daily intake (TDI) approach for calculating the guideline value for chloroform. In a recent IPCS (2004) assessment of chloroform, the Heywood et al. (1979) dog study was chosen as the most appropriate study for risk assessment. In IPCS (2004), a TDI of 0.015 mg/kg of body weight per day was calculated as follows:

$$\frac{12 \text{ mg/litre}}{25} \times \frac{2 \text{ litres}}{64} = 0.015 \text{ mg/kg of body weight per day}$$

where:

- 12 mg/litre is the 95% lower confidence limit for the 5% incidence of hepatic cysts, generated by PBPK modelling (see section 4.7.1.2)
- 25 is the uncertainty factor (10 for intraspecies differences in toxicokinetics and toxicodynamics and 2.5 for differences in interspecies toxicodynamics)[1]
- 2 litres is the amount of drinking-water consumed per day
- 64 is the body weight of an adult.

[1] Since the use of the PBPK model allows for the use of figures based on metabolized tissue dose, the subfactor 4 for possible toxicokinetic differences in humans and laboratory animals is accounted for.

The guideline value for chloroform derived from this TDI is 300 µg/litre (rounded value), based on an allocation of 75% of the total daily intake to drinking-water and assuming a 60-kg adult with 2 litres/day consumption of drinking-water.

Exposure data (see section 2.2) suggest that approximately equal contributions to total chloroform exposure come from four areas: ingestion of drinking-water, inhalation of indoor air largely due to volatilization from drinking-water, inhalation and dermal exposure during showering or bathing, and ingestion of food, with all but food exposure arising primarily from drinking-water (4.61 Ieq/day). This is particularly important in countries with low rates of ventilation in houses and high rates of showering and bathing. In these countries, the guideline value of 300 µg/litre could be, for example, halved (150 µg/litre) to account for the additional exposures.

The guideline value of 300 µg/litre represents an increase from the previous guideline value of 200 µg/litre. The change is a result of the increase in the allocation of exposure in drinking-water from 50% to 75% to account for the fact that chloroform is used less (e.g., as an anaesthetic) now than it was in 1993 when the original guideline was developed.

7.2 BDCM

Genotoxicity studies indicate that BDCM is weakly mutagenic, probably as a result of glutathione conjugation. Carcinogenicity studies show that in rats, BDCM in corn oil, when administered by gavage for 102 weeks at doses ranging from 50 to 100 mg/kg of body weight per day, resulted in increased incidences of renal tubular cell adenomas and adenocarcinomas affecting both sexes and a markedly increased incidence of large intestinal tumours (combined adenomas and carcinomas) in both sexes. In mice, BDCM in corn oil, administered by gavage for 102 weeks at dose levels of 0, 25, or 50 mg/kg of body weight per day or 0, 75, or 150 mg/kg of body weight per day in males and females, respectively, caused renal cytomegaly and hepatic fatty metamorphosis, increased incidences of renal tubular adenomas and carcinomas in males, and an increased incidence of combined hepatocellular adenomas and carcinomas in females. These carcinogenicity studies are supported by epidemiological studies showing an apparent association between the THM group of compounds and colorectal cancer in humans.

BDCM has been classified as possibly carcinogenic to humans (IARC, 1991, 1999a). Among the four THMs commonly found in drinking-water, BDCM appears to be the most potent rodent carcinogen. BDCM caused tumours at lower doses and at more target sites than for any of the other THMs (IPCS, 2000).

The tumours of the large intestine (combined adenomatous polyps and carcinomas) in rats were chosen for cancer risk assessment, as they occurred with the highest frequency and affected both sexes in the study, and because of the apparent epidemiological association of this group of compounds (THMs) with colorectal cancer in humans. Furthermore, these tumours appear most likely to be associated with a mutagenic mechanism, as they were not associated with underlying

cytotoxicity or other non-epigenetic mechanism. The combined large intestinal tumours had high unit risk value, equal to or higher than the unit risks for the other tumour types in rats (kidney and liver) identified in carcinogenicity studies with this compound. A cancer risk assessment was also conducted in mice, although tumours (combined renal adenomas and adenocarcinomas) were observed only in males.

Cancer risks have been estimated on the basis of the results of the only adequate carcinogenesis bioassay in F344/N rats and B6C3F1 mice, which was conducted by the NTP in 1987. It should be noted, however, that the compound was administered by gavage in corn oil in this bioassay and that quantitative risks may be overestimated. Moreover, the increases in hepatocellular adenomas and carcinomas in female mice in the NTP bioassay have not been used for quantitative estimation of the cancer risks, because these increases were confined to one sex and because of the possible contribution of the corn oil vehicle to the induction of liver tumours in mice.

Based on the tumours that were significantly increased in F344/N rats (i.e., intestinal adenomatous polyps and adenocarcinomas; renal tubular cell adenomas and adenocarcinomas) and in male B6C3F1 mice (renal adenomas and adenocarcinomas [combined]) in the NTP (1987) bioassay, unit risks were calculated using the linearized multistage method of Howe (1995). An animal-to-human kinetic adjustment factor, expressed as $(0.35/60)^{1/4}$, was applied to the final unit risks, assuming a rat weighs 0.35 kg and a human weighs 60 kg. The Kaplan-Meier mortality-adjusted data were not used, since using these data generally resulted in a worse fit while not appreciably changing the unit risk. The raw incidence data were used instead.

The multistage model was first fit to the bioassay data. The multistage model is given by:

$$P(d) = 1 - e^{-q_0 - q_1 d - \ldots - q_k d^k}$$

where d is dose, k is the number of dose groups in the study (excluding control), $P(d)$ is the probability of the animal developing a tumour at dose d, and $q_i > 0$, $i = 0,...,k$ are parameters to be estimated.

The unit risk is defined as the increase in excess risk per unit dose, where excess risk is given by:

$$\frac{P(d) - P(0)}{1 - P(0)}$$

The unit risk is applicable at very low doses, presumably in the range where humans will be exposed. For a small dose, d, the excess risk can be shown to be approximately equal to $q_1 d$. Thus, when the background $P(0)$ is small, q_1 represents the slope (i.e., change in risk per increase of unit dose) of the dose–response curve in the low-dose region. In practice, the upper 95% confidence limit on q_1 is used and is denoted by q_1*. This is the unit risk for the linearized multistage method.

A chi-square lack-of-fit test was performed for the model fits. The degrees of freedom for this test are equal to k minus the number of q_is whose estimates are non-zero. A P-value less than 0.05 indicates a significant lack of fit. Some models exhibited a significant lack of fit, but since only three dose groups were present (including control), removing the highest dose group is unadvisable.

The estimated range of concentrations[1] corresponding to an upper-bound excess lifetime cancer risk of 10^{-5} is 25–77 µg/litre for the critical tumour types (i.e., intestinal adenomatous polyps and adenocarcinomas; renal tubular cell adenomas and adenocarcinomas) in rats, based on the model described above, and 21 µg/litre for the critical tumour types (i.e., renal adenomas and adenocarcinomas [combined]) in male mice.

Although a guideline value for BDCM in drinking-water of 21 µg/litre, which is the most conservative of the values noted above, could be derived, the previous guideline value of 60 µg/litre is retained for two reasons. First, and most important, both the previous guideline and the above calculations were based on the same NTP (1987) study. The only differences between the new calculation and the previous guideline are the model and model assumptions used to derive the guideline value. Both calculations support the observation that a guideline based on the mouse tumours is more conservative than a guideline based on the rat tumours. There is therefore no scientific basis on which to justify a change in the guideline value. Second, concentrations of BDCM below 50 µg/litre may be difficult to achieve using currently available technology without compromising the effectiveness of disinfection.

As with chloroform, exposure data (see section 2.2) suggest that approximately equal contributions to total BDCM exposure come from four areas: ingestion of drinking-water, inhalation of indoor air largely due to volatilization from drinking-water, inhalation and dermal exposure during showering or bathing, and ingestion of food, with all but food exposure arising primarily from drinking-water (4.05 Ieq/day). This is particularly important in countries with low rates of ventilation in houses and high rates of showering and bathing. In these countries, the guideline value could be, for example, halved (to 30 µg/litre) to account for the additional exposures, although, as noted above, concentrations of BDCM below 50 µg/litre may be difficult to achieve using currently available technology without compromising the effectiveness of disinfection.

As exposure to BDCM has also been linked to a possible increase in reproductive effects (increased risk for spontaneous abortion or stillbirth) above what can normally be expected, the guideline value will be revisited when new data on possible reproductive effects become available.

[1] Average adult body weight = 60 kg; average daily intake of drinking-water = 2 litres/day.

7.3 DBCM

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 (1991) 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).

7.4 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 (1991) 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, 1989). 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).

7.5 Total THMs

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

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