Trihalomethanes in Drinking Water

Document for Public Comment

Prepared by the Federal-Provincial-Territorial
Committee on Drinking Water

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Public Comment Document
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Purpose of Consultation

For the past several years, the Federal-Provincial-Territorial Committee on Drinking Water has been assessing the available information on trihalomethanes with the intent of revising the current guideline for trihalomethanes in drinking water. The purpose of this consultation is to solicit comments on the proposed guideline, on the approach used for its development, and on the potential economic costs of implementing it, as well as to determine the availability of additional exposure data.

The Committee has requested that this document be made available to the public and open for comment. Comments are appreciated, with accompanying justification, if required. Comments can be sent via E-mail to water_eau@hc-sc.gc.ca or by mail to the Committee on Drinking Water Secretariat, Water Quality and Health Bureau, 4th Floor, Sir Charles Tupper Bldg., A.L. 6604B, Ottawa, Ontario K1A 0K9. All comments must be received before **January 7, 2005.**

It should be noted that this supporting document on trihalomethanes in drinking water will be revised following evaluation of comments received, and maximum acceptable concentrations (MAC) will be established for trihalomethanes and for bromodichloromethane in drinking water. This document should be considered a draft for comment only.

August 2004
Trihalomethanes

1.0   Proposed Guidelines

1.1   Trihalomethanes
The proposed maximum acceptable concentration (MAC) for trihalomethanes[1] (THMs) in drinking water is 0.1 mg/L (100 µg/L) based on an annual average of a minimum of quarterly samples taken at extremities of the distribution system.

1.2   Bromodichloromethane
The proposed maximum acceptable concentration (MAC) for bromodichloromethane (BDCM) in drinking water is 0.016 mg/L (16 µg/L) based on an annual average of a minimum of quarterly samples taken at extremities of the distribution system.

1.3   Other Considerations
Utilities should make every effort to achieve the lowest concentrations possible without compromising the effectiveness of water disinfection.

2.0   Executive Summary
Trihalomethanes are a group of compounds that can form when the chlorine used to disinfect drinking water reacts with naturally occuring organic matter (e.g., decaying leaves and vegetation). The use of chlorine in the treatment of drinking water has virtually eliminated waterborne diseases, because chlorine can kill or inactivate most microorganisms commonly found in water. The majority of drinking water treament plants in Canada use some form of chlorine to disinfect drinking water: to treat the water directly in the treatment plant and/or to maintain a chlorine residual in the distribution system to prevent bacterial regrowth. Disinfection is essential to safeguard drinking water; the health risks from disinfection by-products, including trihalomethanes, are much less than the risks from consuming water that has not been disinfected.

The trihalomethanes most commonly found in drinking water are chloroform, bromodichloromethane (BDCM), dibromochloromethane (DBCM) and bromoform. Of these, chloroform has been most extensively studied, and there is some scientific data available on BDCM. However, insufficient data are available to develop a guideline for either DBCM or bromoform. Because of this, and since chloroform is the trihalomethane most often found in drinking water, and generally at the highest concentrations, the trihalomethane guideline is based on health risks linked to chloroform. This guideline applies to the total concentration of chloroform, BDCM, DBCM and bromoform.

Health Canada recently completed its review of the health risks associated with trihalomethanes in drinking water. This review incorporates multiple routes of exposure to trihalomethanes via drinking water including ingestion, inhalation and skin absorption from showering and bathing. It assesses all identified health risks, taking into account new studies

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[1] Trihalomethanes refers to the total of chloroform, bromodichloromethane, dibromochloromethane and bromoform compounds.

and approaches, and applies appropriate safety factors. Based on this review, the proposed guideline for total trihalomethanes in drinking water is 0.1 mg/L.

Although the concentration of BDCM is included in the concentration of trihalomethanes in the proposed guideline, a separate guideline for BDCM is also proposed. The proposed guideline for bromodichloromethane in drinking water is 0.016 mg/L.

During its April 2004 meeting, the Federal-Provincial-Territorial Committee on Drinking Water reviewed the proposed guidelines for trihalomethanes and for BDCM and approved that these guidelines and the corresponding supporting document undergo public consultations.

2.1 Health Effects

Chloroform is considered to be a possible carcinogen in humans, based on limited evidence in experimental animals, and inadequate evidence in humans. Animal studies have shown links between exposure to specific trihalomethanes and liver tumours in mice and kidney tumours in both mice and rats; some studies in humans show data that are consistent with these findings. Human studies are suggesting a link between exposure to trihalomethanes and colorectal cancers.

Human studies are suggesting a link between reproductive effects and exposure to high levels of trihalomethanes. However, an increase in the concentration of trihalomethanes could not be linked to an increase in risk, suggesting the need for more studies.

Preliminary animal studies indicate that BDCM and other trihalomethanes that contain bromine may be more toxic than chlorinated trihalomethanes such as chloroform. For this reason, and based on the availability of scientific data for BDCM, a separate guideline was also developed for BDCM. BDCM is considered to be a probable carcinogen in humans, with sufficient evidence in animals and inadequate evidence in humans. Animal studies have shown tumours in the large intestine in rats. Among the four trihalomethanes commonly found in drinking water, BDCM appears to be the most potent rodent carcinogen, causing tumours at lower doses and at more target sites that the other three compounds.

Exposure to BDCM at levels higher than the proposed guideline value has also been linked to a possible increase in reproductive effects (increased risk for spontaneous abortion or stillbirth) above what can normally be expected. Further studies are required to confirm these effects as well as their long term significance to human health.

2.2 Exposure

Levels of trihalomethanes, including BDCM, are generally higher in treated surface water than in treated groundwater, because of the high organic content in lakes and rivers, and will be higher in warmer months, because of the increase in organic matter. Trihalomethanes levels are also affected by the choice and design of treatment processes. Recent data indicate that, in general, average trihalomethanes levels in Canadian drinking water supplies are well below the proposed guideline. However, some systems show average levels well above the proposed guidelines; these systems serve only a small proportion of Canadians (less than 4%) and are generally smaller treatment systems with limited ability to remove organic matter before adding the chlorine disinfectant. It should be noted that the presence of brominated by-products such as BDCM will also depend on the presence of bromine in the source water.

2.3 Treatment

Trihalomethanes and haloacetic acids are the two major groups of disinfection by-products found in drinking water and generally at the highest levels. Together, these two groups

can be used as indicators for the presence of all disinfection by-products in drinking water supplies, and their control is expected to reduce the levels of all dinfection by-products and the corresponding risks to health. Guidelines are currently under development for haloacetic acids.

The approach to reduce exposure to trihalomethanes is generally focused on reducing the formation of chlorinated disinfection by-products. The concentrations of trihalomethanes and other disinfection by-products in drinking water can be reduced at the treatment plant by removing the organic matter from the water before chlorine is added, by optimizing the disinfection process or using alternative disinfection methods, or by using a different water source. It is critical that any method used to control trihalomethanes levels **must not** compromise the effectiveness of water disinfection. The Federal-Provincial-Territorial Committee on Drinking Water is also recommending that every effort be made not only to meet the guideline, but to reduce concentrations of trihalomethanes to as low a level as possible.

3.0   Application of the Guidelines
The concentrations of THMs and haloacetic acids (HAAs) can be used as indicators of the total loading of all chlorinated disinfection by-products (CDBPs) which may be found in drinking water supplies. Given the limited information on the risks and uncertainties of other CDBPs, as a precautionary approach, it is recommended that treatment plants strive to achieve THM and BDCM levels as low as possible without compromising disinfection.

When water supplies are expanded or upgraded, every effort should be made not only to meet the guideline, but to reduce concentrations of THMs, including BDCM, to as low a level as possible without compromising the effectiveness of water disinfection. The preferred method of controlling disinfection by-products is precursor removal.

3.1   Monitoring
At minimum, quarterly monitoring of treated water from surface and groundwater sources is recommended for both THMs and BDCM. Increased frequency may be required for facilities using surface water sources[1] during peak by-product formation periods. It is also recommended that monitoring samples be taken at the water treatment plant and at the far end of the distribution system. Monitoring/reporting of BDCM and THMs may be reduced if source waters do not contain bromide and/or drinking water monitoring does not show elevated levels of brominated by-products or THMs within the distribution system.

4.0   Identity, Use and Sources in the Environment
Trihalomethanes (THMs) are halogen-substituted single-carbon compounds with the general formula CHX_3, where X represents a halogen, which may be chlorine, bromine, fluorine, 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 chlorodibromomethane ($CHClBr_2$) (DBCM), and bromoform ($CHBr_3$); consideration of information relevant to the derivation of drinking water guidelines for THMs is restricted to these compounds. THM measurement assesses these four common THMs, with chloroform usually constituting the largest proportion. As well as being the most common THM, chloroform is also the principal disinfection by-product in chlorinated drinking water (LeBel and Williams, 1995).

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[1] Includes groundwater sources that are under the direct influence of surface water

These compounds are formed in drinking water primarily as a result of chlorination of organic matter present naturally in raw water supplies, and they are released into the environment from industrial sources as well as through indirect production in the chlorination of drinking water and municipal sewage. 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). In the presence of bromides, brominated THMs are formed preferentially and chloroform concentrations decrease proportionally (Aizawa *et al.*, 1989).

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). Chloroform decomposes via photochemical oxidation to dichlorocarbonyl (phosgene) and hydrogen chloride (Environment Canada and Health Canada, 2001).

Chloroform has not been manufactured in Canada since 1978, and its use as an anaesthetic has been largely discontinued. The presence of chloroform in dentifrices, liniments, and antitussives has contributed to the exposure of Canadians in the past, but the use of chloroform in these products has now been banned under the *Food and Drugs Act*. Manufacturers are not permitted to import or sell a drug that contains chloroform for human use in Canada (Environment Canada and Health Canada, 2001). Canadian imports of chloroform were 402 tonnes in 1993, 69 tonnes in 1995, and 118 tonnes in 1996, with imports declining in recent years. Chloroform is used as a solvent and in the production of other chemicals (Environment Canada and 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 and 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.

5.0   Exposure

5.1   Water

Although extensively studied, the chemistry of the reactions between chlorine and the organic materials present in water is complex and 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. Consequently, there is a great degree of variation in the measured concentrations of THMs in drinking water.

Levels of chloroform, the most common THM, are generally higher in treated water originating from surface water rather than 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 summer months as during winter months, as a consequence of the higher concentrations of precursor organic materials in the raw water during the warmer period. 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 the winter, when more hot water is required to maintain the shower temperature, as in the summer, so that concentrations of chloroform in the warm water used for showering are relatively constant for both seasons (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 endpoints of the water systems, and the typical methods of analysis were either liquid–liquid extraction or purge-and-trap gas chromatography.

Based on the data received from the eight provinces, the mean THM level was about 66 μg/L in drinking water samples from all systems. Some systems had average values in the 400 μg/L range, and some systems had maximum or peak values in the 800 μg/L range. From the eight provinces, 282 water systems (23% of sampled systems), representing a sampled population of 523 186 (3.4% of sampled population served), reported having mean THM levels greater than 100 μg/L, while 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/L (Water Quality Issues Sub-Group, 2003).

System mean chloroform levels for 1994–2000 were generally less than 50 μg/L, with some single maximum or peak values in the 400 μg/L 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/L, while 425 water systems (39%), serving 1 740 000 (12%) consumers, had a peak concentration greater than 75 μg/L 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/L, though some averages were higher, and several locations reported one-time samples in excess of 200 μg/L. From those suppliers who reported BDCM data, 87 water systems (8% of reporting systems), representing a sampled population of 285 000 (2% of population served), reported having mean BDCM levels greater than 10 μg/L, while 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/L (Water Quality Issues Sub-Group, 2003).

Mean concentrations of bromoform were typically less than the detection limit, or approximately 0.5 μg/L, and individual values were less than 10 μg/L. In a few systems, however, average and maximum bromoform levels exceeded 30 μg/L 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 of systems had average THM levels greater than 75 μg/L, and 45 systems had average BDCM levels greater than 10 μg/L. 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/L, and only one system had an average BDCM level greater than 10 μg/L. 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/L — a value significantly lower than the average of 66 μg/L 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/L (Water Quality Issues Sub-Group, 2003).

5.2   Multi-route 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 litre-equivalents per day (Leq/day), was estimated by evaluating the relative contribution of inhalation and dermal exposures associated with showering and bathing.
        Krishnan (2003) determined Leq/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/L) and BDCM (5 µg/L).[1] The Leq/day values for a 10-minute shower and a 30-minute 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 hours.
        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).
        Leq/day values for the inhalation and dermal routes were higher for the 30-minute bath scenario than for the 10-minute shower for all subpopulations based on the longer exposure time. The highest total exposure values for drinking water were for adults in the 30-minute bath scenario: 4.11 Leq/day (1.5 L ingestion, 1.7 L inhalation, 0.91 L dermal) and 3.55 Leq/day (1.5 L ingestion, 0.67 L inhalation, 1.38 L dermal) for chloroform and BDCM, respectively. Both values are considered to be conservative, since most Canadians do not take a 30-minute bath on a daily basis. In the event that individuals spend more than 10 minutes in a shower or are exposed to chloroform or BDCM via other household activities or additional bathroom time, the above-calculated Leq/day values (which account for inhalation and dermal exposures from a 30-minute bath) are likely to be adequate for assessment.

5.3   Food and Beverages
        Data from the United States 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, pancakes, veal, beef roast, and cheese). The highest concentrations of chloroform have frequently been measured in dairy products (Environment Canada and Health Canada, 2001).
        Maximum concentrations of 2200 µg chloroform/kg and 3 µg BDCM/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

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

sample, at concentrations of 115 and 9 µg/kg, respectively (Ofstad *et al.*, 1981). Neither chloroform nor BDCM was detected in composite samples of meat/fish/poultry (quantitation limits were 18 and 4.5 ng/g, respectively) or oil/fat (quantitation limits were 28 and 8.3 ng/g, respectively) from 39 different foods in the United States (Entz *et al.*, 1982). In the composite sample of dairy foods, concentrations of chloroform and BDCM were 17 and 1.2 µg/L, respectively.

THM concentrations in six different cola and non-cola beverages (five samples of each) in New Jersey ranged from 3.2 to 44.8 µg/L (Abdel-Rahman, 1982). Concentrations of chloroform and BDCM in unspecified beverage composites from the United States averaged 32 and 1.0 µg/L, 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, at a maximum concentration of 14.8 µg/kg in a fruit drink (Environment Canada and Health Canada, 2001).

5.4   Consumer Products

In the United States, 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 and Health Canada, 2001).

5.5   Swimming Pools and Hot Tubs

The use of swimming pools results in inhalation 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. Levesque *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. Limited information suggests that users of hot tubs may have more significant dermal uptake than swimmers due to higher water temperatures (Wilson, 1995).

5.6   Estimates of Total Exposure to Chloroform

Estimates of total chloroform exposure for the general population and the relative contribution of drinking water to total exposure were calculated by the World Health Organization (WHO, 1998). In this estimate, mean intake of chloroform from indoor air was estimated to be 0.3–1.1 µg/kg bw per day. The average intake of chloroform (inhalation and dermal absorption) during showering was 0.5 µg/kg bw per shower. Preliminary results from a study by Benoit *et al.* (1998), based on four volunteers, suggested that showering for 10 minutes

with warm water that has been treated with a chlorinated disinfectant is equivalent to drinking 2.7 L of cold water per day from the same water supply, on an annual average. Dermal absorption accounted 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/L, is less than 0.7 µg/kg bw per day. The estimated intake of chloroform from foodstuffs is approximately 1 µg/kg bw 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 bw 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 bw per day (WHO, 1998).

As described earlier, swimming pools are an additional source of exposure to chloroform among swimmers. The daily dose of chloroform resulting from a 1-hour swim (65 µg/kg bw per day) in conditions found in public indoor swimming pools is much greater than any of the exposures estimated above (WHO, 1998).

The *Canadian Environmental Protection Act, 1999* (CEPA) Priority Substances List assessment report on chloroform (Environment Canada and 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 United States (Table 1). 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. Average total intake was estimated to range from 0.6 to 10.3 µg/kg bw per day. The upper value is for infants 0–6 months of age, assuming exclusive formula feeding.

Table 1. Deterministic estimates of average daily intake of chloroform by the general population (Adapted from Environment Canada and Health Canada, 2001)*

Medium of exposure	Average intake (µg/kg bw per day) by various age groups in the general population					
	0–6 months	7 months – 4 years	5–11 years	12–19 years	20–59 years	60+ years
Outdoor air	0.002–0.034	0.004–0.72	0.003–0.056	0.002–0.32	0.001–0.027	0.001–0.024
Indoor air	0.559–0.744	1.197–1.596	0.933–1.244	0.531–0.708	0.456–0.608	0.396–0.528
Food	–	0.150–1.145	0.105–0.899	0.060–0.612	0.043–0.478	0.028–0.349
Water	1.003–9.536	0.424–4.037	0.334–3.172	0.190–1.806	0.199–1.891	0.209–1.987
SUBTOTAL	1.56–10.31	1.78–6.85	1.38–5.37	0.78–3.16	0.70–3.00	0.63–2.89
Inhalation and dermal intake from daily showering				0.43–4.06	0.36–3.40	0.35–3.35
TOTAL	1.56–10.31	1.78–6.85	1.38–5.37	1.21–7.22	1.06–6.40	0.98–6.24

* Details of measurement criteria available in Environment Canada and Health Canada (2001).

Estimates of exposure using maximum reported concentrations of chloroform range from 40 µg/kg bw per day for the 60+ years age group to 95 µg/kg bw per day for the 7 months to 4 years age group. The highest exposure estimate is for exclusively formula-fed infants consuming the maximum reported concentrations in Canada, at 147.6 µg/kg bw per day, with 130.6 µg/kg bw per day due to ingestion of tap water (Environment Canada and Health Canada, 2001).

In summary, the main pathways of exposure to chloroform for the general population in Canada 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 (Environment Canada and Health Canada, 2001). Most of the chloroform in indoor air is present as a result of volatilization from drinking water (WHO, 1998). In addition, the average daily intake from a single daily 10-minute shower can exceed the intake for all other exposure pathways (Jo *et al.*, 1990b). But a more recent study using PBPK modeling (Krishnan 2003) found the highest chloroform exposure values among adults taking a 30-minute bath daily.

6.0   Analytical Methods

6.1   Formation of THMs During Disinfection

The formation of chlorinated disinfection by-products during disinfection is a function of naturally occurring organic precursor concentration, chlorine dose, chlorination pH, water temperature, contact time, and bromide ion concentration. An important parameter in chlorinated disinfection by-product formation is pH: THM formation increases at high pH and decreases at low pH, whereas the formation of haloacetic acid (the second most common group of disinfection by-products) decreases at high pH and increases at low pH. Therefore, some remedial measures applied to minimize THMs could potentially maximize other chlorinated disinfection by-products.

Results from Health Canada studies (Williams *et al.*, 1995, 1997; LeBel *et al.*, 1996, 1997), including a national survey of chlorinated disinfection by-products in Canadian drinking water (53 systems) and a 1-year monthly survey of three systems using different disinfection processes, indicated that the THMs and haloacetic acids were the major chlorinated disinfection by-products found in all facilities for all treatment processes including chlorine disinfectant, and the haloacetic acid levels often equalled or exceeded the THM concentrations. The chlorinated disinfection by-product levels and variation were also dependent on the disinfection by-product group, temperature (seasonal variation), water sampling location within the distribution system (contact time, spatial variation), and water disinfection agent type (chlorination, chloramination, ozonation). Also, within a chlorinated disinfection by-product group, the bromo-chloro speciation was dependent on the bromide ion level in water.

6.2   Analytical Methods for THMs

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

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 P&T 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 (1993 national survey, 1994 monthly survey, etc.), the THMs were determined using an LLE approach adapted from U.S. Environmental Protection Agency (EPA) Method 551, with analysis of the extract by gas chromatography–electron capture detector (GC-ECD). Samples were also determined using the P&T technique followed by gas chromatography–mass spectrometry (ion trap) detector (GC-ITD).

The LLE approach also allows for the concurrent determination of other disinfection by-products including chloral hydrate, di- and trichloropropanones, haloacetonitriles, and chloropicrin, which are not explicitly covered in this guideline document. The method was later modified to include the concurrent determination of cyanogen chloride (LeBel and Williams, 1996, 1997; LeBel and Benoit, 2000) and other halogenated acetaldehydes (Koudjonou and LeBel, 2003). An essential requirement of the method was the pH adjustment (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 and Williams, 1995).

Both the P&T/GC-ITD and LLE/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 P&T technique gives slightly higher values of chloroform due to breakdown of some chlorinated intermediates (LeBel and Williams, 1995). As well, the P&T technique is not generally amenable to the analysis of the more hydrophilic disinfection by-product analytes targeted by the LLE approach. Therefore, the LLE approach is preferred for its versatility and reliability.

7.0 Treatment Technology

THMs are formed in drinking water primarily as a result of chlorination of organic matter present in raw water supplies. It is therefore important, in assessing the risks associated with the ingestion of THMs in drinking water, to recognize the substantial benefits to health associated with disinfection by chlorination. The use of chlorine has virtually eliminated waterborne microbial diseases because of its ability to kill or inactivate essentially all enteric pathogenic microorganisms, including viruses and bacteria from the human intestinal tract. Chlorine is the most convenient and easily controlled disinfectant; it is a strong oxidant for which a residual can be maintained in the distribution system to prevent bacterial regrowth.

7.1 Municipal-scale Treatment Technologies

Existing treatment facilities and processes should be optimized to reduce the formation of THMs to levels as low as possible without compromising disinfection.

At the municipal level, there are three approaches for reduction of THM concentrations in treated drinking water:
- Removal of THM precursors prior to disinfection
- Modification of disinfection strategies and use of alternative disinfectants
- Use of alternative water supply

Based on experience with these control strategies in Canada, concentrations of THMs produced in the treatment of highly coloured waters can be reduced to a level below the guideline.

7.1.1   Removal of Precursors Prior to Municipal Disinfection

At the municipal level, control technologies for reduction of THM concentrations include optimization of precursor removal using conventional treatment, such as coagulation and sedimentation (Reid Crowther & Partners Ltd., 2000). In some situations, membrane filtration such as nanofiltration and ultrafiltration may be more suitable than conventional treatment, for treatment and economic reasons.

7.1.2   Alternative Municipal Disinfection Strategies

Modification of chlorination practices such as optimizing the chlorine dosage and changing the point of contact for chlorine can help reduce THM concentrations in finished drinking water.

Alternative disinfectants to chlorine include chloramines, ozone and ultraviolet (UV) irradiation. Chloramines are a much weaker disinfectant than chlorine and are not recommended as a primary disinfectant, especially where virus or parasite cyst contamination may be present (NAS, 1987). Moreover, although chloramines do not form significant levels of THMs, they are capable of inducing halogen substitution into organic compounds and thus may produce significant quantities of total organic halogen. Little is known about these oxidant residuals. The nature and toxicity of products formed from the organic base precursor fractions have not been characterized, particularly the organic chloramine portion of the chlorine residual.

Ozone has been used as a primary disinfectant in water treatment plants in some parts of Canada and Europe. Ozone is an excellent disinfectant and does not form chlorinated by-products; however, it must be used in combination with a secondary disinfectant to maintain a residual in the distribution system. Ozonation by-products include bromate, acids, and aldehydes, and chlorination of ozonated drinking water will result in increased levels of chloral hydrate as a result of the chlorination of acetaldehyde. Chloral hydrate may subsequently degrade to chloroform depending on pH, temperature, and maturity (e.g., age) of the water (LeBel and Benoit, 2000).

UV disinfection is a physical process that uses photochemical energy to effectively prevent cellular proteins and nucleic acids (i.e., DNA and RNA) from replicating. As a result, the microorganism cannot infect its host. UV disinfection does not induce any disinfectant residual in the water, therefore, a secondary chemical disinfectant is required to maintain a residual in the distribution system. Since UV disinfection is dependent on light transmission to the microbes, the water quality characteristics affecting the UV transmittance need to be considered in the design of the system. UV irradiation under typical disinfection doses (less than 500 mJ/cm^2) does not form significant levels of disinfection by-products (DBPs), nor does it affect the DBP formation (especially THMs and HAAs) in the subsequent chlorination or chloramination processes (Reid Crowther & Partners Ltd., 2000).

The most effective approach for reduction of THMs in drinking water is the improvement of specific conventional water treatment processes and/or membrane filtration to remove organic compounds prior to disinfection, and the addition of special processes such as carbon adsorption and pre-oxidation. Initial removal of organic precursors precludes the need for reducing contact time, thus improving the efficiency of the disinfection process while still minimizing the formation of chlorinated organic by-products. The formation of THMs can be reduced with the use of granular activated carbon filtration. The level of reduction will be a function of the type and adsorbability of organic matter in the water as well as the process design criteria.

7.2   Residential-Scale Treatment Technologies
          Health Canada does not recommend specific brands of drinking water treatment devices (devices), but it strongly recommends that consumers use devices that have been certified by an accredited certification body as meeting the appropriate NSF International (NSF)/American National Standards Institute (ANSI) drinking water treatment unit (DWTU) standards. These standards have been designed to safeguard drinking water by helping to ensure material safety and performance of products that come into contact with drinking water. Certification organizations provide assurance that a product or service conforms to applicable standards. In Canada, a number of organizations have been accredited by the Standards Council of Canada to certify devices as meeting the above-mentioned NSF/ANSI standards (SCC, 2003).

7.2.1   Filtration Devices
          For households that obtain their drinking water from a municipal system or a private well that chlorinates the water, filtration systems may be installed at the faucet (point of use) or where water enters the home (point of entry) to reduce THMs. Point-of-use and point-of-entry treatment devices as well as some pour-through filters that use activated carbon filters can be effective at removing chlorine and its by-products. It is important that the equipment be monitored and maintained according to the manufacturers' recommendations, in particular the regular replacement of the filter media.
          For a drinking water treatment device to be certified to NSF/ANSI Standard 53 (Drinking Water Treatment Units — Health Effects) , the unit must reduce the concentration of THMs in water, using chloroform as a surrogate chemical, from an influent challenge concentration of 0.300 mg/L (300 µg/L) to less than 0.015 mg/L (15 µg/L), representing a chemical reduction of more than 95% (NSF International, 1999).

7.2.2   Disinfection Devices
          Ultraviolet disinfection is an alternate disinfection technology which can be installed for residential-scale treatment. UV disinfection is dependent on light transmission to the microbes through the raw water. For this reason, some pre-treatment of the raw water may be required to ensure the effectiveness of the UV disinfection.
          The NSF/ANSI Standard 55 covers the certification requirements for ultraviolet disinfection systems. In particular, it addresses the Class A systems which are designed to inactivate and/or remove microorganisms, including bacteria, viruses, *Cryptosporidium* cysts and *Giardia* cysts, from contaminated water. The Class A systems are not designed to treat wastewater or water contaminated with raw sewage, and should be installed in visually clear water (NSF International, 2002).

8.0   Kinetics and Metabolism

8.1   Absorption
          THMs are generally well absorbed, metabolized, and rapidly eliminated by mammals after oral or inhalation exposure (WHO, 1998; IPCS, 2000).

8.1.1   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 (5 hours), uptake of chloroform following administration of 75 mg/kg bw 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.

After exposure to chloroform by inhalation, 60–80% of the inhaled quantity of chloroform was absorbed, with kinetics being dependent upon concentration and species-specific metabolic capacities.

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

8.2   Distribution
8.2.1   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 (WHO, 1998).

8.2.2   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.

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

8.3.1   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 and 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 and 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 and 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 645 mg/m^3 (130 ppm) by inhalation in order to attain levels of active metabolites associated with a concentration of 50 mg/m^3 (10 ppm) 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 hours 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).

8.3.2   Brominated THMs

BDCM is metabolized to phosgene, while 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 currently available literature. 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 hours following intragastric administration of 150 mg/kg bw (rats) or 100 mg/kg bw (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 hours 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 in the mouse to be 4- to 9-fold greater than that 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 and 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.

8.3.3   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).

8.4   Excretion

8.4.1   Chloroform

In animals and humans 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).

8.4.2   BDCM

Mink *et al.* (1986) estimated BDCM half-lives at 1.5 and 2.5 hours 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 minutes for blood were estimated using breath elimination data (Lindstrom *et al.*, 1997; Pleil and Lindstrom, 1997).

9.0　Effects in Humans

9.1　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 which 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 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 (McGeehin *et al.*, 1993), Ontario (King and Marrett, 1996), and Iowa (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 and 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/L)-years[1] THMs in water compared with those exposed to less than 584 (μg/L)-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 (WHO, 1998; IPCS, 2000). In 2002, an expert panel convened by Health Canada to identify critical endpoints 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, 2003a).

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[1] THM-years is a composite of THM levels and years of exposure.

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 focussed on colon, rectal, pancreatic, kidney, brain, and 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.* (2000) 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.* (2000) 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, while 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, while 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 1950 in Finland. 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 (WHO, 1998; IPCS, 2000).

9.2   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 U.S. 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 THMs/L (Gallagher *et al.*, 1998), >80 µg THMs/L (Bove *et al.*, 1992), and >100 µg THMs/L (Bove *et al.*, 1995). Increases in risk for intrauterine growth retardation were also reported at concentrations of chloroform and BDCM ≥10 µg/L, 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 an indicator of socioeconomic status and for 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 and 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 endpoints 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/L of THMs. When specific THMs were considered, only heavy consumption of water containing BDCM (≥18 µg/L) 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 Nova Scotia women exposed to water containing more than 100 µg THMs/L (Dodds *et al.*, 1999). In further analyses of these data, King *et al.* (2000) found dose-dependent increases in adjusted risk for stillbirth with exposure to THMs, chloroform, and BDCM. Exposure to BDCM at levels ≥20 µg/L was associated with a doubling in risk. In New Jersey, Bove *et al.* (1992, 1995) found little evidence for an association with THMs at 80 µg/L, 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/L. Other studies of neural tube defects (Dodds *et al.*, 1999; Klotz and 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.

10.0   Effects on Experimental Animals and *In Vitro*

10.1   Acute Toxicity

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 bw, respectively, in male rats and 1117, 969, 848, and 1147 mg/kg bw, 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 bw, and a LOAEL of 0.5 mmol/kg bw 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 DBCM, BDCM 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).

10.2   Subchronic Toxicity
10.2.1   Trihalomethanes

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/L 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/L 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/L. Food consumption in males was depressed during exposure to 2500 mg bromoform/L 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 chloroform/L, 2500 mg DBCM/L, or 2500 mg bromoform/L. 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/L; the LOAEL is considered to be 2500 mg/L.

10.2.2   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 chloroform/kg bw 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 bw; for males, the LOAEL is 250 mg/kg bw and the NOAEL is 125 mg/kg bw. 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 regime 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 B6C3F$_1$ mice were exposed to 60, 130, or 270 mg/kg bw 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 B6C3F$_1$ mice per dose group at doses of 0, 34, 90, 138, or 277 mg/kg bw 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 bw per day after 4 days of treatment, but these effects were absent at 3 weeks. At 138 and 277 mg/kg bw 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 bw 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 B6C3F$_1$ 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 bw 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 bw per day. The NOAEL for histopathological changes (cytolethality and regenerative hyperplasia) was 10 mg/kg bw per day, and for induced cell proliferation, 34 mg/kg bw per day. In the same study, 14 female B6C3F$_1$ mice per dose group were continuously exposed to chloroform in the drinking water at concentrations of 0, 60, 200, 400, 900, or 1800 mg/L 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 bw 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 bw 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 bw per day. At 200 and 400 mg/kg bw 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 bw 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 bw 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 bw per day) or in the drinking water (0, 60, 200, 400, 900, or 1800 mg/L) for 4 days or 3 weeks. Gavage of 90 or 180 mg/kg bw 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/L were dosed at a rate of 106 mg/kg bw 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 bw (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 (0, 30, or 90 ppm) 6 hours 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 B6C3F$_1$ mice and exposure concentrations of 0, 1.5, 10, 50, 149, and 446 mg/m^3 (0, 0.3, 2, 10, 30, and 90 ppm) for 6 hours 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 as concentrations of 0, 10, 50, 149, 446, or 1490 mg/m^3 (0, 2, 10, 30, 90, or 300 ppm) for 6 hours 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 [300 ppm]) 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 bw per day. At 150 mg/kg bw 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.

10.2.3 *Bromodichloromethane*

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 bw per day) but only hepatotoxic to female mice (75–150 mg/kg bw per day). Munson *et al.* (1982) administered BDCM (50, 125, or

250 mg/kg bw 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 B6C3F$_1$ mice were given BDCM by gavage in corn oil 5 days per week for 13 weeks. Rats (10 per sex per dose) were given 0, 19, 38, 75, 150, or 300 mg/kg bw per day. Male mice (10 per dose) were given 0, 6.25, 12.5, 50, or 100 mg/kg bw per day, and female mice were given 0, 25, 50, 100, 200, or 400 mg/kg bw 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 bw per day. Centrilobular degeneration of the liver was observed at 300 mg/kg bw per day in male and female rats and at 200 and 400 mg/kg bw per day in female mice. Degeneration and necrosis of the kidney were observed at 300 mg/kg bw per day in male rats and at 100 mg/kg bw per day in male mice. The NOAELs in rats were 75 and 150 mg/kg bw per day for body weight reduction and for hepatic and renal lesions, respectively. The NOAEL for renal lesions in mice was 50 mg/kg bw per day (NTP, 1987).

10.2.4   Dibromochloromethane

DBCM-induced cardiotoxicity was reported in male Wistar rats after short-term exposure (4 weeks of daily dosing with 0.4 mmol/kg bw). 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 B6C3F$_1$ mice (10 per sex per dose) were given DBCM by gavage in corn oil at dose levels of 0, 15, 30, 60, 125, or 250 mg/kg bw per day, 5 days per week for 13 weeks. The final body weights of rats that received 250 mg/kg bw 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 bw per day. Kidney and liver toxicity were observed in male and female rats and male mice at 250 mg/kg bw 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 bw 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 bw 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 bw per day in males and at both 50 and 100 mg/kg bw per day in females (Daniel *et al.*, 1990).

10.2.5   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 bw 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 bw 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 bw per day. The NOAELs for hepatocellular vacuolation were 25 and 100 mg/kg bw per day in male rats and male mice, respectively (NTP, 1989).

10.3   Genotoxicity
10.3.1   Trihalomethanes
        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 and 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).

10.3.2   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 and Pueyo (1993) found that chloroform was not mutagenic with or without metabolic activation using several strains in an *S. typhimurium* assay. Shelby and Witt (1995) reported that chloroform was genotoxic in a mouse micronucleus assay in B6C3F$_1$ 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).

10.3.3   Bromodichloromethane
        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, 1999; 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.

10.3.4   Dibromochloromethane
DBCM is mostly positive in genotoxicity tests employing closed systems to overcome the problem of volatility (IARC, 1991, 1999; Pegram *et al.*, 1997). DBCM has given mostly positive results in eukaryotic test systems (Loveday *et al.*, 1990; IARC, 1991, 1999; 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 (WHO, 1996). 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 and Carchman, 1982). It gave positive results for chromosomal aberration in CHO cells with activation (Ishidate *et al.*, 1982) and for SCE in human lymphocytes and mouse bone marrow cells *in vivo* (Morimoto and Koizumi, 1983); it was negative in the micronucleus assay (Ishidate *et al.*, 1982) and UDS in the liver of rats (IPCS, 2000).

10.3.5   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, 1999). 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 and 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 National Toxicology Program (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 bw. As with bacterial assays, bromoform appeared more potent than the other brominated THMs (Morimoto and Koizumi, 1983; Banerji and Fernandes, 1996).

10.4   Chronic Toxicity/Carcinogenicity
10.4.1   Chloroform
Chloroform has been carcinogenic in two animal species in extensive bioassays. In an early study conducted by the National Cancer Institute (NCI), chloroform was administered by gavage in corn oil to groups of 50 male and 50 female Osborne-Mendel rats and B6C3F$_1$ mice. Male rats received 0, 90, or 180 mg/kg bw 5 times per week for 78 weeks; female rats received 0, 125, or 250 mg/kg bw 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 bw were administered to male mice, and 0, 200, or 400 mg/kg bw were administered to female mice. After 18 weeks, the doses were changed to 0, 150, and 300 mg/kg bw for male mice and 0, 250, and 500 mg/kg bw 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%.

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 chloroform/L 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 $B6C3F_1$ 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 bw per day for the rats and from 34 to 263 mg/kg bw 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/L, 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/L) 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 and 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 bw per day in ICI male and female mice) or in toothpaste or arachis oil (0 or 60 mg/kg bw 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 bw 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 B6C3F$_1$ male mice (35 animals per group) ingesting chloroform in drinking water (0, 600, or 1800 mg/L) 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 (DENA) at 10 mg/L for 4 weeks followed by 600 or 1800 mg chloroform/L for up to 52 weeks. There were two control groups: after DENA treatment, the positive control group ingested drinking water containing phenobarbital (500 mg/L), 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 DENA treatment. In contrast, in a study conducted by Deml and Oesterle (1985), chloroform administered in corn oil (100, 200, and 400 mg/kg bw, twice weekly for 11 weeks, 1 week after administration of a single dose of 8 mg DENA) promoted the development of DENA-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 bw 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 bw per day.

10.4.2   *Mechanism of Carcinogenicity for Chloroform*
Since the previous Canadian drinking water guideline was drafted for total THMs (based on chloroform), significant effort has been made to characterize the mechanism of carcinogenicity 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 $B6C3F_1$ mice (males and females) when administered by gavage in corn oil at doses in the range of 138–477 mg/kg bw 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 bw per day in corn oil by gavage (NCI, 1976a,b). However, in this strain, results were similar when the chemical was administered in drinking water, indicating that the response is not entirely dependent on the vehicle used (Jorgenson *et al.*, 1985). It should be noted, however, that at the higher doses in this study, there were significant reductions in body weight. In an early, more limited investigation, kidney tumours were increased in ICI mice but not in CBA, C57BL, or CF1 mice administered chloroform by gavage in toothpaste (Roe *et al.*, 1979). Therefore, the tumorigenic response in the kidney, although observed in both rats and mice (males), is highly strain-specific.

To investigate the possible role of replicative proliferative effects in the carcinogenicity of chloroform, a wide range of studies have been conducted in which replicative proliferative effects have been examined in similar strains of rats and mice exposed to similar doses or concentrations of chloroform, although for shorter periods, as in the principal carcinogenesis bioassays (Larson *et al.*, 1993, 1994a,b,c, 1995a,b, 1996; Lipsky *et al.*, 1993; Pereira, 1994; Templin *et al.*, 1996a,b,c). Most of these studies involved evaluation of histopathological changes and cell proliferation in the kidney and liver, the latter determined as a BrdU labelling index in histological tissue sections. 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 bw) than in the F344 rat (90 mg/kg bw); 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 endpoint for cancer induction (IPCS, 2000).

Environment Canada and 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 re-analyses of the original renal tissues (Hard and 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 regimes, 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 and Gold (1990, 1996), Cohen and Ellwein (1990, 1991, 1996), Preston-Martin *et al.* (1990), Ames *et al.* (1993), Tomatis (1993), Cohen (1995), Cunningham and 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 represent 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 and 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).

10.4.3   Bromodichloromethane

In one carcinogenesis bioassay conducted for BDCM, groups of 50 male and 50 female F344/N rats and B6C3F$_1$ 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 bw per day; male mice received 0, 25, or 50 mg/kg bw per day, while female mice received 0, 75, or 150 mg/kg bw per day (NTP, 1985).

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 alpha-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 incidence of renal cytomegaly and hepatic fatty metamorphosis in male mice was 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 B6C3F$_1$ mice, based on increased incidence 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 B6C3F$_1$ mice for 1 year and evaluated clinical indicators of kidney toxicity. Water containing BDCM concentrations of 0.08, 0.4, and 0.8 g/L for rats and 0.06, 0.3, and 0.6 g/L for mice resulted in average daily doses of 4.4, 21, and 39 mg/kg bw for rats and 5.6, 24, and 49 mg/kg bw 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 B6C3F$_1$ 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.

10.4.4   *Dibromochloromethane*

In a National Toxicology Program (NTP) carcinogenesis bioassay, DBCM was administered in doses of 0, 40, or 80 mg/kg bw 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 bw per day was administered in similar fashion to groups of 50 male and female B6C3F$_1$ 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 B6C3F$_1$ 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 or 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.

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

10.4.5   *Bromoform*

In an NTP carcinogenesis bioassay, 0, 100, or 200 mg bromoform/kg bw 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 B6C3F$_1$ mice (NTP, 1989). Male B6C3F$_1$ mice were administered 0, 50, or 100 mg/kg bw 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 B6C3F$_1$ 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/L for rats and 0.08, 0.4, and 0.8 g/L for mice resulted in average daily doses of 6.2, 29, or 57 mg/kg bw for rats and 8.3, 39, or 73 mg/kg bw 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).

10.5   Reproductive and Developmental Toxicity
10.5.1   Trihalomethanes
The teratogenicity of THMs was investigated in one study in which BDCM, DBCM, or bromoform at doses of 50, 100, or 200 mg/kg bw per day or chloroform at doses of 100, 200, or 400 mg/kg bw 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 bw per day) receiving BDCM and DBCM, but to a lesser extent than

that in the high-dose group for chloroform (400 mg/kg bw per day). Maternal liver weight was also increased at the highest dose of BDCM (200 mg/kg bw 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 bw per day (Ruddick *et al.*, 1983).

A survey of available toxicological literature on reproductive and developmental effects of disinfectant by-products including chloroform and BDCM was conducted for the U.S. 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).

10.5.2   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 bw following administration by gavage in corn oil or emulphor:saline (Thompson *et al.*, 1974; Burkhalter and 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 bw 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 in 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 (0, 3, 10, or 30 ppm) for 7 hours 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 in these animals. A NOAEL of 15 mg/m^3 was established based on the lack of embryotoxicity or teratogenicity (GlobalTox, 2002).

10.5.3   Bromodichloromethane

Narotsky *et al.* (1997) examined the effects of BDCM in F344 rats using doses of 0, 25, 50, or 75 mg/kg bw per day in aqueous or oil gavage vehicles. BDCM induced full-litter resorptions in the 50 and 75 mg/kg bw 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 bw per day, respectively. All vehicle control litters and litters from the group given 25 mg/kg bw 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 bw per day for rats and 0, 1.4, 13.4, 35.6, or 55.3 mg/kg bw 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 bw per day. In rabbits, body weight gain and feed consumption were reduced at ≥35.6 mg/kg bw per day. The maternal NOAELs were 18.4 and 13.4 mg/kg bw 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 bw 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 bw 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/L (equal to 0, 4.1–12.6, 11.6–40.2, or 29.5–109.0 mg/kg bw per day). In the two top 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 bw 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 bw 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 (FLR) after dosing with BDCM. Following aqueous gavage with BDCM at 75 mg/kg bw per day on gestation days 6–10, F344 rats had a 62% incidence of FLR, whereas all SD rats maintained their litters. Additionally, rats treated with BDCM at 75 mg/kg bw per day on gestation days 6–10, the critical period encompassing the luteinizing hormone (LH)-dependent period of pregnancy, had a 75% incidence of FLR, but rats treated on gestation days 11–15 with BDCM at 75 or 100 mg/kg bw per day were unaffected. Twenty-four hours after a single dose, all dams with FLR had markedly reduced serum progesterone levels; however, LH levels were unaffected. The high FLR 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 bw 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.

10.5.4 *Dibromochloromethane*

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/L (0, 17, 171, or 685 mg/kg bw per day) in drinking water for 35 days, then mated; subsequent re-matings occurred 2 weeks after weaning. The F_1 mice were treated with the same test solution for 11 weeks after weaning and then mated; re-mating occurred 2 weeks after weaning. At 17 mg/kg bw 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 bw 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 bw 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 bw per day) and in females at the middle dose (171 mg/kg bw per day). Animals in both these groups exhibited enlarged livers with gross morphological changes. In addition, the gestation index, fertility, and survival of the F_1 generation were significantly reduced. 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 bw per day was identified (Borzelleca and Carchman, 1982).

10.5.5 *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 bw 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 bw 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 bw per day was identified (NTP, 1989).

10.6   **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 and Borzelleca (1982) in adult male mice dosed by aqueous gavage for up to 90 days. Treatment with 1.2 or 11.6 mg/kg bw per day was without effect in various behavioural tests, and dosing for 30 days with 100 mg/kg bw per day did not affect passive avoidance learning. Animals dosed with either 100 or 400 mg/kg bw 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).

10.7   **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).

10.7.1 Chloroform

The 2001 CEPA assessment report on chloroform (Environment Canada and 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:

1. In both experimental animals and humans, metabolism of chloroform by CYP2E1 is responsible for production of the critical reactive metabolite, phosgene.
2. 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.
3. 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 and Health Canada, 2001).

a) *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 (TC_{05}) in humans estimated on the basis of the PBPK model is 3.9 mg/L per hour (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/L in water or 149 mg/m^3 (30 ppm) in air. Respective lower 95% confidence limits for these values are 2363 mg/L and 74 mg/m^3 (15 ppm).

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 re-analysis 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/L per hour (95% lower 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/L in water or 33.8 mg/m^3 (6.8 ppm) in air (Environment Canada and Health Canada, 2001).

b) *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 (2 ppm), 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 (10 ppm); following both short- and long-term exposure to 124–149 mg/m^3 (25–30 ppm), 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 bw 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 bw per day and fatty cysts in the liver of dogs at 15 mg/kg bw 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 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/L per hour (95% lower 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/L in water or 9.9 mg/m^3 (2 ppm) in air.

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 and Health Canada, 2001).

10.7.2 Bromodichloromethane

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).

11.0 Classification and Assessment

There is insufficient data on each of the individual trihalomethanes identified in this document to establish distinct guidelines for each. Rather, the approach chosen is to establish a Maximum Acceptable Concentration for THMs as a group, based on Chloroform data, and a separate Maximum Acceptable Concentration for Bromodichloromethane.

11.1 Trihalomethanes (Chloroform)

As chloroform is the THM present in greatest concentration in drinking water, and the THM for which there is most scientific data available, a guideline developed based on data for this compound should be applicable as a guideline for the THMs identified in this document (chloroform, bromodichloromethane, dibromochloromethane and bromoform). 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, 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).

Chloroform has been classified in Group IIIC in this assessment, possibly carcinogenic to humans based on inadequate evidence for carcinogenicity in humans but limited evidence in experimental animals (Health Canada, 1994). 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 and 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.

PBPK modelling was performed for chloroform in the 2001 CEPA assessment report in order to estimate doses causing toxicity in specific organs. Basing the calculation of a Canadian drinking water guideline on the PBPK approach would lead to a considerable raising of the maximum acceptable concentration (MAC). Given the uncertainties surrounding the health effects of THMs in drinking water in humans and that chloroform is used as a surrogate for THMs, it is considered appropriate to use the more conservative TDI approach for calculating the MAC.[1]

Two key studies were considered in the risk assessment for chloroform: the Heywood *et al.* (1979) study in dogs and the Larson *et al.* (1994b) study in mice. The target organ in both studies was the liver. Although the Heywood *et al.* (1979) study was conducted in a relatively higher mammalian species (dog) and was of a reasonably long duration (7.5 years), it is an older study, used gavage dosing with a toothpaste base in a capsule, and did not cover the full life span of the dog. The Larson *et al.* (1994b) study, on the other hand, was conducted in a relatively lower mammalian species (mouse), used either corn oil vehicle (which may have influenced the pharmacokinetics and toxicity of the test compound) by gavage or drinking water given *ad libitum*, and was of short duration (3 weeks), which is insufficient for proper assessment of a lifetime exposure.

The NOAEL for treatment-related changes in the liver (cytolethality and regenerative hyperplasia) established in the mouse study (Larson *et al.*, 1994b) with chloroform in corn oil vehicle was 10 mg/kg bw per day (corrected to 7 mg/kg bw per day due to 5 days per week dosing). In the same study (Larson *et al.*, 1994b), however, mice treated with chloroform administered via drinking water had no treatment-related changes up to a dose of 329 mg/kg bw per day. In the Heywood *et al.* (1979) dog study, treatment-related liver changes (fatty cysts) were observed at the lowest administered dose (LOAEL = 15 mg/kg bw per day corrected to 13 mg/kg bw per day due to 6 days per week dosing), and no NOAEL was established.

The Heywood *et al.* (1979) dog study was chosen as the most appropriate study for risk assessment, due to its long duration and the possible influence of the corn oil vehicle on the effects observed in the short-term Larson *et al.* (1994b) mouse study. The tolerable daily intake (TDI) is calculated as follows:

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[1] Consistent with this approach, the U.S. EPA did not use PBPK modelling to estimate the delivered dose (i.e., the amount of key chloroform metabolites that actually reach the liver and cause cell toxicity) in their 2001 National Primary Drinking Water Regulations. It was felt that the required toxicokinetic data were not available. Thus, the reference dose used to support their maximum concentration limit goal for drinking water was calculated using the applied dose (i.e., the amount of chloroform ingested) (U.S. EPA, 2001).

TDI = <u>13 mg/kg bw per day</u> ≅ 0.0062 mg/kg bw per day
           2100

where:

- 13 mg/kg bw per day is the LOAEL in the Heywood *et al.* (1979) dog study, corrected from 15 mg/kg bw per day to 13 mg/kg bw per day due to 6 days per week dosing,
- 2100 is the uncertainty factor (×10 for intraspecies variation; ×10 for interspecies variation; ×7 for less-than-lifetime exposure; ×3 for use of a LOAEL instead of a NOAEL). A moderate uncertainty factor of 7 was chosen for the less-than-lifetime exposure because 7.5 years was considered a reasonably long duration in the dog's lifespan. A modest uncertainty factor of 3 was used for the use of a LOAEL, because of the subtle nature of the endpoint (fatty cysts) observed in the dog study. Further support for this uncertainty factor is given by the absence of effects seen in the liver at a considerably higher dose of up to 329 mg/kg bw per day when chloroform was applied in drinking water in what appeared to be a more sensitive species (mouse).

The calculated value for THMs (based on chloroform) is as follows:

<u>6.2 μg/kg bw per day × 70 kg × 0.75</u> ≅ 80 μg/L
           4.11 Leq/d

where:

- 6.2 μg/kg bw per day is the TDI, as derived above,
- 70 kg is the average adult body weight,
- 0.75 is the source allocation factor (CEPA exposure data estimate that approximately equal contributions 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 arises primarily from drinking water; therefore, 75% source allocation to drinking water includes all but food exposure),
- 4.11 Leq/d is the total exposure contribution from drinking water (see section on Multiroute Exposure through Drinking Water).

11.2   Bromodichloromethane

BDCM is used as an indicator of the presence of brominated THMs, but the Maximum Acceptable Concentration developed applies to the level of BDCM in drinking water.

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 bw 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 bw per day or 0, 75, or 150 mg/kg bw 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 in Group II — probably carcinogenic to humans, with sufficient evidence in animals and inadequate evidence in humans (Health Canada, 1994). 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 intestines (combined adenomatous polyps and carcinomas) in rats were chosen for the 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 that the unit risks for the other tumour types (kidney and liver) identified in carcinogenicity studies with this compound.

Cancer risks have been estimated on the basis of the results of the only adequate carcinogenesis bioassay in F344/N rats, 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. Although there has been one carcinogenesis bioassay in which BDCM was administered in a more appropriate vehicle (i.e., drinking water) (Tumasonis *et al.*, 1985), it was considered inadequate for quantitative risk estimation, based on the limitations mentioned in the Chronic Toxicity/Carcinogenicity section. Moreover, the increases in adenomas and adenocarcinomas in the kidney of male mice and 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 in the NTP (1987) bioassay (i.e., intestinal adenomatous polyps and adenocarcinomas; renal tubular cell adenomas and adenocarcinomas), unit risks were calculated using the linearized multistage [LMS] method of Howe (1995). An allometric scaling factor was applied to the final unit risks, assuming a rat weighs 0.35 kg, a mouse weighs 0.03 kg, and a human weighs 70 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

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

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 LMS 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. Unit risks and lack of fit P-values are displayed in Appendix 1 of Health Canada (2003b).

The allometric scaling factor is given by $(0.35/70)^{1/4}$ or $(0.03/70)^{1/4}$, where 0.35 kg is the body weight of a rat, 0.03 kg is the body weight of a mouse, and 70 kg is the body weight of a human. The "raw" unit risks are divided by this factor to obtain the "converted" unit risks in Table 2 of Health Canada (2003b). Using LMS model for the tumours that were significantly increased in F344/N rats in the NTP (1987) bioassay, the estimated calculated unit lifetime human cancer risks associated with the ingestion of 1 µg/L BDCM in drinking water are $2.06 \times 10^{-7}*$ (based on combined adenomatous polyps and carcinomas of the large intestine in females rats) to $6.33 \times 10^{-7}*$ (based on combined adenomatous polyps and carcinomas of the large intestine in males rats).

The estimated concentrations corresponding to lifetime human cancer risks of 10^{-5}, 10^{-6}, and 10^{-7} for these tumour types, based on the model described above and the calculated unit lifetime human cancer risks, are as follows:

Lifetime risk	Concentrations in drinking water (µg/L)
10^{-5}	15.8– 48.5
10^{-6}	1.6– 4.9
10^{-7}	0.2–0.5

Using the most conservative concentration in drinking water estimated for a 10^{-5} lifetime human cancer risk, a value of 16 µg/L (rounded) is derived.

11.3   Chlorodibromomethane
DBCM has been classified in Group IIID, possibly carcinogenic to humans based on limited evidence for carcinogenicity in one species of experimental animals and no data in humans (Health Canada, 1994). An expert panel convened in 2002 by Health Canada to assess the toxicological and epidemiological evidence for the THMs for the purpose of drafting an updated Canadian drinking water guideline concluded that there was insufficient information available to calculate a drinking water guideline for DBCM (Health Canada, 2003b).

11.4   Bromoform
Bromoform has been classified in Group IIID, possibly carcinogenic to humans based on limited evidence for carcinogenicity in one species of experimental animals and no data in humans (Health Canada, 1994). An expert panel convened in 2002 by Health Canada to assess the toxicological and epidemiological evidence for the THMs for the purpose of drafting an updated Canadian drinking water guideline concluded that there was insufficient information available to calculate a drinking water guideline for bromoform (Health Canada, 2003b).

12.0   Rationale
Because THMs are formed in drinking water primarily as a result of chlorination of organic matter present in raw water supplies, it is important to recognize the substantial benefits to health associated with disinfection by chlorination. The use of chlorine has virtually eliminated waterborne microbial diseases, because of its ability to kill or inactivate essentially all enteric pathogenic microorganisms. Chlorine is the most convenient and easily controlled disinfectant; it is a strong oxidant for which a residual can be maintained in the distribution system to prevent bacterial regrowth. Although the use of chlorine can lead to the formation of disinfection by-products such as THMs, efforts to manage THM levels in drinking water **must not** compromise the effectiveness of water disinfection.
THMs and haloacetic acids (HAAs) are the two major groups of disinfection by-products (DBPs) found in drinking water and generally at the highest levels. The concentrations of these contaminants can be used as indicators of the total loading of all DBPs which may be found in drinking water supplies. In the absence of information on other DBPs, control and management of THMs and HAAs should reduce exposure to and risk from other by-products. When drinking water is treated to reduce THMs and HAAs, the levels of other chlorinated disinfection by-products may also be reduced in the process.
Two guidelines for trihalomethanes have been established. The THM guideline is based on health effects of chloroform, and applies to the total concentration of chloroform, BDCM, DBCM and bromoform. A separate guideline for BDCM was also established; BDCM can be used as an indicator of the presence of other brominated THMs in drinking water. Animal data have consistently shown significantly higher level of toxicity for brominated disinfection by-products than chlorinated disinfection by-products.
New information also indicates that inhalation and dermal absorption from drinking water are important exposure routes, and should be considered, resulting in a higher overall exposure to all THMs.

12.1   Trihalomethanes (Chloroform)
Considerable progress has been made since the establishment of the previous Canadian drinking water guideline for THMs which was also based on chloroform. The weight of evidence now suggests that chloroform is a threshold carcinogen mediated through a non-genotoxic mechanisms of action resulting in sustained cytotoxicity by metabolites and ultimately persistent cellular proliferation (i.e., cancer). As such, chloroform has been reclassified from Group II (probably carcinogenic to humans) in the previous guideline to Group III (possibly carcinogenic to humans) in this assessment. Incorporation of additional exposure routes from drinking water such as inhalation and dermal absorption leads to a higher overall exposure (total of 4.11 Leq/day for ingestion and dermal and inhalation exposures from showering and bathing) to THMs than was previously recognized and results in a calculated value of 80 µg/L. Chloroform

was again used as a model THM for the purposes of derivation of a guideline since it is the THM for which there exists the most scientific information on which a guideline could be based, and it is also the predominant THM found in drinking water supplies.

Epidemiological evidence of a possible association between exposure to high levels of THMs and reproductive effects has also been reviewed. However, neither a dose-response pattern of increasing risk with increasing concentration of THMs nor a clear evidence of a threshold has been found.

Since there is no significant expected difference in health risk between the calculated value and the existing MAC, it is proposed that the MAC for THMs in drinking water of 100 µg/L be reaffirmed, based on an annual average. The lower calculated value can already be achieved by many treatment plants through optimization of their processes. Expansion or upgrade of treatment facilities should be designed to achieve the lowest concentrations possible without compromising the effectiveness of water disinfection.

12.2 Bromodichloromethane

Because BDCM is classified in Group II (probably carcinogenic to humans), the MAC is derived based on consideration of the estimated lifetime cancer risk and best available treatment technology. Since the MAC must be measurable by available analytical methods, the Method Quantitation Limit (MQL) is also taken into consideration in derivation of the MAC.
A proposed MAC of 0.016 mg/L (16 µg/L) for BDCM is derived, therefore, on the basis of the following considerations:

(1) The estimated unit lifetime human cancer risk associated with the ingestion of 1 µg/L BDCM[*] in drinking water is 2.06×10^{-7} (based on adenomatous polyps and carcinoma tumours [combined] of the large intestine in female rats) to 6.33×10^{-7} (based on adenomatous polyps and carcinoma tumours [combined] of the large intestine in male rats). Therefore, the estimated lifetime human cancer risk associated with ingestion of 16 µg/L BDCM (i.e., 3.3×10^{-6} to 1.0×10^{-5}) is within a range that is considered to be "essentially negligible."[**]

(2) The MQL (based on the ability of laboratories to measure THMs including BDCM within reasonable limits of precision and accuracy) is 0.1–0.2 µg/L.

(3) The proposed MAC must be measurable and achievable. By optimization of treatment process (i.e., improvement of specific conventional water treatment processes to remove organic [brominated] compounds prior to disinfection and addition of such processes as carbon adsorption and preoxidation), BDCM concentrations can be reduced below 16 µg/L.

Epidemiological studies, partially supported by toxicological studies, have also identified possible associations of reproductive effects (increased risk for spontaneous abortion or stillbirth) with exposure to BDCM. Recent studies suggest that BDCM targets human placental trophoblasts that produce chorionic gonadotrophin, a hormone that plays a vital role in the maintenance of pregnancy. A decrease in bioactive levels of this hormone could lead to adverse effects on pregnancy; however, only limited evidence exists on the biological plausibility of the observed BDCM-induced pregnancy loss. Although the lowest levels of exposure to BDCM associated with possible fetal loss in epidemiological studies are ≥ 20 µg/L, the evidence is

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[*] Average daily intake of drinking water from all sources, including showering and bathing = 3.55 Leq/day; average adult body weight = 70 kg.
[**] Essentially negligible risk is defined by the World Health Organization as one additional cancer per 100 000 (10^{-5}) or per 1 000 000 (10^{-6}) of the population ingesting drinking water containing the substance at the guideline value for 70 years. It is also known as "de minimus" risk.

presently insufficient to determine whether BDCM in drinking water causes reproductive effects in humans. It is recommended that utilities strive to keep levels of brominated THMs as low as possible without compromising the effectiveness of water disinfection.

As part of its on-going guideline review process, Health Canada will continue to monitor new research in this area and recommend any change(s) to the guideline it deems necessary.

13.0   References

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Appendix 1: Provincial[1] Average TTHM Exposure Levels (1994–2000) and Cost Estimates to Meet 100 µg/L

Prov/Terr	Population 1996	Population where average TTHM > 100 µg/L				Total Low Capital ($000)	Total High Capital ($000)	Current Prov/Terr Estimate[2] ($000)
		Based on 1996		Current Prov/Terr Estimate[2]				
		Treated	Distribution	Treated	Distribution			
AB	2,696,826		65,645			$8,665	$24,700	
BC	3,724,500							
MB	1,113,898	83,138	28,885		65,645	$228,905	$242,710	$242,710
NB	738,133		0			$4,600	$10,000	
NL	551,792		44,393	754	85,451	$52,200	$104,400	Unknown
NS	909,282	231,900	71,425		11,300 (BDCM 4,850)	$18,845	$34,580	$21,350
NT & NU	64,402	760	760	0	0	$500	$1,000	$0
ON	10,753,573	71,062	13,612	12,500	12,500	$10,190	$36,330	Unknown
PE	134,557							
QC	7,138,795		251,997					
SK	990,237		47,229			$14,915	$36,670	
YU	30,766							
Total	28,846,761	386,860	523,946	13,254	174,896	$338,320	$89,390	

COSTS: *Table shows the estimated Capital and Operating cost ranges by Province to reduce current maximum levels of THMs to meet 100 µg/L limits. Extrapolated costs for the whole country to meet the 100 µg/L THM limit shows capital costs of $338-$489 million, and annual operating costs of $43-$68 million, based on process modifications to most plants to include optimized coagulation, chloramination, GAC or Nanofiltration. Similar numbers to meet 75 µg/L THM limits for all locations show $388-$574 million capital costs, and $57-$86 million for annual operating costs.*

[1] *Data provided by CDBP Water Quality Subgroup*

[2] *Refer to Provincial/Territorial Notes*

Provincial/Territorial Notes

AB: Exposure data for Alberta (AB) has not changed much from the 1996 data. From a cost impact point of view, costs still remain the same.

BC: Most larger systems in British Columbia (BC) maintain TTHM's well below 100 µg/L. Provincial data systems do not allow ready central access to water chemistry data at this time. Provincial TTHM exposure has remained relatively constant since exposure data was provided to the Secretariat during previous exposure assessments. Some communities have reduced TTHM levels through improved precursor removal or changing source water while others have increased TTHM levels where chlorination practices have changed to accommodate cyst reduction targets. BC policy continues to be to reduce TTHM's to the lowest level achievable while not compromising treatment for pathogen reduction.

NL: For Newfoundland (NL), current estimated population of 85,451 exposed to total trhalomethanes above 100 µg/L baced on December 2003 data.

NS: Based on a review of the trihalomethanes (THMs) database, it is estimated that a population of approximately 11,300 is exposed to total THMs above 100 µg/L and a population of approximately 4,850 is exposed to BDCM levels above 16 µg/L in Nova Scotia (NS).
       Costs to reduce total THM levels, including BDCM, have been updated to 2004 dollars using pro-rated unit costs per cubic metre of capacity required. This approach has been used to account for the fact that small public water systems generally lack economies of scale and require larger unit cost expenditures. Using this approach, costs are expected to be in the order of $21.35 million.
       This estimate is provisional as other options that may be considered include switching to a groundwater supply which may have lower costs.

NT & NU: In 1996 only one community water system in the Northwest Territories (NT) and what is now Nunavut (NU) was identified with TTHM levels above 100 ug/L. In 2002, that community changed it's raw water source and as a result it now is in full compliance with TTHM guideline level of 100 ug/L. Currently there are no drinking water systems identified in the NT or NU that exceed the TTHM guideline level 100 ug/L .

ON: Population exposed estimate for 2003-04 based on systems that will exceed, or are likely to exceed, 100 ug/L TTHMs on a quarterly rolling average basis is 12,500 (treated vs distribution not differentiated). An overall cost estimate is not practical at this time, as any costs associated with system optimization etc. are very site-specific, will have to be dealt with case-by-case.