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Guidelines for Canadian Drinking Water Quality:

Guideline Technical Document

Haloacetic Acids

Canada

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Errata

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Although health-based targets can be established for four of the five HAAs, and considering the technological limitations associated with reducing individual HAA levels in drinking water while maintaining effective disinfection, the Federal-Provincial-Territorial Committee on Drinking Water is establishing a MAC of ~~0.8~~ **0.08** mg/L (80 µg/L) for Total HAA5 in drinking water based on a running annual average rather than individual guidelines. This is consistent with the approach taken by the U.S. EPA, which established a maximum contaminant level based on best available technology for these same HAAs.

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Prepared by the
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Drinking Water
of the
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Health and the Environment

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Other Guideline Technical Documents for the Guidelines for Canadian Drinking Water Quality can be found on the following Web page: http://www.healthcanada.gc.ca/waterquality.

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July 2008

Haloacetic Acids

Part I. Overview and Application

1.0 Guideline

The maximum acceptable concentration (MAC) for total haloacetic acids[*] in drinking water is 0.08 mg/L (80 µg/L) based on a locational running annual average of a minimum of quarterly samples taken in the distribution system.

Utilities should make every effort to maintain concentrations as low as reasonably achievable (or ALARA) without compromising the effectiveness of disinfection.

2.0 Executive summary

Haloacetic acids (HAAs) are a group of compounds that can form when the chlorine used to disinfect drinking water reacts with naturally occurring 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 treatment 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 an essential component of public drinking water treatment; the health risks from disinfection by-products, including haloacetic acids, are much less than the risks from consuming water that has not been appropriately disinfected.

The haloacetic acids most commonly found in drinking water are monochloroacetic acid (MCA), dichloroacetic acid (DCA), trichloroacetic acid (TCA), monobromoacetic acid (MBA) and dibromoacetic acid (DBA). Of these, DCA and TCA have been most extensively studied, and there are some scientific data available on MCA and DBA. However, insufficient data were available to allow the development of an individual guideline for MBA.

This Guideline Technical Document reviews the health risks associated with haloacetic acids in drinking water. It assesses all identified health risks, taking into account new studies and approaches, as well as treatment considerations. Exposure to haloacetic acids from drinking water through inhalation and skin contact has been considered for inclusion, but is not deemed significant. Based on this review, the guideline for total haloacetic acids in drinking water is established at a maximum acceptable concentration of 0.08 mg/L. This guideline takes into consideration the availability of appropriate treatment technologies and the ability of treatment plants to meet the guideline without compromising the effectiveness of disinfection.

[*] Total haloacetic acids refers to the total of monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid and dibromoacetic acid.

2.1 Health effects

The health effects associated with exposure to haloacetic acids will vary with the specific compound. MCA is considered unlikely to be carcinogenic to humans, based on the lack of evidence for carcinogenicity. Changes in body, liver, kidney and testes weights were observed in studies with rats. A health-based target concentration of 0.1 mg/L can be calculated for MCA in drinking water. DCA is considered to be a probable carcinogen to humans, based on sufficient evidence in animals and inadequate evidence in humans. Animal studies have shown links between exposure to DCA and liver tumours in both mice and rats. A health-based target concentration of 0.01 mg/L can be calculated for DCA in drinking water. TCA 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 a link between exposure to TCA and liver tumours in mice only, but it is still uncertain whether the mechanism causing these tumours is relevant to humans. A health-based target concentration of 0.3 mg/L can be calculated for TCA in drinking water. MBA is unclassifiable with respect to carcinogenicity in humans, based on inadequate data from animal studies. DBA is considered to be probably carcinogenic in humans, based on sufficient evidence in animals and inadequate evidence in humans. Animal studies have shown links between exposure to DBA and tumours in several organs in both mice and rats. A health-based target concentration of 0.002 mg/L can be calculated for DBA in drinking water.

There is only one study currently available looking at the incidence or significance of health effects associated with human exposure to haloacetic acids. A small population-based study that was conducted in two eastern provinces did not find a link between exposure to haloacetic acids and risk of stillbirths. Other human studies on the incidence of cancer or reproductive effects have been conducted with chlorinated disinfection by-products, but not specifically with haloacetic acids.

Some animal studies suggest a possible link between developmental effects (heart defects) and exposure to DCA or TCA, whereas other studies fail to show a link. Animal studies also suggest a possible link between male reproductive effects (on sperm and sperm formation) and exposure to DCA or DBA, at levels significantly higher than those found in drinking water. Further studies are required to confirm these effects as well as their long-term significance to human health.

A single guideline for total haloacetic acids is established, based on the health effects of the individual haloacetic acids, and taking into consideration both treatment technology and the ability of treatment plants, particularly smaller ones, to achieve the guideline. The guideline is considered to be protective of health for all haloacetic acids, based on the ratio of haloacetic acids expected to be found in drinking water. The guideline value is primarily designed to be protective of the health effects of DCA, the haloacetic acid that would pose the most significant health concerns and is found at the highest levels in drinking water.

2.2 Exposure

Levels of haloacetic acids are generally higher in treated surface water than in treated groundwater, because of the high organic content in lakes and rivers. Levels of haloacetic acids will be higher in warmer months, because of the higher concentrations of precursor organic materials in the raw water and especially because the rate of formation of disinfection by-products increases at higher temperatures. It should be noted that the presence of by-products such as MBA and DBA will also depend on the presence of bromine in the source water.

Available data suggest that drinking water may be a significant source of exposure to haloacetic acids, but there are few data available to determine the exposure from other media, such as food and air.

2.3 Treatment

Haloacetic acids and trihalomethanes are the two major groups of chlorinated 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 chlorinated disinfection by-products in drinking water supplies, and their control is expected to reduce the levels of all chlorinated disinfection by-products and the corresponding risks to health.

The approach to reduce exposure to haloacetic acids is generally focused on reducing the formation of chlorinated disinfection by-products. The concentrations of haloacetic acids and other chlorinated 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, by using alternative disinfection methods or by using a different water source. It is critical that any method used to control levels of haloacetic acids ***must not*** compromise the effectiveness of disinfection. The Federal-Provincial-Territorial Committee on Drinking Water also recommends that every effort be made not only to meet the guideline, but to maintain concentrations of haloacetic acids as low as reasonably achievable.

3.0 Application of the guideline

Note: *Specific guidance related to the implementation of this guideline should be obtained from the appropriate drinking water authority in the affected jurisdiction.*

The concentrations of haloacetic acids (HAAs) and trihalomethanes (THMs) can be used as indicators of the total loading of all chlorinated disinfection by-products (CDBPs) that may be found in drinking water supplies. The guideline for HAAs is also designed to take into consideration exposure and potential health effects related to other CDBPs, on which very little is known. The guideline is measured as a locational running annual average of quarterly samples, because HAA levels can vary significantly over time, including seasonally, with factors such as the levels of organic matter in the raw water and temperature.

Given the limited information on the risks and uncertainties associated with other CDBPs, it is recommended that treatment plants strive to maintain HAA levels as low as reasonably achievable (ALARA) without compromising disinfection. This should also be considered when changes, upgrades or expansions are made to the treatment plants or distribution systems. Any effort aimed at reducing disinfection by-products, such as changing

disinfection strategies, needs to be considered in light of changes in water quality that may inadvertently increase the levels or leaching of other contaminants, such as lead, in the distributed water.

Table 1 lists the estimated lifetime (70 years) risk of excess liver cancer (in addition to the background lifetime cancer risk) associated with the ingestion of HAAs in drinking water at various concentrations, based on animal studies. It is expressed as a range, which represents estimated proportions of 40–60% of DCA in total HAAs.

Table 1: Estimated lifetime range of risk of excess liver cancer (in addition to the background lifetime cancer risk) from exposure to DCA associated with various concentrations of HAAs in drinking water

Levels of HAAs in drinking water (µg/L)	Estimated lifetime range of risk of excess cancers ($\times 10^{-5}$)[a]
40	1.6–2.4
60	2.4–3.6
80	3.2–4.8
100	4.0–6.0
120	4.8–7.2

[a] The estimated lifetime range of risk of excess cancers above background levels is calculated from the risk associated with ingesting DCA at a concentration of 1 µg/L in drinking water, assuming a proportion of 40–60% of DCA in total HAAs.

3.1 Monitoring

At a minimum, quarterly monitoring of treated water from surface water and groundwater sources is recommended for total HAAs. Increased frequency of monitoring may be required for facilities using surface water sources[*] during periods when water characteristics are more favourable to the formation of by-products, which will vary according to the specific system. Since total HAA concentrations vary within and between distribution systems, depending on different factors, including water quality characteristics (e.g., HAA precursors, pH, season, temperature) and treatment conditions (e.g., disinfectant type, disinfectant dose, contact time), it is recommended that monitoring samples be taken at the water treatment plant and at points in the distribution system where historical data show the highest HAA concentrations.

Where historical data are not available, program should be put in place to monitor HAA levels in the middle and extremities of the distribution system. Areas with extremely low or no disinfectant residual should be avoided, but areas where disinfectant residuals are significantly lower than the system average because of a long residence time (e.g., dead ends, low flow areas) should be targeted. In systems with booster chlorination stations and water tanks or reservoirs, it is expected that higher HAA concentrations would be found downstream of these components.

Monitoring/reporting may be reduced if drinking water monitoring does not show elevated levels of disinfection by-products within the distribution system.

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

Part II. Science and Technical Considerations

4.0 Identity, use and sources in the environment

There are nine common HAAs: MCA, DCA, TCA, MBA, DBA, bromochloroacetic acid, bromodichloroacetic acid, chlorodibromoacetic acid and tribromoacetic acid. This document focuses on the first five HAAs on this list, referred to as either HAA5 or total HAAs.

HAAs belong to the family of halogenated aliphatic carboxylic acids. Although these chemical analogues will in most cases be referred to as "acids" in this document, it should be understood that when present in drinking water at normal pHs, they will in fact be present as salts and strictly should be called acetates (EC, 2003; U.S. EPA, 2003b). The physical-chemical properties of the HAA5 compounds shown in Table 2 apply to the acids.

Table 2: Physical-chemical properties of the HAA5 compounds[a]

Property	MCA	DCA	TCA	MBA	DBA
CAS No.	79-11-8	79-43-6	76-03-9	79-08-3	631-64-1
Formula	$ClCH_2COOH$	$Cl_2CHCOOH$	Cl_3CCOOH	$BrCH_2COOH$	$Br_2CHCOOH$
Molecular weight	94.5	128.942	163.387	138.948	217.844
Boiling point (°C)	189.1[1)]	193–194[1)]	196–197[1)]	208[2)]	195[2)]
Melting point (°C)	63[1)]	13.5[2)]	57–58[1)]	50[2)]	49[2)]
Density (g/cm^3)	1.40 at 25°C[3)]	1.56 at 20°C[1)]	1.62 at 25°C[1)]	1.93[2)]	n/a[c]
Vapour pressure (mmHg)[b]	0.065 at 25°C[3)]	0.179 at 25°C[4)]	0.16 at 25°C[5)]	0.549 at 25°C[6)]	n/a[c]
Dissociation constant (pK_a) at 25°C	2.87[7)]	1.26[8)]	0.66[2)]	2.69[9)]	n/a[c]
Water solubility (g/mL)	1.09 at 25°C[10)]	Miscible[10)]	1.50 at 25°C[10)]	1.75 at 25°C[10)]	2.11 at 25°C[10)]
Log octanol/water partition coefficient	0.22[11)]	0.92[11)]	1.33[11)]	0.41[11)]	1.22[12)]

[a] References are as follows: 1) Budavari et al., 1996; 2) Lide, 2003–2004; 3) Morris and Bost, 2002; 4) Daubert and Danner, 1989; 5) Weast, 1973; 6) Chemada Fine Chemicals, 2002; 7) Serjeant and Dempsey, 1979; 8) Maruthamuthu and Huie, 1995; 9) ZirChrom Separations, Inc., undated; 10) Nikolaou et al., 1999; 11) Hansch et al., 1995; 12) Schultz et al., 1999.

[b] 1 mmHg = 133.3 Pa.

[c] n/a: not available.

MCA is described as a colourless solution or white crystal with a vinegar-like odour (Budavari et al., 1996; CHEMINFO, 2003a, 2003b). It is used mainly as a chemical intermediate in the production of cellulose ethers (mainly carboxymethylcellulose), thioglycolic acid and herbicides (Morris and Bost, 2002). It is also used in the manufacture of glycine, phenoxyacetic acid, sarcosine, amphoteric surfactants, synthetic caffeine, various indigo dyes, pharmaceuticals, preservatives (ethylenediaminetetraacetic acid) and bacteriostats (Lewis, 2001; Koenig et al., 2002; Morris and Bost, 2002).

DCA is a colourless to slightly yellow liquid with a pungent odour (IARC, 1995; Budavari et al., 1996). It is used as a topical astringent, fungicide and medicinal disinfectant, as a test reagent for analytical measurements, to treat lactic acidosis and in the synthesis of organic materials, including pharmaceuticals (Budavari et al., 1996; Koenig et al., 2002; Morris and Bost, 2002).

TCA is a colourless to white deliquescent crystal with a sharp, pungent odour (Ashford, 1994; Budavari et al., 1996). TCA is used as an intermediate in the synthesis of organic chemicals and as a laboratory reagent, herbicide, soil sterilizer and antiseptic (Budavari et al., 1996; Lewis, 2001; Verschueren, 2001; Meister, 2002). It has been used as an etching or pickling agent, a swelling agent and a solvent in plastics and in textile finishing (Koenig et al., 2002). Clinically, TCA has been used in 10–25% aqueous solutions in the treatment of recurrent corneal disease (Grant and Schuman, 1993), for the treatment of external cervical root resorption in dentistry (Heithersay and Wilson, 1988; Lewinstein and Rotstein, 1992) and to treat various skin afflictions (Koenig et al., 2002). TCA has been used as a facial chemical peel and for other therapeutic applications, such as a cauterizing agent, in wart removal and as an astringent (NTP, 2003a).

MBA is a colourless hygroscopic crystalline solid (Ashford, 1994). It has been used in organic synthesis, abscission of citrus fruit in harvesting (Lewis, 2001), commercial letterpress printing and production of plastics, as well as in medical and surgical hospitals (NIOSH, 1990).

DBA is a hygroscopic crystal (U.S. EPA, 2005a). It has no reported industrial use (NIOSH, 1990).

HAAs are formed in drinking water when chlorine disinfectants used in water treatment react with organic matter (e.g., humic or fulvic acids) and inorganic matter (e.g., bromide ion) naturally present in the raw water (IPCS, 2000). HAAs are the second most frequently occurring DBPs, after THMs.

Various water treatment methods lead to the formation of chlorinated and brominated acetic acids, including chlorination, ozonation and chloramination. In the case of chlorination, hypochlorous acid (HOCl) and the hypochlorite ion (OCl^-) are formed, which in turn react with a bromide ion, if present, oxidizing it to hypobromous acid ($HOBr^-$) and hypobromite ion (OBr^-), respectively. Hypochlorous acid and hypobromous acid then react with natural organic matter (NOM) to form different DBPs, including HAAs. The chlorinated HAAs generally dominate; however, in high-bromide waters, the brominated HAAs may be more prevalent (IPCS, 2000). In the case of ozonation, brominated acetic acids (MBA, DBA) can be formed when organic matter and bromide are present in the source waters (U.S. EPA, 2005a). Chloramination also results in HAA production if chloramine is produced by chlorination followed by ammonia addition (IPCS, 2000).

HAA formation can be appreciable when drinking water is chlorinated under conditions of slightly acid pH (IPCS, 2000). Whereas THM formation increases with increasing pH, HAA formation decreases, hydrolysis likely being a significant factor (Krasner et al., 1989; Pourmoghaddas and Stevens, 1995). Despite the fact that HAAs and THMs have different pH dependencies, their formation appears to correlate strongly when treatment conditions are relatively uniform and when the water has a low bromide concentration (Singer, 1993).

Longer contact times and higher water temperatures are contributing factors in HAA formation. At higher water temperatures, reactions are faster and chlorine demand is higher (Nikolaou et al., 1999). Increased concentrations of NOM with aromatic content (humic acids) in raw water favour formation of HAAs (Reckhow et al., 1990; Nikolaou et al., 1999). Increased concentrations of NOM in raw water also increase the chlorine demand and favour the formation of chlorinated DBPs. In the presence of bromide, the chlorination process may also favour the formation of brominated DBPs, depending on the physical and chemical properties of the water. High chlorine concentrations also favour the formation of higher concentrations of TCA compared with MCA and DCA. However, if bromide levels are high in source waters, the formation of brominated and chloro-brominated HAAs is more likely to occur (Nikolaou et al., 1999). Bromide levels in surface water and groundwater may fluctuate seasonally and may occur as a result of saltwater intrusion or pollution as well as from natural sources (Richardson et al., 1999; IPCS, 2000).

The HAA5 compounds may be released into the environment through various waste streams following their production and use. MCA and TCA can be formed as combustion by-products of organic compounds (waste incineration) in the presence of chlorine (Juuti and Hoekstra, 1998). Other potential sources of atmospheric TCA are the photooxidation of tetrachloroethylene (PCE), trichloroethylene (TCE) and 1,1,1-trichloroethane (Reimann et al., 1996; Sidebottom and Franklin, 1996; Juuti and Hoekstra, 1998; Bakeas et al., 2003), as well as biomass burning and natural formation in the marine boundary layer (Hoekstra, 2003). Some atmospheric MCA may also be formed from the hydrolysis of monochloroacetanilide herbicides (Reimann et al., 1996) and directly or indirectly from car exhaust (Bakeas et al., 2003). DCA is believed to be a minor atmospheric degradation product of TCE (Peters, 2003).

HAAs are present in raw water, possibly the result of chlorinated municipal waste effluent, drinking water inputs, precipitation, the degradation of herbicides and industrial inputs involving reactions between chlorine and organic material. Scott et al. (2002) found that HAA levels in raw surface water corresponded with the level of industrial activity in the surrounding area. Concentrations of HAAs in the Detroit River were as follows: MCA, <0.005–0.59 µg/L; DCA, 0.48–1.2 µg/L; TCA, 0.1–2.2 µg/L; MBA, <0.005–0.04 µg/L; and DBA, <0.005–0.26 µg/L. Lake Malawi (Africa), with little industry, had no detectable levels of HAAs, whereas the Laurentian Great Lakes had total concentrations of approximately 0.5 µg/L, consisting of TCA, DCA and MCA; no significant levels of bromoacetic acids were detected at either of these locations (Scott et al., 2002).

4.1 Environmental fate

Volatilization from water surfaces is not expected based upon the low vapour pressures and high water solubilities of the HAA5 compounds. Low pK_a values indicate that these compounds will exist almost entirely in the ionized form at pH values found in drinking water.

Microbial degradation of MCA in water is most likely the main aquatic degradation pathway. MCA was biodegraded in stream water with 73% conversion to carbon dioxide in 10 days at 29°C under laboratory conditions at the highest concentration used (Boethling and Alexander, 1979). In comparison, DCA was found to be more persistent in the aquatic environment. At a concentration of 10 mg/L, only 14% and 8% degradation were reported for river

water and seawater, respectively, after 3 days of incubation (Kondo et al., 1988). TCA is likely to be relatively persistent in water given its high solubility and low vapour pressure. The estimated half-lives of TCA in a model river and a model lake were 1632 and 12 000 days, respectively (HSDB, 2003). In a study to determine the stability of HAAs incubated in river water and seawater (20°C) for 30 and 9 days, respectively, TCA concentrations did not decrease significantly, whereas MCA, DCA, MBA and DBA almost completely disappeared (Hashimoto et al., 1998). The same authors indicated that approximately half the decomposition was due to microbial activity.

5.0 Exposure

Available data suggest that drinking water may be a significant source of exposure to HAAs, but there are few data available to determine the exposure from other media, such as food and air. Since HAAs are neither volatile nor absorbed significantly through the skin, exposure via dermal and inhalation routes is considered negligible.

5.1 Water

In general, levels of HAAs are highest in treated water from sources with high organic matter content, such as rivers and lakes, and low when the source water is groundwater. Within a single distribution system, however, HAA levels can vary greatly, depending on both water quality (e.g., HAA precursors, pH, temperature, ammonia and carbonate alkalinity) and treatment conditions (e.g., disinfectant dose, contact time, removal of NOM before the point of disinfectant application, prior addition of disinfectant) (Nikolaou et al., 1999; CDBP Task Group, 2000; IPCS, 2000).

Health Canada has conducted a series of studies to characterize the presence of CDBPs, including HAAs, in drinking water from treatment plants of various sizes using surface water and groundwater and using different disinfection processes. A 1995 survey investigated 53 sites, covering nine Canadian provinces, to determine the concentrations of HAA5 in drinking water for larger communities (10 000–100 000 people). Treatment plants in the study used one of three disinfection processes: chlorine–chlorine (n = 35), chlorine–chloramine (n = 10) and ozone–chloramine (n = 7). Samples were collected during winter and summer months for raw water, treatment plant water (after final disinfection) and treated water from the distribution system (5–10 km from the treatment plant) (Health Canada, 1995).

All HAAs were non-detectable (<0.01 µg/L) or at very low levels in raw water. DCA and TCA were the major HAAs present in treatment plants and distribution systems (winter and summer) for all treatment processes; concentrations ranged from 0.2 to 163.3 µg/L and from <0.1 to 473.1 µg/L, respectively. MCA, MBA and DBA were detected at concentrations ranging from 0.03 to 9.7 µg/L, from <0.01 to 9.2 µg/L and from <0.01 to 1.9 µg/L, respectively. Most treatment plants and distribution systems had DCA concentrations below 50 µg/L. Generally, mean DCA concentrations in treatment plants and distribution systems were higher for the chlorine–chlorine disinfection process, and mean summer concentrations in treatment plants and distribution systems were slightly higher than those in winter. Most treatment plants and distribution systems had TCA levels below 50 µg/L, although a few facilities using the chlorine–chlorine process had relatively high values (>100 µg/L). As with DCA, mean TCA

concentrations were higher in summer than in winter for all processes. A comparison of TCA concentrations for the chlorine–chlorine process for both seasons indicated that there was a marked increase going from the treatment plant to the distribution system (Health Canada, 1995).

Table 3 shows the results of another Health Canada study (Aranda-Rodriguez et al., 2002; Health Canada, 2003), in which CDBPs (including HAA5) were surveyed in treated drinking water from small systems located in 27 communities (<10 000 people) within nine provinces. Sixteen of the 27 systems used chlorine only, while the balance used chlorine combined with flocculation and filtration processes. A majority of the locations (n = 23) used surface water, whereas two used groundwater and two used a combination of surface water and groundwater. Samples were collected in the warm water season (August to September 1999) and cold water season (January to March 2000) from five sites at each location: raw water, treatment plant (T) and within the distribution system at 0.1–6 km (D1, close to treatment facility), 0.75–16 km (D2, midpoint of system) and 1–23 km (D3, far system site).

No CDBPs were detected in raw water samples. In the treated water, THMs and HAAs accounted for 80% of the CDBPs. DCA and TCA were the most prevalent HAAs, and their concentrations for all locations ranged from <0.3 to 231 µg/L and from <0.1 to 257 µg/L, respectively. Concentrations of MCA, MBA and DBA ranged from <0.3 to 17.4 µg/L, from <0.4 to 18 µg/L and from <0.1 to 4.6 µg/L, respectively.

DCA and TCA concentrations in summer for small treatment plants and distribution systems significantly exceeded those in winter, whereas MCA concentrations in summer slightly exceeded those in winter. In summer, mean concentrations of MCA, DCA and TCA peaked in the treatment plant (T), at the D1 site and at the D2 site, respectively, indicating different formation–degradation patterns for these compounds in warm water conditions (Table 3). In winter, mean concentrations of MCA, DCA and TCA all peaked at the D2 site. Concentrations of MBA and DBA were relatively constant, regardless of the site or season.

Average DCA levels at D2 (midway in the distribution system) during summer (57.4 µg/L, Table 3) were higher than the average system concentrations of DCA in larger facilities (19.0 µg/L, chlorine–chlorine). A similar comparison for the cold water season revealed that average DCA concentrations were higher in the small systems (41.5 µg/L, Table 3) than in the larger systems (15.6 µg/L, chlorine–chlorine). Generally, a greater fraction of small systems had DCA values above 50 µg/L, and concentrations tended to increase in the distribution system after treatment (Table 3), whereas DCA concentrations appeared to level off to a greater extent in the distribution systems of larger facilities (chlorine–chlorine).

Table 3: HAA concentrations in small distribution systems (Health Canada, 2003)

		HAA concentrations (µg/L)			
		Summer		Winter	
Compound	Site	Mean	Range	Mean	Range
MCA	T	3.7	<0.3–17.4	1.6	<0.3–9.2
	D1	3.6	<0.3–16.6	2.0	<0.3–5.2
	D2	3.5	<0.3–12.4	2.4	<0.3–10.1
	D3	2.7	<0.3–8.1	1.8	<0.3–5.7
DCA	T	55.1	0.8–227	26.3	1.8–180
	D1	59.6	0.4–231	31.8	0.5–109
	D2	57.4	<0.3–195	41.5	0.5–188
	D3	43.0	<0.3–134	29.9	0.5–85.2
TCA	T	43.3	0.3–246	22.4	0.4–179
	D1	60.1	<0.1–257	32.8	<0.1–119
	D2	65.9	<0.1–198	42.6	<0.1–192
	D3	56.3	<0.1–230	34.4	<0.1–125
MBA	T	<0.4	<0.4–18	<0.4	<0.4
	D1	<0.4	<0.4–1.5	<0.4	<0.4
	D2	<0.4	<0.4–1.7	<0.4	<0.4–0.5
	D3	<0.4	<0.4–2.2	<0.4	<0.4–0.6
DBA	T	0.4	<0.1–2.8	0.3	<0.1–3.6
	D1	0.3	<0.1–3.4	0.4	<0.1–3.3
	D2	0.5	<0.1–4.6	0.5	<0.1–4.2
	D3	0.3	<0.1–3.7	0.4	<0.1–3.4

Average TCA levels were always higher in the distribution system than in the treatment plant, regardless of system size or season. A comparison of mean TCA values during summer in the distribution systems of small and large facilities indicated that smaller facilities (65.9 µg/L, D2, Table 3) had higher concentrations than large facilities (48.9 µg/L, chlorine–chlorine). In wintertime, there were higher concentrations in the distribution system of larger facilities (56.7 µg/L, chlorine–chlorine) than in the small facilities (42.6 µg/L, D2, Table 3).

The above Health Canada studies indicated that of the five HAAs, DCA and TCA were present in the highest concentrations. DCA and TCA levels ranged from <0.3 to 231 µg/L and from <0.1 to 473 µg/L, respectively, for both studies. Generally, concentrations of both compounds peaked in the distribution system (chlorine treatment) and decreased in the extremities of the system, were higher in summer than in winter and were higher in smaller facilities than in larger ones. Frequently, DCA peaked before TCA, indicating that the former may have a faster rate of formation and degradation. The remaining HAA5 compounds, MCA, MBA and DBA, were found at concentrations ranging from <0.01 to 18 µg/L. A comparison of HAA5 levels for the different disinfection processes indicated that levels were generally higher for plants using chlorine. Since HAA5 concentrations vary within and between distribution systems, depending on different factors, including water quality characteristics (e.g., HAA precursors, pH, season, temperature) and treatment conditions (e.g., disinfectant type, disinfectant dose, contact time), it is recommended that monitoring samples be taken at the water treatment plant and at points in the distribution system where historical data show the highest HAA concentrations.

The spatial variation in HAA5 concentrations in the distribution systems noted in these studies may be explained in part by differences between disinfectant residuals (chlorine versus chloramine) and the susceptibility of individual HAAs to microbial biodegradation. A U.S study (Williams et al., 1998) reported unexpectedly low HAA concentrations at the maximum residence time locations in distribution systems. Analysis of water quality parameters revealed that water at the maximum residence time locations had low levels of free chlorine and high heterotrophic plate counts. Others have previously identified specific bacteria and haloacid dehalogenase as being capable of degrading DCA (Uchiyama et al., 1992; Meusel and Rehm, 1993). Research on haloacid dehalogenase has shown it to have some degree of substrate selectivity, where MCA, DCA, MBA and DBA were degraded while TCA was not (Ploeg et al., 1991). Another factor that may affect the spatial variation of HAA5 in distribution systems is the pH. The rate of formation of TCA is significantly favoured by low pHs (<pH 7), whereas the rate of DCA formation is only slightly higher at low pHs (Miller and Uden, 1983).

Data from 193 communities in Newfoundland and Labrador for the period 1999–2003 indicated that DCA and TCA were the main HAAs present in treated distributed water. Samples had the following concentrations of HAA5: TCA, <1–600 µg/L (average 66.2 µg/L); DCA, <1–499 µg/L (average 50.2 µg/L); MCA, <1–15 µg/L (average 1.1 µg/L); DBA, <1–13 µg/L (average 0.4 µg/L); and MBA, <1–4 µg/L (average 0.1 µg/L). Total HAA5 concentrations for all communities ranged from <1 to 1114 µg/L and averaged approximately 111 µg/L (Newfoundland and Labrador Department of Environment, 2003).

Monitoring data (1999–2003) for 178 Ontario communities similarly indicated that DCA and TCA were the main HAAs present in treated distributed water. Concentration ranges for HAA5 compounds were as follows: TCA, <0.05–141 µg/L; DCA, 0.2–95.9 µg/L; MCA, 0.5–30.5 µg/L; MBA, 0.05–26.6 µg/L; and DBA, 0.05–17.0 µg/L. Total average HAA5 concentrations (based on individual averages for each compound) for all communities ranged from approximately 1.2 to 142.8 µg/L (Ontario Ministry of Environment and Energy, 2003).

Monitoring data from 37 communities in Manitoba for 2000 indicated that DCA, MCA and TCA were the main HAAs present in treated plant water. Samples (n = 47) had the following concentrations: DCA, <0.5–210 µg/L (average 63 µg/L); MCA, <1–51 µg/L (average 7.9 µg/L); TCA, <0.5–35 µg/L (average 6.7 µg/L); DBA, <0.5–5.4 µg/L (average 0.9 µg/L); and MBA, <0.5–3.1 µg/L (average 0.9 µg/L). Total HAA5 concentrations for all communities ranged from 2.5 to 268 µg/L and averaged approximately 80 µg/L (Manitoba Department of Conservation, 2004).

Non-ingestion exposure (dermal and inhalation) to HAAs via showering and bathing was found to be insignificant, because these compounds are neither volatile nor lipophilic (Xu et al., 2002; Xu and Weisel, 2003).

5.1.1 Analysis of HAA5 data

In order to obtain a better understanding of how average HAA5 data for surface water varied in magnitude and if there were any major differences according to community size, an analysis of provincial and territorial monitoring data from 1990 to 2004* was carried out. Average HAA5 values for each location were calculated based on data (n = 1–24) provided for seasonal quarters (January–March, April–June, July–September, October–December) for the

period 1999–2004.* The data used were not necessarily quarterly averages because of data scarcity; some locations had data for only one season, and others had data for many seasons. The location of sampling between sites also varied.

5.1.1.1 Communities with >5000 persons

Health Canada received HAA data from 135 water treatment plants (distribution systems) serving communities of greater than 5000 persons, representing a total population of approximately 19.3 million. The majority of these systems were located in Ontario, Quebec, Nova Scotia and Newfoundland and Labrador, with a few plants located in Alberta, British Columbia, Manitoba, Saskatchewan and the Yukon. Of these, 88% had average HAA5 concentrations below 80 µg/L, while 12% exceeded this level (Table 4). On average, DCA accounted for 46% of the total concentration of HAA5. However, for a significant percentage of systems (26%), DCA accounted for 50–59% of HAA5.

Table 4: Average total HAA concentrations above and below 80 µg/L in distribution systems serving communities with >5000 people, by province/territory

Province/territory	No. of systems per province/territory	No. of systems below 80 µg/L HAA5	No. of systems above 80 µg/L HAA5
Alberta	4	4	0
British Columbia	5	5	0
Manitoba	1	0	1
Newfoundland and Labrador	10	5	5
Nova Scotia	13	11	2
Ontario	74	71	3
Quebec	27	23	4
Saskatchewan	1	1	0
Yukon	1	1	0
Total no. of systems	135	119	16
%		88	12

5.1.1.2 Communities with <5000 persons

Health Canada received HAA data from 312 systems serving communities of fewer than 5000 persons, representing a total population of approximately 333 300. The systems were located in Ontario, Quebec, Nova Scotia and Newfoundland and Labrador. Of these, 56% of systems had average HAA5 concentrations below 80 µg/L, whereas 44% of systems exceeded this level. On average, DCA accounted for 42% of the HAA5. However, for a significant percentage of water treatment plants (25%), DCA accounted for 50–59% of HAA5.

* Nova Scotia data were only from 2005.

Table 5: Average total HAA concentrations above and below 80 µg/L in distribution systems serving communities with <5000 people, by province

Province	No. of systems per province	No. of systems below 80 µg/L HAA5	No. of systems above 80 µg/L HAA5
Newfoundland and Labrador	220	108	112
Nova Scotia	38	22	16
Ontario	32	31	1
Quebec	27	16	11
Total no. of systems	312	174	138
%		56	44

5.2 Air

Stack gases of municipal waste incinerators have been reported to contain 0.37–3.7 µg TCA/m^3 and 3.2–7.8 µg MCA/m^3 (Mowrer and Nordin, 1987).

Air samples taken in rural Scotland and the Netherlands contained DCA and TCA concentrations of ≤0.0007 µg/m^3 (Heal et al., 2003; Peters, 2003), whereas atmospheric particulate measurements of MCA, DCA and TCA from Athens, Greece, ranged from 0.01 to 2.01 ng/m^3, from 0.0006 to 0.46 ng/m^3 and from 0.0009 to 0.125 ng/m^3, respectively (Bakeas et al., 2003).

The detection of chlorinated acetic acids in rain water is an indication of their presence in the atmosphere. Reimann et al. (1996) reported the following levels in rainwater: MCA, 0.05–9 µg/L; DCA, 0.05–4 µg/L; and TCA, 0.01–1 µg/L. Rainwater in Germany contained 1.35 µg DCA/L and 0.1–20 µg TCA/L (IARC, 1995). Sidebottom and Franklin (1996) reported that TCA concentrations in rainwater in remote areas (Antarctic, Arctic and sub-Arctic regions) generally ranged from 10 to 100 ng/L.

No information was available on exposure to MBA or DBA in air (U.S. EPA, 2003b). No Canadian data were available.

5.3 Food

It is speculated that MCA, DCA and TCA may be found in meat and other food products (U.S. EPA, 2003b). This would result from the use of chlorine in food production and processing, including the disinfection of chicken; processing of seafoods, poultry and red meats; sanitizing of equipment and containers; and oxidizing and bleaching in the flour industry (U.S. EPA, 1994).

MCA and TCA can be taken up from cooking water (Raymer et al., 2001). In addition, there is evidence that TCA may be taken up by plants via the roots or by leaves via uptake from the air (Schroll et al., 1994; Sutinen et al., 1995). TCA was found in food (vegetables and fruits) at concentrations of 0.01–0.19 mg/kg following irrigation (Demint et al., 1975). Reimann et al. (1996) examined the concentrations of MCA, DCA and TCA in a limited number of samples of several vegetables, fruits, grain and beer. MCA concentrations ranged from <0.7 to 5.3 µg/kg in vegetables, from 1.7 to 13.2 µg/kg in grains, from 2.3 to 11.8 µg/kg in flours/breads and from

0.2 to 2.6 µg/L in beer. DCA concentrations ranged from <0.9 to 3.5 µg/kg in vegetables, from <0.6 to 11.1 µg/kg in grains, from 0.8 to 19.8 µg/kg in flours/breads and from 1.5 to 15.2 µg/L in beer. TCA concentrations ranged from <0.2 to 5.9 µg/kg in vegetables and from <1.6 to 4.1 µg/kg in grains. TCA was below the detection limit of 1.5 µg/kg in breads and was not analysed in the flours or beer. None of these compounds was detected in fruits or tomatoes.

No information on concentrations of MBA or DBA in food was located (U.S. EPA, 2003b).

5.4 Contribution of drinking water to total exposure

The data for HAA5 in water suggest that drinking water has the potential to be a significant source of these compounds. While data for air and food indicate that these media are also potential sources of HAA5, the data are insufficient to quantify their relative contributions with reasonable certainty. On this basis, a default value of 20% can be used to describe the contribution of drinking water to total daily intake. The U.S. Environmental Protection Agency (U.S. EPA, 2003b) came to a similar conclusion with respect to lack of data for air and food and selected a default relative source contribution of 20% for MCA and TCA. No relative source contribution value was assigned specifically to DCA and DBA because it is classified as a carcinogen.

6.0 Analytical methods

HAAs are relatively non-volatile and hydrophilic organic compounds. These properties make common analytical methods (e.g., purge and trap, headspace and liquid–liquid extraction) less effective for HAA separation. To facilitate analysis using gas chromatography with electron capture detector (GC/ECD), these acids must be chemically converted into methyl esters (methylated HAAs).

Three U.S. EPA methods (EPA Method 552.1, EPA Method 552.2 and EPA Method 552.3) are approved for measuring HAA5 in drinking water (U.S. EPA, 1992, 1995, 2003d). Method detection limits (MDLs) for these methods vary depending on the analyte being measured, as outlined in Table 6. The U.S. EPA also recognizes American Public Health Association (APHA) Standard Method 6251B (APHA et al., 2005) as being equivalent to the U.S. EPA standard methods for measuring HAA5 in drinking water (U.S. EPA, 2003a). All methods include sample extraction, methylation and GC/ECD analysis (using a capillary GC/ECD) steps.

EPA Method 552.1 (U.S. EPA, 1992) employs solid-phase extraction via ion exchange resins. EPA Method 552.2 (U.S. EPA, 1995) and APHA Standard Method 6251B (APHA et al., 2005) both employ micro liquid–liquid extraction with methyl *tert*-butyl ether (MTBE) at acidic conditions. Sodium sulphate and sulphuric acid are added to samples to increase extraction efficiency. EPA Method 552.3 (U.S. EPA, 2003e) provides the option of performing an acidic extraction using either MTBE or tertiary amyl methyl ether (TAME) before adding acidic methanol to the extract, followed by heating.

The MDLs and practical quantitation limits (PQLs) for the various methods are summarized in Table 6.

Table 6: Method detection limits and practical quantitation limits for the various methods for analysis of HAAs

	EPA Method 552.1		EPA Method 552.2		EPA Method 552.3		APHA Standard Method 6251B	
Analyte	**MDL (µg/L)[a]**	**PQL (µg/L)**	**MDL (µg/L)[b]**	**PQL (µg/L)**	**MDL (µg/L)[c]**	**PQL (µg/L)**	**MDL (µg/L)[d]**	**PQL (µg/L)**
MCA	0.21	2	0.273	2.5	0.17	1.7	0.082	1
DCA	0.45	5	0.242	2.5	0.020	0.2	0.054	0.6
TCA	0.07	0.7	0.079	0.8	0.019	0.2	0.054	0.6
MBA	0.24	2	0.204	2	0.027	0.3	0.087	0.5
DBA	0.09	1	0.066	0.7	0.012	0.1	0.065	0.6

[a] Hodgeson and Becker (1992).
[b] Munch et al. (1995).
[c] U.S. EPA (2003e).
[d] APHA et al. (2005).

7.0 Treatment technology

Although HAA formation in water is largely a function of the amount of organic compounds in water and their contact time with chlorine, it is important to recognize that the use of chlorination and other disinfection processes has virtually eliminated waterborne microbial diseases. In order to reduce HAA levels in the finished water, it is important to characterize the source water to ensure that the treatment process is optimized for precursor removal.

7.1 Municipal scale

There are three approaches to limiting the concentrations of HAAs in municipally treated drinking water:

- treatment of water to remove HAA precursors prior to disinfection;
- the use of alternative disinfectants and disinfection strategies; and
- treatment of water to remove HAAs after their formation.

The majority of changes occurring in the water industry now focus on strategies to remove DBP precursors prior to disinfection and the use of alternative disinfectants and alternative disinfection strategies.

7.1.1 Removal of precursors prior to municipal disinfection

The removal of organic precursors is the most effective way to reduce the concentrations of all DBPs, including HAAs, in finished water (U.S. EPA, 1999c; Reid Crowther & Partners Ltd., 2000). These precursors include synthetic organic compounds and NOM, which can react with disinfectants to form HAAs. Removing HAA precursors will also result in the formation of lower concentrations of HAAs (Reid Crowther & Partners Ltd., 2000). Conventional municipal-scale water treatment techniques (coagulation, sedimentation, dissolved air flotation, precipitative softening and filtration) can reduce the amount of HAA precursors, but are ineffective in removing HAAs once they are formed. Granular activated carbon (GAC),

membranes and ozone–biofiltration systems can also remove organic matter from water. The U.S. EPA has identified precursor removal technologies such as GAC and membrane filtration, specifically nanofiltration, as Best Available Technologies (BAT) for controlling DBP formation (U.S. EPA, 2005b).

Potassium permanganate can be used to oxidize organic precursors at the head of the treatment plant, thus minimizing the formation of by-products at the disinfection stage (U.S. EPA, 1999a). The use of ozone for oxidation of precursors is currently being studied. Early work has shown that the effects of ozonation, prior to chlorination, depend on treatment design and raw water quality and thus are unpredictable. The key variables that seem to determine the effect of ozone are dose, pH, alkalinity and the nature of the organic material in the water. Ozone has been shown to be effective at reducing precursors at low pH. However, at pH levels above 7.5, ozone may actually increase the production of CDBP precursors (U.S. EPA, 1999a).

7.1.2 Alternative municipal disinfection strategies

The use of alternative disinfectants, such as chloramines (secondary disinfection only), ozone (primary disinfection only) and chlorine dioxide (primary and secondary disinfection), is increasing. However, each of these alternatives has also been shown to form its own set of DBPs. Combinations of disinfectants, when optimized, can help control HAA formation. Pre-ozonation is feasible for water sources that have turbidity levels below 10 nephelometric turbidity units (NTU) and bromide concentrations below 0.01 mg/L, to minimize the formation of bromate (Reid Crowther & Partners Ltd., 2000). Ultraviolet (UV) disinfection is also being used as an alternative disinfectant. Since UV disinfection is dependent on light transmission to the microbes, water quality characteristics affecting UV transmittance must be considered in the design of the system. UV irradiation at typical doses and wavelengths does not affect HAA formation in subsequent chlorination or chloramination steps (Reid Crowther & Partners Ltd., 2000). Neither ozone nor UV disinfection leaves a residual disinfectant, and both must therefore be used in combination with a secondary disinfectant to maintain a residual in the distribution system.

It is recommended that any change made to the treatment process, particularly when changing the disinfectant, be accompanied by close monitoring of lead levels in the distributed water. A change of disinfectant has been found to affect the levels of lead at the tap; in Washington, DC, for example, a change from chlorine to chloramines resulted in significantly increased levels of lead in the distributed drinking water. When chlorine, a powerful oxidant, is used as the disinfectant, lead dioxide scales formed in distribution system pipes reach a dynamic equilibrium in the distribution system. In Washington, DC, switching from chlorine to chloramines decreased the oxidation–reduction potential of the distributed water and destabilized the lead dioxide scales, which resulted in increased lead leaching (Schock and Giani, 2004). Subsequent laboratory experiments by Edwards and Dudi (2004) and Lytle and Schock (2005) confirmed that lead dioxide deposits could be readily formed and subsequently destabilized in weeks to months under realistic conditions of distribution system pH, oxidation–reduction potential and alkalinity.

7.1.3 Removal of HAAs after formation

Although precursor removal is considered the most effective approach to reduce HAA concentrations, removal of HAAs is also possible. Adsorption onto activated carbon is widely used to remove organic compounds such as HAAs from drinking water. This method involves pumping water through a bed of activated carbon onto which HAA molecules become attached (adsorbed). If an activated carbon filter bed is deep enough to allow sufficient contact time, it can be effective in removing HAAs from drinking water. Biofiltration may be an effective process for removing biodegradable organic matter and biodegradable DBPs from water. GAC, anthracite, sand and garnet are common media that support colonization by bacteria and can be used as biological filters. Information on the use of biofiltration for HAA removal is limited, although work has shown that bacteria-colonized GAC (biologically active carbon) is an effective process for HAA removal (Xie, 2004).

7.2 Residential scale

Generally, it is not necessary to use drinking water treatment devices with municipally treated water. In cases where municipal treatment has produced low concentrations of HAAs in drinking water, some residential-scale point-of-entry or point-of-use treatment technologies such as activated carbon, reverse osmosis or distillation systems may remove the HAAs from the drinking water. At this time, however, none is certified specifically for HAA removal.

NSF International (NSF) has developed several standards for residential water treatment devices designed to reduce the concentrations of various types of contaminants in drinking water, but HAAs are not currently included in any NSF standard. Research is ongoing in the private and public sectors to test and adopt efficient methods for the reduction of HAAs in drinking water. Products that use adsorption or reverse osmosis technology can lose removal capacity through usage and time and need to be maintained and/or replaced. Consumers should verify the expected longevity of the adsorption media or membrane in their treatment device as per the manufacturer's recommendations and service it when required.

Health Canada does not recommend specific brands of drinking water treatment devices, but it strongly recommends that consumers look for a mark or label indicating that the device has been certified by an accredited certification body as meeting the appropriate NSF/American National Standards Institute (ANSI) standards. These standards have been designed to safeguard drinking water by helping to ensure the material safety and performance of products that come into contact with drinking water. Certification organizations provide assurance that a product conforms to applicable standards and must be accredited by the Standards Council of Canada (SCC). In Canada, the following organizations have been accredited by the SCC to certify treatment devices and materials as meeting NSF/ANSI standards:

- Canadian Standards Association International (www.csa-international.org);
- NSF International (www.nsf.org);
- Water Quality Association (www.wqa.org);
- Underwriters Laboratories Inc. (www.ul.com);
- Quality Auditing Institute (www.qai.org); and
- International Association of Plumbing & Mechanical Officials (www.iapmo.org).

An up-to-date list of accredited certification organizations can be obtained from the SCC (www.scc.ca).

8.0 Kinetics and metabolism

In the following studies, and in those in subsequent sections, HAAs were administered as the free acid, as the sodium salt or as a neutralized solution, depending on the study methodology. The sodium salt and neutralized solution of the HAA both result in the salt being formed and are designated as, for example, "MCA (sodium salt)." The free acid is designated as, for example, "MCA (acid)." The form of HAA used in each study is noted, because the form can influence the effects seen in the test systems. When it is described in the following studies that the HAA was neutralized, it signifies that sodium hydroxide was the base used to adjust the pH. In the event that another base was used, this is noted in the description of the study.

8.1 Absorption

8.1.1 Monochloroacetic acid

Experiments with buffered solutions of MCA at pH 7 across human skin using *in vitro* diffusion chambers failed to show evidence of significant dermal absorption (Xu et al., 2002). The authors stated that ionization may be the most significant factor limiting the permeability of HAAs.

ECETOC (1999) reported that MCA given orally to rats or mice was rapidly and extensively absorbed.

8.1.2 Dichloroacetic acid

DCA is also readily absorbed into the bloodstream from the gastrointestinal tract following oral exposure in both rats and humans (Stacpoole et al., 1987, 1998a; James et al., 1998; Schultz et al., 1999). Dermal absorption in humans is minor both *in vivo* (Kim and Weisel, 1998) and *in vitro* (using diffusion chambers with a buffered solution of DCA) (Xu et al., 2002). DCA exists primarily as an ionic species in drinking and swimming pool waters that are kept within a neutral pH range (Kim and Weisel, 1998), which limits its dermal absorption (Xu et al., 2002).

8.1.3 Trichloroacetic acid

TCA is readily absorbed from the gastrointestinal tract following oral exposure in both rats and humans (Kim and Weisel, 1998; Schultz et al., 1999). The concentration of TCA in blood in rats following oral ingestion peaked at approximately 2 hours post-dosing (Schultz et al., 1999). No evidence of significant dermal absorption was seen with TCA in humans *in vivo* (Kim and Weisel, 1998) or using diffusion chambers *in vitro* (Xu et al., 2002).

8.1.4 Monobromoacetic acid

No specific studies measuring the absorption of MBA following different routes of exposure were undertaken; however, acute oral studies have shown that MBA is absorbed and causes adverse effects (see Section 10.1).

8.1.5 Dibromoacetic acid

DBA is rapidly absorbed into the bloodstream from the gastrointestinal tract following oral exposure in rats; the blood concentration peaked at approximately 1 hour post-dosing (Schultz et al., 1999). Schultz et al. (1999) estimated the oral bioavailability of DBA (using only a single high dose) at only 30%. They postulated that it was due to first-pass metabolism. No other doses were used to confirm this value.

Other short-term studies (Linder et al., 1994a,b, 1995, 1997b; Parrish et al., 1996; Cummings and Hedge, 1998; Vetter et al., 1998; NTP, 1999b) report effects on the liver, kidney, spleen and male reproductive system, demonstrating that DBA is sufficiently absorbed to have caused adverse effects.

No evidence of significant dermal absorption was seen with DBA at pH 7 across human skin using *in vitro* diffusion chambers (Xu et al., 2002). The authors stated that ionization may be the most significant factor limiting the permeability of HAAs, including DBA.

8.2 Metabolism

8.2.1 Monochloroacetic acid

Two different pathways have been proposed for the breakdown of MCA in biological systems (ECETOC, 1999):

- formation of *S*-carboxymethyl glutathione and subsequently *S*-carboxymethyl cysteine, which is then metabolized to thiodiacetic acid (main route); and
- formation of glycolic acid following hydrolysis of the C–Cl bond; subsequent oxidation leads to the formation of oxalic acid and carbon dioxide.

Other metabolic pathways suggested are via dehalogenation to form oxalate and glycine and/or dehalogenation and reduction to thiodiacetic acid via glutathione conjugation (Bhat et al., 1990). MCA (acid) has also been reported to bind to lipids (Yllner, 1971; Bhat and Ansari, 1989; Kaphalia et al., 1992).

8.2.2 Dichloroacetic acid

DCA's principal metabolic pathway occurs via oxidative dechlorination to form glyoxylate (Keys et al., 2004). Glyoxylate can be further biotransformed to oxalate (by oxidation), to glycine (by transamination) and subsequently to glycine conjugates such as serine and/or 5,10-methylene tetrahydrofolate, or to glycolate (by reduction); all of these metabolites are excreted in variable quantities in the urine (Stacpoole, 1989; James et al., 1998; Stacpoole et al., 1998a; U.S. EPA, 2003c). Some DCA is also converted to carbon dioxide and eliminated via exhaled air (James et al., 1998). DCA can also be metabolized through reductive dechlorination to form MCA and subsequently thiodiacetate (James et al., 1998).

The enzyme that initially catalyses the glutathione-dependent oxygenation of DCA has been identified as glutathione-*S*-transferase-zeta (GST-zeta) and is found primarily in the cytosol (Tong et al., 1998a,b). Appreciable differences in the metabolism of DCA exist between species.

The half-lives of DCA in mice and rats following oral dosing were 1.5 hours and 0.9 hour, respectively (Larson and Bull, 1992). Repeat dosing with DCA has also shown an increased plasma elimination half-life in both rats and humans (Anderson et al., 1999).

Toxicokinetics studies indicate that DCA is able to inhibit its own metabolism (also known as suicide inhibition) by irreversibly inactivating the GST-zeta enzyme (U.S. EPA, 2003c; Keys et al., 2004). Prior treatment with DCA has been shown to inhibit the metabolic clearance of subsequent doses of DCA in rats (James et al., 1998), mice (Schultz et al., 2002) and humans (Curry et al., 1985; Stacpoole et al., 1998a).

Species- and age-related differences in GST-zeta activity were observed. The relative rate of DCA transformation was greater in mice and rat cytosol than in human hepatic cytosol (Tong et al., 1998a). Reduced liver metabolism was seen in young mice, accompanied by a decrease in immunoreactive GST-zeta, whereas the levels of that protein remained unchanged in aged mice (Schultz et al., 2002).

Pharmacokinetic models, created to help estimate concentrations of DCA in the liver, may be useful to refine the tissue dose–response for liver tumours. However, these models are limited, as they can provide estimates of liver concentrations only where the metabolism is not inhibited or is at its maximum inhibition. Partial inhibition is difficult to model, since concentrations may vary depending on GST-zeta activity (U.S. EPA, 2003c).

Carcinogenic and genotoxic effects have been associated with high doses of DCA, where its metabolism is inhibited (U.S. EPA, 2003c).

However, as reported in the U.S. EPA (2003c) Toxicological Review of DCA, there are still many unanswered questions regarding DCA's metabolism, including whether there is more than one metabolic pathway, and its relevance to toxicity in laboratory animals and humans.

8.2.3 Trichloroacetic acid

A relatively small proportion of TCA is metabolized in the liver. The formation of carbon dioxide, glyoxylic acid, oxalic acid, glycolic acid and DCA was observed in rats and mice following oral administration of radiolabelled TCA (neutralized). It was suggested that TCA was metabolized by reductive dehalogenation to DCA (Larson and Bull, 1992). Further reductive dehalogenation of DCA to MCA and ultimately to thiodiglycolate has been proposed as a metabolic pathway (Bull, 2000). However, other investigators have suggested that metabolism to DCA may have been over-reported in earlier studies due to analytical methodologies that convert TCA to DCA due to the presence of a reagent (Ketcha et al., 1996; Lash et al., 2000).

8.2.4 Monobromoacetic acid

As part of a larger metabolism study (Jones and Wells, 1981), a group of three male Sprague-Dawley rats was orally administered MBA (sodium salt), equivalent to 50 mg MBA/kg bw, and the urine was collected for 24 hours. Unchanged MBA was excreted in the urine within 24 hours, along with *N*-acetyl-*S*-(carboxymethyl)cysteine. No other details were provided in the study.

8.2.5 Dibromoacetic acid

An *in vitro* metabolism study conducted by Tong et al. (1998a) demonstrated that GST-zeta enzyme catalysed the oxygenation of DBA to glyoxylic acid, a pathway shared by DCA. WHO (2004c) reported that glyoxylic acid can be metabolized to glycine, glycolate, carbon dioxide or oxalic acid, based on a study by Stacpoole et al. (1998b).

8.3 Distribution

8.3.1 Monochloroacetic acid

After oral administration of a single toxic dose (225 mg/kg bw) of MCA (acid) to rats, levels initially remained below those seen following administration of a subchronic dose (10 mg/kg bw), because most of the toxic dose was retained in the stomach for up to 8 hours (a spasm of the pyloric sphincter prohibited further flux for several hours) (Saghir and Rozman, 2003).

Kaphalia et al. (1992) found that the liver, kidney, intestine and spleen were the organs containing the highest MCA levels when it was orally administered (as an acid) to rats as a single dose.

8.3.2 Dichloroacetic acid

DCA, when administered via gavage, is distributed initially to the liver and muscle and subsequently to other target organs (Evans, 1982; James et al., 1998). James et al. (1998) administered a single radiolabelled oral dose of DCA (sodium salt) to young adult rats, and the dose distributed mostly to the muscles (11.9%), liver (6.19%), gastrointestinal tract (3.74%), fat (3.87%) and kidney (0.53%). Other tissues, including plasma, spleen, heart, skin, bone, brain, lung and testes, accounted for 9.5% of the administered dose. DCA also exhibited low binding to plasma when given intravenously (Schultz et al., 1999). Schultz et al. (1999) noted that the lipophilicity of DCA was low when measured at a pH value close to that of blood (pH 7.4), which indicates that DCA would not tend to accumulate in fat.

8.3.3 Trichloroacetic acid

Following oral and intravenous administration in rats, TCA appears to bind significantly to plasma proteins and is also distributed to the liver (Templin et al., 1993; Schultz et al., 1999; Yu et al., 2000). Because of the significant binding to plasma, only the free TCA is available to tissues for uptake and elimination (Yu et al., 2000). Plasma protein binding has been found to vary across species and is highest in humans (Lumpkin et al., 2003).

8.3.4 Monobromoacetic acid

No specific studies measuring the distribution of MBA in various tissues following different routes of exposure were undertaken; however, acute oral studies have shown that MBA is absorbed and causes adverse effects (see Section 10.1).

8.3.5 Dibromoacetic acid

DBA (acid) was detected in the plasma of male and female Sprague-Dawley rats following exposure via deionized drinking water in a range-finding reproductive/developmental study (Christian et al., 2001). DBA was not detected in the plasma of female B6C3F1 mice administered DBA in drinking water for 28 days (NTP, 1999b). This may be due to extensive metabolism and excretion and not to limited absorption (U.S. EPA, 2005a). Detectable and quantifiable levels of DBA were also found in the placenta, amniotic fluid and milk (Christian et al., 2001). According to Christian et al. (2001), no apparent accumulation of DBA was observed. DBA was also detected in testicular interstitial fluid when male Sprague-Dawley rats (number

not given) were gavaged for 5 days with DBA (neutralized) at 250 mg/kg bw (Holmes et al., 2001). The concentration of DBA peaked at 30 minutes and exhibited a half-life of 1.5 hours.

The lipophilicity of DBA was low when measured at a pH value close to that of blood (pH 7.4), indicating that DBA would not tend to accumulate in fat. DBA also exhibited low binding to plasma when given intravenously (Schultz et al., 1999).

8.4 Excretion

8.4.1 Monochloroacetic acid

Urination is reported as the major route of MCA elimination in rats when dosed orally or dermally with MCA (acid) (Saghir and Rozman, 2003). Approximately 90% of a single oral dose of MCA (acid) given to rats was excreted in the urine within 24 hours (Kaphalia et al., 1992).

8.4.2 Dichloroacetic acid

DCA is mostly eliminated either unchanged or by metabolic transformation primarily in expired air or in the urine. In the urine of rodents, the amount eliminated as unmetabolized DCA or as metabolites varies according to the dose. At low doses, DCA is almost completely eliminated in the urine as metabolites, while a higher percentage of unmetabolized DCA was seen using higher or repeat doses of DCA (Lukas et al., 1980; Lin et al., 1993; Gonzalez-Leon et al., 1997; Cornett et al., 1999), possibly due to the inhibition of its metabolism. In rodents and humans, variable levels of metabolites are found in the urine.

DCA is also eliminated from the lungs as carbon dioxide, but the levels may differ between species. Studies with rats and mice showed that carbon dioxide represented 24–30% and 2–45% of the total dose, respectively (Larson and Bull, 1992; Lin et al., 1993; Xu et al., 1995). Less than 2% of DCA was recovered in the faeces in animal studies (Larson and Bull, 1992; Lin et al., 1993).

DCA is a metabolite of TCE in humans and has been detected in the seminal fluid of some workers exposed to TCE (Forkert et al., 2003).

8.4.3 Trichloroacetic acid

The primary route of excretion for TCA given orally or intravenously is the urine (Templin et al., 1993; Schultz et al., 1999; Yu et al., 2000).

In a limited metabolism study, three volunteers ingested TCA (sodium salt) at a dose of 3 mg/kg bw; the elimination half-life from the blood was 50.6 hours (Muller et al., 1974). In a longitudinal exposure pilot study, Bader et al. (2005) measured the elimination half-life of TCA in the urine of five volunteers. These volunteers were provided tap water (concentrations of TCA ranged from 50 to 180 µg/L) for the first 2 weeks and then TCA-free bottled water for the last 2 weeks. Individual TCA urinary elimination rates ranged from 2.1 to 6.3 days. TCA appears to persist for several days when a steady state is almost reached within the plasma, therefore reflecting average exposure over several days. The authors inferred that TCA in plasma may be a viable biomarker for drinking water exposure.

Since TCA is one of the major metabolites of TCE and PCE in humans (Monster et al., 1979; IARC, 1995; ACGIH, 2001; Forkert et al., 2003), several metabolism studies looked at the half-life of the parent compounds as well as their metabolites, such as TCA, and were included in this review.

After volunteers inhaled 50–100 ppm TCE (6 hours/day for 5–10 days), the elimination half-life of TCA from the blood ranged from 85 to 99 hours, a higher value than that obtained following ingestion of TCA (sodium salt) (Muller et al., 1974).

Allen and Fisher (1993) developed a physiologically based pharmacokinetic model for humans exposed to TCE with emphasis on the metabolite, TCA, and compared it with findings in mice and rats (Allen and Fisher, 1993). The volume of TCA distribution in humans was found to be lower than in rats or mice. The model estimated that, in humans, 93% of total TCA eliminated was excreted unchanged in the urine, while the remainder may be metabolized or eliminated by other routes (Allen and Fisher, 1993).

Another inhalation study looked at the comparative excretion rates of inhaled PCE and its metabolites in rats and humans (Volkel et al., 1998). The mean elimination half-life of TCA in the urine was 45.6 hours in humans, compared with 11.0 hours in rats, suggesting that elimination of TCA in rats is more rapid and may be due to differences in PCE metabolism (Volkel et al., 1998).

TCA, being one of the major metabolites of TCE and PCE in humans, has been used as a biomarker of occupational exposure to these chemicals (Monster et al., 1979; IARC, 1995; ACGIH, 2001; Forkert et al., 2003). TCA is also a metabolite of 1,1,1-trichloroethane, 1,1,2,2-tetrachloroethane and chloral hydrate (IARC, 1995).

8.4.4 Monobromoacetic acid

No studies on MBA excretion were identified.

8.4.5 Dibromoacetic acid

Only one study on DBA excretion was located. Schultz et al. (1999) exposed rats intravenously to a single dose of 109 mg DBA/kg bw, and the elimination half-life was calculated at 0.72 hour. The major route of elimination was believed to be via biotransformation. The urine and faeces were very minor contributors to overall blood clearance when DBA was given intravenously to rats, with the urine representing less than 1% of total clearance and the amount in the faeces being negligible (Schultz et al., 1999).

9.0 Health effects in humans

A limited population-based case–control study conducted in Nova Scotia and eastern Ontario did not find an association between HAA exposures and stillbirth risk when controlling for total THM exposures (King et al., 2005). The analysis included 112 stillbirth cases and 398 live birth controls that occurred between 1999 and 2001. No other specific information was available on the teratogenic, reproductive or embryotoxic effects of chlorinated HAAs in humans (CHEMINFO, 2003a,b,c,d,e). Epidemiological studies have been conducted with CDBPs to determine if exposure contributes to reproductive and developmental effects in humans (Bove et al., 1995; Mills et al., 1998). No other information was available to assess the effects of

individual HAAs, nor have HAAs been satisfactorily dissociated from the many other by-products (hundreds, if not thousands) that are produced by chlorination. Therefore, it is difficult to infer causality between specific HAAs and adverse reproductive and developmental health outcomes in humans based on one limited study.

Epidemiological studies on the incidence of cancer have been conducted with CDBPs, but not specifically with chlorinated HAAs. Cohort and descriptive studies have also been conducted with TCE and PCE. Biological monitoring of exposure to both these compounds was done by measuring the urinary levels of DCA or TCA, which have been identified as metabolites of these two compounds (TCE and PCE) in humans (IARC, 1995). TCA has also been identified as a metabolite of other chlorine-containing ethanes and ethylenes (ACGIH, 2001).

Most chlorinated HAAs are found in drinking water (where the pH range of 6–9 is close to neutral) almost exclusively in the ionized form (anion) due to their very low pK_a values (IPCS, 2000). HAAs are found in drinking water at very low concentrations, which means that the dilution of HAAs in drinking water and the buffer capacity of drinking water would mostly counter the irritative properties described below for concentrated acid solutions of HAAs.

9.1 Monochloroacetic acid

The probable lethal oral dose for MCA (acid) in humans is in the range of 50–500 mg/kg bw (Gosselin et al., 1984). In one fatal case, a 5-year-old girl ingested a teaspoon (5–6 mL) of a wart remover composed of 80% MCA (acid); she began vomiting, collapsed and died 8 hours later. The cause of her death was attributed to metabolic acidosis and cardiac arrhythmia. The autopsy revealed that the liver was damaged, and the stomach had signs of marked irritation (Feldhaus et al., 1993; Rogers, 1995).

The sodium salt of MCA is not corrosive to the skin and has limited skin absorption (ECETOC, 2001). MCA does not easily form a vapour (CHEMINFO, 2003a,b). No cases of acute intoxication from MCA (sodium salt) have been located in the literature (ECETOC, 2001).

Three adult volunteers who drank 300 mL of a 0.05% aqueous solution of MCA (acid) daily for a period of 60 days (corresponding to 2 mg/kg bw per day) experienced no adverse health effects (Morrison and Leake, 1941; NTP, 1992). Dilute solutions of MCA (acid) (i.e., up to 1% aqueous) failed to produce irritation of human skin (Morrison and Leake, 1941).

9.2 Dichloroacetic acid

Patients diagnosed with genetic disorders, such as familial hypercholesterolaemia (a common disorder of lipid metabolism associated with a high risk of early mortality from coronary artery disease) or various mitochondrial disorders and, as a result, treated daily with DCA (sodium salt) for periods longer than 4 months were found to develop peripheral neuropathy (loss of reflexes and muscle weakness) and in one case hepatomegaly (enlarged liver) (Moore et al., 1979; Spruijt et al., 2001; Izumi et al., 2003).

Humans exposed in an occupational setting to concentrated DCA mist or vapour may develop pulmonary oedema several hours after exposure (CHEMINFO, 2003c).

9.3 Trichloroacetic acid

In an occupational setting, concentrated solutions and/or mists of TCA (acid) have caused skin effects (redness, swelling, pain and possibly burns), eye effects (severe irritation or corrosive injury) or damage to the gastrointestinal tract due to accidental ingestion (CHEMINFO, 2003d,e). According to CHEMINFO (2003d,e), the severity of the injury (dermal, oral, ocular) increases with the concentration of the acid and the duration of exposure.

TCA did not induce chromosomal aberrations in human lymphocytes (IARC, 1995). No reports were available on *in vivo* mutagenicity studies in humans (CHEMINFO, 2003d,e).

No long-term studies on humans exposed to TCA were located in the literature (IARC, 1995; OEHHA, 1999).

9.4 Monobromoacetic acid

No studies reporting human health effects from exposure to MBA were reported.

9.5 Dibromoacetic acid

No studies were located in the literature on the health effects of DBA in humans (IPCS, 2000).

10.0 Health effects in laboratory animals and *in vitro* test systems

10.1 Acute toxicity

Details on the available acute oral and dermal toxicity studies are provided in Tables 7 and 8.

Table 7: Summary table of acute oral toxicity data of the various HAA compounds

	Oral LD_{50} (mg/kg bw)[a]				
Compound	**Rats**	**Mice**	**Rabbits**	**Guinea pigs**	**Clinical observations**
MCA acid	90.4 for a 1% solution[1)] ~200 for a 6% solution[1)]	260[2)]	n/a[b]	n/a	Apathy, hypoactivity, disorders of balance, lacrimation, dyspnoea, cyanosis[1)]
MCA salt	76[3)]	165–255[3), 4)]	n/a	80[3)]	Apathy, weight loss[3)]
DCA salt	4480[3)]	4845–5500[3), 5)]	n/a	n/a	Semi-narcosis, narcosis[3)]
TCA acid	400[6)]	n/a	n/a	n/a	n/a
TCA salt	3320–5000[3), 7)]	3640–4870[3), 7), 8)]	4000[7)]	n/a	Semi-narcosis, narcosis[3)]
MBA salt	177[9)]	n/a	n/a	n/a	Excessive drinking, hypomobility, laboured breathing, mild diarrhoea[9)]
DBA salt	1737[9)]	n/a	n/a	n/a	Excessive drinking, hypomobility, laboured breathing, mild diarrhoea[9)]

[a] References are as follows: 1) ECETOC, 1999; 2) Berardi et al., 1987; 3) Woodard et al., 1941; 4) Morrison, 1946; 5) Yount et al., 1982; 6) Tomlin, 1994; 7) WSSA, 1983; 8) Meier et al., 1997; 9) Linder et al., 1994a.

[b] n/a: not available.

Table 8: Summary table of acute dermal toxicity data of the various HAA compounds

Compound	Dermal LD_{50} (mg/kg bw)[a]		Clinical observations
	Rats	Rabbits	
MCA acid	>400 (5% solution)[1)] 305–800 (40–50% solution)[1)] 145 (acid)[2)]	250 (50% solution)[1)]	Apathy, hypoactivity, lacrimation, piloerection, panting, prone position[1)]
MCA salt	>2000 (no deaths)[1)]	n/a[b]	Decreased activity, squatting posture, stilted and staggering gait, irregular breathing, moist rales, diarrhoea[1)]
TCA salt	>2000[3), 4)]	n/a	

[a] References are as follows: 1) ECETOC, 1999; 2) Saghir and Rozman, 2003; 3) Tomlin, 1994; 4) Hoechst AG, 1974.

[b] n/a: not available.

10.2 Short-term exposure

10.2.1 Monochloroacetic acid

In a 13-week gavage study, B6C3F1 mice (20 per sex per dose) received MCA (as an acid in deionized water) at doses of 0, 25, 50, 100, 150 or 200 mg/kg bw per day, 5 days per week. Interim evaluations were done on five mice per dose after 4 and 8 weeks of treatment. Mortality was increased only at the highest dose and mostly in male mice. The final mean body weight and the mean weight gain were significantly less in the 200 mg/kg bw per day females compared with controls. In female mice only, absolute and relative liver weights were significantly increased at the highest dose. Cholinesterase levels were decreased at the two highest dose groups in female mice. Hepatocellular cytoplasmic vacuolations were found following histopathological examination and were related to metabolic derangement occurring in moribund animals. No evidence of peroxisome proliferation in the liver was reported. The authors set the no-observed-adverse-effect level (NOAEL) at 100 mg/kg bw per day (Bryant et al., 1992; NTP, 1992).

In the same study as above, six groups of F344 rats (20 per sex per dose) received MCA (as an acid in deionized water) by gavage at 0, 30, 60, 90, 120 or 150 mg/kg bw per day, 5 days per week, for 13 weeks. Interim evaluations were done on five rats per dose group after 4 and 8 weeks of treatment. Toxic effects were observed at every dose level. Almost all animals died at 90 mg/kg bw per day and above. Relative heart weights were significantly decreased in both sexes at 60 mg/kg bw per day and only in females at 30 mg/kg bw per day. A dose-related increase in the incidence and severity of cardiomyopathy was observed in both sexes at 60 mg/kg bw per day and above. In male rats, relative liver and kidney weights were increased at 30 and 60 mg/kg bw per day; in female rats, relative liver weight was increased at 60 mg/kg bw per day only. During the treatment, blood urea nitrogen levels were increased in male rats at 90 mg/kg bw per day and up and in female rats at 60 mg/kg bw per day and up; however, there was no microscopic evidence of kidney or liver damage. Significant dose-related increases in serum levels of alanine transaminase (ALT) and aspartate transaminase (AST) were seen in both

sexes at 60 mg/kg bw per day and above. Lymphocyte counts were decreased at 30 mg/kg bw per day and above, but were related to stress. The authors set the NOAEL at 30 mg/kg bw per day based on cardiac effects in both sexes (Bryant et al., 1992; NTP, 1992).

10.2.2 Dichloroacetic acid

In a 7-week drinking water study, male Sprague-Dawley rats received DCA (sodium salt) at dose levels of 50 or 1100 mg/kg bw per day (Stacpoole et al., 1990). The high-dose rats had severe hind limb weakness; however, light microscopic examination of the peripheral nerves did not detect any changes. Thiamine deficiency was also detected at the high dose, as measured by transketolase activity in red cells. No clinical signs or effects on transketolase activity were seen at the low dose. The authors stated that the toxicity seen in rats in this study is associated with signs typical of thiamine deficiency and that hind limb weakness and other neuropathic manifestations of chronic thiamine deficiency in animals are considered to be due to changes in central, rather than peripheral, nervous system structure and function.

In a 12-week dietary study, hind limb weakness and abnormal gait also appeared in male Wistar rats (n = 6) exposed to DCA (neutralized). Approximate doses varied from 4 mmol/kg bw per day (516 mg/kg bw per day) at the beginning of the study to about 2.5 mmol/kg bw per day (323 mg/kg bw per day) at the end of the study (Yount et al., 1982). Decreased nerve conduction velocity was also detected in several nerves (sural and motor), as well as a decrease in the diameter of the tibial nerves. Decreased weight gain as a result of decreased food consumption and the presence of hepatomegaly were among the other toxic effects observed.

In a 3-month oral gavage study, DCA (sodium salt) was administered to Sprague-Dawley rats (10 per sex per dose) at dose levels of 0, 125, 500 or 2000 mg/kg bw per day, with an additional five rats per sex dosed with 0 or 2000 mg/kg bw per day allowed a 4-week recovery period (Katz et al., 1981). Death was seen in two rats per sex at the high dose. Hind limb paralysis occurred in 27% of rats of both sexes at the high dose. In the 4-week recovery group, one rat per sex afflicted by the paralysis seemed to have recovered completely. Histopathological examination showed the brain and testes as the main target organs. Oedematous brain lesions, characterized by vacuolation of the myelinated white tracts, were seen in the cerebrum and, to a lesser extent, the cerebellum. Combined incidence rates for these were 60% at the low dose and 100% at the middle and high doses. In the high-dose recovery group, brain lesions persisted in three of eight rats. Body weight was decreased in all treated rats and was associated with reduced food consumption. A significant increase was observed in relative liver weight (both sexes, all doses), relative kidney weight (females, all doses) and relative adrenal weight (males, 500 mg/kg bw per day and above; females, 2000 mg/kg bw per day). Clinical chemistry showed a mild depression of the erythroid parameters at the middle and high doses and a decrease in blood glucose and lactate levels at all levels. Testicular effects were also seen and are discussed in Section 10.5.2.

In a 90-day drinking water study, male Sprague-Dawley rats (n = 10) were treated with DCA (neutralized) at doses of 0, 50, 500 or 5000 mg/L (0, 4, 35 or 350 mg/kg bw per day) (Mather et al., 1990). Decreased body weight and water consumption were observed at 35 mg/kg bw per day and above. Relative liver and kidney weights were decreased at doses of 35 mg/kg bw per day and above. However, histological and biochemical signs of liver and kidney damage,

as well as an increase in the hepatic peroxisomal beta-oxidation activity, were seen only in the highest dose group. Increases in relative spleen weight were seen at the top dose in the absence of histopathological effects. No effects on immunological functions were seen.

In a 13-week drinking water study (NTP, 2000), B6C3F1 mice (10 per sex per dose) were exposed to DCA (neutralized to pH 5) doses of 0, 67, 125, 250, 500 or 1000 mg/L (0, 9, 16, 32, 61 and 124 mg/kg bw per day for males and 0, 10, 18, 38, 72 and 132 mg/kg bw per day for females). Dose-related increases were seen in liver weight and incidence of cytoplasmic vacuolation change in hepatocytes (females at 10 mg/kg bw per day and above; males at 32 mg/kg bw per day and above). No clinical signs or deaths were observed during the study. In the high-dose group, mild leukopenia, neutropenia and monocytopenia were observed in some male mice, which were potentially chemical related. The authors set NOAELs of <32 mg/kg bw for males and <10 mg/kg bw for females, both based on microscopic liver lesions.

NTP (2000) also dosed Fischer-344 rats (10 per sex per dose) with the same protocol and doses as above (calculated doses: males: 0, 5, 9.3, 18.8, 39.2 and 81.4 mg/kg bw per day; females: 0, 5.9, 10.0, 20.9, 43.8 and 94.7 mg/kg bw per day). A significant decrease in body weight gain was noted in high-dose males during weeks 4–13. No other significant effects were observed.

In a 13-week subchronic study (Katz et al., 1981), 10- to 12-month-old beagle dogs (3–4 per sex per dose) received DCA (sodium salt) in gelatin capsules at doses of 0, 50, 75 or 100 mg/kg bw per day, with one additional dog per sex dosed with 0 or 100 mg/kg bw per day and allowed a 4-week recovery period. In female dogs only, food consumption was reduced at all doses; however, dose-dependent weight losses at all dose levels were observed in both sexes during the treatment but reversed upon cessation. One female and one male died at the 75 and 100 mg/kg bw per day dose, respectively, and signs of adverse effects observed prior to their deaths included anorexia, ataxia, hind limb weakness and reduced activity. Other adverse effects related to the treatment include emesis (75 and 100 mg/kg bw per day), bloody stools (100 mg/kg bw per day) and paralysis (100 mg/kg bw per day). Also reported in all dose groups (both sexes) were a high incidence of ocular anomalies: bilateral lenticular opacities, injected bulbar conjunctivae and superficial corneal vascularization with a tendency for keratoconjunctivitis sicca. Haematological parameters were depressed at all dose levels in both sexes. Liver and kidney parameters were not affected by the DCA treatment in dogs. An increase in the incidence of lung consolidation was observed in all doses (both sexes). Histopathological examination of the brain revealed that dogs (in all treated groups) suffered slight to moderate vacuolization of white myelinated tracts in the cerebrum and, to a lesser extent, in the cerebellum. Increases in the incidence of haemosiderin-laden Kupffer cells in the liver and cystic mucosal hyperplasia in the gall bladder were observed at all dose levels, even 5 weeks after cessation of the treatment. Prostate and testicular changes were also seen at 50 mg/kg bw per day and above and are discussed in Section 10.5.2. The authors noted that beagle dogs are more susceptible to cataract formation than any other species (Katz et al., 1981).

In another subchronic dog study (Cicmanec et al., 1991), 4-month-old male and female beagle dogs (five per sex per dose) received DCA (neutralized to pH 7.4) in gelatin capsules at 0, 12.5, 39.5 or 72 mg/kg bw per day for 90 days. The controls received encapsulated distilled water. Three high-dose animals died as a result of dehydration and pneumonia. Overt clinical

signs such as dyspnoea (middle and high doses) and partial paralysis (high dose only) were observed in both sexes. Diarrhoea was present in the mid- and high-dose animals; some dogs that were highly dehydrated required fluid therapy. Inflammation of the ocular membranes was accompanied by swelling; discharge was clear at the low and middle doses and became purulent in the high-dose group. A dose-related decrease in body weight gain was observed in all treated animals. Relative liver weights were increased in all female dose groups; however, kidney and lung weights were increased only in the high-dose group. In male dogs, the effects in these organs were less consistent. Apparent increases in ALT, AST and lactate dehydrogenase were observed in both sexes in the high-dose group. Erythrocytes and haemoglobin concentrations were significantly reduced in both sexes in the high-dose group from day 30 and beyond. Upon microscopic examination, lesions were apparent in the liver, brain, lung, pancreas and testes. Hepatic vacuolization was observed in most of the treated dogs (both sexes) as well as in a few control animals. In the brain, mild vacuolization of the white myelinated tracts of the cerebrum, cerebellum and/or spinal cord was present in all exposed groups. Pneumonia and bronchopneumonia was observed in most treated dogs; more severe effects were seen in the mid- and high-dose groups. Pancreatic acinar associated with chronic inflammation was observed in many mid- and high-dose animals (both sexes). Testicular (all doses) and prostatic effects (middle and high doses) were seen and are discussed in Section 10.5.2.

10.2.3 Trichloroacetic acid

In a 90-day study (Mather et al., 1990), male Sprague-Dawley rats (10 per dose) were treated with TCA (neutralized) in their drinking water at dose levels of 0, 50, 500 or 5000 mg/L (0, 4.1, 36.5 or 355 mg/kg bw per day). Water consumption was decreased (statistically significant) at the middle and high doses compared with controls. A decrease in body weight was observed for all dose groups, but was not statistically significant. The high-dose group also had an increase in relative liver and kidney weights and a significant increase in hepatic peroxisomal activity.

10.2.4 Monobromoacetic acid

No subchronic studies on MBA were located in the literature.

10.2.5 Dibromoacetic acid

To determine the effects on the liver, male B6C3F1 mice (five per dose) were administered DBA (neutralized) at 0, 300, 1000 or 2000 mg/L in drinking water for up to 12 weeks (Kato-Weinstein et al., 2001). All animals were sacrificed at 20 weeks of age. Decreased water consumption and body weight were seen at the highest dose. A dose-related increase in relative liver weights was seen at 1000 mg/L and above at 12 weeks. Increased absolute and relative liver weights were seen at all time points (4, 8 and 12 weeks) at the highest dose. A significant increase in the total liver glycogen content was seen at the highest dose at 12 weeks. Dose-related decreases in serum glucose and serum insulin concentrations were seen at 1000 mg/L and above at 12 weeks (Kato-Weinstein et al., 2001). DBA displayed effects similar to those of DCA; both caused an increase in glycogen content and a depression of serum insulin concentrations. This may suggest that common mechanisms are involved with dihaloacetates.

A 13-week drinking water study in both B6C3F1 mice and F344 rats was reported by Melnick et al. (2007) and in greater detail in NTP (2007). Animals (10 per dose per sex per species) were exposed to DBA (neutralized to pH 5) in drinking water at doses of 0, 125, 250, 500, 1000 or 2000 mg/L (equivalent to 0, 10, 20, 40, 90 and 166 mg/kg bw per day in male rats and 0, 12, 23, 48, 93 and 181 mg/kg bw per day in female rats; 0, 16, 30, 56, 115 and 230 mg/kg bw per day in male mice and 0, 17, 34, 67, 132 and 260 mg/kg bw per day in female mice). No clinical signs or deaths were observed. A significant decrease in final mean body weights were seen in both species and sexes at the highest dose, however only a slight decrease in water consumption was seen at weeks 1 and/or 13. Dose-related increases in liver weights were noted in both sexes of mice at 500 mg/L and above and in rats at 125 mg/L and above. Dose-related increases in the severity of cytoplasmic vacuolation in hepatocytes were seen in mice (both sexes at 1000 mg/L and above), although these lesions were present in both controls and treated mice. It was noted that the increase in severity corresponded to increased liver weights. In rats, an increased incidence of cytoplasmic vacuolation in hepatocytes was noted in males at 500 mg/L and above and in females at 2000 mg/L. Haematopoietic cell proliferation (mild to minimal) was observed in the spleen of high-dose female rats. A significant increase in the incidence of cellular hypertrophy of the pituary gland was observed in high-dose male rats; however, these effects were considered secondary to testicular atrophy. Testicular effects were seen in rats and mice and are outlined in Section 10.5.5.

Moser et al. (2004) and Phillips et al. (2002) examined the neurotoxic potential of DBA. Adolescent male and female F344 rats (12 per sex per group) were administered DBA (acid) at 0, 200, 600 and 1500 mg/L (estimated as 0, 20, 72 and 161 mg/kg bw per day) for 6 months in drinking water. No treatment-related deaths were noted. A decrease in weight gain was noted in the high-dose rats (both sexes). Functional observational battery test results revealed dose-related neuromuscular toxicity at the middle and high doses, such as limb weakness, mild changes in gait and hypotonia. Sensorimotor responses were depressed at all treatment levels. Decreased activity and chest clasping were also noted at the highest dose. Neuropathological evaluation revealed degeneration of spinal cord nerve fibres in the mid- and high-dose groups. Neuronal vacuolization of the spinal cord (mostly in grey matter and occasionally in white matter) increased in severity and incidence at the middle dose and above. The authors set a no-observed-effect level (NOEL) of 20 mg/kg bw per day based on neuropathological effects but were unable to establish one for neurobehavioural effects due to sensorimotor changes at that level.

Clinical signs of neurotoxic effects were also seen in a male reproductive study (Linder et al., 1995). Male rats (n = 10) were dosed with 250 mg DBA/kg bw daily for up to 42 days, after which dosing was discontinued due to severe toxicity; clinical signs consisted of abnormal posturing, light tremor, atypical movement of limbs and difficulty in moving hind limbs. Rats were allowed to recover for a 6-month interval; some of the effects either diminished (abnormal gait) or disappeared (tremors). No other overt signs of toxicity were seen at the lower doses in male rats (Linder et al., 1995).

NTP (1999a) examined the immunotoxicity of DBA in female mice in four separate studies investigating different end-points. Groups of female B6C3F1 mice (eight per dose) were exposed to DBA in drinking water at concentrations of 0, 125, 250, 500, 1000 or 2000 mg/L per

day (calculated as 0, 14–20, 33–39, 68–73, 132–150 or 236–285 mg/kg bw per day) for a period of 28 days. No signs of overt toxicity were seen, and water consumption was unaffected. Only high-dose females had a 40% decrease in body weight gain compared with controls. In one experiment, an increase in spleen weight was observed, while none was seen in a second experiment using similar doses. Several indicators of immunological response were affected. A statistically significant dose-related increase in the number of spleen macrophages was seen at 500 mg/L and above, indicating an immunotoxic response in the spleen. Humoral immunity was also affected, as seen by a decrease of spleen immunoglobulin M antibody-forming cell response to sheep erythrocytes at doses of 500 mg DBA/L and above. A lowest-observed-adverse-effect level (LOAEL) of 500 mg/L (68–73 mg/kg bw per day) and a NOAEL of 250 mg/L (33–39 mg/kg bw per day) were established for immunotoxicity by U.S. EPA (2005a).

McCay et al. (2000) reported in an abstract that no effects on the immune system were seen in a 28-day study in which female B6C3F1 mice were dosed daily via drinking water with DBA concentrations of 0, 250, 500 or 1000 mg/L. Changes in relative and/or absolute liver and thymus weights were the only effects seen.

10.3 Long-term exposure and carcinogenicity

Long-term studies with MCA failed to produce tumours in rodents. In contrast, DCA, TCA and DBA produced liver tumours in laboratory animals. However, these liver tumours were seen only in mice, and not in rats, exposed to TCA and DBA, whereas both rats and mice were affected when exposed to DCA. However in rats treated with DBA, tumours in other organs were detected. No adequate long-term/carcinogenicity studies on MBA were identified. The results of these carcinogenicity studies relating to liver tumours are summarized in Table 9, while the details of these studies are described in the sections below.

Table 9: Summary table: carcinogenicity studies relating to liver tumours for MCA, DCA and TCA[a]

HAA	Dosing route	Doses (mg/kg bw per day)	Duration	Acid or salt	Strain/ species	Sex	Liver tumours found	Study author
MCA	Gav	0, 15, 30	2 years	A	F344 rats	M & F	None	NTP, 1992
	Gav	0, 50, 100	2 years	A	B6C3F1 mice	M & F	None	NTP, 1992
	DW	0, 3.5, 26, 59.9	2 years	S	F344 rats	M	None	DeAngelo et al., 1997
DCA	DW	0, 140, 280	52 weeks	S	B6C3F1 mice	M	HA, HC	Bull et al., 1990
	DW	0, 7.6, 77, 410, 486	60–75 weeks	?[b]	B6C3F1 mice	M	HA, HC	DeAngelo et al., 1991; U.S. EPA, 1991
	DW	0, 93	104 weeks	S	B6C3F1 mice	M	HA, HC	Daniel et al., 1992

HAA	Dosing route	Doses (mg/kg bw per day)	Duration	Acid or salt	Strain/ species	Sex	Liver tumours found	Study author
	DW	0, 8, 84, 168, 315, 429	90–100 weeks	S	B6C3F1 mice	M	HA, HC	DeAngelo et al., 1999
	DW	0, 40, 120, 330	360 or 576 days	S	B6C3F1 mice	F	HA, HC	Pereira, 1996
	DW	0, 3.6, 40.2, 139.1	100 weeks	S	F344 rats	M	HA, HC	DeAngelo et al., 1996
TCA	DW	0, 178, 319	Up to 52 weeks	S	B6C3F1 mice	M	HA, HC	Bull et al., 1990
	DW	0, 7, 71, 595	60 weeks	possible S	B6C3F1 mice	M	HA, HC	DeAngelo and Daniel, 1990; U.S. EPA, 1991
	DW	0, 64, 212, 640	360 or 576 days	S	B6C3F1 mice	F	HA, HC	Pereira, 1995, 1996; Pereira and Phelps, 1996
	DW	0, 71, 583	104 weeks	?[b]	B6C3F1 mice	F	HA, HC	U.S. EPA, 1991
	DW	0, 3.6, 32.5, 364	104 weeks	S	F344 rats	M	None	DeAngelo and Daniel, 1992; DeAngelo et al., 1997
DBA	DW	0, 4, 35, 65	104 weeks	S	B6C3F1 mice	F	HA, HC	Melnick et al. 2007; NTP, 2007
	DW	0, 4, 45, 87	104 weeks	S	B6C3F1 mice	M	HA, HC	Melnick et al. 2007; NTP, 2007
	DW	0, 2, 25 45	104 weeks	S	F344 rats	F	None[b]	Melnick et al. 2007; NTP, 2007
	DW	0, 2, 20, 40	104 weeks	S	F344 rats	M	None[b]	Melnick et al. 2007; NTP, 2007

[a] A: acid; DW: drinking water; F: female; Gav: gavage; HA = hepatocellular adenomas; HC = hepatocellular carcinomas; M: male; S: salt; ? = form unknown.

[b] Although no liver tumours were found, tumours in other organs were detected.

10.3.1 Monochloroacetic acid

No tumours were observed in two different strains of mice (18 per sex per strain) gavaged with MCA (acid) in distilled water at 46 mg/kg bw per day from the age of 7 days to 4 weeks, after which MCA was mixed directly in the diet at 149 mg/kg for a total of 18 months (Innes et al., 1969).

In a 2-year carcinogenicity bioassay, F344/N rats (70 per sex per dose) were dosed with MCA (acid) in deionized water at 0, 15 or 30 mg/kg bw per day by gavage, 5 days per week (NTP, 1992). The mean body weight of high-dose male rats was reduced after 30 weeks. Survival rates were significantly reduced for the high-dose male and low- and high-dose female rats, but were not treatment related. Overall, there were no treatment-related increases for non-neoplastic lesions or neoplasia reported. The authors concluded that "there was *no evidence of carcinogenic activity*" for MCA in male and female F344/N rats at the dose levels used in the study.

In the same 2-year bioassay, B6C3F1 mice (60 per sex per dose) were dosed with MCA (acid) in deionized water by gavage at 0, 50 or 100 mg/kg bw per day, 5 days per week (NTP, 1992). A significant reduction was noted for mean body weight in high-dose females (after 52 weeks) and in survival rates for high-dose males. The incidences of acute nasal inflammation ranged from mild to minimal in severity but were not treatment related. The incidence of metaplasia of the olfactory epithelium was significantly greater in high-dose females. No other significant increases in non-neoplastic lesions were observed in either sex. No treatment-related increases for any neoplasia were reported. The authors stated that "there was *no evidence of carcinogenic activity*" for MCA in B6C3F1 mice (both sexes) at the dose levels used in the study.

In a 104-week drinking water study, male F344/N rats (50 per dose) were exposed to MCA (neutralized) at 0, 50, 500 or 1100 mg/L[*] (0, 3.5, 26 or 59.9 mg/kg bw per day) (DeAngelo et al., 1997). MCA treatment had no significant effect on survival, but drinking water consumption and final mean body weights were significantly reduced in the two highest dose groups. Absolute and relative spleen weights were significantly increased (74% and 80% over controls, respectively) for rats consuming 3.5 mg MCA/kg bw per day; however, this increase was seen in the absence of gross or microscopic lesions. Although decreases in absolute and relative spleen weights were observed at the two highest doses, only the absolute weight was statistically lower at the highest dose (59.9 mg/kg bw per day). Exposure to 26 mg/kg bw per day significantly decreased absolute liver and kidney weights and relative liver weights and increased relative testes weights. At the 59.9 mg/kg bw per day dose, absolute kidney and absolute and relative liver weights were reduced, while relative testes weights were increased. Serum enzyme analysis revealed no treatment-related effects on either AST or ALT, and MCA treatment did not enhance peroxisome proliferation or hepatocyte proliferation. No MCA-induced liver pathology was observed during the study, nor were there any significant lesions found in any non-hepatic tissues. Most common age-related spontaneous changes present in rat tissues were within historical controls except for myocardial degeneration and chronic/active inflammation of the nasal cavities, which were seen at an increased incidence at 104 weeks but not at earlier sacrifice periods or in the lower dose groups. There were no significant increases in either the prevalence or multiplicity of hepatocellular adenomas, carcinomas or hyperplastic nodules in any of the MCA-treated animals compared with controls. Similarly, neoplastic changes in non-hepatic

[*] The highest dose administered was originally 2000 mg/L but was lowered to 1500 mg/L after 8 weeks and to 1000 mg/L after 24 weeks due to a severe reduction in body weight gain. Therefore, the time-weighted mean MCA concentration of the high-dose group over the course of study was 1100 mg/L.

tissues were consistent with those reported in historical controls. The authors concluded that the decreased body weights and other pathology observed in the mid-dose groups were not significant and set the NOEL for carcinogenicity at 500 mg/L (26 mg/kg bw per day) for MCA (DeAngelo and Daniel, 1992; DeAngelo et al., 1997).

However, Health Canada did not agree with the decision to ignore statistically significant changes in body weight and liver, kidney and testes weights, particularly since these changes are consistent with a dose-related trend. At the next lower dose of 50 mg/L (3.5 mg/kg bw per day), the only significant change was an increase in absolute and relative spleen weights. This change can be considered idiosyncratic (a physiological peculiarity), since there is no dose-related trend towards an increase in spleen weight. At the next dose, 500 mg/L (26 mg/kg bw per day), the absolute and relative spleen weights were lower than control, while the absolute weight was significantly lower at the highest dose. Based on these considerations, Health Canada derived a NOAEL of 3.5 mg/kg bw per day.

10.3.2 Dichloroacetic acid

Several studies on the potential carcinogenicity of DCA have been conducted and have been summarized in Table 9. Increased incidences of either liver tumours (adenomas and carcinomas) or preneoplastic lesions (e.g., hyperplastic nodules and altered hepatic foci) were reported when mice and rats were exposed to drinking water containing 0.5–5 g DCA/L for periods ranging from 52 to 104 weeks. The details of the studies are reported below.

In a 52-week drinking water study (Bull et al., 1990), groups of male B6C3F1 mice (n = 61, 11, 50) were exposed to DCA (neutralized) at 0, 1000 or 2000 mg/L (0, 140 or 280 mg/kg bw per day; WHO, 2005), and a group of 10 females was exposed to 2000 mg DCA/L for up to 52 weeks. Interim sacrifices of five male mice from the 2000 mg/L group were carried out at 15, 24 and 37 weeks. All other animals were sacrificed on week 52, including 11 males that had their exposure terminated at 37 weeks, followed by 15 weeks of recovery before sacrifice. A significant dose-related increase in the absolute and relative liver weights was noted in treated males at 37 or 52 weeks. Livers from all DCA-treated animals were enlarged (hepatomegaly), with a uniform distribution of marked cytomegaly and extensive accumulation of glycogen in hepatocytes as well as multi-focal areas of necrosis with lymphocyte infiltration throughout the liver. Glycogen-poor basophilic foci of cellular alteration were observed at 24 and 37 weeks in the central lobes of livers from males treated with 2000 mg DCA/L. After 52 weeks of treatment, hyperplastic nodules, adenomas and carcinomas were observed in the livers of males in the 2000 mg/L group. In contrast, the only hepatoproliferative lesions observed in the other groups were one hyperplastic nodule in a male mouse from each of the control and 1000 mg/L dose groups and hyperplastic nodules in three females. No hepatocellular carcinomas were reported in males where DCA treatment was terminated at 37 weeks; by 52 weeks, two animals had adenomas, and six had hyperplastic nodules. The relationship between mean total dose of DCA and total number of hepatic lesions (nodules + adenomas + carcinomas) per mouse was non-linear, with the number of lesions increasing sharply as the dose increased from 1000 to 2000 mg/L (Bull et al., 1990).

Male B6C3F1 mice (30 per dose) were administered DCA in their drinking water at 50, 500 or 5000 mg/L for 60 and/or 75 weeks (DeAngelo et al., 1991; U.S. EPA, 1991). The control

group received 2 g sodium chloride/L. In a concurrent study, mice were exposed to 3500 mg DCA/L and killed after 60 weeks. In this experiment, the control group received acetic acid. Time-weighted mean daily doses of 7.6, 77, 410 and 486 mg/kg bw per day were calculated for the 50, 500, 3500 and 5000 mg/L concentrations, respectively. Mice exposed to 3500 and 5000 mg/L had their final body weights reduced compared with the control group: 87% and 83% of the control value, respectively. The relative liver weights were increased at the three highest dose groups compared with the control value: 118%, 230% and 351% for 500, 3500 and 5000 mg/L, respectively.

Hyperplastic liver nodules (a non-neoplastic nodular lesion) were observed primarily at the two highest doses: 58% and 83% incidence at 3500 and 5000 mg/L, respectively. Mice receiving 5000 mg/L (after 60 weeks) had a 90% prevalence of liver neoplasia (carcinomas and adenomas), with a mean multiplicity of 4.50 tumours per animal; those receiving 3500 mg/L had a 100% prevalence, with a tumour multiplicity of 4.0 tumours per animal. The tumour prevalence and multiplicity in the two lowest dose groups at 75 weeks did not significantly differ from the control value. The control group had no liver tumours. The authors concluded that there was a significant positive dose-related trend in age-adjusted prevalence of liver tumours. The authors concluded that DCA exhibited a threshold of at least 500 mg/L (77 mg/kg bw per day) for tumour response in mice. A steep increase in tumour incidence occurred at 3500 mg/L (410 mg/kg bw per day), which also represents the maximum incidence attained (DeAngelo et al., 1991; U.S. EPA, 1991).

In a 104-week drinking water study (Daniel et al., 1992), DCA (neutralized) was administered to a group of 33 male B6C3F1 mice at a concentration of 500 mg/L (93 mg/kg bw per day), with one interim sacrifice (n = 5) at 30 weeks. There were no significant treatment-related effects on drinking water consumption, relative body weight gain or relative or absolute spleen, kidney or testes weight. Absolute and relative liver weights were increased at both 30 and 104 weeks. The most notable treatment-related non-neoplastic hepatic effects observed were cytomegaly, necrosis and chronic active inflammation. After 104 weeks, there was a significant increase in the incidence of hepatocellular carcinomas and hepatocellular adenomas. The combined incidences of carcinoma + adenoma and carcinoma + adenoma + nodule were significantly increased in treated animals compared with controls. Mice killed at the end of the study had hepatocellular carcinomas (15/24, compared with 2/20 in the control groups); hepatocellular adenomas (10/24, compared with 1/20 in the controls); and carcinomas or adenomas (18/24, compared with 3/20 in the controls). Other effects observed were hyperplastic nodules in 2/24 treated mice, hepatocellular necrosis in 8/24 treated mice (compared with 1/20 in the controls) and cytomegaly in 22/24 treated mice (compared with 1/20 in the controls) (Daniel et al., 1992). (Data were not provided for interim necropsies at week 30.)

In a 90- to 100-week drinking water study, male B6C3F1 mice (n = 35–71) were exposed to DCA (neutralized) at 50, 500, 1000, 2000 or 3500 mg/L (8, 84, 168, 315 and 429 mg/kg bw per day) (DeAngelo et al., 1999). The control group consisted of 88 mice. Interim sacrifices were performed throughout the study, except at the lowest dose. At final sacrifice, the body weights were decreased at the two highest doses. A dose-dependent increase in liver weight at 84 mg/kg bw per day and above was seen at 26 and 52 weeks and at 315 mg/kg bw per day and above at

100 weeks. Liver toxicity was demonstrated at 168 mg/kg bw per day and above, as indicated by an increase in serum liver enzymes and histopathology. Mortality at the two highest doses was statistically significant when compared with the control group. There was a significant increase in the incidence of hepatocellular carcinoma in male mice. At 26 weeks, no tumours were seen in the livers of any of the mice, but at 52 and 78 weeks, the incidence of hepatocellular carcinoma was significantly ($P < 0.05$) elevated in the highest dose group (Table 10). At the terminal sacrifice, the incidence of hepatocellular carcinoma was significantly elevated ($P < 0.05$) at the three highest dose groups. Hepatocellular adenomas were first observed at the high dose at 26 weeks; however, only at 100 weeks was the incidence of hepatocellular adenomas significantly ($P < 0.05$) increased (no dose–response) in the three highest dose groups (Table 10).

Table 10: Prevalence of hepacellular carcinomas and adenomas in mice (DeAngelo et al., 1999)

Dose (mg/kg bw per day)	Prevalence of male mice with hepatocellular carcinomas (%)			Prevalence of male mice with hepatocellular adenomas (%)		
	Week 52	Week 78	Week 100	Week 52	Week 78	Week 100
0 (water control)	0	10	26	0	10	10
8	n/r[a]	n/r	33	n/r	n/r	n/r
84	0	0	48	10	10	20
168	0	20	71[b]	10	20	51.4[b]
315	20	50	95[b]	0	50	42.9[b]
429	50	70[b]	100[b]	50	50	45[b]

[a] n/r = not reported.

[b] Statistically significant ($P \leq 0.05$) when compared with the water control.

Liver peroxisome proliferation was significantly elevated only in the high-dose group after 26 weeks, but not at 52 weeks. In contrast, hepatocyte proliferation was not significantly different from the control rates at any of the doses that produced tumours. The authors could not determine a NOEL for hepatocarcinogenicity based on the significant increase in hepatocellular carcinoma multiplicity at the lowest dose (0.58 compared with 0.28 in the control), but concluded that DCA produced a dose-related increase in the incidence of hepatocellular carcinoma that was not associated with either liver peroxisome or hepatocyte proliferation. Endocrine disruption and liver cell necrosis were proposed as playing important roles in the carcinogenesis (DeAngelo et al., 1999).

Groups of 7- to 8-week-old B6C3F1 female mice (n = 40–90 and n = 134 in the control) were administered DCA (neutralized) in drinking water for either 360 or 576 days at concentrations of 0, 2.0, 6.67 or 20.0 mmol/L (0, 40, 120 or 330 mg/kg bw per day) (Pereira, 1996; IPCS, 2000). An additional group of animals (n = 50) was exposed intermittently to 20.0 mmol DCA/L in drinking water in a 72-day cycle, 24 days with exposure and 48 days without. The 72-day cycle was repeated until the mice were sacrificed, so that the total dose was the same as for those continuously exposed to 6.67 mmol DCA/L. Body weights were reduced in the high-dose group after 35 weeks. There was a dose-related increase in relative liver weight and vacuolated

hepatocytes. For the 576-day exposure groups, there was a significant increase in hepatic foci, hepatocellular adenomas and hepatocellular carcinomas at the high dose and an increased incidence of foci and adenomas (but not carcinomas) at the middle dose, compared with controls. The intermittent DCA group also displayed a significantly increased incidence of altered hepatic foci at 576 days, but no significant increases in neoplastic response (Pereira, 1996).

In a modified carcinogenesis bioassay (DeAngelo et al., 1996), 28-day-old male F344 rats (n = 50–78) were exposed to DCA (neutralized) at 0, 50, 500 or 1600* mg/L (estimated time-weighted mean daily doses: 0, 3.6, 40.2 and 139.1 mg/kg bw) in drinking water for 100 weeks. There were no significant differences in water consumption or survival for any of the treatment groups when compared with controls. Terminal body weights and relative liver and kidney weights were reduced only in the 1600 mg/L group. Testicular effects are reported in Section 10.5.2. The only non-neoplastic treatment-related hepatic change was hepatocellular cytoplasmic vacuolization, attributed to DCA-induced increases in glycogen deposition. The combined hepatocellular adenoma and hepatocellular carcinoma prevalence was significantly increased ($P < 0.05$) in the 500 mg/L group compared with controls, as was the total hepatoproliferative lesion prevalence (hyperplastic nodules, adenomas and carcinomas). Significant dose-related trends were observed for hepatocellular adenoma and hepatocellular carcinoma ($P < 0.05$ for each) prevalence, combined hepatocellular adenoma and hepatocellular carcinoma prevalence and total hepatoproliferative lesions. In the 1600 mg/L group, increased prevalences of hepatocellular carcinoma, combined hepatocellular carcinoma and hepatocellular adenoma and total hepatoproliferative lesions were observed. At the high dose, DCA induced hepatocyte peroxisome proliferation. DCA treatment depressed hepatocyte proliferation at 14 weeks; at the other time periods, it remained depressed, but did not differ significantly from the control group. The authors concluded that DCA is a hepatocarcinogen in male F344 rats. Male F344 rats were found to be more sensitive to DCA exposure than male B6C3F1 mice based on a previous study by DeAngelo et al. (1991). The authors set a NOEL of 3.6 mg/kg bw per day (DeAngelo et al., 1996).

The ability of DCA to act as a promoter of carcinogenesis in the liver was observed in several studies. According to Pereira (1995), “promotion is defined as the enhancement of the progression of initiated cells to precancerous lesions and tumors.”

A tumour promotion study was conducted in male B6C3F1 mice with DCA (neutralized) (Herren-Freund and Pereira, 1986; Herren-Freund et al., 1987). Tumour incidences (hepatocellular adenomas and carcinomas) were significantly elevated for mice with and without initiation compared with the controls. The authors concluded that DCA is a complete hepatocarcinogen in B6C3F1 mice (Herren-Freund et al., 1987).

* Originally, this dose was set at 5000 mg/L and was reduced to 2500 mg/L at 9 weeks, to 2000 mg/L at 23 weeks and to 1000 mg/L at 52 weeks. This group of rats was sacrificed at 60 weeks due to irreversible peripheral hind leg neuropathy and was not used in the analysis. In a follow-up bioassay, a group of 78 animals was exposed to 2500 mg DCA/L in drinking water for 103 weeks, with the dose lowered to 1500 mg/L at 8 weeks and to 1000 mg/L at 26 weeks to alleviate a mild transient neurotoxicity. The mean daily concentration was determined to be 1600 mg DCA/L for this dose group.

The potential of DCA (neutralized) to promote tumours was also investigated in female B6C3F1 mice (Pereira, 1995; Pereira and Phelps, 1996). An increased incidence of hepatic foci and adenomas was seen in initiated mice exposed to the highest dose of DCA for either 31 or 52 weeks compared with the corresponding animals receiving initiation or DCA alone. A second-order relationship (i.e., exponential relationship) was observed between the concentration of DCA and the yield of total lesions (foci + adenomas + carcinomas) at both 31 and 52 weeks. Upon termination of treatment, the foci of altered liver hepatocytes and adenomas regressed.

Two separate initiation–promotion studies, conducted by Bull et al. (2004) and Pereira et al. (1997), looked at the interaction between DCA and TCA (both tumour promoters involving different mechanisms) in B6C3F1 mice. Different results between the two studies may be related to doses used.

Bull et al. (2004) demonstrated that the interactions between TCA and DCA were limited by additivity: the lowest effective doses with TCA and DCA showed additivity, whereas the effects tended to be inhibitory (suppression of overall growth rate) as the doses increased. DCA and TCA selectively promote the growth of different types of initiated cells, by stimulating the clonal expansion in one cell type while inhibiting it in another cell type. Such a process would explain the presence of a mixed phenotype of tumours as well as the suppression of the overall tumour growth rate in the present study (Bull et al., 2004).

In contrast, Pereira et al. (1997) observed synergistic effects with a mixture of TCA and DCA at high doses; the yields of foci of altered hepatocytes and total lesions produced were greater than the sums of the yields produced by the two HAAs administered alone, whereas additive or inhibitory effects were seen with the mixtures at lower doses. The mixtures of DCA–TCA produced more foci of altered hepatocytes than adenomas, similar to the effects seen when DCA was administered alone. In addition, the phenotype of these lesions resembled those of DCA-induced tumours, suggesting that DCA may predominate in determining the characteristics of the proliferative lesions.

10.3.2.1 Mechanisms of carcinogenicity

Based on the results of the studies described above and summarized in Table 9, DCA has been shown to be a liver carcinogen in two species of rodents, mice and rats. DCA is thought to be a “complete carcinogen,” since it has induced tumours at both low doses in long-term assays and high doses in shorter-term assays when administered alone; doses from these studies ranged from 50 to 5000 mg/L. More than one mode of action may explain DCA-induced carcinogenicity, and several hypotheses have been put forward, as described below. In all likelihood, a number of events would be significant to tumour development in the rodent under bioassay conditions. Uncertainty exists, however, as to which events may be relevant to human exposure to DCA at environmental levels.

10.3.2.1.1 Genotoxicity

Mixed reviews were seen with regard to DCA-induced carcinogenicity mediated through a genotoxic mechanism. Previous reviews by IARC (1995) and ILSI (1997) reported that DCA was not genotoxic, whereas a more recent review by IPCS (2000) reported that DCA had some ability to induce genotoxic effects but only at high concentrations; it concluded that at low doses,

DCA was either not acting or acting minimally through a genotoxic mechanism. The U.S. National Center for Environmental Assessment (U.S. EPA, 2003c) reported that more recent literature indicated that DCA is a direct-acting genotoxic agent. IARC (2004) recently reported that DCA is genotoxic (*in vivo* and *in vitro*) but may be acting indirectly via an epigenetic mechanisn. Owing to the lack of causal data, U.S. EPA (2003c) took the cautious position that DCA might be genotoxic at high doses while uncertainty remained for the lower doses.

10.3.2.1.2 Peroxisome proliferation

Some hepatocarcinogens have been shown to increase the number and/or size of liver peroxisomes (peroxisome proliferation) in rodents (U.S. EPA, 2003c). Peroxisome proliferator-activated receptors (PPARs) are a class of nuclear receptors that regulate this proliferation and are believed to be responsible for the initiation of certain cellular events leading to transformation (as well as other reported effects) for liver carcinogens. Although peroxisome proliferation has been correlated with carcinogenesis, the actual mechanism of carcinogenesis as it is related to peroxisome proliferation is unknown (Bull, 2000). Species differences are seen with relation to the expression of various PPARs; humans seem less responsive to a variety of peroxisome proliferators than rodents, and, as a result, controversy exists as to whether these compounds are even carcinogenic to humans (Bull, 2000; U.S. EPA, 2003c).

Studies conducted by DeAngelo et al. (1989, 1999), Daniel et al. (1992), Mather et al. (1990) and Pereira (1995, 1996) showed that DCA was a weak peroxisome proliferator in rodents.

Seemingly, much lower doses of DCA are needed to induce liver tumours compared with those that cause a significant increase in peroxisome proliferation (U.S. EPA, 2003c). In conclusion, U.S. EPA (2003c), Thai et al. (2003) and Bull (2000) do not consider this mechanism an important route in DCA tumorigenesis.

10.3.2.1.3 Down-regulation of insulin

The glycogen content of liver cells of DCA-treated mice can vary depending on the state of the cell; altered hepatic foci and tumours are glycogen poor, whereas normal liver cells accumulate large amounts of glycogen (Lingohr et al., 2001), thus suggesting a possible link between glycogen and hepatic tumours. Glycogen levels are mediated primarily by insulin via metabolism in the liver (Lingohr et al., 2001). Insulin may have other roles, such as acting as an agent that triggers mitosis (mitogen) for normal and malignant liver cells and suppressing apoptosis (Lingohr et al., 2001).

Lingohr et al. (2001) investigated the effects of DCA on insulin levels and expression of insulin-controlled signalling proteins in normal liver tissue and DCA-induced liver tumour tissues of male B6C3F1 mice treated with DCA (neutralized) at 100–2000 mg/L in drinking water for 2–10 weeks. Decreases were seen in insulin receptor proteins, serum insulin levels and protein kinase B expression after 2 weeks in DCA-treated mice. In contrast, increases in glycogen (which preceded these effects) were seen as early as 1 week, suggesting that DCA-induced alterations in insulin, insulin receptors and possibly protein kinase B resulted from a compensatory down-regulation of the insulin pathway triggered by high glycogen levels in the

liver. An *in vitro* study by Lingohr et al. (2002) using isolated hepatocytes showed similar results: increased glycogen levels (independent of insulin) and effects on insulin signalling proteins.

These studies show an apparent down-regulation of insulin and insulin receptor activity after relatively short durations of treatment.

10.3.2.1.4 Tumour promotion, alterations in cell replication and death

Stauber and Bull (1997) studied the differences in phenotype and cell replicative behaviour of liver tumours induced by DCA and TCA in male B6C3F1 mice. Clear differences in phenotype were seen between DCA- and TCA-induced tumours. The study also showed the extent to which changes in cell replication within tumours and normal hepatocytes were influenced by DCA to increase tumour formation. Stauber et al. (1998) also conducted *in vitro* studies, which duplicated these results.

Several mechanisms of action are proposed for tumour promoters. Bull et al. (2004) suggested that promoters should influence mainly tumour size rather than tumour numbers. This behaviour can be observed with DCA. Miller et al. (2000) investigated, by means of magnetic resonance imaging, the growth rates of liver tumours in male B6C3F1 mice given DCA (neutralized) at 2000 mg/L in their drinking water for 48 weeks — a treatment period ensuring induction of small liver tumours. Results showed that continued growth of the tumours was entirely dependent upon DCA treatment. The authors suggested that DCA's main effect in tumour induction is mediated through accelerated growth of spontaneously initiated cells and that it may be due to the suppression of apoptosis and modification of cell replication rates.

Another hypothesis for promoters is the suppression of apoptosis. Snyder et al. (1995) studied the frequency of spontaneous apoptosis in liver hepatocytes of male B6C3F1 mice when given DCA at dose levels of 0, 500 or 5000 mg/L in their drinking water for 5–30 days. DCA significantly reduced apoptosis in treated mice in a dose-dependent manner relative to the untreated controls. The authors suggest that disrupting the apoptosis process can result in the outgrowth of initiated cells (by suppressing the ability of the liver to remove initiated cells, preneoplastic cells) and thus leading to the formation of tumours, rather than by induction of selective proliferation of initiated cells.

In a review of TCE and its metabolites (Bull, 2000), the data suggest that the modification of cell signalling pathways resulting in cell replication, selection and apoptosis may be an important contributor to the hepatocarcinogenicity of DCA.

In a hepatic cell proliferation study (Pereira, 1995, 1996), groups of 10 female B6C3F1 mice were exposed to DCA (neutralized) in drinking water at concentrations of 0.26, 0.86 or 2.6 g/L (52, 172 or 520 mg/kg bw per day) until sacrifice at day 5, 12 or 33. A concentration-related increase in cell proliferation was seen after 5 days of treatment with DCA but not at 12 and 33 days, except for an apparent increase at the high dose at 12 days. Therefore, DCA treatment caused a transient increase in hepatic cell proliferation (Pereira, 1995, 1996).

Increases in hepatocyte proliferation were observed in other short-term studies (Carter et al., 1995; Stauber and Bull, 1997). Decreased cell proliferation was seen at higher doses and with chronic dosing periods (Bull, 2000; U.S. EPA, 2003c).

Carter et al. (2003) conducted a histopathological analysis of hepatic lesions from male mice from the carcinogencity study by DeAngelo et al. (1999) in order to identify and quantify the different phenotypes of hepatocellular lesions, such as altered hepatic foci, large foci of cellular alteration, adenomas and carcinomas. As a result of the analysis, three different lesion sequences were proposed during mouse liver carcinogenesis — altered hepatic foci, large foci of cellular alteration and adenomas — which demonstrated neoplastic progression with time. The analysis also demonstrated that some toxic adaptive changes in non-involved liver were related to this neoplastic progression, regardless of the dose and length of exposure. According to Carter et al. (2003), the homeostasis of liver cells is altered by DCA, which leads to negative selection of cells (by suppressing apoptosis, a natural process for eliminating initiated cells), with a new state of differentiation resistant to DCA toxicity, thereby allowing the growth and/or survival of these initiated cells (i.e., preneoplastic cells and lesions). These dose-related effects occur at less than 1 g/L, which the authors considered as the inflection point of the dose–response curve for carcinogenesis.

10.3.2.1.5 Other mechanisms: hypomethylation

Another hypothesis for non-genotoxic carcinogens may be through an epigenetic mechanism involving DNA methylation (Pereira et al., 2004). The reduction in the level of DNA methylation (hypomethylation) is a common event in most cancers, including liver cancer (Pereira et al., 2004).

DNA methylation, a DNA modification that occurs naturally, takes place when a methyl group is added to the 5-position carbon of the cytosine ring to form 5-methylcytosine; the methyl group is supplied by *S*-adenosylmethionione, while the reaction is catalysed by DNA methyl-transferase (Ge et al., 2001; Pereira et al., 2004). Disruption of these different processes of DNA methylation may lead to hypomethylation (Tao et al., 2000).

DCA (neutralized) reduced the level of DNA methylation (hypomethylation) in the liver and as a result induced foci of altered hepatocytes and hepatocellular adenomas when given to female B6C3F1 mice in drinking water (Pereira et al., 2004). The authors suggested that there may be a correlation between the prevention of carcinogen-induced DNA methylation and prevention of liver tumours and that DNA hypomethylation may be critical for the carcinogenic effects of DCA.

Tao et al. (1998) showed in a promotion study that DCA reduced the levels of 5-methyl-cytosine in DNA of liver tumours and that the neoplastic progression of the liver lesions, from adenomas to carcinomas, seemed associated with a decrease in the level of 5-methylcytosine in DNA. In another study, Tao et al. (2000) reported that DCA decreased the methylation in the promoter regions for 2 proto-oncogenes, *c-jun* and *c-myc*, and increased the expression of their mRNA and proteins, while the addition of methionine prevented these changes. Both these proto-oncogenes are known to participate in the control of cell proliferation (U.S. EPA, 2003c).

Stauber and Bull (1997) identified that DCA-induced lesions had a phenotype that was *c-jun* immunoreactive.

Uncertainty exists regarding the actual importance that decreased methylation associated with an increase in the expression of the mRNA bears on the mediation of tumorigenicity of DCA (U.S. EPA, 2003c).

10.3.3 Trichloroacetic acid

Several studies on the potential carcinogenicity of TCA have been conducted and have been summarized in Table 9. Increased incidences of liver tumours (adenomas and carcinomas) were reported when mice were exposed to drinking water containing 500–5000 mg TCA/L for periods ranging from 52 to 104 weeks. No liver tumours were found in rats. The details of the studies are reported below.

In a 1-year chronic drinking water study (Bull et al., 1990), groups of male B6C3F1 mice (n = 61, 11 and 50) were exposed to TCA (neutralized) at 0, 1000 or 2000 mg/L (0, 178 and 319 mg/kg bw per day, respectively; WHO, 2004b), and a group of 10 females was exposed to 2000 mg/L in drinking water for up to 52 weeks. Interim sacrifices were made throughout the study, but all animals were sacrificed by week 52. Dose-related accumulation of lipofuscin (indicative of intracellular lipid peroxidation) in the liver was seen in mice at 52 weeks but not in males terminated at 37 weeks. Dose-related increases in the incidence of hepatoproliferative lesions — namely, hyperplastic nodules, adenomas and hepatocellular carcinomas — were seen in mice at 1000 mg/L and above at 52 weeks. Large concentrations of lipofuscin were found in areas surrounding the hepatoproliferative lesions but were absent from the lesions themselves. There was a linear relationship between the mean total dose of TCA consumed over 52 weeks and the number of hepatic lesions (nodules + adenomas + carcinomas) per mouse, although the incidence of lesions per mouse for animals in the 37-week exposure group was less than what would have been predicted on the basis of the total dose administered. No hyperplastic or neoplastic lesions were observed in any of the female mice treated with TCA. In rats treated with TCA, the liver showed only small increases in hepatic cell size, modest accumulation of glycogen, absence of focal necrosis and only marginal induction of cell proliferation and organ hypertrophy (Bull et al., 1990).

In a 60-week drinking water study (DeAngelo and Daniel, 1990), groups of male B6C3F1 mice (number not specified) were administered TCA (possibly neutralized based on a similar study with DCA by DeAngelo et al., 1991) at 0, 50, 500 or 5000 mg/L (0, 7, 71 and 595 mg/kg bw per day). A dose-related increase in the incidences of hyperplastic nodules, adenomas and carcinomas was seen. The hepatocellular tumour incidence was 55.2% at the high dose, 37.9% at the middle dose and 13.37% in the untreated control group. At the lowest dose (50 mg/L), no significant difference in tumour prevalence or multiplicity was observed compared with the control group. Hyperplastic nodules of the liver were seen in the highest dose only (prevalence: 24.1%), while none was seen at the lower dose or in the control group. Chronic inflammation and liver necrosis were observed in the two highest dose groups near the end of the study.

In another chronic study, female B6C3F1 mice (n = 10–40) received TCA (neutralized) at 2.0, 6.67 or 20.0 mmol/L (64, 212 and 640 mg/kg bw per day) in their drinking water for 360 or 576 days (Pereira, 1996; Pereira and Phelps, 1996). The control group received 640 mg sodium chloride/kg bw per day. At the high dose, after 360 days of exposure, a significant increase in hepatocellular carcinoma incidence and multiplicity was seen; after 576 days of exposure, significant increases in altered hepatic foci, hepatocellular adenoma and hepatocellular carcinoma incidences and yields were seen. After 576 days, the middle dose also had increased incidences and yields of foci and carcinomas. The combined numbers of total lesions (foci +

adenomas + carcinomas) and total tumours (adenomas + carcinomas) appeared to be linearly related to TCA dose. The authors concluded that TCA is a peroxisome proliferator and that the basophilic staining of the tumours is consistent with other peroxisome proliferators (Pereira, 1995, 1996; Pereira and Phelps, 1996).

In a review of the carcinogenicity of TCA in rodents by the U.S. EPA (1991), the results from a carcinogenicity study of TCA (form unknown) in female B6C3F1 mice (number unknown) were reported. Groups of female mice were administered 0, 500 or 4500 mg/L (0, 71 or 583 mg TCA/kg bw per day; WHO, 2004b) in drinking water for 104 weeks. The combined liver tumour incidence (adenomas + carcinomas) was significantly increased in the high-dose group. The high dose had a 64% hepatocellular tumour incidence, and the lowest dose had a tumour incidence of 16.7%; the incidence was 7.7% in the untreated control group. The LOAEL as derived by U.S. EPA (1991) in female mice is 71 mg/kg bw per day based on the presence of tumours.

There was no evidence of increased liver tumours in male F344 rats in a 2-year carcinogenicity study in which groups (50 per dose) were administered TCA (neutralized) at 0, 50, 500 or 5000 mg/L (0, 3.6, 32.5 or 364 mg/kg bw per day) in drinking water (DeAngelo and Daniel, 1992; DeAngelo et al., 1997). In the high-dose group, significant decreases were seen in body weights and in absolute liver weights, while increases in serum levels of ALT and palmitoyl conenzyme A activity (a marker of hepatic peroxisome proliferation) were seen. The authors set a NOEL of 364 mg/kg bw per day. A NOAEL based on non-neoplastic effects is derived at 32.5 mg/kg bw per day.

The ability of TCA to act as a promoter of carcinogenesis in the liver was also examined in several studies. Parnell et al. (1986, 1988) studied the initiation and promotion abilities of TCA (neutralized) using rat hepatic enzyme-altered foci bioassays. The authors concluded that TCA was a weak peroxisome proliferator and a liver tumour promoter, but not an initiator of liver tumours in Sprague-Dawley rats (Parnell et al., 1986, 1988).

The potential of TCA (neutralized) to promote tumours was also investigated in male B6C3F1 mice (Herren-Freund and Pereira, 1986; Herren-Freund et al., 1987). The incidence of hepatocellular adenomas and carcinomas was significantly increased for both types of treatment: those pretreated with an initiator or not. The authors concluded that TCA was hepatocarcinogenic, regardless of pretreatment with an initiator (Herren-Freund and Pereira, 1986; Herren-Freund et al., 1987).

In a second promotion study, Pereira (1995) and Pereira and Phelps (1996) used groups of female B6C3F1 mice. A small increase in the incidence of neoplastic changes was noted at the highest dose in TCA groups without prior initiation. In contrast, when mice were pretreated with an initiator and then exposed to TCA, a significant increase in the incidence and multiplicity of adenomas and carcinomas was seen at the higher doses. The total number of neoplastic lesions per mouse was linearly related to the TCA dose with time.

10.3.4 Monobromoacetic acid

No long-term studies on MBA were identified.

10.3.5 Dibromoacetic acid

Although an abstract published by Bull (1995) referred to a 2-year limited drinking water study on the carcinogenic potential of DBA and other brominated haloacetates in B6C3F1 mice and F344 rats, the final version has yet to be published.

However, a more recent 2-year carcinogenicity drinking water study on DBA was published by Melnick et al. (2007) and with greater detail by NTP (2007). Groups of F344/N rats (50 per sex per dose) and B6C3F1 mice (50 per sex per dose) were administered DBA (neutralized to pH 5) in drinking water at 0, 50, 500 or 1000 mg/L (equivalent to 0, 2, 20 and 40 mg/kg bw per day for male rats and 0, 2, 25 and 45 mg/kg bw per day for female rats; 0, 4, 45 and 87 mg/kg bw per day for male mice and 0, 4, 35 and 65 mg/kg bw per day for female mice). No effect on survival was seen in either species, nor were there any changes in body weight in mice. However, in rats (both sexes), mean body weight was decreased at the two highest doses compared with controls, whereas water consumption was decreased at the highest dose (both sexes). Neoplastic lesions were observed at multiple sites in both rats and mice.

In male rats, a significant increase in malignant mesotheliomas of the abdominal cavity was observed at the highest dose. In female rats, a positive increasing trend in the incidence of monocellular cell leukaemia, a haematopoietic (involved in the formation of blood cells) neoplasm, was noted that was significant at the highest dose. In contrast, male rats showed a significant increase in monocellular cell leukaemia at the lowest dose and no increase at the highest dose (which was similar to concurrent controls and historical controls), but incidences seen at the low and middle doses exceeded historical controls. Other non-cancer effects included significant increased incidence of lesions in the liver (cystic degeneration, minimal to mild) of all exposed groups of males, in the lung (alveolar epithelial hyperplasia) of the two highest dose groups in females, and in the kidney (nephropathy) of all exposed groups of females.

In mice, neoplasms were observed in both the liver and lung. A significant increase in the incidence of multiple hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) was observed in all treated males and in females at 500 mg/L and above, whereas a significant increase in the incidence of hepatocellular carcinoma was observed only in high-dose males and in mid-dose females. A significant increase in the incidence of hepatoblastoma was observed in male mice at 500 mg/L and above. The incidence of alveolar/bronchiolar adenoma exceeded the historical controls in males at 500 mg/L and 1000 mg/L, although the increase was significant only in males at 500 mg/L. In females, a non-significant increase in lung neoplasms was observed. Non-cancer effects, non-significant increases in the incidence of alveolar epithelial hyperplasia, were observed at all doses in male mice. An increased incidence of splenic haematopoieisis was also observed in high-dose male mice (NTP, 2007).

NTP (2007) indicates that there was clear evidence of carcinogenic activity of DBA in mice, based on increased incidences of hepatocellular neoplasm (both sexes) and hepatoblastomas (male only). It also finds some evidence of carcinogenic activity in rats based on an increased incidence of malignant mesothelioma in males and an increased incidence and positive trend of mononuclear cell leukaemia in females.

10.4 Mutagenicity and genotoxicity

10.4.1 Monochloroacetic acid

There was no evidence of genotoxic potential in mutagenicity studies in bacteria using *Salmonella typhimurium* (Rannug et al., 1976; NTP, 1992; BG Chemie, 1993; Giller et al., 1997; ECETOC, 1999). Mostly positive results were seen in assays using mammalian cells (mouse lymphoma assay) (Amacher and Turner, 1982; McGregor et al., 1987) , but these may be due to changes in pH or cytotoxicity (ECETOC, 1999). *In vitro* DNA repair/damage assays involving *Escherichia coli, S. typhimurium* and cultured mammalian cells were largely negative (Gross et al., 1982; Ono et al., 1991; Chang et al., 1992; NTP, 1992; BG Chemie, 1993; Giller et al., 1997; ECETOC, 1999), except for one study looking at DNA strand breaks in Chinese hamster ovary cells, which was positive (Plewa et al., 2002). Clastogenic studies were mostly negative with MCA (Galloway et al., 1987; Sawada et al., 1987; Giller et al., 1997), except for one study in which MCA (acid) induced sister chromatid exchange in Chinese hamster ovary cells without S9 only (Galloway et al., 1987). In an *in vivo* bone marrow assay, positive results were seen via the intraperitoneal route, but not by the oral or subcutaneous route (Bhunya and Das, 1987). Details were lacking in this study. Sperm shape abnormalities were seen in an intraperitoneal injection study in mice only at the top two doses, but this study was poorly reported (Bhunya and Das, 1987).

10.4.2 Dichloroacetic acid

There was mostly negative evidence of genotoxic potential in mutagenicity studies in bacteria using *S. typhimurium* (Herbert et al., 1980; Matsuda et al., 1991; DeMarini et al., 1994; Fox et al., 1996; Giller et al., 1997; Meier et al., 1997), and equivocal results were seen with mammalian cells (Fox et al., 1996; Harrington-Brock et al., 1998). Clastogenic studies demonstrated largely negative results (Fox et al., 1996; Giller et al., 1997; Meier et al., 1997). DNA repair assays with bacteria were generally positive, but involved DCA as a free acid. DNA damage assays (strand breaks) involving mammalian cells (*in vitro* and *in vivo*) were largely negative (Chang et al., 1992; Plewa et al., 2002), except for one oral study involving mice and rats where the acid was used (Nelson and Bull, 1988; Nelson et al., 1989). Dose-related sperm head abnormalities were seen in mice following oral gavage with DCA (sodium salt) at doses of 1125–4500 mg/kg bw per day (Meier et al., 1997).

10.4.3 Trichloroacetic acid

There was no evidence of genotoxic potential in mutagenicity studies in bacteria (IARC, 1995; Kargalioglu et al., 2002; NTP, 2003a), except in one modified Ames study, which was weakly positive (Giller et al., 1997). Weakly positive results were also seen in the one gene mutation study with mammalian cells (Harrington-Brock et al., 1998). *In vitro* DNA damage assays with mammalian cells were negative (Chang et al., 1992; Plewa et al., 2002), but mixed results were seen when TCA was administered *in vivo* (Nelson and Bull, 1988; Nelson et al., 1989; Chang et al., 1992). TCA gave mostly negative results for DNA damage/repair with bacterial systems (Ono et al., 1991; Giller et al., 1997).

Clastogenic studies (*in vitro* and *in vivo*) also demonstrated equivocal results (Bhunya and Behera, 1987; MacKay et al., 1995; Giller et al., 1997; Meier et al., 1997). Harrington-Brock

et al. (1998) reported that positive results for clastogenicity and mutagenicity may be seen in *in vitro* mammalian assays as a result of low pH, especially in the presence of metabolic activation.

Sperm shape abnormalities in mice were equivocal with intraperitoneal administration but negative with oral dosing (Bhunya and Behera, 1987). Gap-junctional intercellular communication was seen in liver cells of mice (*in vitro*) (IARC, 1995).

10.4.4 Monobromoacetic acid

There is some evidence to suggest that MBA is weakly mutagenic. Mixed results were seen in the Ames assay with MBA (Saito et al., 1995; Kohan et al., 1998; Kargalioglu et al., 2002; NTP, 2003b). Kargalioglu et al. (2002) reported in their Ames assay that MBA was more mutagenic than DBA or MCA. There was also slight evidence of genotoxic potential in *in vitro* DNA damage assays. MBA (acid) failed to induce primary DNA damage in an SOS chromotest (*E. coli*) with and without S9 and failed to increase the frequency of micronuclei in a newt micronucleus test (Giller et al., 1997). In contrast, MBA induced DNA strand breaks in the absence of S9, following a 1-hour treatment, using L-1220 mouse leukaemia cells (Stratton et al., 1981). The number of strand breaks increased further when the chemical was removed (Stratton et al., 1981). The authors suggested that MBA may act as an alkylating agent due to the presence of strand breaks (indicator of direct DNA damage).

10.4.5 Dibromoacetic acid

There is some evidence to suggest that DBA is weakly mutagenic. Mixed results were seen with the Ames test with *S. typhimurium* (Saito et al., 1995; Giller et al., 1997; Morita et al., 1997; Kohan et al., 1998; Kargalioglu et al., 2002; NTP, 2007). Positive results were seen for genotoxic potential in *in vitro* DNA damage assays. DBA induced primary DNA damage in an SOS chromotest using *E. coli* with and without metabolic activation (Giller et al., 1997), and induced DNA strand breaks in Chinese hamster ovary cells as measured in a comet assay (Plewa et al., 2002). There was also some evidence of clastogenicity (increased frequencies of micronucleated normochromatic erythrocytes using peripheral blood) in male mice, but not in female mice, when DBA was administered orally via drinking water as part of a 13-week study (NTP, 2007). No clastogenic activity was seen in the newt micronucleus test (Giller et al., 1997).

10.5 Reproductive and developmental toxicity

10.5.1 Monochloroacetic acid

No reproductive studies were identified. No adequate developmental studies were conducted with MCA. Two studies are reported below. However, they lack important information; one study originates from an abstract, and the other one uses only one dose.

One developmental assay (oral gavage with MCA acid in distilled water at 0, 17, 35, 70 or 140 mg/kg bw per day from gestation days 6 to 15) using Long-Evans rats was published in an abstract by Smith et al. (1990). This study suggested developmental effects (laevocardia in the high-dose group) in the presence of maternal toxicity. No statistical data were provided in the abstract, and no final study was published to confirm these results.

In a second developmental study (using only one dose), a group of 10 pregnant Sprague-Dawley rats was given MCA (neutralized) in drinking water at a concentration of 1570 mg/L

(193 mg/kg bw per day) on gestation days 1–22 (Johnson et al., 1998). The control group consisted of 55 females. A significant decrease in body weight gain was observed in exposed dams relative to controls. The average amount of drinking water consumed on a daily basis per maternal rat was lower in treated dams than in the control group. No adverse reproductive, developmental or teratogenic effects were reported; however, a complete fetal examination for internal or skeletal abnormalities was not conducted.

10.5.2 Dichloroacetic acid

10.5.2.1 Developmental studies

Several studies on the potential developmental toxicity of DCA in female rats have been conducted and are detailed below and summarized in Table 11.

Table 11: Summary of DCA (salt) developmental toxicity in rats

Doses (mg/kg bw per day)	Species	Developmental effects	LOAEL/ NOAEL	Reference
0, 14, 140, 400, 900, 1400, 1900 or 2400 (GD[a] 6–15)	Long-Evans hooded rats (19–21/dose)	- increased post-implantation resorptions at 900+ mg/kg bw per day - reduced fetal body weights at 400+ mg/kg bw per day - maternal toxicity at 14+ mg/kg bw per day - malformations: cardiovascular at 400+ mg/kg bw per day soft tissue at 140+ mg/kg bw per day urogenital at 1400+ mg/kg bw per day	NOAEL for developmental toxicity: 14 mg/kg bw per day	Smith et al., 1992
Four studies: 1) 1900 (GD 6–8, 9–11 or 12–15) 2) 2400 (GD 10, 11, 12 or 13) 3) 3500 (GD 9, 10, 11, 12 or 13) 4) 1900 (GD 6–15)	Long-Evans rats (7–11/dose)	- no significant maternal toxicity - fetal heart malformations seen at: 1) 1900 mg/kg bw per day (GD 9–11 and 12–15) 2) 2400 mg/kg bw per day (GD 10, 12) 3) 3500 mg/kg bw per day (GD 9, 10, 12) 4) 1900 mg/kg bw per day (GD 6–15)		Epstein et al., 1992
0 or 300 (GD 6–15)	Sprague-Dawley rats (19–20/dose)	- no malformations of the heart		Fisher et al., 2001

[a] GD = gestation day.

In a developmental study (Smith et al., 1992), pregnant Long-Evans hooded rats (19–21 per dose) were administered DCA (neutralized) by gavage in two separate studies at dose levels of 0, 14, 140 or 400 or of 0, 900, 1400, 1900 or 2400 mg/kg bw per day on days 6–15 of gestation, inclusively. There were dose-related reductions in adjusted maternal weight gain (140 mg/kg bw per day and above), dose-related increases in relative liver weights (all doses) and dose-related increases in relative kidney and spleen weights (400 mg/kg bw per day and above). Treatment-related maternal lethality was seen at 1400 mg/kg bw per day and above

(1/19 [5.3%], 2/19 [10.5%] and 5/21 [24%], respectively). The pregnancy rates, total number of implants per litter and preimplantation losses were not affected by treatment. There was a dose-related increase in post-implantation loss rate (900 mg/kg bw per day and above), and the number of live fetuses per litter was reduced at the highest dose (at which significant maternal toxicity [lethality] was observed). A dose-related decrease in fetal body weight and crown–rump length was observed at 400 mg/kg bw per day and above. Dose-related increases in external (1400 mg/kg bw per day and above), total soft tissue (140 mg/kg bw per day and above), cardiovascular (400 mg/kg bw per day and above), urogenital (1400 mg/kg bw per day and above) and orbital (900 mg/kg bw per day and above) malformations were observed. Lower frequencies of urogenital (bilateral hydronephrosis, renal papilla, stage one) and orbital anomalies were seen compared with other malformations. The main fetal target for DCA was the heart and major vessels. The most common heart defect in fetuses occurred between the ascending aorta and the right ventricle and was identified as high interventricular septal defect. The second most common heart malformation was overt interventricular septal defect. The authors set the NOAEL for developmental toxicity at 14 mg/kg bw per day.

Four studies were performed with pregnant Long-Evans rats (7–11 per dose) exposed to DCA (neutralized) to determine the most sensitive developmental period and also to characterize heart defects (Epstein et al., 1992). In the first study, rats were treated by oral intubation with 1900 mg DCA/kg bw per day on gestation days 6–8, 9–11 or 12–15; in the second study, with 2400 mg/kg bw per day on gestation day 10, 11, 12 or 13; and in the third study, with 3500 mg/kg bw per day on gestation day 9, 10, 11, 12 or 13. No significant maternal toxicity was observed in the first three studies. The mean percentages of heart malformations were significantly higher (statistically) in the group treated with 1900 mg DCA/kg bw per day on gestation days 9–11 (7.2% per litter) and 12–15 (15.1% per litter) compared with the combined controls. The cardiac defects seen were either high interventricular septal defect or a conventional interventricular septal defect. Lower incidences of cardiac malformations were observed with the 2400 mg DCA/kg bw per day dose (gestation day 10: 2.5% per litter; gestation day 12: 3.3% per litter; gestation days 11 and 13: 0%). The incidences on gestation days 10 and 12 were significantly different from the combined controls. At the higher dose, 3500 mg/kg bw per day, the incidences of heart defects were not increased, but this study showed that dosing on gestation days 9, 10 and 12 (3.6%, 2.9% and 2.9% per litter, respectively) would produce these defects, and the incidences were significantly different from combined control (0.5% per litter). No heart defects were seen on gestation day 11 or 13 (Epstein et al., 1992).

In a fourth study (Epstein et al., 1992) to characterize heart defects, six dams were orally intubated with 1900 mg DCA/kg bw per day on days 6–15 of pregnancy, and 56 fetuses from the six litters as well as eight control fetuses from four litters were harvested for light microscopic examination of the heart. Examination revealed that 25 of 56 fetuses (45%) had cardiovascular defects; 24 had hearts characterized by high interventricular septal defects (five of these hearts also presented a membranous-type interventricular septal defect), and one had an isolated occurrence of an interventricular septal defect, membranous type.

In a more recent study, Fisher et al. (2001) gavaged a group of 20 pregnant female Sprague-Dawley rats with DCA (neutralized) at 300 mg/kg bw per day during gestation days 6–15. A control group (n = 19) was dosed with water. No malformations of the heart were seen,

contrary to those reported in previous studies by Smith et al. (1992) and Epstein et al. (1992). It is possible that this study was not sensitive enough, given the high background of heart malformations (on a per litter basis) seen in the water controls (31.6%; higher than in the treated animals: 30%), which might have masked the effects in the DCA treatment group. The Smith et al. (1992) study showed only one or two cardiovascular defects at doses below 300 mg/kg bw per day. It is also possible that the strain differences in the rats and differences in the purity of the test agents used may account for the incongruent findings between the two set of studies.

10.5.2.2 Reproductive studies

Adult Sprague-Dawley male rats (n = 24 per dose) were given single oral doses of DCA (neutralized) at 0, 1500 or 3000 mg/kg bw to study testicular toxicity and then sacrificed (n = 8 per time period) on day 2, 14 or 28 (Linder et al., 1997a). No clinical signs of toxicity were seen in treated rats. Body weights were not statistically different from controls at any of the three time points. Mild effects on spermiation (i.e., delays) and changes in the degree of resorption of residual bodies were seen at both doses; these effects persisted to varying degrees throughout the observation period (Linder et al., 1997a).

In a parallel study by the same author, another group of male rats (n = 8–26) was given multiple oral doses of DCA (neutralized) at 0, 18, 54, 160, 480 or 1440 mg/kg bw per day for up to 14 days (Linder et al., 1997a). No clinical signs of toxicity were observed in the treated animals, except for reduced weight gain, which was seen at the three top doses by day 14. Delayed spermiation and formation of atypical residual bodies were observed in all treated males, except at the lowest dose. An increased number of fused epididymal sperm occurred after day 5 at doses of 160 mg/kg bw per day and above. A decrease in percentage of motile sperm was seen on day 9 at 480 mg/kg bw per day and above and on day 14 at 160 mg/kg bw per day and above. On day 14, epididymal sperm count was decreased at 160 mg/kg bw per day and above, while a decrease in epididymal sperm weight was seen at 480 mg/kg bw per day and above. Distorted sperm heads and acrosomes were seen in step 15 spermatids after 14 days at 480 mg/kg bw per day and above. Testicular lesions developed with greater severity as the duration of the dosing and dose levels increased.

Long-Evans male rats (n = 8–19) were gavaged daily with DCA (sodium salt) at 0, 31.3, 62.5 or 125 mg/kg bw for 10 weeks (Toth et al., 1992). On day 70, each male was mated overnight with one untreated, pro-estrous female to assess fertility. Males were then sacrificed on day 75 and females on day 14 of gestation. Pregnancy and implantation rates were not significantly different from the female controls. However, in high-dose females, a reduction in the number of live implants was observed. In males, decreased weights were seen in the epididymis and preputial glands at 31.3 mg/kg bw per day and above and in the accessory organs (prostate and seminal vesicles) at the highest dose (125 mg/kg bw per day). At the two highest doses, sperm morphology was affected and epididymal sperm counts were decreased. Effects on the testis and spermiation (a reduction in late-step spermatid head counts) were seen only in the highest dose group. The authors set a LOAEL of 31.3 mg/kg bw per day for DCA (sodium salt) (equivalent to 26.7 mg DCA/kg bw per day), based on adverse male reproductive effects on the accessory organs and sperm (Toth et al., 1992).

In contrast, in a shorter 7-week subchronic drinking water study, Sprague-Dawley rats had normal testicular histopathology and sperm production when exposed to DCA (sodium salt) at 1100 mg/kg bw per day (Stacpoole et al., 1990). Normal histopathology and testis weights were also seen in male Sprague-Dawley rats (10 per dose) when treated with DCA (neutralized) in their drinking water at lower doses of 0, 50, 500 or 5000 mg/L (0, 4, 35 or 350 mg/kg bw per day) as part of a 90-day drinking water study (Mather et al., 1990).

In a 3-month gavage study (Katz et al., 1981), DCA (sodium salt) was administered to groups of male and female Sprague-Dawley rats (10 per sex per dose) at dose levels of 0, 125, 500 or 2000 mg/kg bw per day. An additional five rats per sex dosed with 0 or 2000 mg/kg bw per day were allowed a recovery period of 4 weeks. No adverse reproductive effects were observed in female rats, whereas adverse effects were observed in males only at the two highest doses. Testicular germinal epithelial degeneration was observed in 40% of mid-dose males and 100% of high-dose males. High-dose males showed aspermatogenesis and formation of syncytial giant cells in the germinal epithelium, whereas no spermatozoa were seen in the epididymis ducts. Syncytial giant cells were also seen in 20% of males from the mid-dose group. In the high-dose recovery group (n = 5), half of the males showed germinal epithelium regeneration, 75% were aspermatogenic and all showed loss of germinal epithelium.

As part of another 90-day study (Cicmanec et al., 1991), 4-month-old male and female beagle dogs (five per sex per dose) received DCA (neutralized) in gelatin capsules at 0, 12.5, 39.5 or 72 mg/kg bw daily. No significant weight changes were seen in the ovaries or the testes compared with controls. No effects of DCA on uterine histopathology in treated females were seen. Testicular changes (syncytial giant cell formation and degeneration of testicular germinal epithelium) were observed in all treated males, and prostatic glandular atrophy was observed in the mid- and high-dose males. A dose-related increase in severity of testicular lesions were seen at the mid- and high-dose groups.

In a 13-week subchronic study (Katz et al., 1981), similar testicular effects were observed in 10- to 12-month-old male beagle dogs (3–4 per dose) administered DCA (sodium salt) in capsules at doses of 0, 50, 75 or 100 mg/kg bw per day. Testicular changes in the germinal epithelium (degeneration of germinal epithelium, vacuolation of Leydig cells and formation of syncytial giant cells) as well as prostate gland atrophy were seen in all treated males. One male from the high-dose group was allowed to recover for 4 weeks post-treatment; the prostate appeared normal, and there was evidence of germinal epithelium regeneration with spermatogenesis.

DeAngelo et al. (1996) reported, as part of a modified carcinogenesis bioassay with male F344 rats, that testicular and relative testicular weights were slightly increased in the mid-dose group (500 mg/L), but absolute testes weights were decreased in the high-dose group (1600 mg/L).

10.5.3 Trichloroacetic acid

No reproductive studies were identified

In a developmental study, pregnant Long-Evans rats (n = 20–26) were treated with TCA (neutralized) at 0, 330, 800, 1200 or 1800 mg/kg bw per day (as sodium salt) by gavage on gestation days 6–15. No treatment-related maternal deaths were observed during the study. A

dose-dependent increase in the frequency of resorptions per litter (34%, 62% and 90% of implants resorbed at dose levels of 800, 1200 and 1800 mg/kg bw per day, respectively) was observed at maternally toxic doses (decreased weight gain and dose-related increases in spleen and kidney weights). At all dose levels, a dose-related reduction in fetal weight and length as well as a dose-related increase in the frequency of soft tissue anomalies (from 9% at 330 mg/kg bw per day to 97% at 1800 mg/kg bw per day) were seen. Soft tissue anomalies were found mostly in the cardiovascular system and consisted of interventricular septal defects and laevocardia; however, the authors reported that this strain of rats is somewhat susceptible to laevocardia. At the two highest doses, an increase in skeletal malformations (principally in the orbit) was also observed. The maternal LOAEL is considered to be 330 mg/kg bw per day based on weight changes in the spleen and kidney. The developmental LOAEL is considered to be 330 mg/kg bw per day based on an increase in the frequency of soft tissue anomalies (Smith et al., 1989). No NOAEL was determined; however, a benchmark dose of 218 mg/kg bw per day was calculated for developmental toxicity (Health Canada, 2004b) for purposes of comparison with other end-points seen with TCA.

Previous developmental studies with TCE have shown an increase in congenital cardiac lesions in rats; however, it was suggested that metabolites of TCE were possibly responsible for these effects (Johnson et al., 1998). As a result, researchers conducted developmental studies with several metabolites of TCE, including TCA, to identify the responsible metabolites. The two studies that included TCA are described below but are inconclusive for the observed heart defects, since they used different methodologies and obtained conflicting results.

In a study by Johnson et al. (1998), pregnant Sprague-Dawley rats were given TCA (neutralized) in drinking water at concentrations of 0 mg/L (n = 55) or 2730 mg/L (n = 11) (0 or 290 mg/kg bw per day) on gestation days 1–22. A significant decrease in body weight gain was observed in treated dams, relative to controls. Developmental effects included a statistically significant increase in the number of resorptions, in the number of implantation sites and in cardiac soft tissue malformations (10.53% versus 2.15% for controls).

Fisher et al. (2001) gavaged a group of 19 pregnant female Sprague-Dawley rats with TCA (neutralized) at 300 mg/kg bw per day during gestation days 6–15. A control group (n = 19) was dosed with water. Mean maternal body weight gain was significantly less than controls on gestation days 7–15 and 18–21. A statistically significant reduction in fetal body weight (on a per fetus basis and on a per litter basis) was seen in the treated group. No malformations of the heart were seen at 300 mg/kg bw per day, contrary to those reported in the previous study by Smith et al. (1989).

10.5.4 Monobromoacetic acid

No well-conducted developmental studies were done with MBA. Randall et al. (1991) published an abstract on a study with MBA (acid) in Long-Evans rats suggesting possible soft tissue malformations in pups in the presence of maternal toxicity; however, the final study was never published.

No adverse reproductive effects were observed in male Sprague-Dawley rats when administered MBA (neutralized) at 0 or 100 mg/kg bw in a single dose or at 0 or 25 mg/kg bw per day for 14 days (Linder et al., 1994a).

10.5.5 Dibromoacetic acid

No effect on early pregnancy was seen in groups of mature female Holtzman rats (eight per dose per experiment) when gavaged with DBA (neutralized) at 0, 62.5, 125 or 250 mg/kg bw per day during gestation days 1–8. A group of dams was sacrificed on gestation day 9 or 20. The only effect, an increase in serum 17β-estradiol at the highest dose, was seen in dams sacrificed on gestation day 9 (Cummings and Hedge, 1998).

No adequate developmental studies were conducted. However, two developmental screening assays (oral gavage with neutralized DBA) using CD-1 mice were published in abstracts by Narotsky et al. (1996, 1997). Although these studies suggested developmental effects in the presence/absence of maternal toxicity, no statistical data were provided in either abstract, and no final study was published to confirm these results.

Dose-related alterations of estrous cyclicity in female Sprague-Dawley rats were observed at doses of 90 and 270 mg/kg bw per day when rats were dosed for 14 consecutive days in drinking water with DBA (neutralized) (Balchak et al., 2000), but no alterations were seen at lower doses (10 or 30 mg/kg bw per day) in the same study or during a 20-week exposure to a dose of 5, 16 or 33 mg/kg bw per day (Murr and Goldman, 2005).

In a two-generation drinking water study, groups of Sprague-Dawley rats (30 per sex per dose) were treated with DBA (97% purity in deionized water) continuously via the drinking water at concentrations of 0, 50, 250 or 650 mg/L (equivalent to 0, 4.4–11.6, 22.4–55.6 and 52.4–132.0 mg/kg bw per day, respectively) (Christian et al., 2002). Doses were determined based on a range-finding reproductive/developmental study by Christian et al. (2001). Reduced water consumption was seen at all dose levels of the parental (P) and F1 generations. In the top dose group of the P and F1 generations, clinical signs associated with reduced water consumption, reduced body weights and weight gains and reduced food consumption were observed. Food consumption was also decreased in the F1 mid-dose group. Body weight decreases were also seen with all doses of the F1 generation during lactation; as a result, weaning was delayed until day 29. Small delays in sexual maturation (preputial separation, vaginal patency) at the top dose in F1 rats and a significant decrease in anogenital distance on lactation day 22 in F2 male pups at the middle and high doses were also attributed to a general retardation of growth associated with a significant reduction in body weight. Reproductive performance and development of female rats were unaffected at all doses in both P and F1 generations. In males, sperm motility, count and density and abnormal sperm were also unaffected by treatment. However, histopathology of the reproductive organs of P and F1 males in the mid- and high-dose groups revealed altered sperm production (a dose-related increase in retained step 19 spermatids in Stage IX and X tubules, and the presence of abnormal residual bodies in affected seminiferous tubules) and some epididymal tubule changes. Reproductive effects were seen in high-dose F1 males: a significant increase in the unilateral malformation of the reproductive tract (small or absent epididymides, and small testis) was seen. The authors identified a parental NOAEL for general toxicity of 50 mg/L (4.4–11.6 mg/kg bw per day) based on an increase in absolute and relative kidney and liver weights in the absence of histopathology. A U.S. EPA draft report (U.S. EPA, 2005a) has proposed a reproductive/developmental LOAEL of 250 mg/L (or 22.4–55.6 mg/kg bw per day) based on abnormal spermatogenesis in P and F1 males and a reproductive/developmental NOAEL at 50 mg/L (4.4–11.6 mg/kg bw per day).

Testicular effects were observed in a 13-week drinking water study (Melnick et al., 2007; NTP, 2007) in which male B6C3F1 mice and F344 rats were exposed to DBA (neutralized to pH 5) at doses of 0, 125, 250, 500, 1000 or 2000 mg/L (equivalent to 0, 16, 30, 56, 115 and 230 mg/kg bw per day for mice, and 0, 10, 20, 40, 90 and 166 mg/kg bw per day for rats). Testicular atrophy of the germinal epithelium as well as significant reductions in testicular weight, sperm motility and sperm concentrations were observed in high-dose male rats, along with a significant increase in hypospermia. Delayed spermiation with atypical residual bodies was observed at the two highest doses in male mice and only in the middle doses (500 and 1000 mg/L) in rats. The lowest dose showing testicular effects in rats is 500 mg/L. NTP (2007) reported a NOEL for testicular lesions of 250 mg/L in rats. Similar effects (such as decreased testis weights, delayed spermiation or atypical residual bodies) were observed when rats (1000 mg/L and above) and mice (500 mg/L and above) were dosed for two weeks (Melnick et al., 2007; NTP, 2007).

Spermatotoxic/testicular effects consisting of testicular atrophy and sperm alteration (motility, morphology, count, fused or abnormal sperm, increased retention of step 19 spermatids, atypical residual bodies) were also observed in Sprague-Dawley rats given single or repeated oral doses of TCA (neutralized or acid) (Linder et al., 1994a,b, 1995; Vetter et al., 1998; Tsuchiya et al., 2000; Holmes et al., 2001).

Reproductive ability was compromised in male rats when gavaged with DBA (neutralized) (Linder et al., 1995). Abnormal sperm morphology, decreased sperm counts and motility and behaviour changes (during mating) in the males were noted.

Preliminary results of recently published abstracts (Klinefelter et al., 2000; Veeramachaneni et al., 2000; Bodensteiner et al., 2001; Veeramacheneni, 2002) suggest disruption in pubertal development, reproductive function, spermatogenesis and fertility in male rats and/or rabbits and a reduction of the population of primordial follicles in female rabbits when DBA (neutralized) is administered with the first exposure *in utero* from gestation day 15 throughout life. No final study was published to confirm these results.

11.0 Classification and assessment

11.1 Monochloroacetic acid

There was no evidence of carcinogenicity of MCA in mice or rats in a lifetime study (NTP, 1992). Tests for mutagenicity and genotoxicity of MCA were also largely negative. Based on the lack of evidence for carcinogenicity, MCA has therefore been classified in Group IV.D in this assessment, unlikely to be carcinogenic to humans (Health Canada, 1994).

MCA has not been evaluated by the International Agency for Research on Cancer (IARC). The U.S. EPA (2003b) reported that, under the 1999 Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999b), the data for MCA were considered “inadequate for an assessment of human carcinogenic potential.”

Several long-term studies conducted with MCA failed to show carcinogenicity. The lowest NOAEL of all the studies was from the 104-week drinking water study with rats by DeAngelo et al. (1997), for which a NOAEL of 3.5 mg/kg bw per day was derived by Health Canada for treatment-related changes in body, liver, kidney and testes weights. This study was

chosen for the risk assessment, based on the appropriateness of the vehicle used (drinking water), the absence of significant effects at the low dose, the length of the study as well as the form of MCA administered (i.e., neutralized solution).

The tolerable daily intake (TDI) for MCA is calculated as follows:

$$\text{TDI} = \frac{\text{3.5 mg/kg bw per day}}{300} = \text{0.0117 mg/kg bw per day}$$

where:

- 3.5 mg/kg bw per day is the NOAEL in the DeAngelo et al. (1997) chronic rat study, as derived by Health Canada,
- 300 is the uncertainty factor (×10 for interspecies variation, ×10 for intraspecies variation and ×3 for database deficiencies, including lack of reproductive/developmental studies).

Using the TDI derived from the NOAEL, a health-based target can be calculated as follows:

$$\frac{\text{0.0117 mg/kg bw per day} \times \text{70 kg} \times 0.2}{\text{1.5 L}} = \text{0.1 mg/L (rounded)}$$

where:

- 0.0117 mg/kg bw per day is the TDI, as derived above,
- 70 kg is the average body weight of an adult,
- 0.2 is the proportion of the daily intake allocated to drinking water; this is a default value, since there are insufficient data to calculate the actual value,
- 1.5 L is the average daily consumption of drinking water by an adult.

11.2 Dichloroacetic acid

Liver tumours in both mice and rats have been reported in several carcinogenicity bioassays (Herren-Freund et al., 1987; Bull et al., 1990; Daniel et al., 1992; Richmond et al., 1995; DeAngelo et al., 1996, 1999; Pereira and Phelps, 1996) and are considered sufficient evidence to classify DCA as an animal carcinogen. The exact mechanism for tumorigenicity has not been identified, and tests for mutagenicity and genotoxicity have been mostly negative or equivocal in bacterial and mammalian test systems. It is unknown at this time if the carcinogenicity of DCA is mediated by a non-genotoxic mechanism. DCA did not induce peroxisome proliferation (Tong et al., 1998b).

DCA has been classified in Group II, probably carcinogenic to humans, with sufficient evidence in animals and inadequate evidence in humans (Health Canada, 1994). IARC (2004) recently classified DCA as Group 2B, possibly carcinogenic to humans on the basis of sufficient evidence of its carcinogenicity in experimental animals and inadequate evidence of its carcinogenicity in humans. In the 2006 edition of the U.S. drinking water standards and health advisories, U.S. EPA (2006) considers DCA as “likely to be carcinogenic to humans” based on the 2005 U.S. EPA Guidelines for Carcinogen Risk Assessment. This is “based on current data

and the lack of conclusive data regarding the mode of action of DCA at environmentally relevant doses" (U.S. EPA, 2003c). There is no information available on the mutagenic effects of DCA in humans (CHEMINFO, 2003c).

Liver tumours in both mice and rats have been reported in several carcinogenicity bio-assays with DCA. Liver tumours (hepatocellular carcinomas) in mice were chosen for the cancer risk assessment, as the only rat study available used a high dose that was not well tolerated by the rats and had to be decreased several times. Therefore, cancer risks have been estimated on the basis of results of an adequate long-term drinking water study (90–100 weeks) in male B6C3F1 mice, which was conducted by DeAngelo et al. (1999). Liver tumours (hepatocellular carcinomas) seen in male mice showed an appropriate dose–response relationship. Other considerations for choosing the study for the risk assessment were the appropriateness of the vehicle used (drinking water), the form of DCA administered (i.e., neutralized solution), the length of the study as well as the use of numerous dose groups (five doses and a control group). A NOEL could not be determined for hepatocarcinogenicity because of the significant increase in hepatocellular carcinoma multiplicity observed at the lowest dose, 8 mg/kg bw per day (0.58 compared with 0.28 in the control) (DeAngelo et al., 1999).

Based on the classification of probable carcinogen and the uncertainty surrounding whether or not the carcinogenicity of DCA is mediated by a non-genotoxic mechanism, Health Canada has chosen to use a low-dose linear risk extrapolation for calculating the cancer risk. This is consistent with the most recent (2005) U.S. EPA Guidelines for Carcinogen Risk Assessment, which state that "when the weight of evidence evaluation of all available data are insufficient to establish the mode of action for a tumour site and when scientifically plausible based on the available data, linear extrapolation is used as a default approach, because linear extrapolation generally is considered to be a health-protective approach. Nonlinear approaches generally should not be used in cases where the mode of action has not been ascertained."

Therefore the unit risk can be assessed by the linearized multistage (LMS) method (Health Canada, 2004a), using the number of male B6C3F1 mice in each dose group having hepatocellular carcinomas following exposure to DCA in drinking water for 90–100 weeks (DeAngelo et al., 1999).

In this method, the multistage model is fit to the dose–response data, and the upper 95% confidence limit on the linear term is taken to be the unit risk. The multistage model is given by:

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

where d is dose, k is the number of dose groups in the study (excluding control), $P(d)$ is the probability of the animal developing a tumour at dose d and $q_i > 0$, $i = 0, \ldots, k$ 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 would 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.

The multistage model was fit using THRESH (Howe, 1995), and the unit risk was calculated by the LMS method without prior application of the kinetic adjustment factor. The P-value for the chi-square lack of fit test was 0.87, indicating that the model fit the data adequately. The units of the unit risks were converted to concentrations in drinking water by multiplying by the drinking rate of a human (1.5 L/day) and dividing by the standard body weight of a human (70 kg).

An animal-to-human kinetic adjustment factor (KA) (also known as allometric scaling factor, which corrrects for body weight differences between animals and humans) can also be applied. The animal-to-human kinetic adjustment factor (KA) is given by:

$$KA = \left(\frac{\text{animal weight kg}}{70\text{kg}} \right)^{1/4}$$

where 70 kg is the standard body weight of a human. Since the allometric scaling factor is applied to the unit risk after modelling, a mouse body weight of 43.9 g was used in the above formula. This was the average body weight in the control group.

The estimated calculated unit lifetime human cancer risk associated with the ingestion of DCA at 1 µg/L in drinking water is 1.02×10^{-6} (based on liver tumours, hepatocellular carcinomas).

In the context of drinking water guidelines, Health Canada has defined the term "essentially negligible" as a range from one new cancer above background per 100 000 people to one new cancer above background per 1 million people (i.e., 10^{-5} to 10^{-6}) over a lifetime. The estimated concentrations for these tumour types, based on the model described above, and the corresponding calculated unit lifetime human cancer risks are as follows:

Lifetime risk	**Concentration in drinking water (µg/L)**
10^{-4}	98.1 (rounded to 100)
10^{-5}	9.81 (rounded to 10)
10^{-6}	0.98 (rounded to 1)

Using the most conservative concentration in drinking water estimated for a 10^{-5} lifetime human cancer risk, a health-based target of 0.01 mg/L (10 µg/L) is derived for DCA in drinking water.

An additional analysis was undertaken in which the kinetic adjustment factor was applied to the experimental doses before modelling the data. This was done in order to facilitate comparisons with the U.S. EPA's (2003c) methodology. The multistage model was fit using THRESH (Howe, 1995), and unit risks were calculated by LMS, with prior application of the kinetic adjustment factor. The results demonstrated that applying the animal-to-human kinetic adjustment factor before or after fitting the model had no impact on the resulting unit risks or concentrations.

11.3 Trichloroacetic acid

TCA administered in drinking water has consistently been shown to produce liver tumours in mice but not in rats. The mechanism underlying the mouse liver tumours was determined to be based on peroxisome proliferation, which may or may not be relevant to humans (Cattley et al., 1998). TCA was seen as weakly genotoxic.

Based on evidence of carcinogenicity being limited to one species of rodents, the mouse, and inadequate evidence in humans, TCA has been classified in Group III in this assessment, possibly carcinogenic to humans (Health Canada, 1994). According to IARC (1995), there is inadequate evidence for its carcinogenicity in humans and limited evidence for its carcinogenicity in experimental animals. U.S. EPA (1986) classified TCA as a possible human carcinogen, whereas U.S. EPA (2003b), under the 1999 Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999b), stated that there is suggestive evidence for TCA carcinogenicity, but the data are not sufficient to assess human carcinogenicity.

Long-term drinking water studies produced liver tumours in mice, but not in rats. Since it is not known whether the underlying mechanism is relevant to humans, a long-term rat study with a non-cancer end-point was chosen for the risk assessment. The 104-week drinking water study by DeAngelo et al. (1997) also showed the lowest NOAEL of 32.5 mg/kg bw per day based on treatment-related changes (decreased body weight, increased liver serum enzyme activity and liver histopathology compared with control animals). Other considerations for choosing the study included the appropriateness of the vehicle used (drinking water), the absence of significant effects at the low dose, the length of the study and the form of TCA administered (i.e., neutralized solution).

The TDI for TCA can be calculated as follows:

$$\text{TDI} = \frac{\text{32.5 mg/kg bw per day}}{1000} = \text{0.0325 mg/kg bw per day}$$

where:

- 32.5 mg/kg bw per day is the NOAEL in the DeAngelo et al. (1997) chronic rat study,
- 1000 is the uncertainty factor (×10 for interspecies variation, ×10 for intraspecies variation and ×10 for database deficiencies, including lack of a multigenerational reproductive study, and possible carcinogenicity).

Using the TDI derived from the NOAEL, a health-based target can be calculated as follows:

$$\frac{0.0325 \text{ mg/kg bw per day} \times 70 \text{ kg} \times 0.2}{1.5 \text{ L/d}} = 0.3 \text{ mg/L (rounded value)}$$

where:

- 0.0325 mg/kg bw per day is the TDI, as derived above,
- 70 kg is the average body weight of an adult,
- 0.2 is the proportion of the daily intake allocated to drinking water; this is a default value, since there are insufficient data to calculate the actual value,
- 1.5 L/d is the average daily consumption of drinking water by an adult.

11.4 Monobromoacetic acid

There are insufficient data on the toxicity of MBA identified in this document to establish a health-based target. Acute oral studies have shown MBA to be acutely toxic. However, no subchronic, chronic or carcinogenic studies were conducted or published with MBA, nor was a standard developmental or multigeneration study conducted. Mixed results were seen with regards to mutagenicity/genotoxicity studies.

MBA has been classified in Group VI in this assessment, unclassifiable with respect to carcinogenicity in humans, based on inadequate data from animal studies (Health Canada, 1994). U.S. EPA (2005a) reported that, under the 1999 Draft Revised Guidelines for Carcinogen Risk Assessment, the data for MBA were considered "inadequate for an assessment of human carcinogenic potential." MBA has not been evaluated or classified by IARC.

11.5 Dibromoacetic acid

Based on the only oral acute toxicity study, DBA has been shown to be moderately toxic. Available subchronic and chronic studies suggest that the liver is the target organ. Reproductive studies suggest male-mediated effects, whereas the reporting of developmental studies is limited to abstracts (limited data) but may suggest fetotoxicity. Additional studies need to be conducted in order to adequately assess the developmental toxicity of DBA. Available mutagenicity studies are limited and show mixed results.

In a recent 2-year drinking water study in mice and rats conducted by NTP (2007) (also published as a summary by Melnick et al., 2007), DBA was shown to be a multiple-organ carcinogen in laboratory animals, with tumour induction seen in the liver and lung of mice, in the abdominal cavity (mesotheliomas) of male rats and in the haematopoietic system (monocellular cell leukaemia) in female rats. As a result, Health Canada has classified DBA as Group II, probably carcinogenic to humans, based on sufficient evidence in animals and inadequate evidence in humans (Health Canada, 1994). DBA has not been evaluated or classified by IARC. In 2005, before the publication of these new studies, the U.S. EPA (2005a) had reported that, based on the 1999 Draft Revised Guidelines for Carcinogen Risk Assessment, the data for DBA were considered "inadequate for an assessment of human carcinogenic potential."

Based on DBA's new classification as a probable carcinogen, the linearized multistage method was chosen to calculate unit risks. The application of the multistage model is described in Section 11.2.

The multistage models were fit using THRESH (Howe, 1995) and the unit risks were calculated (Health Canada, 2007a). An animal-to-human kinetic adjustment factor (KA) 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 KA formula is also described in Section 11.2. 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_i's whose estimates are non-zero. A p-value less than 0.05 indicates a significant lack of fit. Based on this criterion, a few models exhibited a significant lack of fit due to an uneven dose–response. Although no simple model will adequately describe these data, these models do provide a reasonable visual fit. This is in contrast to DCA, where the model fit the data adequately. The calculations for the unit risks (raw and converted using the allometic scaling factor) for DBA and lack of fit p-values are displayed in Health Canada (2007b).

The estimated unit lifetime risks associated with ingestion of 1 µg/L of DBA in drinking water were estimated to range from 0.14×10^{-6} to 4.26×10^{-6}. The unit risk range was derived from mesotheliomas observed in male rats (0.14×10^{-6}) as its lower bound (least sensitive) and hepatocellular adenoma/carcinoma in male mice (4.26×10^{-6}) as its upper bound (most sensitive).

The estimated concentrations for these tumour types and the corresponding calculated unit lifetime human cancer risks are as follows:

Lifetime risk	Concentration in drinking water (µg/L)
10^{-4}	23.5 - 701.6
10^{-5}	2.3 - 70.2
10^{-6}	0.23 - 7.0

Using the most conservative concentration in drinking water estimated for a 10^{-5} lifetime human cancer risk, a health-based target of 0.002 mg/L (2 µg/L) (rounded) is derived for DBA in drinking water.

11.6 International considerations

Several agencies have reviewed HAAs and have established guidelines or standards as a function of various factors, such as best available technology and/or health effects.

WHO (2004a,b, 2005) has established separate guideline values for three of the HAAs: MCA (20 µg/L), DCA (provisional guideline value of 50 µg/L) and TCA (200 µg/L). Although the World Health Organization (WHO) calculated a health-based guideline value of 40 µg/L for DCA based on a 10^{-5} upper-bound excess lifetime cancer risk, the guideline is provisional because "the data on treatment are insufficient to ensure that the 40 µg/litre value is technically achievable under a wide range of circumstances" (WHO, 2005). In regards to MBA and DBA, WHO (2004c) considered the databases inadequate for the derivation of guideline values.

The U.S. EPA (2006) has used a different approach and established a single maximum contaminant level (MCL) for all five HAAs (HAA5) of 0.06 mg/L based on best available

technology, as well as individual non-enforceable maximum contaminant level goals of 0.03 mg/L for MCA, 0 for DCA (based on its carcinogenicity) and 0.02 mg/L for TCA.

12.0 Rationale

Because haloacetic acids 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, 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. Although the use of chlorine can lead to the formation of DBPs such as HAAs, efforts to manage HAA levels in drinking water ***must not*** compromise the effectiveness of disinfection.

HAAs and THMs are the two major groups of 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 that may be found in drinking water supplies. In the absence of information on other DBPs, control and management of HAAs and THMs should reduce exposure to and risk from other by-products. When appropriate drinking water treatment strategies are implemented to reduce HAAs and THMs, the levels of other halogenated DBPs may also be reduced in the process.

There are sufficient scientific data available to derive health-based targets for four HAAs: MCA, DCA, TCA and DBA. MCA is classified as Group IV (unlikely to be carcinogenic to humans). A health-based target concentration of 0.1 mg/L can be calculated for MCA in drinking water, based on changes in body, liver, kidney and testes weights observed in rats. DCA is classified in Group II (probably carcinogenic to humans), based on sufficient evidence in animals and inadequate evidence in humans. A health-based target concentration of 0.01 mg/L can be calculated for DCA in drinking water, based on liver tumours observed in both mice and rats. TCA is classified in Group III (possibly carcinogenic to humans), based on limited evidence of carcinogenicity in experimental animals and inadequate evidence in humans. A health-based target concentration of 0.3 mg/L can be calculated for TCA in drinking water. Although animal studies have shown a link between exposure to TCA and liver tumours in mice only, it is still uncertain whether the mechanism causing these tumours is relevant to humans. MBA is classified in Group VI (unclassifiable with respect to carcinogenicity in humans), based on inadequate data from animal studies. No health-based target concentration can be established for MBA at this time. DBA is classified in Group II (probably carcinogenic to humans), based on sufficient evidence in animals and inadequate evidence in humans. A health-based target concentration of 0.002 mg/L can be calculated for DBA in drinking water, based on tumours in several organs observed in both mice and rats.

Recent Canadian exposure data for surface water sources show that the HAAs consistently found at the highest concentrations in Canadian distribution systems were DCA and TCA.

Although the proportion of each HAA will vary according to conditions, DCA can commonly represent 40–60% of the total HAA concentration. DBA makes up only a small fraction (generally less than 6%)* of the total HAA concentration.

The removal of HAAs after their formation in drinking water supplies is not considered to be the best approach to reduce exposure to HAAs. The most efficient and practical way to reduce HAA concentrations in finished waters is to prevent their formation, primarily through the removal of organic precursors. Although pH adjustments may help reduce HAA formation, they may cause a corresponding increase in the formation of other DBPs, including THMs.

Although health-based targets can be established for four of the five HAAs, and considering the technological limitations associated with reducing individual HAA levels in drinking water while maintaining effective disinfection, the Federal-Provincial-Territorial Committee on Drinking Water is establishing a MAC of ~~0.8~~ 0.08 mg/L (80 µg/L) for Total HAA5 in drinking water based on a running annual average rather than individual guidelines. This is consistent with the approach taken by the U.S. EPA, which established a maximum contaminant level based on best available technology for these same HAAs.

The MAC of 0.08 mg/L for total HAAs in drinking water is established, using a risk management approach based on the following considerations:

1a. Although the health-based target for DBA is the lowest value calculated, it is also found as a very small proportion of the HAA mixture and is not considered to be the appropriate driver for a health-based guideline. Mean levels of DBA observed are well below the health-based target of 0.002 mg/L for DBA.

1b. The health-based target for DCA is the lowest value that should be considered. The concentration of DCA in drinking water representing an "essentially negligible" risk is 0.01 mg/L, but this level cannot be achieved in distribution systems without compromising the effectiveness of disinfection.

2. The PQLs, based on the capability of laboratories to routinely measure HAAs within reasonable limits of precision and accuracy, vary according to the individual HAAs and the method used. However, all the PQL values are well below the MAC.

3. The MAC must be achievable at reasonable cost. Based on the limited available Canadian data, it is estimated that 88% of existing treatment plants serving populations over 5000 and 56% of existing treatment plants serving populations less than 5000 can be expected to achieve HAA concentrations of 0.08 mg/L. By optimization of treatment processes and the use of advance treatment technologies to remove organic compounds prior to disinfection, it is possible to reduce HAA5 below 0.08 mg/L.

The estimated lifetime cancer risk associated with the ingestion of drinking water containing HAAs at 0.08 mg/L is greater than the range that is considered generally to be "essentially negligible" (i.e., between 10^{-5} and 10^{-6}). Based on the incidence of liver cancer in animal studies for DCA, the estimated lifetime risk associated with ingestion of water containing HAAs at 0.08 mg/L is 3.2×10^{-5} to 4.8×10^{-5} for DCA proportions of 40 % to 60%, respectively, of total HAAs. Although exposure to HAAs at the guideline level may carry a

* based on table 3 - small system data from 1999-2000

lifetime risk from DCA that is higher than would normally be considered negligible, this risk is calculated using a very conservative approach that typically overestimates potential risk.

It is recommended that utilities strive to maintain HAA levels as low as reasonably achievable without compromising the effectiveness of disinfection. As part of its ongoing guideline review process, Health Canada will continue to monitor new research in this area and recommend any change(s) to the guideline that it deems necessary.

13.0 References

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Appendix A: List of acronyms

ALARA	as low as reasonably achievable
ALT	alanine transaminase
ANSI	American National Standards Institute
APHA	American Public Health Association
AST	aspartate transaminase
CAS	Chemical Abstracts Service
CDBP	chlorinated disinfection by-product
DBA	dibromoacetic acid
DBP	disinfection by-product
DCA	dichloroacetic acid
DNA	deoxyribonucleic acid
ECD	electron capture detection
EPA	Environmental Protection Agency (USA)
GAC	granular activated carbon
GC	gas chromatography
GST-zeta	glutathione-*S*-transferase-zeta
HAA	haloacetic acid
HAA5	total haloacetic acids; refers to the total of monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid and dibromoacetic acid
IARC	International Agency for Research on Cancer
KA	kinetic adjustment factor
kg bw	kilogram body weight
LD_{50}	median lethal dose
LMS	linearized multistage
LOAEL	lowest-observed-adverse-effect level
MAC	maximum acceptable concentration
MBA	monobromoacetic acid
MCA	monochloroacetic acid
MDL	method detection limit
mRNA	messenger ribonucleic acid
MTBE	methyl *tert*-butyl ether
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NOM	natural organic matter
NSF	NSF International
NTP	National Toxicology Program (USA)
NTU	nephelometric turbidity unit
PCE	perchloroethylene (tetrachloroethene)
PPAR	peroxisome proliferator activated receptor
ppm	parts per million
PQL	practical quantitation limit

SCC	Standards Council of Canada
TCA	trichloroacetic acid
TCE	trichloroethylene
TDI	tolerable daily intake
THM	trihalomethane
UV	ultraviolet
WHO	World Health Organization