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SOME CHEMICALS PRESENT IN INDUSTRIAL AND CONSUMER PRODUCTS, FOOD AND DRINKING-WATER

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

DIBROMOACETONITRILE

Dibromoacetonitrile was considered by previous IARC Working Groups in June 1990 and February 1998 (IARC, 1991, 1999). Since that time, new data have become available and have been incorporated into this *Monograph,* and taken into consideration in the present evaluation.

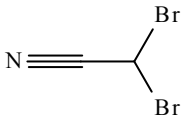
1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 3252-43-5
EC Number: 221-843-2
Chem. Abstr. Name: Acetonitrile, 2,2-dibromo-
IUPAC Systematic Name: 2,2-Dibromoacetonitrile
Synonyms: Acetonitrile, dibromo-; dibromocyanomethane

1.1.2 Structural and molecular formulae and relative molecular mass



C_2HBr_2N
Relative molecular mass: 198.84

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless to pale-yellow liquid with an organohalide odour (NTP, 2010)
Boiling-point: bp_{760} 169 °C; bp_{24} 68 °C (Lide, 2005)
Density: 2.369 at 20 °C (Lide, 2005)
Spectroscopy data: Infrared and magnetic resonance spectra (proton and C-13) have been reported (IARC, 1991; NTP, 2010).
Solubility: Slightly soluble in water
Vapour pressure: 0.3 mm Hg at 25 °C (HSDB, 2010)
Octanol/water partition coefficient (P): log P, 0.420 (IARC, 1991)
Conversion factor: $mg/m^3 = 8.13 \times ppm$, calculated from: mg/m^3 = (relative molecular mass/24.45) ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

1.1.4 Technical products and impurities

No information on technical products and impurities was available to the Working Group.

1.1.5 Analysis

Dibromoacetonitrile can be determined in drinking-water by gas chromatography with electron capture detection following liquid-liquid extraction, with a limit of detection of 0.034 µg/L (EPA, 1990).

1.2 Production and use

1.2.1 Production

Dibromoacetonitrile can be produced by treatment of cyanoacetic acid with *N*-bromosuccinimide (Wilt, 1956).

Information available in 2010 indicated that dibromoacetonitrile was manufactured by eight companies in the USA, two companies in the United Kingdom and one company in Germany (Chemical Sources International, 2010).

1.2.2 Use

Dibromoacetonitrile has been used as an antimicrobial component (≤ 3%) of a metalworking fluid (DOW, 2006).

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Dibromoacetonitrile is not known to occur naturally.

1.3.2 Occurrence and exposure in drinking-water

(a) Formation of halogenated acetonitriles disinfection by-products

Halogenated acetonitriles are formed during water disinfection as a result of the reaction of chlorinated oxidizing compounds (e.g. chlorine gas, hypochlorous acid and hypochlorite) with natural organic matter, such as algae, humic substances and proteinaceous material, present in water (IARC, 1991), and particularly nitrogen-containing organic compounds in water that contains bromide; it is also a by-product of disinfection by ozonation (Huang *et al.*, 2003, 2004).

Halogenated acetonitriles form rapidly, but then decay in the distribution system as a result of hydrolysis (IPCS, 2000); they have not been detected in raw (untreated) water sources (Trehy & Bieber, 1981).

Plants that used chloramines (with or without chlorination) had the highest levels of halogenated acetonitriles in their finished drinking-water. Higher levels were also observed in distribution-system waters treated by chloramination compared with free chlorine. However, the increased levels following chloramination may be the result of the higher levels of total organic carbon in the source waters (McGuire *et al.*, 2002).

Factors that affect the formation of halogenated acetonitriles in drinking-water supplies include water temperature, pH, the dose and type of disinfectant and contact time (IPCS, 2000; Huang *et al.*, 2003; Liang & Singer, 2003; Huang *et al.*, 2004; WHO, 2004).

(b) Concentrations in drinking-water

Haloacetonitriles have been measured in several studies of occurrence (Richardson *et al.*, 2007).

Chloro-, bromochloro-, dibromo- and trichloroacetonitrile are the most commonly measured halogenated acetonitrile species and have been included in a survey of 35 water utilities in the USA conducted in 1988–89 with a broad range of source water qualities and treatment processes (Krasner *et al.*, 1989; IPCS 2000). Median concentrations of total halogenated acetonitrile over the four seasons ranged from 2.5 to 4 µg/L, with median concentrations of dibromoacetonitrile ranging from 0.46 to 0.54 µg/L. At a drinking-water utility with high levels of bromide, clearwell effluent contained concentrations of dibromoacetonitrile ranging from

5.9 µg/L to 6.7 µg/L according to the season. At a utility with seasonally variable levels of bromide, concentrations of dibromoacetonitrile ranged from 4.6 µg/L to 11 µg/L.

Water collected from 53 Canadian drinking-water treatment facilities in the winter of 1993 was found to contain dibromoacetonitrile (Williams *et al.*, 1997). When bromide concentrations were very low (< 0.01 mg/L), the water contained < 0.1 µg/L dibromoacetonitrile; when they were low (0.06 mg/L), the water contained 0.6 µg/L dibromoacetonitrile; and when they were moderate (0.5 mg/L), the water contained 1.2 µg/L dibromoacetonitrile.

A nationwide study of the occurrence of disinfection by-products in diverse geographical regions of the USA was conducted between October 2000 and April 2002 (Weinberg *et al.*, 2002). Twelve water-treatment plants that had different source water quality and levels of bromide and used the major disinfectants chlorine, chloramines, ozone and chlorine dioxide were sampled. Concentrations of dibromoacetonitrile in the finished water ranged from 0.6 to 2.0 µg/L.

Dibromoacetonitrile was identified in stored, chlorinated Rhine water in the Netherlands. The concentration of dibromoacetonitrile was less than 0.1 µg/L before chlorination and 1 µg/L after chlorination (Zoeteman *et al.*, 1982).

Treatment plant samples collected in 1984 and 1985 from 29 community water systems in the USA (that used free chlorine disinfection) contained dibromoacetonitrile at levels of < 0.2–11 µg/L (14 of 29 sites). Samples from the distribution system contained dibromoacetonitrile at < 0.2–2.5 µg/L (11/26 sites) (Reding *et al.*, 1989).

Water samples were collected in 1985 from 10 utilities in the USA that used free chlorine disinfection (one of which also added ammonia before distribution). Dibromoacetonitrile was detected at concentrations of < 10 µg/L at three of the seven sites and was not detected in the others (Stevens *et al.*, 1989).

Groundwater samples were collected from utilities in Taiwan, China, which are subject to saltwater intrusion and, therefore, have high levels of bromide (up to 1.5 mg/L) (Huang *et al.*, 2003). Concentrations of dibromoacetonitrile resulting from ozonation of such groundwater — when detected — ranged from 3.1 to 18.1 µg/L (eight of 28 samples).

Seasonal variation in concentrations of haloacetonitriles was investigated in tap-water samples collected from five sampling points (one groundwater and four surface water sources) in İzmir, Turkey, between July 2006 and April 2007 (Baytak *et al.*, 2008). Dibromoacetonitrile was detected in 95% of samples (n = 217) with a mean concentration of 4.23 µg/L (median, 2.77 µg/L; range, not detected–16.4 µg/L; 90th percentile, 9.72 µg/L; 95th percentile, 11.4 µg/L). The limit of detection for dibromoacetonitrile was 0.073 µg/L. The highest concentrations of total haloacetonitriles were detected in spring and the lowest in summer and autumn at all locations. The highest levels of dibromoacetonitrile were detected at the groundwater sampling point, most probably due to bromide ion intrusion from seawater.

In a national survey in Canada, concentrations of dibromoacetonitrile ranged from < 0.1 µg/L (minimum quantifiable limit) to 1.2 µg/L in groundwater and surface water distribution systems (Health Canada, 1995). Samples were collected in 1993 during the winter (February–March) and summer (August–September), when levels of disinfection by-products were expected to be lowest and highest, respectively.

Dibromoacetonitrile was measured in water samples taken from a water-treatment plant in Barcelona between November 1997 and March 1998 (Cancho *et al.*, 1999). Dibromoacetonitrile was detected in pre-chlorinated water (mean, 2.5 µg/L; range, 0.6–7.6 µg/L), sand-filtered water (mean, 4.6 µg/L; range, 4.6–8.7 µg/L), ozonated

water (mean, 7 µg/L; range, 5.5–9.9 µg/L) and post-chlorinated water (mean, 1.5 µg/L; range, 0.6–3.1 µg/L).

(c) Dietary exposure from drinking-water

To assess exposure to disinfection by-products through drinking-water, WHO uses a default consumption value of 2 L drinking-water per capita per day and a typical body weight (bw) of 60 kg (WHO, 2008). The underlying assumption is that of a total water consumption of 3 L per capita per day, including water contained in food, which usually represents a conservative value (WHO, 2003).

The mean concentrations and ranges of dibromoacetonitrile concentrations from all available references were used by the Working Group to assess dietary exposure in adults and infants (60 and 5 kg bw, respectively) assuming a consumption of 2 and 0.75 L drinking-water, respectively, i.e. 33 mL/kg bw and 150 mL/kg bw, respectively (Table 1.1). The infant scenario (expressed in mL/kg bw) would correspond to the consumption of 9 L drinking-water per day in a 60-kg adult and therefore cover any possible scenario of physically active persons and increased temperature.

Based on concentrations of dibromoacetonitrile reported in the literature, average dietary exposure through drinking-water in a standard 60-kg adult ranges from 0.02 to 0.14 µg/kg bw per day; high observed concentration values would lead to a dietary exposure of 0.02–0.60 µg/kg bw per day. Similarly, average dietary exposure through drinking-water in a 5-kg infant ranges from 0.09 to 0.63 µg/kg bw per day; and high observed concentration values would lead to a dietary exposure of 0.08–2.71 µg/kg bw per day (Table 1.1).

An estimate of dietary exposure to dibromoacetonitrile arising from the measured consumption of drinking-water was performed by the Joint FAO/WHO expert meeting for Europe, the USA and Australia (FAO/WHO, 2009). The estimate for Europe was based on the mean consumption of 'tap-water' observed in adults in the 15 countries for which these data were available in the Concise European Food Consumption Database developed by the European Food Safety Authority (EFSA, 2008). The highest observed mean consumption of tap-water was 11 mL/kg bw per day (average consumption of 0.84 and 0.886 L per day for an average body weight of 74 and 77 kg, respectively, in Denmark and Finland). Estimated mean dietary exposure to dibromoacetonitrile was therefore up to 0.007 µg/kg bw per day in Europe. For the USA and Australia, mean dietary exposure to dibromoacetonitrile was estimated to be 0.009 µg/kg bw per day (assuming a mean body weight of 65 and 68 kg and a mean consumption of drinking-water of 0.926 and 0.969 L per day, respectively, in the USA and Australia).

(d) Other dietary sources

No data on the levels of dibromoacetonitrile in foods (other than drinking-water) could be identified. Extrapolations from values in drinking-water to values in food are difficult to achieve because the conditions of the chemical interactions, dosages, temperatures, contact times and especially the precursors differ considerably (FAO/WHO, 2009).

1.3.3 Exposure through inhalation or dermal contact

Dibromoacetonitrile occurs in water used for showering and bathing due to its presence in household water distribution systems (see Section 1.3.2). Dibromoacetonitrile was also detected in the water of two large public swimming pools disinfected with either chlorine or bromine in Barcelona, Spain (Richardson *et al.*, 2010).

No data were available to the Working Group in relation to dermal absorption of or inhalation exposure to dibromoacetonitrile.

Table 1.1 Dietary exposure to dibromoacetonitrile in drinking-water

Reference (country) *Source*	Concentration (µg/L)			Estimated exposure in adults (µg/kg bw per day)			Estimated exposure in children (µg/kg bw per day)		
	Mean	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**
Weinberg *et al.* (2002) (USA)		0.60	2.0		0.02	0.07		0.09	0.30
Krasner *et al.* (1989); IPCS (2000) (USA)									
Distribution systems		0.46	0.54		0.01	0.02		0.07	0.08
Clearwell effluent with high bromide levels		5.90	6.70		0.20	0.22		0.89	1.00
Utility with seasonally-varying bromide levels		4.60	11		0.15	0.37		0.69	1.65
Baytak *et al.* (2008) (Turkey)									
Groundwater and surface water	4.23	ND[b]	16.4	0.14	-	0.55	0.63	-	2.46
Health Canada (1995) (Canada)									
Groundwater and surface water		< 0.1[c]	1.2		-	0.04		-	0.18
Cancho *et al.* (1999) (Spain)									
Post-chlorinated water (considered as finished water)[d]	1.5	0.6	3.1	0.05	0.02	0.10	0.23	0.09	0.47
Williams *et al.* (1997) (Canada)									
Very low bromide concentrations (< 0.01 mg/L)	< 0.1[c]			-			-		
Low bromide concentrations (0.06 mg/L)	0.6			0.02			0.09		
Moderate bromide concentrations (0.5 mg/L)	1.2			0.04			0.18		
Zoeteman *et al.* (1982) (Netherlands)									
Before chlorination	< 0.1			-			-		
After chlorination			1			0.03			0.15
Reding *et al.* (1989) (USA)									
Distribution system		< 0.2	2.5		< 0.01	0.08		0.03	0.37
Stevens *et al.* (1989) (USA)			< 10			< 0.33	< 1.5		
Huang *et al.* (2003) (Taiwan, China)									
Groundwater after ozonation (high bromide levels: 1.5 mg/L)		3.1	18.1		0.10	0.60		0.47	2.71

[a] Calculated by the Working Group assuming a daily intake and a body weight of 2 L and 60 kg for adults, and 0.75 L and 5 kg for children, respectively.
[b] Detection limit, 0.073 µg/L
[c] Minimum quantifiable limit, 0.1 µg/L
[d] The study reported the levels of dibromoacetonitrile according to different water treatments (e.g. chlorinated water, sand-filtered water, ozonated water, granulated activated carbon-filtered water). For dietary exposure assessment, the chlorinated water values were considered as finished water.
ND, not detected

1.3.4 Environmental occurrence

Halogenated acetonitriles have been identified in the environment only as by-products of the disinfection of ground- and surface waters for drinking-water supplies. Therefore, the only known route of their environmental release is as a constituent of potable water supplies.

Halogenated acetonitriles such as dibromoacetonitrile undergo hydrolysis in water, which occurs at a faster rate in alkaline waters and in the presence of chlorine. Approximately 5 and 20% of dibromoacetonitrile are lost via hydrolysis within 10 days at pH 6 and 8, respectively. Volatilization losses are expected to be minimal, and adsorption to sediment and bioconcentration in aquatic organisms are not expected. In the atmosphere, dibromoacetonitrile reacts extremely slowly with photochemically produced hydroxyl radicals, with a resulting half-life of 696 days (WHO, 2004; HSDB, 2010).

1.3.5 Occupational exposure

No data were available to the Working Group.

1.4 Regulations and guidelines

WHO (2004) has established a tolerable daily intake of 11 µg/kg bw per day for dibromoacetonitrile. A guideline value of 70 µg/L (rounded figure) was calculated by allocating 20% of the tolerable daily intake to drinking-water and assuming a body weight of 60 kg and a daily drinking-water intake of 2 litres (WHO, 2004; 2008).

The Dow Chemical Company has established an industrial Hygiene Guide value of 0.1 ppm (0.8 mg/m^3) (time-weighted average, skin) for dibromoacetonitrile, and has set this value as an occupational exposure ceiling value based on their assessment of its toxicology in the absence of an industry-accepted value or a government-regulated level.

2. Cancer in Humans

See the Introduction to the *Monographs* on Bromochloroacetic Acid, Dibromoacetic Acid and Dibromoacetonitrile.

3. Cancer in Experimental Animals

Studies on the carcinogenicity of dibromoacetonitrile after oral administration and skin application in mice were reviewed by a previous IARC Working Group (IARC, 1991). The only additional carcinogenicity studies since that time are those conducted by the NTP (2010). Significant results are summarized in Table 3.1.

3.1 Oral administration

3.1.1 Mouse

Groups of 40 female A/J mice were administered dibromoacetonitrile by gavage at a dose of 0 (controls) or 10 mg/kg bw three times a week for 8 weeks and were then held until they reached 9 months of age. No treatment-related increases in lung adenoma incidence or multiplicity were observed (Bull & Robinson, 1985).

In a 2-year study, groups of 50 male and 50 female B6C3F$_1$ mice were administered dibromoacetonitrile in the drinking-water at doses of 0 (controls), 50, 100 or 200 mg/L (corresponding to average daily doses of approximately 0, 4, 7 or 13 and 0, 3, 6 or 11 mg/kg bw in males and females, respectively). Dibromoacetonitrile significantly increased the incidence of forestomach squamous-cell papilloma or carcinoma (combined) in males and forestomach squamous-cell papilloma in females (NTP, 2010). [Squamous-cell tumours of the forestomach are rare spontaneous tumours in experimental animals.]

Table 3.1 Carcinogenicity studies of exposure to dibromoacetonitrile in experimental animals

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 105–106 wk NTP (2010)	Drinking-water 0 (control), 50, 100, 200 mg/L (daily dose of ~0, 4, 7, 13 mg/kg bw) 50/group	Forestomach (squamous-cell papilloma): 0/50, 1/50, 0/50, 3/50	$P = 0.042$ (trend)	98.6% pure
		Forestomach (squamous-cell carcinoma): 0/50, 0/50, 0/50, 2/50	NS	
		Forestomach (squamous-cell papilloma or carcinoma, combined)[a]: 0/50, 1/50, 0/50, 5/50	$P = 0.031$ (high dose), $P = 0.003$ (trend)	
Mouse, B6C3F$_1$ (F) 105–106 wk NTP (2010)	Drinking-water 0 (control), 50, 100, 200 mg/L (daily dose of ~0, 3, 6, 11 mg/kg bw) 50/group	Forestomach (squamous-cell papilloma)[b]: 1/50, 0/50, 5/50, 14/50	$P < 0.001$ (high dose), $P < 0.001$ (trend)	98.6% pure; survival: 36/50, 36/50, 43/50*, 47/50*
Mouse, Sencar (F) 1 yr Bull (1985); Bull *et al.* (1985); IARC (1991)	Skin application (initiation–promotion) 0, 200, 400, 800 mg/kg bw (in 0.2 mL acetone), 3 times/wk for 2 wk followed 2 wk later by 1.0 µg 12-*O*-tetradecanoylphorbol 13-acetate (in 0.2 mL acetone) 3 times/wk for 20 wk and observed for 1 yr	Skin (squamous-cell papilloma or carcinoma, combined): 9/105, 8/36, 33/70, 10/74 Skin (squamous-cell papilloma): 4/105, 6/36, 16/70, 6/74) Skin (squamous-cell carcinoma): 5/105, 2/36, 17/70, 4/74		96% pure (4% tribromoacetonitrile); survival: 105/120, 36/40, 70/80, 74/80; data compiled from 3 separate experiments; limited reporting of the study

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 105–106 wk NTP (2010)	Drinking-water 0 (control), 50, 100, 200 mg/L (daily dose of ~0, 2, 4, 7 mg/kg bw) 50/group	Oral cavity (oral mucosa or tongue) (squamous-cell papilloma or carcinoma, combined)[c]: 0/50, 0/50, 2/50, 5/50	$P = 0.035$ (high dose), $P = 0.003$ (trend)	98.6% pure; glandular stomach (gland hyperplasia): 0/50, 0/50, 2/50, 2/50
		Oral cavity (oral mucosa or tongue) (squamous-cell carcinoma)[d]: 0/50, 0/50, 1/50, 3/50	$P = 0.021$ (trend)	
		Glandular stomach (adenoma)[e]: 0/50, 0/50, 0/50, 2/50	$P = 0.046$ (trend)	

* $P \leq 0.05$

[a] Historical incidence for 2-year drinking-water studies (mean ± standard deviation): 3/249 (1.2% ± 1.8%); range, 0–4%

[b] Historical incidence for 2-year drinking-water studies (mean ± standard deviation): 3/300 (1.0% ± 1.1%); range, 0–2%

[c] Historical incidence for 2-year drinking-water studies (mean ± standard deviation): 1/300 (0.3% ± 0.8%); range, 0–2%

[d] Historical incidence for 2-year drinking-water studies: 0/300

[e] Historical incidence for 2-year drinking-water studies: 0/300

bw, body weight; F, female; M, male; NS, not significant; wk, week or weeks; yr, year or years

3.1.2 Rat

In a 2-year study, groups of 50 male and 50 female F344/N rats were administered dibromoacetonitrile in the drinking-water at doses of 0 (controls), 50, 100 or 200 mg/L (corresponding to average daily doses of approximately 0, 2, 4 or 7 and 0, 2, 4 or 8 mg/kg bw in males and females, respectively). A significant increase in the incidence of squamous-cell papilloma or carcinoma (combined) and squamous-cell carcinoma of the oral cavity (oral mucosa or tongue) was observed in males, as well as an increased incidence of adenoma of the glandular stomach (NTP, 2010). [Squamous-cell carcinomas of the oral cavity are rare spontaneous tumours in experimental animals.]

3.2 Skin application

Groups of 40 female Sencar mice received skin applications of 0 (controls) or 400 mg/kg bw dibromoacetonitrile in 0.2 mL acetone three times a week for 24 weeks [total duration of the study not reported]. Dibromoacetonitrile did not induce skin tumours (Bull *et al.*, 1985). [The Working Group noted the limited reporting of the study.]

3.3 Co-exposure with modifying agents

In a series of three initiation–promotion studies, female Sencar mice received skin applications of 0 (controls), 200, 400 or 800 mg/kg bw dibromoacetonitrile three times a week for 2 weeks. Two weeks after the final dose, 1.0 µg 12-*O*-tetradecanoylphorbol 13-acetate was applied three times a week for 20 weeks and the animals were then observed for 1 year. Treatment with 200 and 400 mg/kg bw dibromoacetonitrile plus 12-*O*-tetradecanoylphorbol 13-acetate increased the incidence of squamous-cell papilloma or carcinoma (combined), but not that with 800 mg/kg (Bull, 1985; Bull & Robinson, 1985; Bull *et al.*, 1985). [The Working Group noted the limited reporting of the study and that these data were compiled from three separate, independent studies.]

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

(a) Absorption, distribution and excretion

Disposition studies in F344/N rats and B6C3F$_1$ mice were conducted after oral (0.2, 2.0 or 20 mg/kg bw) and intravenous (2.0 mg/kg bw) administration of [2-^{14}C]-dibromoacetonitrile. The compound was well absorbed in both species. Approximately 60% of the oral radiolabelled dose was excreted in the urine (none as the parent compound) within 24 hours in rats and 72 hours in mice; 8–17% was excreted in the faeces and 10–13% was exhaled as carbon dioxide ($^{14}CO_2$). At 72 hours after oral administration, 5–6% was recovered in the tissues of rats and approximately 2–3% in the tissues of mice. Most of the radiolabel remained in the stomach and liver and was not extractable with organic solvents, suggesting covalent binding in these tissues. At 72 hours after intravenous administration, 3% was recovered in the faeces of rats, and retention in the tissues was three to four times greater (19% in rats and 10% in mice) than that after oral administration in both species. The parent compound accounted for less than 6% of circulating radiolabel in rats and was not detected in mouse blood; at 24 hours, 50–80%

was not extractable into acetone (Mathews *et al.*, 2010; NTP, 2010).

Within 24 hours, 8% of a single oral dose (0.75 mmol/kg bw; 149 mg/kg bw) of dibromoacetonitrile administered to male Sprague-Dawley rats was excreted in the urine as thiocyanate (Pereira *et al.*, 1984).

Mathews *et al.* (2010) showed that dibromoacetonitrile reacts rapidly with rat blood *in vitro*, and binds covalently. Absorption of oral radiolabelled doses was about 90%. At 72 hours after intravenous administration, the amount of radioactivity recovered in mouse and rat tissues was 10 and 20% of the dose, respectively, while that recovered after oral dosing was three to four times less and was mostly covalently bound in the stomach. Excretion was higher in the urine than in the faeces. Within 72 hours, 9–15% was exhaled as $^{14}CO_2$ and 1–3% as volatile compounds.

(b) Metabolism

The metabolism of dibromoacetonitrile has been reviewed (NTP, 2010). The finding by Pereira *et al.* (1984) that 8% of a single oral dose in rats was excreted as thiocyanate, the product of a reaction of cyanide with thiosulfate that is catalysed by rhodanese, suggests that haloacetonitriles are metabolized to hydroxyacetonitriles by direct displacement of a halide ion by a hydroxyl group or by cytochrome P450-mediated oxidation. Moreover, subsequent release of cyanide or halide ion might result in the formation of formylhalide or cyanoformaldehyde. Dibromoacetonitrile is also transformed to cyanide by the hypoxanthine/xanthine oxidase/iron system *in vitro* (Mohamadin & Abdel-Naim, 2003).

In-vitro studies have suggested some additional metabolic pathways for haloacetonitriles. For example, dichloroacetonitrile was oxidized with the release of cyanide in a system that generates hydroxyl free radicals (a Fenton-like reaction involving ferrous salts and hydrogen peroxide); the oxidation of dichloroacetonitrile was sensitive to hydrogen peroxide scavengers (e.g. catalase), an iron chelator (desferroxiamine), or free radical scavengers (e.g. mannitol) (Mohamadin, 2001).

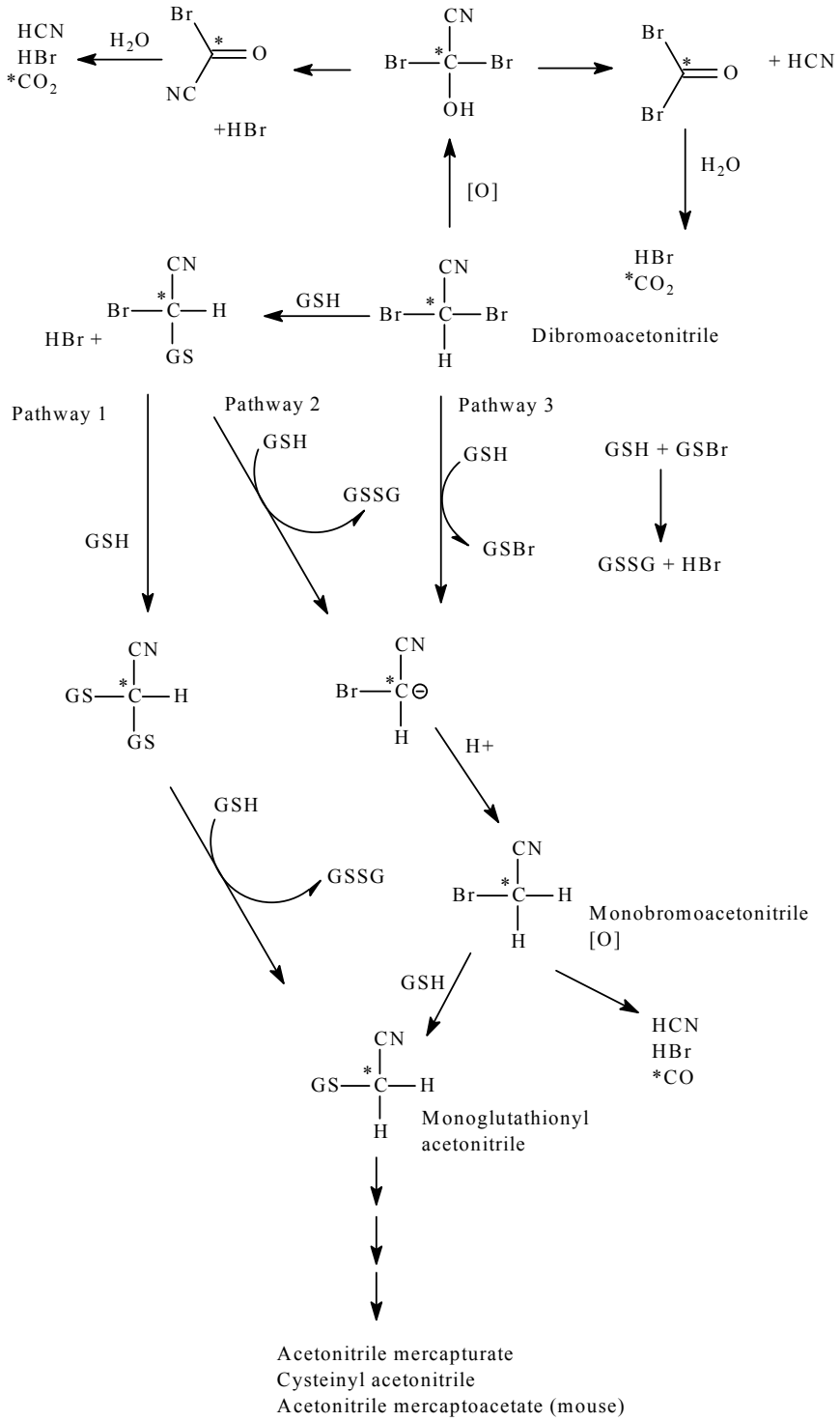
Because dibromoacetonitrile was also oxidized by a hydroxyl radical generated *in vitro* in a hypoxanthine/xanthine oxidase/iron system (Mohamadin & Abdel-Naim, 2003), oxidative activation of haloacetonitriles may occur via a reactive oxygen-mediated mechanism (Mohamadin, 2001).

The major metabolite extracted with acetone and methanol from rat stomach or from rat or mouse liver was monoglutathionyl acetonitrile. The profiles of rat and mouse urinary metabolites were unaffected by incubation with glucuronidase or sulfatase, but were altered by acylase. The major urinary metabolites identified were acetonitrile mercapturate in rats, and acetonitrile mercaptoacetate, acetonitrile mercapturate and cysteinyl acetonitrile in mice. Because one equivalent of dibromoacetonitrile reacted with 2.5–2.7 equivalents of glutathione (GSH) *in vitro* (Lin & Guion, 1989) and because bromoacetonitrile was detected in reaction media containing both dibromoacetonitrile and GSH, it was suggested that monoglutathionyl conjugate derivatives are formed via a GSH-dependent reduction of dibromoacetonitrile to bromoacetonitrile, followed by the reaction of bromoacetonitrile with another GSH (NTP, 2010).

Results from *in vitro* studies indicate that dibromoacetonitrile: (1) reacts directly with glutathione, but not with lysine, to form an intermediate that can alkylate histidine; (2) reacts with rat caecal contents to form polar products; and (3) reacts rapidly with rat blood to form polar metabolites and a large non-extractable fraction that may represent covalent protein adducts (NTP, 2010).

Mathews *et al.* (2010) studied the metabolism of radiolabelled dibromoacetonitrile in male rats and mice after oral and intravenous administration. It was noted that the prior depletion of

Fig. 4.1 Proposed pathways for the metabolism of [^{14}C]dibromoacetonitrile in mice and rats



* Denotes position of [^{14}C] label.
GSSG, glutathione disulfide; GSH, glutathione
Adapted from Mathews *et al.* (2010)

GSH markedly diminished the loss of dibromoacetonitrile and that the chemical reaction with GSH led immediately to glutathionyl acetonitrile. Thus, the main pathway of dibromoacetonitrile metabolism is via GSH, and covalent binding may be due to a reaction with tissue sulfhydryls (Mathews *et al.*, 2010; see Fig. 4.1).

(c) Toxicokinetic models

No data were available to the Working Group.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

The genetic and related effects of dibromoacetonitrile are summarized in Table 4.1.

(a) DNA damage

Dibromoacetonitrile induced sister chromatid exchange in cultured Chinese hamster ovary cells (Bull *et al.*, 1985), DNA strand breaks in human lymphoblast cell lines (Daniel *et al.*, 1986), a dose-related increase in DNA damage in HeLa S3 cells (Muller-Pillet *et al.*, 2000) and primary DNA damage in *Escherichia coli* strain PQ37 (Le Curieux *et al.*, 1995).

Two studies found that the potency to induce DNA damage was directly related to the number of halogen atoms present, and that bromine-substituted compounds produced stronger responses than chlorinated compounds (Daniel *et al.*, 1986; Muller-Pillet *et al.*, 2000).

(b) Mutations

Dibromoacetonitrile was shown to be a weak mutagen in *Salmonella typhimurium* strains TA100 and TA1535 and in the presence of induced hamster liver metabolic activation enzymes; equivocal responses were observed in these strains in the presence of rat liver metabolic activation (Mortelmans *et al.*, 1986; NTP, 2010). Another study showed no increase in gene mutations in TA100, TA1535, TA1537, TA1538 or TA98 exposed to dibromoacetonitrile in the presence and absence of metabolic activation (Bull *et al.*, 1985). The responses for these end-points were found to be weakly positive in a review of these studies (NTP, 2010). Mutagenic activity was observed in *Escherichia coli* strain WP2 *uvrA*/pKM101 in the presence of metabolic activation, particularly hamster liver metabolic activation (NTP, 2010), but not in *S. typhimurium* TA100 in the Ames-fluctuation assay (Le Curieux *et al.*, 1995; Muller-Pillet *et al.*, 2000). No induction of sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* after feeding or injection of dibromoacetonitrile (Valencia *et al.*, 1985).

(c) Chromosomal effects

Dibromoacetonitrile induced mitotic recombination in *Saccharomyces cerevisiae* (Zimmermann & Mohr, 1992), did not induce aneuploidy in the oocytes of female *Drosophila melanogaster* after inhalation (Osgood & Sterling, 1991) and increased the frequencies of micronucleated erythrocytes in newt (*Pleurodeles waltl*) larvae after 12 days of exposure in water (Le Curieux *et al.*, 1995). It did not induce micronuclei in the bone marrow of mice (Bull *et al.*, 1985).

4.3 Mechanistic data

Several studies have demonstrated that dibromoacetonitrile induces oxidative stress both *in vitro* and *in vivo*. In cultured glioma cells, it induced the generation of reactive oxygen species, lipid peroxidation and the accumulation of oxidized proteins (Ahmed *et al.*, 2008). In male mice, dibromoacetonitrile caused GSH depletion and inhibition of GSH *S*-transferase (GST), superoxide dismutase and catalase activity in

Table 4.1 Genetic and related effects of dibromoacetonitrile

Test system	Results		Dose[a] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Primary DNA damage, *Escherichia coli* strain PQ37 (SOS chromotest)	+	–	10	Le Curieux *et al.* (1995)
Salmonella typhimurium TA100, TA1535, TA1538, TA98 reverse mutation	–	–	0.58	Bull *et al.* (1985)
Salmonella typhimurium TA100, TA1535 reverse mutation	–	(+)	10 000	Mortelmans *et al.* (1986)
Salmonella typhimurium TA100, reverse mutation, Ames-fluctuation	–	–	30	Le Curieux *et al.* (1995)
Salmonella typhimurium TA100, reverse mutation, Ames-fluctuation	–	NT	5.00	Muller-Pillet *et al.* (2000)
Salmonella typhimurium TA100, reverse mutation	(+)	(+)	67 [c]	NTP (2010)
Salmonella typhimurium TA1535, reverse mutation	–		200	NTP (2010)
Salmonella typhimurium TA1535, reverse mutation		(+)	67 [b]	NTP (2010)
Salmonella typhimurium TA1537, reverse mutation	–		166	NTP (2010)
Salmonella typhimurium TA1537, reverse mutation		–	333	NTP (2010)
Salmonella typhimurium TA98, reverse mutation	–		200	NTP (2010)
Salmonella typhimurium TA98, reverse mutation		–	750	NTP (2010)
Salmonella typhimurium TA97, reverse mutation	(+)		33	NTP (2010)
Salmonella typhimurium TA97, reverse mutation		(+)	100 [c]	NTP (2010)
Escherichia coli WP2 *uvrA*/pKM101, reverse mutation	NT	+	100 [b]	NTP (2010)
Saccharomyces cerevisiae, mitotic recombination	+	NT	11.42	Zimmermann & Mohr (1992)
Drosophila melanogaster, sex-linked recessive lethal mutation *in vivo*	–		200 ppm	Valencia *et al.* (1985)
Drosophila melanogaster, aneuploidy in oocytes *in vivo*	–		0.30 ppm	Osgood & Sterling (1991)
DNA strand breaks (Comet assay), HeLa S3 cells *in vitro*	+		0.02	Muller-Pillet *et al.* (2000)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	+	17.33	Bull *et al.* (1985)
DNA strand breaks, human lymphoblast cell line *in vitro*	+	NT	3	Daniel *et al.* (1986)
Micronucleus test, CD-1 mouse erythrocytes *in vivo*	–		50	Bull *et al.* (1985)
Micronucleus test, male and female B6C3F1 mouse peripheral erythrocytes, *in vivo*	–		200, dw, 3 mo	NTP (2010)
Micronucleus test, *Pleurodeles waltl* erythrocytes *in vivo*	+		0.12	Le Curieux *et al.* (1995)
Sperm morphology, B6C3F1 mice *in vivo*	–		50	Meier *et al.* (1985)

[a] *in vitro* test, µg/mL; *in vivo* test, mg/kg bw per day

[b] Active with 10% hamster liver metabolic activation, not with 10% rat liver metabolic activation

[c] Active with 10% hamster liver metabolic activation, usually not with 10% rat liver metabolic activation

+, positive; (+), weakly positive; –, negative; bw, body weight; d, day or days; dw, drinking-water; HID, highest ineffective dose; LED, lowest effective dose; mo, month or months; NT, not tested

stomach tissues after a single oral dose (Abdel-Wahab *et al.*, 2002), and GSH depletion in the testis after a single intraperitoneal dose (Abdel-Wahab, 2003). In rat primary hepatocytes, it inhibited aldehyde dehydrogenase and GST activity (Poon *et al.*, 2003). *In vivo*, no significant change in the hepatic activity of these enzymes or in the level of hepatic GSH occurred after 13 weeks of exposure to dibromoacetonitrile via the drinking-water, while it increased the levels of peroxisomal enzymes in both sexes and lipid peroxidation in males (Poon *et al.*, 2003).

4.4 Susceptibility

No data were available to the Working Group.

4.5 Mechanisms of carcinogenesis

The mechanisms that lead to the carcinogenicity of dibromoacetonitrile are not known.

Some findings suggest that covalent binding occurs in tissues such as the stomach and liver, possibly following GSH-mediated activation. Oxidative stress associated with reduced GSH levels and deficiency in GST activity and/or binding to protein may also be involved. Dibromoacetonitrile may also act via a genotoxic mechanism.

5. Summary of Data Reported

5.1 Exposure data

Dibromoacetonitrile is formed as a by-product during the disinfection of water by chlorination in the presence of natural organic matter (e.g. algae) and bromide. Concentrations of dibromoacetonitrile up to 18 µg/L were measured in drinking-water. Maximum daily human exposure to dibromoacetonitrile through drinking-water, estimated from such measurements, is at the low microgram per kilogram of body weight level.

Dibromoacetonitrile is also produced for use as an antimicrobial component in metalworking fluids. Occupational exposure may occur during its production or use.

5.2 Human carcinogenicity data

No epidemiological studies were identified that evaluated exposure specifically to dibromoacetonitrile. This chemical occurs in mixtures in disinfected water. Studies on disinfected water are reviewed in the Introduction to the *Monographs* on Bromochloroacetic Acid, Dibromoacetic Acid and Dibromoacetonitrile.

5.3 Animal carcinogenicity data

In one study in mice, administration of dibromoacetonitrile in the drinking-water increased the incidence of squamous-cell papilloma or carcinoma (combined) of the forestomach in males and of squamous-cell papilloma of the forestomach in females. In one study in rats, administration of dibromoacetonitrile in the drinking-water increased the incidence of glandular stomach adenoma, and of squamous-cell papilloma or carcinoma (combined) and squamous-cell carcinoma of the oral cavity (oral mucosa or tongue) in males. Squamous-cell tumours of the forestomach and squamous-cell carcinomas of the oral cavity are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

No data were available to the Working Group on the toxicokinetics of dibromoacetonitrile in humans. Dibromoacetonitrile was well absorbed (almost 90%) after oral administration in mice and rats, and there is evidence that covalent binding occurs in the stomach and liver. It can

be metabolized via several pathways, including direct displacement of bromide by a hydroxyl group, and cytochrome P450-mediated or -independent oxidation. The main metabolite identified in rat stomach and rat or mouse livers was monoglutathionyl acetonitrile. The major urinary metabolites identified were acetonitrile mercapturate in rats and acetonitrile mercaptoacetate, acetonitrile mercapturate and cysteinyl acetonitrile in mice. Thiocyanate was also identified in rats.

Dibromoacetonitrile induced DNA damage in bacteria and in human cell lines, mutations in bacteria and micronuclei in newt larvae, but not in mice. No mutations were found in *Drosophila*.

The mechanisms that lead to the carcinogenicity of dibromoacetonitrile are not known, but there is weak evidence that oxidative stress and/or genotoxicity may lead to cancer in rodents exposed to dibromoacetonitrile.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of dibromoacetonitrile.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of dibromoacetonitrile.

6.3 Overall evaluation

Dibromoacetonitrile is *possibly carcinogenic to humans (Group 2B)*

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