Bromodichloromethane

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Bromodichloromethane*
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Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van broomdichloormethaan. Het maakt deel uit van een uitgebreide reeks waarin kankerverwekkende stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld.

Het advies is opgesteld door een vaste subcommissie van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS), de Subcommissie Classificatie van carcinogene stoffen. Het advies is voorgelegd aan de Commissie GBBS en vervolgens getoetst door de Beraadsgroep Gezondheid en omgeving van de Gezondheidsraad.

Ik heb dit advies vandaag ter kennisname toegezonden aan de minister van Volksgezondheid, Welzijn en Sport en de minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Hoogachtend,

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Subcommittee on the classification of carcinogenic substances of the
Dutch Expert Committee on Occupational Standards,
a committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2007/05OSH, The Hague, December 12, 2007

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues...” (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature & Food Quality. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

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Contents

Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld. De evaluatie en beoordeling worden verricht door de subcommissie Classificatie van Carcinogene Stoffen van de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen van de Raad, hierna kortweg aangeduid als de commissie. In het voorliggende advies neemt de commissie broomdichloormethaan onder de loep. Broomdichloormethaan is een stof dat onder andere wordt gebruikt bij de synthese van organische stoffen.

Op basis van de beschikbare gegevens leidt de commissie af dat broomdichloormethaan beschouwd moet worden als kankerverwekkend voor de mens. Dit komt overeen met een classificatie in categorie 2 volgens de richtlijnen van de Europese Unie. De commissie concludeert verder dat broomdichloormethaan een stochastisch genotoxisch werkingsmechanisme heeft.

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates and judges the carcinogenic properties of substances to which workers are occupationally exposed. The evaluation is performed by the subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter called the committee. In this report, the committee evaluated bromodichloromethane. Bromodichloromethane is an agent that is used in the synthesis of organic chemicals.

Based on the available information, the committee is of the opinion that bromodichloromethane should be considered as carcinogenic to humans. This recommendation corresponds to the EU classification in category 2. The committee concludes furthermore that bromodichloromethane acts by a stochastic genotoxic mechanism.

12 Bromodichloromethane

Chapter 1

Scope

1.1 Background

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the carcinogenic properties of substances, and to propose a classification with reference to an EU-directive (see annex A and F). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substance in question. The assessment and the proposal for a classification are expressed in the form of standard sentences (see annex E). This report contains the evaluation of the carcinogenicity of bromodichloromethane.

1.2 Committee and procedure

The evaluation is performed by the subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter called the committee. The members of the committee are listed in annex B. The first draft was prepared by MI Willems, from the Department of Occupational Toxicology of the TNO Nutrition and Food Research, by contract with the Ministry of Social Affairs and Employment.

In 2007 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are

listed in annex C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The evaluation and recommendation of the committee is standardly based on scientific data, which are publicly available. The starting points of the committees' reports are, if possible, the monographs of the International Agency for Research on Cancer (IARC). This means that the original sources of the studies, which are mentioned in the IARC-monograph, are reviewed only by the committee when these are considered most relevant in assessing the carcinogenicity and genotoxicity of the substance in question. In the case of bromodichloromethane, such an IARC-monograph is available, of which the summary and conclusion of IARC is inserted in annex D.

More recently published data were retrieved from the online databases Medline, Toxline, Chemical Abstracts, and RTECS. The last updated online search was in March 2007. The new relevant data were included in this report.

Chapter 2

General information

2.1 Identity, and physical and chemical properties

Bromodichloromethane (BDCM) is used in the synthesis of organic chemicals and as a reagent in laboratory research.[1-3] It has also been used to: separate minerals and salts; as a flame retardant; in fire extinguishers; and, as a solvent for waxes, fats and resins. Furthermore, it is found as a by-product of chlorinated drinking- and swimming pool water.

The major source of occupational exposure is when the compound is used in laboratory research, or when the agent is inhaled in drinking water facilities and swimming pool facilities. The general population is mainly exposed through consumption of contaminated drinking water, or through ingestion of chlorinated swimming pool water.[1-3]

Below is given the identity and some of its physical and chemical properties.

Chemical name	:	bromodichloromethane
CAS registry number	:	75-27-4
EC/EINECS number	:	200-856-7
Synonyms	:	dichlorobromomethane; dichloromonobromomethane; monobromodichloromethane; BDCM
Description	:	colourless liquid
Molecular formula	:	$CHBrCI_2$

Structure	:	Cl | H – C – Br | Cl
Molecular weight	:	163.83
Boiling point	:	90.1 °C
Melting point	:	-57.1 °C
Relative density of vapour/ air mixture at 20 °C	:	1.3
Vapour pressure	:	6.7 kPa at 20 °C
Solubility	:	Soluble in water (4.5 g/L at 20 °C); acetone; ethanol; benzene; chloroform; and, diethyl ether
N-octanol/water partition coefficient as log Pow	:	2.10
Conversion factors (25°C, 760 mm Hg)	:	1 ppm = 0.15 mg/m^3 1 mg/m^3 = 6.70 ppm

2.2 IARC classification

In 1999, IARC concluded that there is sufficient evidence for the carcinogenicity of BDCM in experimental animals, but that there were no carcinogenicity data available from studies in humans.[2] Therefore, according to the IARC guidelines, it classified BDCM in Group 2B, which means that the agent is possibly carcinogenic to humans.

Chapter 3

Carcinogenicity studies

3.1 Observations in humans

Several population-based case control studies suggested that exposure to chlorinated water, that predominantly contained BDCM, is associated with increased risk of colorectal and bladder cancer in humans.[1,2,4-6] However, a problem in these studies is that bromodichloromethane exposure is almost always accompanied by exposure to other trihalomethane agents, such as chloroform, dibromochloromethane, bromoform, and many other by-products that can be present in chlorinated water. Therefore, it is far from certain whether BDCM was the responsible agent.

No epidemiological data or case reports are available on the carcinogenicity of BDCM alone.

3.2 Carcinogenicity studies in animals

The American National Toxicology Program (NTP) carried out three long-term animal carcinogenicity studies. [6-8] In the first, groups of 50 male and female Fischer F344 rats and B6C3F_1 mice, were given BDCM in corn oil by gavage, five days per week for 102 weeks (Dunnick *et al*. 1993).[7] Control groups of 50 male and female animals received normal diet containing an empty capsule alone. The results are shown in Tables 3.1 (rats) and 3.2 (mice).

Table 3.1 Tumour development in Fischer rats, given BDCM in corn oil by gavage for two years (Dunnick *et al.* 1993).

	Males			Females		
Dose in mg BDCM/kg bw	Control	50	100	Control	50	100
Survival at 104 weeks	28/50	36/50	28/50	34/50	27/50	41/50
No. of tumour bearing animals						
Large Intestines:						
• Adenomatous polyps	0/50	3/50	33/50	0/46	0/50	7/47
• Adenocarcinomas	0/50	11/50	38/50	0/46	0/50	6/47
The kidneys						
• Tubular cell adenomas	0/50	1/50	3/50	0/50	1/50	6/50
• Tubular cell adenocarcinomas	0/50	0/50	10/50	0/50	0/50	9/50
• Adrenal medullary phaeochromocytomas	18/50	14/50	5/50	-	-	-
Anterior pituitary neoplasms	-	-	-	31/49	20/49	14/49
Mammary gland fibroadenomas	-	-	-	20/50	15/50	1/50

-, no evidence for compound-related neoplasms.

Table 3.2 Tumour development in B6C3F$_1$ mice, given BDCM in corn oil by gavage for two years (Dunnick *et al.* 1993).

	Males			Females		
Dose in mg BDCM/kg bw	Control	25	50	Control	75	150
Survival at 104 weeks	34/50	32/50	42/50	26/50	13/50	15/50
No. of tumour bearing animals						
The liver						
• Hepatocellular adenomas	-	-	-	1/50	13/48	23/50
• Hepatocellular carcinomas	-	-	-	2/50	5/48	10/50
The kidneys						
• Tubular cell adenomas	1/49	2/50	6/50	-	-	-
• Tubular cell adenocarcinomas	0/50	0/50	4/50	-	-	-
Anterior pituitary neoplasms	-	-	-	17/44	8/43	3/38
The thyroid						
• Follicular cell hyperplasia	0/48	3/44	5/49	6/50	18/45	21/48

-, no evidence for compound-related neoplasms.

Regarding male and female rats, the numbers of tumour-bearing animals having neoplasms in the large intestines and in the kidneys were significantly greater in the high dose group than in controls ($p<0.001$). On the other hand, the number of female animals with anterior pituitary neoplasms and mammary gland fibroadenomas was significantly lower in the high dose group than in controls ($p<0.001$). This was also the case for adrenal medullary phaeochromocytomas (benign and malignant) in males ($p=0.003$).

In mice, the numbers of tumour-bearing animals having neoplasms in the liver (males) and in the kidneys (females) were significantly greater in the high dose groups than in controls ($p<0.05$). Also follicular cell hyperplasia in the thy-

roid was more common in the high dose groups than in controls. But again, the number of female animals with anterior pituitary neoplasms was significantly lower in the high dose group than in controls (p=0.006).

In the second study, groups of 50 male F344/N rats and B6C3F$_1$ mice were given BDCM in drinking water for up to 100 or 104 weeks (George *et al*. 2002).[8] Control groups of 50 male animals received normal drinking water with 0.25% Emulphor, a nontoxic dissolvent. In rats and mice, no changes in feed consumption, final body weight and survival were observed. In both animal species, BDCM did not increase cancer in the large bowel, kidney, spleen, bladder, or in any other organ tissues examined, except in the liver of rats. As shown in Table 3.3, in rats receiving the lowest dose, the prevalence of hepatocellular adenomas was significantly increased compared to controls. However, in the mid dose group the increase was marginal when hepatocellular adenomas were combined with carcinomas, while in the high dose group no clear increase in prevalence was observed. In the kidneys of the rats, an increase in the prevalence of renal tubular hyperplasia was observed in the highest dose group compared to controls (15.8% versus 8.7%). Overall, the authors concluded that under the study conditions, BDCM in drinking water was not carcinogenic in male B6C3F$_1$ mice, but that it was carcinogenic in male F344/N rats.

To compare exposure doses to reality, concentrations of BDCM in chlorinated drinking water are reported to be between 1 and 50 µg/L, with a maximum detected over 200 g/L.[9]

Table 3.3 Tumour prevalence of male F344/N rats and B6C3F$_1$ mice, given BDCM in drinking water for two years (George *et al*. 2002).

	F344/N rats				B6C3F$_1$ mice			
BDCM concentration in drinking water (mg/L)	Control	70	350	700	Control	50	250	500
Mean daily dose (mg BDCM/kg bw/day)	0	3.9	20.6	36.3	0	8.1	27.2	43.4
Survival at 104 weeks	32/50	31/50	33/50	33/50	36/50	32/50	36/50	40/50
Prevalence (percent of animals with a tumour)								
Hepatocellular adenomas	2.2	15.5*	6.2	4.1	20.5	22.2	14.3	15.9
Hepatocellular carcinomas	2.2	2.2	8.3	4.1	23.1	25.0	19.0	15.9
Hepatocellular adenomas and carcinomas	4.4	17.8*	14.6**	8.2	n	n	n	n

* p< 0.05; ** p<0.10; n, not reported.

In the third study, which had the same design as the second study, no increased incidences of neoplasms to BDCM concentrations of 0, 175, 350, and 700 mg/L was observed in male F344/N rats and B6C3F$_1$ mice (NTP 2006).[6] In fact, in mice a negative trend was observed of hepatocellular adenomas and carcinomas (combined). Furthermore, in the mid-dose group, the incidence of hemangiosarcomas in all organs was significantly decreased compared to control mice. Survival rates did not differ among the exposed and control groups.

The American National Toxicology Program analysed the different outcomes of its studies. Using a pharmacokinetic model, NTP concluded that "The different responses observed in these studies were attributed to differences in organ dosimetry by these routes of exposure and possible influences of dietary factors and differences in body weight on neoplasm development".[6]

In the IARC Monograph of 1991, the Working Group evaluated two other animal carcinogenicity studies, but it noted the incomplete reporting and diagnosis of these studies.[9] One study concerned life-long exposure of BDCM in drinking water of male and female Wistar rats (Tumasonis *et al*. 1985).[10] In female animals increased number of tumour-bearing animals were observed for neoplastic nodules and adenofibrosis in the liver compared to controls, while the proportion of lymphosarcomas was increased in males, but decreased in females. Furthermore, in exposed female animals, the prevalence of pituitary gland or mammary gland tumours was decreased compared to controls. The other study concerned CBA x C57B1/6 hybrid mice, which were given BDCM in drinking water for a life-long period (Voronin *et al.* 1987)[11] No treatment-related tumours were observed in any of the mice.

Aida *et al.* (1992) carried out a carcinogenicity study on male and female Wistar rats, which were given microencapsuled BDCM in the diet for up to two years.[12] The mean daily intake was calculated to be 6-8, 25-32, and 138-168 mg/kg bw for males and females, respectively. Control groups were given the diet containing an empty capsule alone. At several time points (6, 12 and 18 months) interim kills were scheduled, leaving a maximum of 20 animals per group alive for the total exposure period. Data on neoplasms were only presented for the initial number of animals at the start of the experiment. No significant treatment-related increases in the number of neoplastic lesions were observed in any of the groups and in any of the organs examined, including the liver and kidneys. Regarding non-neoplastic lesions in the liver, dose-related changes included: fatty degeneration (mid dose, males); fatty degeneration and granulomas (two highest doses, females); and, bile duct proliferation and cholangiofibrosis (highest dose, males and females). No non-neoplastic lesions were observed in the kidneys of either sex.

In 2007 the National Toxicology Program published a report on the toxicity and carcinogenicity of BDCM in genetically modified mice, which were susceptible for developing cancer.[13] Tg.AC mice (hemizygous for mutant v-Ha-*ras* transgene; Ha-ras is an oncogene), and p53 haploinsufficient mice were given BDCM in drinking water, by gavage, or dermally. In the drinking water studies, groups of 15 male and 15 female mice (study duration, 26 weeks), or 10 male and 10 female mice (study duration, 42 weeks) received daily 0, 175, 350 or 750 mg BDCM per liter drinking water; in the gavage studies, mice received 0, 25, 50 or 100 mg BDCM/kg bw for 5 days per week; and, in the dermal study (study duration, 26 and 39 weeks; Tg.AC strain only), doses of 0, 64, 128, or 256 mg BDCM/kg bw were administered for 5 days per week. Survival of all the exposed animals were similar to that of there respective vehicle controls. Overall, no treatment-related neoplasms were observed in any of the exposed animals. In both drinking water and gavage studies in Tg.AC and p53 haploinsufficient mice, BDCM-exposed males had increased renal tubule degeneration, and BDCM-exposed females had fatty changes of the hepatocytes. These non-neoplastic changes were not observed in vehicle controls. Since BDCM caused cancer in other animal studies, the authors suggested that the genetically modified mice, which were used in their studies, may not be as sensitive for detecting cancer-causing agents.

In 2002 and 2003, a few animal studies have been published by Hooth *et al*. and McDorman *et al*. of the same research group.[14-16] In these studies, male and female *Tsc2* mutant Long-Evans (Eker) rats received BDCM in their drinking water at 70 and 700 mg/L for 4 or 10 months. The concentrations corresponded to a mean daily intake of 3,5 and 35 mg BDCM/kg bw, and to 6.5 and 56 mg BDCM/kg bw, for male and females, respectively. Rats carrying a mutation in the *Tsc2* tumour suppressor gene readily develop renal preneoplastic and neoplastic lesions, and are highly susceptible to the effects of renal carcinogens. In all studies, no more than eight animals per group were used. The number of tumour-bearing animals, and the total number of renal adenomas and carcinomas per animal, did not differ from the data obtained from control animals. Furthermore, no lesions were observed in the urinary bladder, while the incidence of aberrant crypt foci in the colon was non-significantly increased compared to control group.

DeAngelo *et al*. (2002) administered BDCM in the drinking water of male F344/N rats, and B6C3F_1 mice at a dose of 700 mg/L for 13 weeks.[17] They also used A/J mice, which were given 500 mg BDCM/L for 13 and 30 weeks. The study was performed to investigate the development of aberrant crypt foci, which are considered early putative preneoplastic lesions in the colon. No aberrant

crypt foci were observed in the two mouse strains at any time. In rats, BDCM induced a low but significant increase in aberrant crypt foci compared to controls. In an additional study, male F344/N rats were given the same concentration in drinking water, but also a group was added, in which the animals received BDCM in corn oil by gavage (50 mg/kg bw) for 26 weeks.[18] In all BDCM-treated groups, the incidence of aberrant crypt foci was significantly increased compared to negative controls. Corn oil did not promote BDCM-induced aberrant crypt foci.

Toussaint *et al*. (2001) allowed three groups of Japanese medaka (*Oryzias latipes*) fish plus a control group to swim in BDCM containing water for up to nine months.[19] The three groups were exposed to 18, 143, and 1424 μg/L, respectively. At the end of the exposure period, in none of the exposed fish treatment-related neoplastic lesions in the liver or any other organs were observed. The main non-neoplastic lesions observed, were a statistically significant increase in gall bladder hyperplasia and bile duct abnormalities in fish exposed to the highest dose at six and nine months.

Chapter 4

Mutagenicity and genotoxicity

4.1 *In vitro* assays

Conflicting findings have been reported concerning the mutagenic potential of BDCM in *Salmonella typhimurium* assays. For instance, it did induce reverse mutations in tester strains TA100, TA98, and TA97, all in the presence of a metabolic activation system, but no mutations were observed in TA102, TA1535, TA1537, and in some tests using TA100, and TA98.[2,20] Le Curieux *et al*. (1995) used a normal TA100 strain in an Ames-fluctuation assay.[21] This is a modified *Salmonella* assay, in which bacteria are exposed in a liquid medium instead of an agar plate. However, the test results were negative. Also no treatment-related reverse mutations in strain TA1535 was observed by Pegram *et al*. (1997).[22] But in the same study, in a TA1535 strain, which was transfected with a glutathione transferase gene (TA1535+GST), a clear dose-dependent increase in mutation frequencies was observed.[22,23]

In 2006, the American National Toxicology Program repeated mutagenicity testing using the standard *Salmonella* assay and various tester strains, in the presence and absence of a metabolic activation system.[6] Again conflicting results were found. For instance, equivocal results were obtained for strains TA100, TA97, and TA98, without a metabolic activation system (one of the two trials only). No reverse point mutations were found for strain TA1535 and TA1537. Also, no treatment-related mutations were found when a metabolic activation system was used in any of the strains, except a weak positive outcome in strain

TA1535 (hamster S9; one of the two trials only). Based on its data and from data presented by others, The National Toxicology Program suggested that the conflicting results may, in some cases, be related to improper control for the volatility of the agent.

In cultured animal cells, BDCM induced gene mutations in the *tk* locus of L5178Y mouse lymphoma cells.[2] Using the same lymphoma assay, inconclusive outcomes were obtained in an international collaborative study, because a marginal response was found in one laboratory and a negative response in the other (Sofuni *et al*. 1996).[24] BDCM induced trifluorothymidine resistance in L5178Y mouse lymphoma cells, in the presence of a metabolic activation system, but not without such a metabolic activation (NTP 2006).[6]

Regarding DNA damage, BDCM induced primary DNA damage in *Escherichia coli* PQ37, when the SOS chromotest was used (Le Curieux *et al*. 1995).[21] Furthermore, exposure of primary rat and human kidney cells to 0.5 – 4.0 mM BDCM resulted in a statistically significant increase in DNA damage (Robbiano *et al*. 2004).[25]

Bromodichloromethane provoked a slight increase in chromosomal aberrations in Chinese hamster lung fibroblasts, in the presence and absence of a metabolic activation system (Matsuoka *et al*. 1996).[26] However, in the same study, it did not induce polyploidy. In another study, no increased frequencies in chromosomal aberrations due to BDCM exposure could be detected in Chinese hamster ovary cells.[6]

Conflicting outcomes were reported of sister chromatid exchanges (SCEs). In its Monograph, IARC reported on two studies using Chinese hamster ovary and FAF cells with negative outcomes.[2] In the absence of a metabolic activation system, BDCM significantly induced SCEs in a clear dose-dependent manner in rat erythroblastic leukemia cells (Fujie *et al.* 1993).[27] Also, a small increase in SCEs was observed in one of the four trials, using Chinese hamster ovary cells, but only in the presence of a metabolic activation system (NTP 2006).[6]

In a micronucleus assay, using primary rat and human kidney cells, a significant increase in micronucleated cells occurred after BDCM exposure.[25]

4.2 *In vivo* assays

No data were available on the induction of gene mutations by bromodichloromethane.

No unscheduled DNA synthesis occurred in the liver cells of male Sprague-Dawley rats, which were given a single dose of 135 and 450 mg BDCM/kg bw by gastric intubation (Stocker *et al.* 1997).[28]

In another study, using the same animal species and the Comet assay, a single oral administration of 458 mg BDCM/kg bw (half of the LD_{50} dose) did induce DNA damage in the kidney cells of the rats (Robbiano *et al*. 2004).[25]

Potter *et al*. (1996) did not find increased frequencies of DNA strand breaks in the kidneys of male F344 rats, which were given daily oral doses of 0.75 and 1.5 mmol BDCM/kg bw by gavage for 7 days.[29]

Regarding its clastogenic potential, in the bone marrow cells of male and female Long-Evans rats, statistically significantly increased frequencies of chromosomal aberrations were observed after a single intraperitoneal injection of 164 mg BDCM/kg bw, but not at lower doses (Fujie *et al.* 1990).[30] However, when the agent was given orally by gastric intubation at the same dose levels, five times on five successive days, no treatment-related chromosomal aberrations in the bone marrow cells could be detected.

Morimoto and Koizumi (1983) reported on the induction of sister chromatid exchanges in bone marrow cells of mice, which were given orally 50 mg BDCM/kg bw, four times on four different days.[2,31]

Conflicting results were obtained in showing chromosomal fragmentations by micronucleus tests. For instance, no BDCM-induce increases in micronucleated cells were observed in: the bone marrow cells and peripheral blood lymphocytes of mice, given intraperitoneal injections of 200 or 500 mg BDCM/kg bw (Hayashi *et al*. 1988; IARC 1999)[2,32]; in bone marrow polychromatic erythrocytes of male $B6C3F_1$ mice, which were given intraperitoneal injections of 200 to 500 mg BDCM/kg bw, three times at 24-hour intervals (killing 24 hours after last injection; NTP 2006)[6]; and, in peripheral blood erythrocytes of female $B6C3F_1$ mice, given 44 – 700 mg BDCM per liter drinking water for 3 weeks (NTP 2006)[6]. However, significant positive outcomes were found in: the kidney cells of Sprague-Dawley rats, which were given a single oral administration of 458 mg BDCM/kg bw (Robbiano *et al*. 2004)[25]; and, in peripheral blood lymphocytes of the amphibian *Pleurodeles waltl* larvae, which were exposed to 50 µg BDCM/mL for 12 days (Le Curieux *et al*. 1995).[21]

Torti *et al.* (2002) investigated the induction of micronuclei in peripheral blood cells of wild type and heterozygous p53 C57BL/6 and FVB/N mice by inhalation exposure.[33] In short, groups of five to six animals were daily exposed to up to 30 or 300 ppm BDCM (≈ 4.5 or 45 mg/m^3 at 25°C) for 6 hours per day

for one, three or thirteen weeks. The outcome indicated a weak induction of micronuclei by BDCM at concentrations higher than 30 ppm.

4.3 Carcinogenic mode of action

The mechanisms through which bromodichloromethane may exert its genotoxic and carcinogenic potential are not clarified yet. Nevertheless, some ideas are given in the literature, as stated below.

Firstly, the biotransformation of BDCM. There are three potential (competing) metabolic pathways for BDCM suggested, which give rise to the formation of reactive intermediates (NTP 2006).[6] The first is oxidative metabolism by a cytochrome P450 mixed function oxidase system (predominantly the cytochrome P450 2E1 isoform), resulting in highly reactive dihalocarbonyl intermediates that are further detoxified into CO_2 (hydrolysis) or CO (reduction by glutathione binding). The second is reductive metabolism mediated by cytochrome P450, which results in free radical intermediates. Thirdly, BDCM may undergo glutathione-S-transferase-catalyzed conjunction with glutathione, resulting in DNA reactive S-dihalomethyl metabolites. Reactive intermediates may bind to proteins and even DNA. That BDCM is able to covalently modify DNA and to form DNA (2'-deoxyguanosine) adducts, after conjunction with glutathione, is shown by Ross *et al*. (2004).[23] Based on their results and those from others, these authors suggested that the carcinogenicity of BDCM could depend on the bioactivation capacity, in particular the activity of P450 2E1 and glutathione-S-transferase (GSTT1-1) isoenzymes.

The rate of activity of these enzymes are genetically determined and species dependent. Such differences between species may explain the different outcomes between rats and mice in carcinogenicity studies. Furthermore, the central role of biotransformation in the toxicity of BDCM may explain why toxic effects are restricted to a few organs, such as the liver and kidneys, since biotransformation of xenobiotics is mainly localized in these (and some other) organs.

Secondly, in a few animal studies using $B6C3F_1$ mice or F344 rats, it was shown that BDCM induced DNA hypomethylation, when the compound was given in the drinking water or by gavage (Coffin *et al*. 2000; Pereira *et al*. 2004; Tao *et al*. 2005).[35-37] In $B6C3F_1$ mice, it was furthermore shown that BDCM caused DNA hypomethylation of the *c-myc* tumour promotor gene. DNA methylation is a key epigenetic mechanism that results in the silencing or down regulation of genes, without changing their coding sequence. As such, DNA methylation plays an important role in DNA repair and genome instability.[38,39] It

is hypothesized that hypomethylation may contribute to oncogenesis, for instance by activating oncogenes or stimulating chromosome instability.[38]

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

No data on the genotoxicity and carcinogenicity in humans were available.

The main routes of BDCM-exposure in animal carcinogenicity studies were by gavage and drinking water. This plus the use of different animal species resulted in mixed results. For instance, in mice, liver and kidney tumours were found when BDCM was given by gavage, but not in drinking water. Also tumour development in rats depended on the route of exposure. For instance, liver tumours were found after exposure by gavage diet and drinking water, but kidney tumours and tumours in the large intestines were only found when BDCM was given by gavage. Furthermore, under comparable study and exposure conditions, conflicting results were obtained in that in one study tumours were observed, but not in another study. However, despite the somewhat contradictory findings, the study was well designed, and no reasons could be found that the tumours found in the animals were not relevant. All in all, the animal studies give sufficient evidence that exposure to bromodichloromethane can result in cancer development.

Also mutagenicity and genotoxicity testing revealed mixed results. For instance, gene mutations were observed in certain bacterial *Salmonella* strains and mammalian cells, but not in all cases. DNA damage was observed *in vitro* and *in vivo*, but in at least one *in vivo* study no damage (strand breaks) was found. Also testing for its clastogenic potential (*i.e.*, chromosomal aberrations, sister chromatid exchanges, micronuclei) resulted in mixed outcomes in both *in*

vitro and *in vivo* assays. It seems that the conflicting results could partly be explained by differences in experimental conditions, such as the route of exposure, and the absence or presence of certain biotransformation enzymes, such as glutathione-S transferase (GSTT1-1). At the moment it is unclear how bromodichloromethane exerts its genotoxic and carcinogenic effects, although it is hypothesized that through biotransformation, reactive intermediates are formed, which can bind to DNA and thus cause DNA damage. Another possible (non-genotoxic) route is DNA hypomethylation. Based on the available mutagenicity and genotoxicity data, the committee is of the opinion that there are sufficient indications that BDCM could exert its carcinogenic effect by a stochastic genotoxic mechanism.

The committee did not find indications that the observations in animals, and the proposed carcinogenic mechanism would not occur in humans.

5.2 Recommendation for classification

The committee is of the opinion that bromodichloromethane should be considered as carcinogenic to humans. This recommendation corresponds to the EU classification in category 2. The committee concludes furthermore that bromodichloromethane acts by a stochastic genotoxic mechanism.

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34 Bromodichloromethane

Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a ‘exposure versus tumour incidence range’ and a calculated concentration in air corresponding with reference tumour incidences of 10-4 and 10-6 per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council.

Annex **B**

The committee

- G..J. Mulder, *chairman*
  emeritus professor of toxicology, Leiden University, Leiden
- P.J. Boogaard
  toxicologist, SHELL International BV, The Hague
- Ms. M.J.M. Nivard
  Molecular biologist and genetic toxicologist, Leiden University Medical Center, Leiden
- G.M.H. Swaen
  epidemiologist, Dow Chemicals NV, Terneuzen
- R.A. Woutersen
  toxicologic pathologist, TNO Nutrition and Food Research, Zeist
- A.A. van Zeeland
  professor of molecular radiation dosimetry and radiation mutagenesis, University Medical Center, Leiden
- E.J.J. van Zoelen
  professor of cell biology, Radboud University Nijmegen, Nijmegen
- J.M. Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

The committee consulted an additional expert, Prof dr G Mohn, working at Department of Radiation Genetics and Chemical Mutagenesis of the University of Leiden, with respect to the genotoxic data.

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2007 for public review. The following organisations and persons have commented on the draft document:

- G. Jonkers, Vereniging van Verf en Drukinktfabrikanten, the Netherlands;
- E. González-Fernández, Ministerio de Trabajo y Asuntos Sociales, Spain;
- R.D. Zumwalde, National Institute for Occupational Safety and Health, the USA.

Annex **D**

IARC Monograph

D.1 VOL: 52 (1991) (p. 179)[9]

CAS No.: 75-27-4

Summary of Data Reported and Evaluation

Exposure data

Bromodichloromethane is found in chlorinated drinking-water as a consequence of the reaction between chlorine, added during water treatment, and natural organic substances in the presence of bromide. The major route of human exposure to bromodichloromethane is *via* drinking-water. It has been detected in chlorinated drinking-water in many parts of the world; it has also been detected in some untreated waters, but at much lower levels. Bromodichloromethane is a major component of the organohalides produced by marine algae.

Experimental carcinogenicity data

Bromodichloromethane was tested for carcinogenicity in two-year studies in male and female Fischer 344 rats and B6C3F_1 mice by oral gavage, in life-span studies in male and female Wistar rats and in CBA x C57Bl/6 hybrid mice by administration in drinking-water. In the gavage studies, bromodichloromethane

increased the incidences of adenomatous polyps and adenocarcinomas of the large intestine and of tubular-cell adenomas and adenocarcinomas of the kidney in male and female rats, of tubular-cell adenomas and adenocarcinomas of the kidney in male mice and of hepatocellular adenomas and carcinomas in female mice. In the study by administration in drinking-water, it induced neoplastic nodules and adenofibrosis of the liver in rats; no increase in tumour incidence was seen in mice. In a screening test for lung adenomas by intraperitoneal injection, bromodichloromethane did not increase the incidence of lung tumours in strain A mice.

Human carcinogenicity data

No relevant data were available to the Working Group.

Other relevant data

Repeated exposure of rats and mice to bromodichloromethane resulted in toxic effects in several organs, including the liver and kidney.

A study of developmental toxicity in rats given bromodichloromethane throughout the period of major organogenesis showed skeletal variations in the presence of maternal toxicity but no teratogenic effect.

Bromodichloromethane induced mutations in some studies with bacteria and, in a single study, in cultured mammalian cells. Chromosomal aberrations but not sister chromatid exchange were observed in cultured mammalian cells. In single studies, sister chromatid exchange was observed in cultured human cells and in mouse bone marrow *in vivo*. In one study, bromodichloromethane did not induce micronuclei in bone-marrow cells of mice treated *in vivo*.

Evaluation

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

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

Overall evaluation

Bromodichloromethane is *possibly carcinogenic to humans (Group 2B)*.

D.2 VOL: 71 (1999) (p. 1295)[2]

Evaluation

No epidemiological data relevant to the carcinogenicity of bromodichloromethane were available.

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

Overall evaluation

Bromodichloromethane is *possibly carcinogenic to humans (Group 2B)*.

Annex **E**

Carcinogenic classification of substances by the committee

The committee expresses its conclusions in the form of standard phrases:

Judgment of the committee	*Comparable with EU class*
This compound is known to be carcinogenic to humans • It is stochastic or non-stochastic genotoxic • It is non-genotoxic • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	1
This compound should be regarded as carcinogenic to humans • It is stochastic or non-stochastic genotoxic • It is non-genotoxic • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	2
This compound is a suspected human carcinogen. • This compound has been extensively investigated. Although there is insufficient evidence for a carcinogenic effect to warrant a classification as ‘known to be carcinogenic to humans’ or as ‘should be regarded as carcinogenic to humans’, they indicate that there is cause for concern. • This compound has been insufficiently investigated. While the available data do not warrant a classification as ‘known to be carcinogenic to humans’ or as ‘should be regarded as carcinogenic to humans’, they indicate that there is a cause for concern.	3 (A) (B)
This compound cannot be classified • There is a lack of carcinogenicity and genotoxicity data. • Its carcinogenicity is extensively investigated. The data indicate sufficient evidence suggesting lack of carcinogenicity.	not classifiable

Annex **F**

Guideline 93/21/EEG of the European Union

4.2 Criteria for classification, indication of danger, choice of risk phrases

4.2.1 Carcinogenic substances

For the purpose of classification and labelling, and having regard to the current state of knowledge, such substances are divided into three categories:

Category 1:

Substances known to be carcinogenic to man.

There is sufficient evidence to establish a causal association between human exposure to a substance and the development of cancer.

Category 2:

Substances which should be regarded as if they are carcinogenic to man.

There is sufficient evidence to provide a strong presumption that human exposure to a substance may result in the development of cancer, generally on the basis of:

- appropriate long-term animal studies
- other relevant information.

Category 3:

Substances which cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment.

There is some evidence from appropriate animal studies, but this is insufficient to place the substance in Category 2.

4.2.1.1 The following symbols and specific risk phrases apply:

Category 1 and 2:

T; R45 May cause cancer

However for substances and preparations which present a carcinogenic risk only when inhaled, for example, as dust, vapour or fumes, (other routes of exposure e.g. by swallowing or in contact with skin do not present any carcinogenic risk), the following symbol and specific risk phrase should be used:

T; R49 May cause cancer by inhalation

Category 3:

Xn; R40 Possible risk of irreversible effects

4.2.1.2 Comments regarding the categorisation of carcinogenic substances

The placing of a substance into Category 1 is done on the basis of epidemiological data; placing into Categories 2 and 3 is based primarily on animal experiments.

For classification as a Category 2 carcinogen either positive results in two animal species should be available or clear positive evidence in one species; together with supporting evidence such as genotoxicity data, metabolic or biochemical studies, induction of benign tumours, structural relationship with other known carcinogens, or data from epidemiological studies suggesting an association.

Category 3 actually comprises 2 sub-categories:

a substances which are well investigated but for which the evidence of a tumour-inducing effect is insufficient for classification in Category 2. Additional experiments would not be expected to yield further relevant information with respect to classification.

b substances which are insufficiently investigated. The available data are inadequate, but they raise concern for man. This classification is provisional; further experiments are necessary before a final decision can be made.

For a distinction between Categories 2 and 3 the arguments listed below are relevant which reduce the significance of experimental tumour induction in view of possible human exposure. These arguments, especially in combination, would lead in most cases to classification in Category 3, even though tumours have been induced in animals:

- carcinogenic effects only at very high levels exceeding the 'maximal tolerated dose'. The maximal tolerated dose is characterized by toxic effects which, although not yet reducing lifespan, go along with physical changes such as about 10% retardation in weight gain;
- appearance of tumours, especially at high dose levels, only in particular organs of certain species is known to be susceptible to a high spontaneous tumour formation;
- appearance of tumours, only at the site of application, in very sensitive test systems (e.g. i.p. or s.c. application of certain locally active compounds);
- if the particular target is not relevant to man;
- lack of genotoxicity in short-term tests *in vivo* and *in vitro*;
- existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g. hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation;
- existence of a species - specific mechanism of tumour formation (e.g. by specific metabolic pathways) irrelevant for man.

For a distinction between Category 3 and no classification arguments are relevant which exclude a concern for man:

- a substance should not be classified in any of the categories if the mechanism of experimental tumour formation is clearly identified, with good evidence that this process cannot be extrapolated to man;
- if the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories;
- particular attention should be paid to cases where the only available tumour data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence.