ETHYLENE DIBROMIDE (1,2-DIBROMOETHANE)

Data were last reviewed in IARC (1977) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

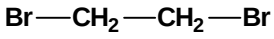
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 106-93-4
Chem. Abstr. Name: 1,2-Dibromoethane
IUPAC Systematic Name: 1,2-Dibromoethane
Synonym: EDB

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_4Br_2$ Relative molecular mass: 187.86

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid with a sweetish, chloroform-like odour (Lewis, 1993; Budavari, 1996)
(*b*) *Boiling-point*: 131.6°C (Lide, 1995)
(*c*) *Melting-point*: 9.9°C (Lide, 1995)
(*d*) *Solubility*: Miscible with acetone, benzene, diethyl ether and ethanol; slightly soluble in water (0.43 g/100 mL at 30°C) (Lide, 1995; Verschueren, 1996)
(*e*) *Vapour pressure*: 1.5 kPa at 25°C; relative vapour density (air = 1), 6.5 (Budavari, 1996; Verschueren, 1996)
(*f*) *Conversion factor*: $mg/m^3 = 7.69 \times ppm$

1.2 Production and use

Production of ethylene dibromide in the United States in 1982 was reported to be 77 100 tonnes (United States National Library of Medicine, 1997).

Ethylene dibromide has been used as a scavenger for lead in gasoline, as a general solvent, in waterproofing preparations, in organic synthesis and as a fumigant for grain and tree crops (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 9000 workers in the United States were potentially exposed to ethylene dibromide (see General Remarks). Occupational exposures to ethylene dibromide occur in pest control occupations, petroleum refining and waterproofing. In addition, car mechanics and other workers handling leaded gasoline may be dermally exposed to ethylene dibromide.

1.3.2 *Environmental occurrence*

Ethylene dibromide enters the atmosphere primarily from fugitive emissions and exhaust associated with its use as a scavenger in leaded gasoline. Another important but localized source is emissions from fumigation centres for citrus and grain and soil fumigation operations. It has been detected at low levels in groundwater, drinking-water, wastewater, ambient water, urban air and ambient air samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for ethylene dibromide in workplace air. The ACGIH (1991) lists ethylene dibromide as an animal carcinogen. Until 1979, the 8-h time-weighted average threshold limit value was 154 mg/m^3. Values ranging between 1 mg/m^3 and 145 mg/m^3 have been used as standards or guidelines in many countries (International Labour Office, 1991).

The World Health Organization has determined that there are no adequate data to permit recommendation of a health-based guideline value for ethylene dibromide in drinking-water (WHO, 1993).

2. Studies of Cancer in Humans

In one study, the mortality of 161 men exposed to ethylene dibromide in two factories since the mid-1920s and 1942, respectively, was investigated. By January 1976, 36 workers had died, seven of them from cancer (5.8 expected) (Ott *et al*., 1980). In another study, the mortality of 2510 male workers employed at a chemical plant was investigated. Ethylene dibromide was one of the several chemicals used and was apparently a minor component of the mixed exposure. No significant excess of cancer at any site was found (Sweeney *et al*., 1986).

In the United States, ethylene dibromide has been used as a fumigant in the grain industry since the 1940s. Alavanja *et al*. (1990) analysed mortality during 1955–85 in 22 938 white men who were enrolled in the life insurance programme of the American Federation of Grain Millers. Among a subset of 9660 who worked in flour mills (where

pesticides were used more frequently), 1914 deaths were recorded, giving a standardized mortality ratio (SMR) of 0.9 based on national rates ($p < 0.05$). These included 25 deaths from leukaemia (SMR, 1.4, not significant) and 21 from non-Hodgkin lymphoma (SMR, 1.5, not significant). In a nested case–control study, having ever been employed in a flour mill was significantly associated with mortality from non-Hodgkin lymphoma (21 cases; odds ratio, 4.2; 95% confidence interval (CI), 1.2–14.2) and pancreatic cancer (33 cases; odds ratio, 2.2; 95% CI, 1.1–4.3) but not leukaemia (25 cases; odds ratio, 1.8; 95% CI, 0.8–3.9). [The Working Group noted that interpretation was difficult in the absence of information about individual exposures to specific fumigants.]

3. Studies of Cancer in Experimental Animals

Ethylene dibromide was administered orally to mice and rats and produced squamous-cell carcinomas of the forestomach (IARC, 1977).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F_1 mice, five weeks of age, were administered daily time-weighted average doses of 62 and 107 mg/kg bw technical-grade ethylene dibromide (purity, 99.1%) in corn oil by gavage on five days per week for 53 weeks followed by observation for 24–37 weeks. A group of 20 males and 20 females received corn oil alone and served as vehicle controls and a further group of 20 males and 20 females served as untreated controls. Squamous-cell carcinomas of the forestomach were observed in both sexes (males: vehicle control, 0/20; low dose, 45/50; high dose, 29/49; females, 0/20, 46/49, 28/50). The incidence of alveolar/bronchiolar adenomas was significantly higher in treated mice of each sex than in vehicle controls (males, 0/20, 4/45, 10/47 ($p = 0.02$); females, 0/20, 11/43 ($p = 0.009$), 6/46) (United States National Cancer Institute, 1978).

Groups of 30 male and 30 female B6C3F_1 mice were administered 4 mmol/L ethylene dibromide (purity, > 99%), a dose equivalent to 116 mg/kg bw for males and 103 mg/kg bw for females) in distilled drinking-water for 450 days. A control group of 60 males and 60 females was given distilled drinking-water. Ethylene dibromide induced squamous-cell carcinomas of the forestomach in 26/28 males and 27/29 females and squamous-cell papilloma of the oesophagus in 3/30 females compared with none in 45 male and 50 female controls (Van Duuren *et al.*, 1985).

3.1.2 *Rat*

Groups of 50 male and 50 female Osborne-Mendel rats, five weeks of age, were administered daily time-weighted average doses of 38 or 41 (males) and 37 or 39 (females) mg/kg bw technical-grade ethylene dibromide (purity, 99.1%) in corn oil by gavage on five days per week for 36–57 weeks followed by observation for 2–13 weeks.

A group of 20 males and 20 females received corn oil alone and served as vehicle controls. Squamous-cell carcinomas of the forestomach were observed in 45/50 low-dose males, 33/50 high-dose males, 40/50 low-dose females and 29/50 high-dose females, while none was observed in controls. The lesions, seen as early as week 12, were locally invasive and eventually metastasized. A significantly higher incidence of haemangiosarcomas of the spleen was observed in low-dose males (0/20 controls, 10/50 low-dose and 3/49 high-dose) (United States National Cancer Institute, 1978).

3.2 Inhalation exposure

3.2.1 *Mouse*

Groups of 50 male and 50 female $B6C3F_1$ mice, five weeks of age, were exposed by whole-body inhalation to air containing 0 (control), 10 or 40 ppm [0, 77 or 308 mg/m^3] ethylene dibromide (purity, 99.3–99.4%) for 78–106 weeks. The incidence of alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas was significantly higher in exposed male and female mice than in controls. The incidence of haemangiosarcomas of the circulatory system, fibrosarcomas in subcutaneous tissue, carcinomas of the nasal cavity and adenocarcinomas of the mammary gland was significantly increased in females (see Table 1) (United States National Toxicology Program, 1982).

3.2.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, five weeks of age, were exposed by whole-body inhalation to air containing 0 (control), 10 or 40 ppm [0, 77 or 308 mg/m^3] ethylene dibromide (purity, 99.3–99.4%) for 88–106 weeks. The incidence of carcinomas, adenocarcinomas and adenomas of the nasal cavity and haemangiosarcomas of the circulatory system was significantly increased in exposed male and female rats. The incidence of mesotheliomas of the tunica vaginalis and adenomatous polyps of the nasal cavity in males and of fibroadenomas of the mammary gland and alveolar/bronchiolar adenomas and carcinomas (combined) in females was also significantly increased (see Table 2) (United States National Toxicology Program, 1982).

Groups of 48 male and 48 female Sprague-Dawley weanling rats were exposed by whole-body inhalation to 0 or 20 ppm [154 mg/m^3] ethylene dibromide (purity, 99%) for 7 h per day on five days per week for 18 months. Rats inhaling 20 ppm ethylene dibromide vapour had significantly higher mortality than the controls. Among treated rats, 10/48 males and 6/48 females developed haemangiosarcomas of the spleen compared with 0/48 male and 0/48 female controls. Mammary tumours (benign and malignant combined) occurred in 25/48 treated females compared with 2/48 controls. Subcutaneous mesenchymal tumours were found in 11/48 males compared with 3/48 controls (Wong *et al*., 1982).

3.3 Skin application

Mouse: Groups of 30 female Ha:ICR Swiss mice, six to eight weeks of age, received thrice-weekly skin applications of 25 or 50 mg per animal ethylene dibromide (purity,

Table 1. Incidence of tumours in mice exposed to ethylene dibromide by inhalation exposure

Tumour type	Males			Females		
	0	10 ppm	40 ppm	0	10 ppm	40 ppm
Lung, alveolar/bronchiolar						
Adenoma	0/41	0/48	11/46**	3/49	7/49	13/50*
Carcinoma	0/41	3/48	19/46**	1/49	5/49	37/50**
Circulatory system						
Haemangiosarcoma				0/50	11/50**	23/50**
Subcutaneous tissue						
Fibrosarcoma				0/50	5/50*	11/50**
Nasal cavity						
Carcinoma				0/50	0/50	6/50*
Mammary gland						
Adenocarcinoma				2/50	14/50**	8/50*

From United States National Toxicology Program (1982)
* $p < 0.05$
** $p \leq 0.001$

Table 2. Incidence of tumours in rats exposed to ethylene dibromide by inhalation exposure

Tumour type	Males			Females		
	0	10 ppm	40 ppm	0	10 ppm	40 ppm
Nasal cavity						
Adenoma	0/50	11/50**	0/50	0/50	11/50**	3/50
Carcinoma	0/50	0/50	21/50**	0/50	0/50	25/50**
Adenocarcinoma	0/50	20/50**	28/50**	0/50	20/50**	29/50**
Circulatory system						
Haemangiosarcoma	0/50	1/50	15/50**	0/50	0/50	5/50*
Tunica vaginalis						
Mesothelioma	0/50	7/50**	25/50**			
Mammary gland						
Fibroadenoma				4/50	29/50**	24/50**
Lung alveolar/bronchiolar						
Adenoma and carcinoma				0/50	0/48	5/47*

From United States National Toxicology Program (1982)
* $p < 0.05$
** $p \leq 0.001$

> 99%) in 0.2 mL acetone on the shaved dorsal skin, or applications of acetone alone or served as untreated controls. The times to the first appearance of skin tumour (papilloma) were 434 days for the 25-mg group and 395 days for the 50-mg group. In comparison with controls, both groups showed a significantly increased incidence of lung papillary adenomas (24/30 low-dose, 26/30 high-dose) and, in the 50-mg group, a significant increase in the incidence of skin papillomas (8/30) (Van Duuren *et al*., 1979).

3.4 Other systems

Fish: Groups of 200 (males and females combined) Shasta strain rainbow trout, eight weeks of age, were fed a diet containing 0 or 2000 ppm ethylene dibromide [purity unspecified]. Eighty fish were killed after nine months of feeding the test diet and 120 fish were killed after 18 months. Liver neoplasms (adenoma and carcinoma combined), occurred at nine months in 0/66 and 1/65 fish (males and females combined) in the control and ethylene dibromide-treated groups, respectively; at 18 months, the incidences were 0/113 and 6/117, respectively. The incidences of stomach papillomas at nine months were 0/66 and 0/65 for the control and ethylene dibromide-treated groups, respectively and at 18 months were 0/113 and 36/117, respectively. A higher incidence of stomach tumours was seen in males than in females ($p < 0.05$, Mantel–Haenszel test) (Hendricks *et al*., 1995).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

Various aspects of the toxicokinetics and metabolism of ethylene dibromide have recently been reviewed (Guengerich, 1994; WHO, 1996).

4.1.1 *Humans*

Human liver preparations metabolize ethylene dibromide to water-soluble and irreversibly protein- and DNA-bound metabolites by both cytochrome P450 and glutathione *S*-transferase (GST) enzymes (Wiersma *et al*., 1986). DNA adduct formation occurs also in isolated human hepatocytes (Cmarik *et al*., 1990).

There is convincing evidence that CYP2E1 is a major enzyme metabolizing ethylene dibromide. Among heterologously expressed human cytochromes P450, only CYP2E1 (low K_m enzyme), CYP2B6 and CYP2A6 (high K_m enzymes) metabolized ethylene dibromide to 2-bromoacetaldehyde (Wormhoudt *et al*., 1996), CYP2E1 having the highest intrinsic clearance. Interindividual variation in P450-catalysed microsomal metabolism, reflecting presumably variable amounts of CYP2E1 enzyme, was almost 50-fold.

Human fetal liver cytosol and several GST forms from human fetal liver catalyse the conjugation of ethylene dibromide (Kulkarni *et al*., 1992; Mitra *et al*., 1992). The α-class GST enzymes from human liver are especially active in the conjugation of ethylene dibromide (Cmarik *et al*., 1990).

4.1.2 *Experimental systems*

After intraperitoneal administration of radiolabelled ethylene dibromide to guinea-pigs (30 mg/kg bw) or mice (40 mg/kg bw), the largest portion of the radioactivity was excreted in urine. The highest levels of radioactivity were found in kidney, liver and stomach. Enzymatic reaction with glutathione (GSH) *in vitro* and *in vivo* as well as excretion of glutathione-derived metabolites in urine of rats and mice have been demonstrated (IARC, 1977).

Ethylene dibromide was absorbed rapidly through the skin of guinea-pigs and reached maximal blood levels at 1 h (Jakobson *et al*., 1982). In rats, 24-h urinary excretion of radiolabelled ethylene dibromide was > 70 % and the highest amount at 24 h was found in the liver and kidneys (Plotnick *et al*., 1979).

The metabolic pathways of ethylene dibromide are known in detail. In rodents, the major routes are oxidation by CYP2E1 and conjugation by GST (Guengerich, 1994; Wormhoudt *et al*., 1996). The primary metabolite formed by CYP2E1 is 2-bromoacetaldehyde, which can be conjugated with glutathione and enter the mercapturic acid pathway (Guengerich, 1994). The excretion of thiodiacetic acid in urine has been suggested as a biomarker for P450-catalysed oxidation (Wormhoudt *et al*., 1997).

The CYP2E1-catalysed pathway is responsible for the major part of protein binding and consequent tissue toxicity, although glutathione conjugates also play a role (Khan *et al*., 1993; Wormhoudt *et al*., 1996). The ratio between the oxidation pathway and the GST pathway in rodents *in vitro* and *in vivo* is about 4. Debromination during oxidative metabolism may result in increased bromine concentrations, which may be of significance in initiating lipid peroxidation (Guha *et al*., 1993).

The GSH conjugation pathway is responsible for the formation of DNA adducts and bacterial mutagenicity (Sipes *et al*., 1986). The major (> 95%) adduct is *S*-[2-(*N*7-guanyl)ethyl]glutathione (Cmarik *et al*., 1990). Three minor guanyl or adenyl adducts (1% or less) are also formed. Various forms of GST differ in their catalytic activities. The amount of the major adduct formed *in vivo* in liver and kidney DNA is directly proportional to the dose in rats (Kim & Guengerich, 1989). The amount of the adduct can be modulated by inducers of GST or inhibitors of CYP2E1 (Kim & Guengerich, 1990; Guengerich, 1994), with resultant consequences for hepatic tumorigenesis (Wong *et al*., 1982). More DNA adduct was formed in the livers of rats than in those of mice (Kim & Guengerich, 1990).

In whole-body autoradiographic studies, covalently bound radioactivity from ethylene dibromide was detected in the surface epithelia of the entire respiratory and the upper alimentary tracts of mice and rats (Brandt, 1986), in the epithelia of the oral cavity, oesophagus and forestomach of fetal mouse (Kowalski *et al*., 1986), and in vaginal epithelium of mice and rats (Brittebo *et al*., 1987).

Covalent binding of ethylene dibromide to albumin has been demonstrated after in-vivo administration of ethylene dibromide to rats and after in-vitro incubation of ethylene dibromide with human albumin (Kaphalia & Ansari, 1992).

4.1.3 *Comparison of human and rodent data*

Both human and rodent livers contain significant levels of enzymes necessary for the two major pathways of ethylene dibromide metabolism, although some qualitative (especially with respect to GST) and quantitative (CYP2E1) differences may exist.

The rate of metabolism of ethylene dibromide by human liver cytosol (three individuals examined) is about half that in rat cytosol (Kim & Guengerich, 1990).

A physiologically based pharmacokinetic model for predicting ethylene dibromide kinetics and consequent toxicity, based on in-vitro metabolic parameters of rodents and humans and on the use of scaling factors, has been presented (Ploemen *et al*., 1997). Its most important prediction is that the GST pathway is significantly active even at low ethylene dibromide concentrations, which has important implications for risk assessment.

4.2 Toxic effects

4.2.1 *Humans*

Several cases of fatalities following acute exposure of humans to ethylene dibromide have been reported. Two workers died following inhalation exposure while cleaning a tank used to temporarily store fertilizer mixtures in the field during application. Neither worker had respiratory or skin protection. The air inside the tank was sampled approximately 20 h after the accident and ethylene dibromide concentrations ranged from 15 to 41 ppm [115–315 mg/m^3] with an average of 28 ppm [215 mg/m^3]. The oxygen concentration inside the tank was 21%. The first worker was exposed for approximately 5 min and the second for approximately 20–30 min. The first worker died approximately 12 h after exposure and the second died 64 h after entering the tank. These two cases provided evidence that ethylene dibromide can produce metabolic acidosis, acute renal and hepatic failure and necrosis of skeletal muscle and many other organs (Letz *et al*., 1984).

Another fatal poisoning occurred in a woman who intentionally ingested a capsule containing 6480 mg ethylene dibromide [140 mg/kg]. On admission to hospital, the patient was drowsy, disoriented and jaundiced with mild hepatomegaly. She died eight days later and a post-mortem liver biopsy revealed congestion and focal liver cell necrosis (Singh *et al*., 1993).

4.2.2 *Experimental systems*

Male and female Fischer 344 rats were exposed to 0, 3, 10 or 40 ppm [0, 23, 77 or 308 mg/m^3] ethylene dibromide for 6 h per day on five days per week for 13 weeks for a total of 67–68 exposures in 95–96 days. Animals were killed after one, six or 13 weeks of exposure and after a recovery period of 88–89 days. At 10 ppm, ethylene dibromide caused slight epithelial hyperplasia of the nasal turbinates in animals killed after one, six or 13 weeks of exposure. However, 88 days after the last exposure, nasal turbinate changes were not observed. Rats exposed to 40 ppm ethylene dibromide had increased liver and kidney weights, hyperplasia and non-keratinizing squamous metaplasia of the respiratory epithelium of the nasal turbinates. After the recovery period of 88 days, the turbinates had reverted to normal histology. The most sensitive response associated with

repeated subchronic exposure of rats to 10 or 40 ppm ethylene dibromide involved pathological changes in the respiratory epithelium of the nasal turbinates (Nitschke *et al*., 1981). In these studies, 3 ppm was defined as the no-observable-effect level (NOEL).

Male and female Fischer 344 rats and B6C3F_1 mice were exposed to 3, 15 or 75 ppm [23, 115 or 577 mg/m^3] ethylene dibromide for 6 h per day on five days per week for 13 weeks. Rats and mice examined after 13 weeks of exposure showed severe necrosis and atrophy of the olfactory epithelium in the nasal cavity after inhalation of 75 ppm ethylene dibromide. Lower concentrations induced squamous-cell metaplasia, hyperplasia and cytomegaly of the epithelium of the respiratory nasal turbinates. Metaplasia, hyperplasia and epithelial cytomegaly were also seen in other respiratory tissues (larynx, trachea, bronchi, bronchioles) at this dose (Reznik *et al*., 1980).

The characteristics of the nasal lesions in mice following chronic inhalation of ethylene dibromide were investigated. Male and female B6C3F_1 mice were exposed to 10 or 40 ppm [77 or 308 mg/m^3] ethylene dibromide for 6 h per day on five days per week for 103 (10 ppm) or 90 (40 ppm) weeks. The incidence of hyperplastic lesions was related to the dose of ethylene dibromide and was equivalent in males and females. Lesions consisted of focal areas of cuboidal to columnar cells arranged in a glandular pattern with foci of hyperplastic squamous epithelium also seen occasionally. Lesions were usually located in the anterior (respiratory turbinates) of the nasal cavities. A broad spectrum of proliferative lesions was observed (Stinson *et al*., 1981).

Female B6C3F_1 mice were administered 100, 125, 160 or 200 mg/kg bw ethylene dibromide in corn oil by gavage daily for 14 days. Host resistance was not altered after challenge with a variety of agents. Decreases were seen in relative thymus and spleen weights, red blood cells, haemoglobin, haematocrit and responses of immunological cells in culture. Increases in relative weights of liver and kidney were seen. The authors concluded that even in animals exhibiting clinical signs of toxicity, short-term exposure to ethylene dibromide did not alter the immune integrity of mice as measured by host resistance assays *in vivo*. However, in-vitro assessments of immune integrity were altered in a dose-dependent fashion (Ratajczak *et al*., 1994).

Male and female Fischer 344 rats were given intraperitoneal injections of 40 mg/kg bw ethylene dibromide in corn oil twice daily for two consecutive days and were killed on the third day. No hepatotoxic effects were observed and impairment of renal function, as measured by in-vitro accumulation of *para*-aminohippurate by slices of renal cortex, was only observed only in male rats (Kluwe *et al*., 1981a).

Induction of renal cell proliferation following administration by gavage of a single dose of 100 mg/kg bw ethylene dibromide in corn oil was investigated in male Wistar rats. Incorporation of ^{3}H measured in extracted DNA was used to quantitate renal cell proliferation and was five times greater than in controls 20–30 h after treatment. No tubular necrosis was observed on histological examination (Ledda-Columbano *et al*., 1987).

Livers of male Sprague-Dawley rats were evaluated for foci and nodules either 90 days or 16 months after one or two oral doses of 75 mg/kg bw ethylene dibromide. Doses

were given within a 24-h period. Cell proliferation was stimulated by partial hepatectomy at approximately one day or 90 days after dosing and 0.05% phenobarbital in drinking-water for four months beginning at one year. At 90 days, no changes were noted. At 16 months, the incidence of nodules in the animals receiving two doses of ethylene dibromide was twice that of animals receiving one dose and three times that of the control group. Animals receiving ethylene dibromide had higher incidence of eosinophilic foci and γ-glutamyltranspeptidase-positive foci, suggesting that both hepatocyte foci and nodules can be initiated by limited exposure to ethylene dibromide (Moslen, 1984).

The cell cycle-dependent expression of proto-oncogenes in response to the proliferative stimuli induced by the mitogenic action of ethylene dibromide was investigated. Male Wistar rats were given a single dose of 100 mg/kg bw ethylene dibromide in corn oil by gavage. Hepatic cell proliferation was assessed using a single injection of tritiated thymidine 22 h after ethylene dibromide administration. Although there was a measurable increase in cell proliferation as measured by incorporation of tritiated thymidine into hepatic DNA extracted after 1 h, there was no increase in the expression of c-*fos* mRNA, although there was elevated expression of c-*myc* mRNA. Increased expression of c-Ha-*ras* mRNA and c-Ki-*ras* mRNA was also observed (Coni *et al*., 1990, 1993).

Male ICR mice and Fischer 344 rats were given a single intraperitoneal injection of 33, 100 or 330 mg/kg ethylene dibromide in corn oil and were killed 2 h later. Tissues were removed and assayed for non-protein sulfhydryl content (largely glutathione). Hepatic and renal non-protein sulfhydryl concentrations were depleted in mice in a dose-related manner. Lung, testis and stomach non-protein sulfhydryl concentrations were also decreased. The degree of depletion was not as great in the other organs as in kidney and liver, being significant only at the highest dose. In general, the conclusion of the authors was that there was a poor correlation between reported organ sensitivities to ethylene dibromide and tissue-specific depletion of non-protein sulfhydryls (Kluwe *et al*., 1981b).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

A retrospective assessment of the potential antifertility influence of ethylene dibromide was conducted by studying the reproductive performance of men exposed to ethylene dibromide in the workplace. Data were obtained from four chemical plants manufacturing ethylene dibromide located in the southern part of the United States (Arkansas and Texas). Exposures in the plants ranged from less than 0.5 ppm to 5 ppm [3.8–38 mg/m^3]. Evaluations were made exclusively on the basis of the men's reproductive histories of live births to their wives, subsequent to their occupational exposure. The number of live births was compared with the expected number derived from national fertility tables. One of the four plants studied showed a significant decrease in fertility; however, when data from the four plants were combined, there was no significant effect of ethylene dibromide exposure on reproductive performance (Wong *et al*., 1979).

The effect of long-term exposure to ethylene dibromide on semen quality was studied among 46 men employed in the papaya fumigation industry in Hawaii, United States,

with an average duration of exposure of five years and an average exposure to ethylene dibromide of 8 ppb [0.06 mg/m^3] as an 8-h time-weighted average, with peak exposures up to 262 ppb [2.0 mg/m^3]. The comparison group was 43 unexposed men from a sugar refinery. Significant decreases in sperm count, viable and motile sperm and increases in sperm with morphological abnormalities were observed among exposed men. The authors suggested that exposure to ethylene dibromide may increase the risk of reproductive impairment in workers at exposure levels near the recommended limit of 45 ppb [0.35 mg/m^3] and far below the current permissible exposure limit of 20 ppm [154 mg/m^3] (Ratcliffe *et al*., 1987).

A longitudinal study was conducted in 10 forestry employees and six unexposed men in Colorado, United States, with an exposure time of approximately six weeks. Sperm velocity decreased in all 10 exposed men and in only two unexposed men. Semen volume was also decreased. The time-weighted average exposure of these men was 60 ppb [0.46 mg/m^3] with peak exposures in the order of 2165 ppb [16.6 mg/m^3]. The authors suggested that the exposure may have effected the accessory sex glands and that ethylene dibromide may have multiple sites of action (Schrader *et al*., 1988).

4.3.2 *Experimental systems*

The effect of ethylene dibromide on reproduction was studied in male and female CD rats exposed to 0, 19, 39 or 89 ppm [0, 146, 300, 684 mg/m^3] ethylene dibromide for 7 h per day on five days per week for 10 weeks. Morbidity and mortality were observed at the highest concentration. Males in this group had reduced testicular weight, reduced serum testosterone concentration and failed to impregnate any females during a two-week mating period. Atrophy of the testes, epididymis, prostate and seminal vesicles was also observed. Reproductive performance of males exposed to the lower doses (19 or 39 ppm) was not impaired. Females in the highest-dose group did not cycle normally until several days after termination of exposure. However, the reproductive performance of females in the lower-dose groups was normal (Short *et al.*, 1979).

Male New Zealand white rabbits were given subcutaneous injections of 15, 30 or 45 mg/kg bw ethylene dibromide per day for five days. Semen samples were taken before exposure, during treatment and during 12 weeks after exposure and analysed for serum concentration, number, morphology, viability and motion parameters. Fertility was assessed by artificial insemination. Mortality, hepatotoxicity and alterations in measured semen parameters were observed in the highest-dose group. Fertility and fetal structural development were unaffected. The authors noted that semen parameters (velocity, percentage motility, amplitude of lateral head displacement) were affected only at doses close to the LD_{50} (55 mg/kg) (Williams *et al*., 1991).

The effect of exposure to ethylene dibromide on oestrous cycling was investigated in female B6C3F$_1$ mice given by gavage 31.25, 62.5 or 125 mg/kg bw on five days per week for 12 weeks. Vaginal smears showed that the oestrous cycle was significantly longer at the highest dose (Ratajczak *et al*., 1995). The effect of inhaled ethylene dibromide during the gestation period in rats and mice was investigated by exposing pregnant CD rats and

CD-1 mice to 20, 38 and 80 ppm [154, 292, and 615 mg/m^3] ethylene dibromide for 23 h per day over 10 days, beginning on day 6 of gestation. Rats and mice were killed on gestational days 20 and 18, respectively. Ethylene dibromide was more toxic to pregnant mice than pregnant rats. All of the mice exposed to 80 ppm died during the study. A significant increase in adult mortality occurred in rats exposed to 80 ppm and in mice exposed to 38 ppm or 80 ppm ethylene dibromide. Ethylene dibromide produced adverse effects on maternal welfare as measured by weight change, feed consumption and survival in both species at all doses tested. Fetal mortality was increased in rats exposed to 80 ppm and in mice exposed to 38 ppm. Reduced body weights were observed in fetuses from rats exposed to 38 ppm and in mice exposed to 20 or 38 ppm ethylene dibromide. Signs of fetal toxicity occurred at ethylene dibromide concentrations that adversely affected the dam (Short *et al*., 1978).

The effects of ethylene dibromide exposure in male rats were studied through behavioural assessments of their F_1 progeny. Fischer 344 male rats were treated by subacute intraperitoneal injection of a daily dose of 1.25, 2.5, 5 or 10 mg/kg bw ethylene dibromide on five successive days. Four weeks or nine weeks after the last injection, males were crossed with virgin females. Behavioural assessment of motor reflexes and motor coordination were examined in the offspring up to 21 days of age. Significant differences in the development of motor coordination and motor activity were observed in the F_1 progeny (Fanini *et al*., 1984). In a review of experimental male-mediated behavioural and neurochemical disorders, Nelson *et al*. (1996) noted that, although the above study is suggestive of effects in offspring following paternal exposures, only one laboratory has studied these effects.

4.4 Genetic and related effects

4.4.1 *Humans*

There have been two studies of ethylene dibromide workers for cytogenetic effects upon peripheral lymphocytes. In one of these (Steenland *et al.*, 1985), full working shift breathing zone samples of 14 sprayers of felled pine trees in Colorado, United States, indicated an average eight-hour time weighted average concentration of ethylene dibromide of 60 ppb [0.46 mg/m^3], with a range of 5 to 281 ppb [0.04 to 2.16 mg/m^3]; short-term samples taken over 4 to 15 min in the breathing zone during times of peak exposures averaged 463 ppb [3.6 mg/m^3], with a range of 8 to 2165 ppb [0.06 to 17 mg/m^3]. Exposure was for a few months and blood samples were taken before and after exposure. Six nonexposed controls were available who provided blood samples at the same time. In the other study (Steenland *et al.*, 1986), full working shift breathing zone samples of 60 papaya-packing workers at six different plants in Hawaii, United States, indicated geometric mean exposures to ethylene dibromide ranging from 16 to 175 ppb [0.12 to 1.35 mg/m^3]. Controls consisted of 42 sugar mill workers from a plant in the same area. In this study, there was control for sex, age, smoking, alcohol use, prescription and nonprescription drug use and recent illness. There were no increases in levels of either sister chromatid exchanges or total chromosomal aberrations as a result of exposure in either study.

4.4.2 *Experimental systems* (see Table 3 for references)

Ethylene dibromide was mutagenic in bacteria, *Streptomyces coelicolor*, *Aspergillus nidulans*. *Salmonella typhimurium* TA1535 expressing human GST1-1 showed greatly enhanced mutagenicity when treated with ethylene dibromide. Ethylene dibromide was highly mutagenic in *Salmonella typhimurium* NM5004, which has high levels of GST and inducible *umuC* gene expression (Oda *et al*., 1996).

Ethylene dibromide induced delayed sex-linked recessive lethal mutations in spermatozoa and spermatids of adult *Drosophila* males. Mutations were detected in F_3 generations as well as in the conventional F_2 generations.

Ethylene dibromide was mutagenic to *Drosophila melanogaster* and studies in repair-proficient and -deficient strains suggested that the compound is mutagenic through modification of ring nitrogens of purines (N7 of guanine and N1 of adenine).

Ethylene dibromide induced gene mutations, sister chromatid exchanges, chromosomal aberrations and cell transformation in animal cells. It induced mutations in two human lymphoblastoid cell lines, AHH-1 and TK6 in the absence of exogenous metabolic activation. Administration of radiolabelled ethylene dibromide to Wistar rats and BALB/c mice resulted in binding to DNA, RNA and proteins. [The nature of the binding was not characterized.]

Ethylene dibromide gave rise to micronuclei in binucleated peripheral human lymphocytes after a 4-h exposure, whereas a comparable effect in mononucleated cells was observed only after continual exposure.

Ethylene dibromide caused a dose-dependent increase in liver DNA alkaline-labile sites and single-strand breaks (as determined by alkaline elution assay) in female Sprague-Dawley rats. It was positive in an unscheduled DNA synthesis assay in rat spermatocytes *in vitro* but was negative in the spermatocytes of rats dosed *in vivo*. Ethylene dibromide gave positive results in an amphibian (*Pleurodeles waltl*) micronucleus test but gave negative results in dominant lethal tests.

The binding of ethylene dibromide to DNA of human and rat hepatocytes is mediated by GST-catalysed conjugation to glutathione (see Section 4.1.2).

Administration of a single intraperitoneal dose of ethylene dibromide gave rise to *S*-[2-(*N*7-guanyl)ethyl]glutathione DNA adducts in livers of several strains of rats (Fischer 344, Sprague-Dawley and Osborne-Mendel) and mice ($B6C3F_1$, ICR and A/J), with levels in rats being four to five times higher than those in mice.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to ethylene dibromide (1,2-dibromoethane) may occur in pest control, petroleum refining and waterproofing. Dermal exposure is possible when handling leaded gasoline containing ethylene dibromide. It has been detected at low levels in air and water.

Table 3. Genetic and related effects of ethylene dibromide

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links	+	NT	65 µg/assay	Quillardet *et al.* (1985)
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage	+	NT	442	Nakamura *et al*. (1987)
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage	+	NT	0.16% in air	Ong *et al*. (1987)
PRB, SOS *umu* test, *Salmonella typhimurium* NM5004 expressing GST 5-5	+	NT	1.9	Oda *et al.* (1996)
PRB, SOS *umu* test, *Salmonella typhimurium* TA1535/pSK1002	–	NT	19	Oda *et al*. (1996)
ERD, *Escherichia coli pol*A-deficient, differential toxicity	(+)	NT	22000	Brem *et al.* (1974)
SAF, *Salmonella typhimurium* BA13, forward mutation	+	+	54.5	Roldán-Arjona *et al*. (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	NT	2174	McCann *et al.* (1975)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	188	van Bladeren *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	94	Stolzenberg & Hine (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	1300	Barber *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	1100	Principe *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	250	Moriya *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	50	Dunkel *et al*. (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	0.725 in air	Simula *et al*. (1993)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	25	Novotná & Duverger-van Bogaert (1994)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	+	NT	470 µg/disk	Brem *et al.* (1974)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	NT	1880 µg/disk	Brem *et al.* (1974)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	NT	2174	McCann *et al.* (1975)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	47	Rannug *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	10	Elliott & Ashby (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	1300	Barber *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	1100	Principe *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	94	Kerklaan *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	17	Dunkel *et al.* (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	25	Novotná & Duverger-van Bogaert (1994)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	220000	Principe *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1667	Dunkel *et al.* (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	1880 µg/disk	Brem *et al.* (1974)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	220000	Principe *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1667	Dunkel *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	1300	Barber *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	220000	Principe *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	167	Dunkel *et al.* (1985)
SAS, *Salmonella typhimurium* TA1535 with decreased GSH levels, reverse mutation	+	NT	94	Kerklaan *et al.* (1983)
SAS, *Salmonella typhimurium* TA100 expressing GSTA1-1 or GST1-1, reverse mutation	+	NT	0.544 in air	Simula *et al.* (1993)
SAS, *Salmonella typhimurium* TA1535 expressing GST1-1, reverse mutation	+[c]	NT	18.8	Thier *et al.* (1996)
ECF, *Escherichia coli* (excluding K12), forward mutation	(+)	NT	NG	Izutani *et al.* (1980)
ECK, *Escherichia coli* K12, forward or reverse mutation	+	+	75	Mohn *et al.* (1984)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	+	+	167	Dunkel *et al.* (1985)
EC2, *Escherichia coli* WP2, reverse mutation	(+)	(+)	8800	Scott *et al.* (1978)
STF, *Streptomyces coelicolor*, forward mutation	+	NT	220000	Principe *et al.* (1981)
ANR, *Aspergillus nidulans*, reverse mutation	(+)	(+)	8800	Scott *et al.* (1978)
ANR, *Aspergillus nidulans*, reverse mutation	+	NT	110000	Principe *et al.* (1981)
DMM, *Drosophila melanogaster*, somatic mutation (and recombination)	+		7 ppm inh	Ballering *et al*. (1993)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		56 inh	Vogel & Chandler (1974)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		125 ppm/h inh	Kale & Baum (1979a)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		2.3 ppm/h inh	Kale & Baum (1979b)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		31 ppm/h inh	Kale & Baum (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		1 ppm 3 h inh	Kale & Baum (1983)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		94 feed	Ballering *et al*. (1993)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		25 ppm feed	Foureman *et al.* (1994)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		94 feed	Ballering *et al*. (1994)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		125 ppm inh	Kale & Kale (1995)
DIA, DNA strand breaks, cross-links or related damage, rat hepatocytes *in vitro*	+	NT	5.6	Sina *et al.* (1983)
DIA, DNA strand breaks, cross-links or related damage, rat testicular germ cells *in vitro*	+	NT	117	Bradley & Dysart (1985)
URP, Unscheduled DNA synthesis, Fischer 344 rat primary hepatocytes *in vitro*	+	NT	22	Williams *et al.* (1982)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	9.4	Working *et al*. (1986)
UIA, Unscheduled DNA synthesis, rat spermatocytes *in vitro*	+	NT	18.8	Working *et al*. (1986)
GCO, Gene mutation, Chinese hamster ovary CHO cells *in vitro*	+	+	9.4	Tan & Hsie (1981)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
GCO, Gene mutation, Chinese hamster ovary CHO cells *in vitro*	+	+	7.5	Brimer *et al.* (1982)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	(+)	50	Clive *et al.* (1979)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	94	Tezuka *et al.* (1980)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	5	Ivett *et al*. (1989)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	+	NT	380	Tezuka *et al.* (1980)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	+	125	Ivett *et al*. (1989)
TBM, Cell transformation, BALB/c 3T3 mouse cells	+	+	23	Perocco *et al.* (1991)
TBM, Cell transformation, BALB/c 3T3 mouse cells	+	NT	3	Colacci *et al*. (1995)
GIH, Gene mutation, human epithelial-like (EUE) cells *in vitro*	+	NT	19	Ferreri *et al.* (1983)
GIH, Gene mutation, human lymphoblastoid cell lines (AHH-1 and TK6) *in vitro*	+	NT	5	Crespi *et al*. (1985)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	1.8	Tucker *et al.* (1984)
MIH, Micronucleus test, human lymphocytes *in vitro*	+	NT	188	Channarayappa *et al.* (1992)
DVA, DNA strand breaks, cross-links or related damage, rat liver cells *in vivo*	+		75 po × 1	Nachtomi & Sarma (1977)
DVA, DNA strand breaks, cross-links or related damage, Swiss-Webster mouse liver cells *in vivo*	+		50 ip × 1	White *et al.* (1981)
DVA, DNA strand breaks, cross-links or related damage, $B6C3F_1$ mouse liver *in vivo*	+		90 ip × 1	Storer & Conolly. (1983)
DVA, DNA strand breaks, cross-links or related damage, male Fischer 344 rat testicular germ cells *in vivo*	+		234 ip × 1	Bradley & Dysart (1985)
DVA, DNA strand breaks, cross-links or related damage, female Sprague-Dawley rat liver cells *in vivo*	+		1.8 po × 1	Kitchin & Brown (1994)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
RVA, DNA repair exclusive of unscheduled DNA synthesis, Swiss Webster mouse liver *in vivo*	–		50 ip × 1	White *et al.* (1981)
UPR, Unscheduled DNA synthesis, male Fischer 344 rat hepatocytes *in vivo*	+		100 ip × 1	Working *et al.* (1986)
UPR, Unscheduled DNA synthesis, male Fischer 344 rat hepatocytes *in vivo*	(+)		100 po × 1	Working *et al.* (1986)
UVR, Unscheduled DNA synthesis, male Fischer 344 rat spermatocytes *in vivo*	–		100 ip × 1	Working *et al.* (1986)
UVR, Unscheduled DNA synthesis, male Fischer 344 rat spermatocytes *in vivo*	–		100 po × 1	Working *et al.* (1986)
UVR, Unscheduled DNA synthesis, male Fischer 344 rat spermatocytes *in vivo*	–		150 ip × 1	Bentley & Working (1988)
SVA, Sister chromatid exchange, CD1 mouse bone-marrow cells *in vivo*	(+)		84 ip × 1	Krishna *et al.* (1985)
Micronucleus test, *Pleurodeles waltl in vivo*	+		1 feed	Fernandez *et al.* (1993)
MVM, Micronucleus test, CD1 mouse bone-marrow cells *in vivo*	–		168 ip × 1	Krishna *et al.* (1985)
MVM, Micronucleus test, ddY mice *in vivo*	–		200 ip × 1	Asita *et al.* (1992)
CBA, Chromosomal aberrations, CD1 mouse bone-marrow cells *in vivo*	–		168 ip × 1	Krishna *et al.* (1985)
DLM, Dominant lethal test, ICR/Ha Swiss mice	–		100 po × 1	Epstein *et al.* (1972)
DLM, Dominant lethal test, BDF_1 mice	–		150 po × 5	Teramoto *et al.* (1980)
DLM, Dominant lethal test, male DBA/2J mice	–		100 ip × 1	Barnett *et al.* (1992)
DLR, Dominant lethal test, Sprague-Dawley rats	–		30 po × 5	Teramoto *et al.* (1980)
DLR, Dominant lethal test, Fischer 344 rats	–		75 inj × 1	Teaf *et al.* (1990)
BID, Binding (covalent) to DNA *in vitro*	+	+	10.7	Arfellini *et al.* (1984)
BID, Binding (covalent) to DNA *in vitro*	NT	+	10	Colacci *et al.* (1985)
BID, Binding (covalent) to DNA *in vitro*	+	NT	94	Inskeep *et al.* (1986)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BID, Binding (covalent) to calf thymus DNA *in vitro*	NT	+	11	Prodi *et al.* (1986)
BID, Binding (covalent) to DNA, rat hepatocytes *in vitro*	+	NT	94	Cmarik *et al.* (1990)
BIH, Binding (covalent) to DNA, human hepatocytes *in vitro*	+	NT	94	Cmarik *et al.* (1990)
BIP, Binding (covalent) to human albumin, *in vitro*	NT	+	28.9	Kaphalia & Ansari (1992)
BIP, Binding (covalent) to RNA or protein *in vitro*	+	+	10.7	Arfellini *et al.* (1984)
BVD, Binding (covalent) to liver, kidney, stomach lung DNA, BALB/c mice *in vivo*	+		1.6 ip × 1	Arfellini *et al*. (1984)
BVD, Binding (covalent) to liver, kidney, stomach lung DNA, Wistar rats *in vivo*	+		1.6 ip × 1	Arfellini *et al*. (1984)
BVD, Binding (covalent) to DNA, Sprague-Dawley rat hepatocytes *in vivo*	+		37 ip × 1	Inskeep *et al.* (1986)
BVD, Binding (covalent) to DNA, Sprague-Dawley rat hepatocytes *in vivo*	+		37 ip × 1	Kim & Guengerich (1990)
BVD, Binding (covalent) to DNA, Fischer 344 rat hepatocytes *in vivo*	+		37 ip × 1	Kim & Guengerich (1990)
BVD, Binding (covalent) to DNA, Osborne-Mendel rat hepatocytes *in vivo*	+		37 ip x 1	Kim & Guengerich (1990)
BVD, Binding (covalent) to DNA, ICR Swiss mouse hepatocytes *in vivo*	+		37 ip × 1	Kim & Guengerich (1990)
BVD, Binding (covalent) to DNA, $B6C3F_1$ mouse hepatocytes *in vivo*	+		37 ip × 1	Kim & Guengerich (1990)
BVD, Binding (covalent) to liver, kidney, stomach, lung DNA, Wistar rats *in vivo*	+		1.2 ip × 1	Prodi *et al.* (1986)
BVD, Binding (covalent) to liver, kidney, stomach, lung DNA, BALB/c mice *in vivo*	+		1.2 ip × 1	Prodi *et al.* (1986)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BVP, Binding (covalent) to RNA or protein, BALB/c mouse liver, kidney, stomach, lung *in vivo*	+		1.6 ip × 1	Arfellini *et al*. (1984)
BVP, Binding (covalent) to RNA or protein, Wistar rat liver, kidney, stomach, lung *in vivo*	+		1.6 ip × 1	Arfellini *et al*. (1984)
BVP, Binding (covalent) to RNA, or proteins, BALB/c mice *in vivo*	+		1.2 ip × 1	Prodi *et al.* (1986)
BVP, Binding (covalent) to RNA, or proteins, Wistar rats *in vivo*	+		1.2 ip × 1	Prodi *et al.* (1986)
BVP, Binding (covalent) to albumin, Sprague-Dawley rats *in vivo*	+		25 po × 2	Kaphalia & Ansari (1992)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inh, inhalation; po, oral; ip, intraperitoneal; inj, injection

[c] Results were negative for strain TA1535 not expressing GST1-1 at doses of up to 0.5 mM (94 µg/mL)

5.2 Human carcinogenicity data

Three cohort studies have included workers exposed to ethylene dibromide, but because of their low statistical power and/or lack of information about individual exposures, little can be concluded about the carcinogenicity of this compound in humans.

5.3 Animal carcinogenicity data

Ethylene dibromide has been tested for carcinogenicity by oral administration in mice, rats and fish, by inhalation in mice and rats and by skin application in mice. Following its oral administration, it produced squamous-cell carcinomas of the forestomach in rodents of both species, an increased incidence of alveolar/bronchiolar lung tumours in mice of each sex, haemangiosarcomas in male rats, oesophageal papillomas in female mice and liver and stomach tumours in fish. Following its inhalation, ethylene dibromide produced adenomas and carcinomas of the nasal cavity, haemangiosarcomas, mammary gland tumours, subcutaneous mesenchymal tumours, an increased incidence of alveolar/bronchiolar lung tumours in animals of each species and an increased incidence of peritoneal mesotheliomas in male rats. It induced skin and lung tumours in mice after skin application.

5.4 Other relevant data

In rodents and humans, ethylene dibromide is metabolized both by cytochrome P450 and GST enzymes; the latter seem to be responsible for DNA adduct formation. In rodents, covalently bound radioactivity has been detected in the epithelial lining of a number of organs.

In humans, acute high-dose exposure leads to liver and kidney damage. In rodents, inhalation exposure causes primarily proliferative lesions in nasal cavities. After intragastric administration, liver and kidney were the main target organs. Some evidence of adverse effects on reproduction was observed both in humans and rodents.

Ethylene dibromide is mutagenic in bacteria and *Drosophila*, and in rodent and human cells *in vitro*. It induced DNA breakage but not chromosomal aberrations or micronuclei *in vivo* in rodents. It gave negative results in dominant lethal tests in mice and rats. It did not induce either chromosomal aberrations or sister chromatid exchange in humans *in vivo*.

Ethylene dibromide binds to DNA *in vitro* and *in vivo* in rodents.

5.5 Evaluation

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

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

Overall evaluation

Ethylene dibromide is *probably carcinogenic to humans (Group 2A)*.

In making the overall evaluation, the Working Group took into consideration that ethylene dibromide is genotoxic in a broad range of in-vitro and in-vivo assays and binds covalently with DNA *in vivo*.

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