1,3-BUTADIENE

1,3-Butadiene was considered by previous IARC Working Groups in 1991, 1998, and 2007 (IARC, 1992, 1999, 2008). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 106-99-0
Chem. Abstr. Serv. Name: 1,3-Butadiene
IUPAC Systematic Name: 1,3-Butadiene

C_4H_6
Relative molecular mass: 54.09
Description: Colourless gas
Solubility: Sparingly soluble in water (1 g/L at 20 °C); slightly soluble in ethanol and methanol; soluble in benzene, carbon tetrachloride, and diethyl ether
Conversion factor: $mg/m^3 = 2.21 \times ppm$
From O'Neil (2006) and Lide (2008)

1.2 Use

Butadiene is used primarily in the production of synthetic rubbers and polymers, which are used in a wide variety of industrial and consumer products (e.g. automobiles, construction materials, appliance parts, computers and telecommunication equipment, protective clothing, packaging and household articles). The advantages of butadiene-based polymers include improved functionality, performance and safety, and lower costs. Synthetic rubbers that are produced from butadiene include styrene-butadiene rubber, poly-butadiene rubber, styrene-butadiene latex, chloroprene rubber and nitrile rubber. Important plastics that contain butadiene as a monomeric component are shock-resistant polystyrene, a two-phase system that consists of polystyrene and poly-butadiene; polymers that consist of acrylonitrile, butadiene and styrene; and a co-polymer of methyl methacrylate, butadiene and styrene, which is used as a modifier for polyvinyl chloride. Butadiene is also used as an intermediate in the production of chloroprene, adiponitrile and other basic petrochemicals (White, 2007).

1.3 Human exposure

1.3.1 Occupational exposure

The highest exposures to butadiene occur in occupational settings. The potential for exposure exists in several industrial activities, such as petroleum refining and related operations (production of C4 fractions containing butadiene, and production and distribution of gasoline), production of purified butadiene monomer,

Table 1.1 Estimated numbers of workers exposed to 1,3-butadiene in the European Union (top 10 industries)

Industry, occupational activity	
Manufacture of industrial chemicals	8300
Manufacture of rubber products	7100
Manufacture of plastic products not elsewhere classified	7000
Petroleum refineries	2200
Construction	1600
Manufacture of other chemical products	1300
Education services	700
Manufacture of transport equipment	700
Wholesale and retail trade and restaurants and hotels	600
Manufacture of machinery except electrical	500
TOTAL	31600

From CAREX (1999)

production of various butadiene-based rubber and plastic polymers and other derivatives, and manufacture of rubber and plastic products, such as tyres, hoses and a variety of moulded objects (IARC, 1999).

Estimates of the number of workers potentially exposed to 1,3-butadiene have been developed by CAREX (CARcinogen EXposure) in Europe. CAREX is an international information system that provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Based on occupational exposure to known and suspected carcinogens collected from 1990 to 1993, the CAREX database estimates that 31 600 workers were exposed to 1,3-butadiene in the European Union (EU). Table 1.1 presents the number of exposed workers for 1,3-butadiene (top 10 industries) in the EU by industry (CAREX, 1999).

From the US National Occupational Exposure Survey (1981–1983) it was estimated that approximately 52 000 workers (including approximately 1400 women) were potentially exposed to 1,3-butadiene (NIOSH, 1990).

No measurements of exposure in butadiene-monomer production before the 1970s are available, but exposure levels have decreased from up to 20 mg/m^3 to less than 2 mg/m^3 between the late 1970s and the early 2000s (IARC, 2008). In styrene-butadiene polymer production, the estimated median levels of exposure to butadiene in earlier decades varied in the range 8–20 mg/m^3, while current exposure measurements in modern facilities in North America and western Europe generally show values below 2 mg/m^3. Concentrations of butadiene-in-air reported from the People's Republic of China are somewhat higher (~4 mg/m^3) (IARC, 2008). Butadiene is not usually found in detectable concentrations in workplace air during the manufacture of finished rubber and plastic products (IARC, 1999). Regardless of the type of factory, production process, or country, some tasks are still characterized by very high exposures (~200 mg/m^3), which are typically short in duration (IARC, 2008). For a detailed description of studies on occupational exposure to butadiene and Tables with data summarizing the results, the reader is referred to *IARC Monographs* Volumes 71 and 97 (IARC, 1999, 2008).

The utility of haemoglobin adducts as biomarkers of human exposure to butadiene has been investigated in several molecular epidemiological studies that often included the measurement of urinary metabolites and personal-air

monitoring of butadiene, as well as genotoxicity end-points and metabolic phenotypes (IARC, 2008).

1.3.2 Non-occupational exposure

Butadiene has been widely detected in ambient air but at much lower levels (μg/m^3) than reported in some occupational settings (mg/m^3). Elevated concentrations may occur in the vicinity of point sources, such as municipal structural fires, wood and brush fires; cigarette smoking; vehicle emissions and gasoline volatilization (IARC, 2008). Studies on non-occupational exposures to 1,3-butadiene have been reviewed in previous *IARC Monographs* (IARC, 1999, 2008).

In a study conducted between 1990 and 1994, concentrations of butadiene were determined in 1611 samples of outdoor air from 25 sites within 14 cities, towns or rural locations in Ontario, Canada. The mean concentration in all samples was 0.1 μg/m^3 (maximum, 1.7 μg/m^3) (Health Canada, 2000).

Dollard *et al.* (2007) measured butadiene concentrations at rural, urban background (UB), urban industry-influenced (UI) and 'busy-road-traffic' (BR) locations in the United Kingdom from 1993 to 2004. Mean rural levels dropped from 0.39 to 0.02 μg/m^3 between 1995 and 2004; mean UB levels decreased from 0.64 to 0.15 μg/m^3 in 1993–2004; mean UI levels came down from 0.85 to 0.35 μg/m^3 in 1995–2000; and mean BR levels went from 3.3 to 0.57 μg/m^3 in the period 1997–2004.

2. Cancer in Humans

In *IARC Monograph* Volume 97 (IARC, 2008) three cohort studies of workers in the butadiene-monomer industry were reviewed (Ward *et al.*, 1995; Divine & Hartman, 2001; Tsai *et al.*, 2001), along with two cohort studies of workers in the styrene–butadiene rubber (SBR) industry (McMichael *et al.*, 1974, 1976; Meinhardt *et al.*, 1982; Matanoski & Schwartz, 1987; Matanoski *et al.*, 1990, 1993; see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-21-Table2.1.pdf). The excess of mortality from leukaemia in one of the butadiene-monomer industry cohorts, which did not increase with duration of exposure or with cumulative exposure, was more pronounced among workers who had been exposed during the Second World War, when exposures to butadiene had probably been higher (Divine & Hartman, 2001). These cohorts were subsequently combined, although data from one styrene-butadiene plant were excluded because the information was incomplete (Delzell *et al.*, 1996; Macaluso *et al.*, 2004; Graff *et al.*, 2005; Sathiakumar *et al.*, 2005; Delzell *et al.*, 2006; Cheng *et al.*, 2007). A series of overlapping analyses examined the mortality of approximately 17 000 male workers from eight SBR-manufacturing facilities in the USA and Canada (see Table 2.1 online). A limiting factor was that diagnosis and classification of lymphatic and haematopoietic malignancies are extremely complex, and that these underwent several changes over the course of time. Although mortality from leukaemia was only slightly elevated in the most recent updates (Sathiakumar *et al.*, 2005; Delzell *et al.*, 2006; Cheng *et al.*, 2007), large excesses of mortality from leukaemia were seen in workers in the most highly exposed areas of the plants and among hourly-paid workers, especially those who had been hired in the early years and had been employed for more than ten years. These excesses were attributable to both chronic lymphocytic and chronic myelogenous leukaemia, with significant exposure–response relationships between cumulative exposure to butadiene and mortality from both leukaemia types. The most recent analyses showed that the exposure–response for butadiene and leukaemia was independent of exposures to benzene,

styrene and dimethyl-dithiocarbamate (Delzell *et al.*, 2006; Cheng *et al.*, 2007).

The strongest evidence of an association between exposure to butadiene and non-Hodgkin lymphoma comes from studies in the butadiene-monomer industry (Ward *et al.*, 1995, 1996; Divine & Hartman, 2001). Although this association did not become stronger with duration of exposure, it was more pronounced among workers who had been exposed during the Second World War, when exposures had presumably been higher.

Whitworth *et al.* (2008) conducted an ecological study in South-eastern Texas that assessed whether census tracts with the highest ambient-air concentrations of benzene or 1,3-butadiene have a higher incidence of lymphohaematopoietic cancer in children. The analysis included 977 cases of childhood lymphohaematopoietic cancer diagnosed from 1995–2004. Estimates of airborne concentrations of benzene and 1,3-butadiene were obtained for 886 census tracts by use of the models proposed in 1999 by the US Environmental Protection Agency. Poisson-regression models were used to explore the associations between pollutant levels and census-tract cancer rates. Adjustments were made for age, sex, race/ethnicity, and socioeconomic status at the community level. Census tracts with the highest 1,3-butadiene concentrations had rate ratios of 1.4 (95%CI: 1.1–1.8), 1.7 (95%CI: 0.8–3.4), and 1.3 (95%CI: 1.0–1.8) for all leukaemia, acute myeloid leukaemia and acute lymphocytic leukaemia, respectively. A statistically significant dose–response trend was noted for all leukaemia. No association was found between 1,3-butadiene concentrations and lymphoma incidence. An excess of leukaemia was also found in association with environmental exposure to benzene, but analyses that examined both exposures simultaneously indicated that the effects were independent. [The Working Group noted that environmental levels of butadiene are considerably lower than in industrial settings but children may be a more sensitive group. However, potential confounding factors have not been fully addressed and the findings need to be confirmed in future studies.]

The mortality of women in the styrene-butadiene cohort has also been evaluated (Sathiakumar & Delzell, 2007, 2009; see Table 2.1 online). No increased risks were found for leukaemia or lymphoma. Statistically significant positive SMRs were seen for cancers of the lung and bladder. [Unlike in the male cohorts, the female cases were not confirmed pathologically, the exposure level was low, most women were short-term workers (median duration of employment was approximately 1.7 years; 70% had worked during less than four years), and only 30% of women were exposed to butadiene and styrene.]

In a further analysis of this SBR cohort, the lung-cancer risk among men and women was evaluated. Among men there was no indication of an increased risk for lung cancer and no evidence for an internal dose–response. Among women there was evidence of an increased risk for lung cancer, although there was no evidence for an internal dose–response in the exposed group (Sathiakumar & Delzell, 2009).

Overall, the epidemiological evidence from the styrene-butadiene and the butadiene-monomer industries clearly indicates an increased risk for haematolymphatic malignancies. Studies from the styrene-butadiene industry show an excess of leukaemia, and a dose–response relationship with cumulative exposure to butadiene, while studies from the monomer industry show an excess of haematolymphatic malignancies in general, attributable both to leukaemia and malignant lymphoma. The evidence for an association between exposure to butadiene and cancer of the haematolymphatic organs has gained some support by findings of an association between environmental levels of butadiene and risk for leukaemia in children.

The epidemiological evidence for an association with specific subtypes of haematolymphatic malignancies is weaker, mainly since numbers are lower, giving imprecise risk estimates. However, when malignant lymphomas and leukaemias are distinguished, the evidence is strongest for leukaemia.

3. Cancer in Experimental Animals

3.1 1,3-Butadiene

Studies on the carcinogenesis of 1,3-butadiene in rats and mice have been reviewed in previous IARC *Monographs* (IARC, 1999, 2008) and by Grosse *et al.* (2007). The results of adequately conducted carcinogenicity studies are summarized in Table 3.1. There were no additional studies reported in the published literature since *IARC Monograph* Volume 97 (IARC, 2008).

1,3-Butadiene was tested for carcinogenicity by inhalation exposure in one study in rats and four studies in mice.

Inhalation of 1,3-butadiene induced tumours in rats at exposure concentrations ranging from 1000 to 8000 ppm [2200–17650 mg/m^3], and in multiple organs in mice at exposure concentrations ranging from 6.25 to 1250 ppm [13.8–2760 mg/m^3]. In rats, 1,3-butadiene caused a significantly increased incidence of carcinomas of the Zymbal gland, sarcomas of the uterus, adenomas and carcinomas (combined) of the mammary gland, and follicular cell adenomas of the thyroid gland in females. In males, it caused malignant gliomas and adenomas of the pancreas and testes in males (Owen *et al.*, 1987; Owen & Glaister, 1990; Melnick *et al.*, 1993; Melnick & Huff, 1993). In mice of both sexes, 1,3-butadiene caused a significantly increased incidence of Harderian gland adenomas and carcinomas, heart haemangiosarcomas, lymphoid tissue neoplasms (lymphoma, histiocytic sarcoma), lung adenomas and carcinomas, hepatocellular adenomas and carcinomas, and fore-stomach papillomas and carcinomas. It caused mammary gland cancers, benign tumours and carcinomas of the ovary, and skin sarcomas in females. It also caused preputial gland carcinomas and kidney tubule adenomas in males (NTP, 1984, 1993; Huff *et al.*, 1985; Miller *et al.*, 1989; Melnick *et al.*, 1990a, b, 1993; Melnick & Huff, 1993; Hong *et al.*, 2000; Melnick & Sills, 2001; Kim *et al.*, 2005). No increased incidence of tumours was observed in one study in mice exposed once to 1,3-butadiene at concentrations up to 10 000 ppm [22000 mg/m^3] (Bucher *et al.*, 1993).

3.2 Diepoxybutane

Diepoxybutane, a metabolite of 1,3-butadiene, was tested for carcinogenicity by inhalation in one study in rats and one study in mice, by four skin-application studies in mice, by one subcutaneous injection study in rats and two such studies in mice, and by one gavage and one intraperitoneal injection study in mice (Tables 3.1, 3.2, 3.3, 3.4).

Diepoxybutane increased the incidence of adenomas of the Harderian gland in female mice, and of squamous cell carcinoma of the nose in female rats after inhalation exposure (Henderson *et al.*, 1999, 2000). Subcutaneous injection resulted in an increased incidence of fibrosarcomas in female rats and female mice. The gavage study in mice did not produce any tumours (Van Duuren *et al.*, 1966). Intra-peritoneal injection led to an increased incidence of lung tumours in strain A/J mice (Shimkin *et al.*, 1966). Two skin-application studies in mice resulted in an increased incidence of dermoid carcinomas (Van Duuren *et al.*, 1963, 1965).

Table 3.1 Carcinogenicity studies in experimental animals exposed to 1,3-butadiene and diepoxybutane by inhalation

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
1,3-Butadiene				
Rat, Sprague-Dawley (M, F) killed at 52 wk, remainder killed when survival was approximately 20% (105 wk for F, 111 wk for M) Owen & Glaister (1990), Melnick *et al.* (1993), Melnick & Huff (1993)	0, 1 000, 8 000 ppm, 6 h/d, 5 d/wk 110/group	Pancreas (exocrine adenomas): 3/100, 1/100, 10/100 (M); 2/100, 0/100, 0/100 (F)	$P \leq 0.001$ (high-dose M) $P \leq 0.001$ (trend M)	99.2% pure 16 deaths occurred during the first yr. During the second yr mortality increased with increasing dosage. Increased mortality in females was due to mammary tumours and in males due to renal lesions. The incidence of uterine sarcomas and Zymbal-gland tumours were similar to the historical laboratory control. Zymbal-gland tumours were noted between 76 and 90 wk.
		Uterus (sarcomas): 1/100, 4/100, 5/100 (F)	$P \leq 0.005$ (trend F)	
		Zymbal gland (adenomas): 1/100, 1/100, 1/100(M); 0/100, 0/100, 0/100 (F) Zymbal gland (carcinomas): 0/100, 0/100, 1/100 (M); 0/100, 0/100, 4/100 (F)	Carcinoma: $P \leq 0.05$ (trend F)	
		Mammary gland (benign): 0/100, 2/100, 0/100 (M); 32/100, 64/100, 55/100 (F)	NS	
		Mammary gland (malignant): 1/100, 0/100, 0/100 (M); 18/100, 15/100, 26/100 (F)	NS	
		Mammary gland (total combined benign and malignant mammary tumours): 1/100, 2/100, 0/100 (M); 50/100, 79/100, 81/100 (F)	$P \leq 0.001$ (trend F)	
		Thyroid (follicular cell adenomas): 3/100, 5/100, 1/100 (M); 0/100, 2/100, 10/100 (F)	$P \leq 0.01$ (trend F)	
		Thyroid (carcinomas): 1/100, 0/100, 0/100 (M); 0/100, 2/100, 1/100 (F)	NS	
		Testis (leydig cell tumours): 0/100, 3/100, 8/100 (M)	$P \leq 0.001$ (trend M)	
		Brain (glial cell tumours (malignant)): 1/100, 4/100, 5/100 (M)	$P \leq 0.05$ (trend M)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 60–61 wk NTP (1984), Huff *et al.* (1985), Miller *et al.* (1989), Melnick *et al.* (1993), Melnick & Huff (1993), Hong *et al.* (2000), Melnick & Sills (2001), Kim *et al.* (2005)	0, 625, 1 250 ppm 6 h/d, 5 d/wk. 50/group	Lung: (alveolar/bronchiolar adenomas): 2/50, 12/49, 11/49 (M); 3/49, 9/48, 20/49 (F)	$P < 0.001$ (trend), $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	> 99% pure The survival of both dose-groups of mice of each sex was significantly less than that of the corresponding controls. The study was planned for 103 wk, but was terminated after 60 wk for males and 61 wk for females, because of poor survival ($P < 0.01$) in all exposed groups due to malignant tumours in multiple organs. Malignant lymphomas and haemangiosarcomas were considered the major cause of early death in these studies.
		Lung (alveolar/bronchiolar carcinomas): 0/50, 2/49, 5/49 (M); 0/49, 6/48, 8/49 (F)	$P < 0.001$, $P = 0.018$, $P < 0.001$ (M); $P = 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Lung (alveolar/bronchiolar adenomas or carcinomas): 2/50, 14/49, 15/49 (M); 3/49, 12/48, 23/49 (F)	$P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Lymphoma (all lymphomas): 0/50, 23/50, 29/50 (M); 1/50, 10/49, 10/49 (F)	$P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P = 0.006$, $P < 0.001$, $P = 0.003$ (F)	
		Heart (haemangiosarcomas): 0/50, 16/49, 7/49 (M); 0/50, 11/48, 18/49 (F)	$P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Fore-stomach (all papillomas): 0/49, 5/40, 0/44; (M) 0/49, 4/42, 10/49 (F)	$P = 0.036$, $P < 0.001$, – (M); $P < 0.001$, $P = 0.001$, $P < 0.001$ (F)	
		Fore-stomach (squamous cell carcinomas): 0/49, 2/40, 1/44 (M); 0/49, 1/42, 1/49 (F)	NS	
		Fore-stomach (all papillomas or carcinomas): 0/49, 7/40, 1/44 (M); 0/49, 5/42, 10/49 (F)	$P = 0.006$, $P < 0.001$, $P = 0.248$ (M); $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Liver (hepatocellular adenomas): 0/50, 1/47, 4/49 (F)	$P = 0.015$, $P = 0.278$, $P = 0.030$ (F)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 60–61 wk NTP (1984), Huff *et al.* (1985), Miller *et al.* (1989), Melnick *et al.* (1993), Melnick & Huff (1993), Hong *et al.* (2000), Melnick & Sills (2001), Kim *et al.* (2005) Contd.		Liver (hepatocellular carcinomas): 0/50, 1/47, 1/49 (F)	NS	
		Liver (hepatocellular adenomas or carcinomas): 0/50, 2/47, 5/49 (F)	$P = 0.009$, $P = 0.048$, $P = 0.015$ (F)	
		Mammary gland (acinar cell carcinomas): 0/50, 2/49, 6/49 (F)	$P = 0.004$, $P = 0.048$, $P = 0.007$ (F)	
		Mammary gland (adenosquamous carcinomas): 0/50, 4/49, 0/49 (F)	$P = 0.575$, $P = 0.030$, – (F)	
		Ovary (benign granulosa cell tumours): 0/49, 6/45, 12/48 (F)	$P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Preputial gland (carcinomas): 0/50, 3/50, 2/50 (M)	NS	
		Brain (gliomas): 0/50, 2/50, 1/50 (M)	NS	
		Zymbal gland (carcinomas): 0/50, 0/50, 2/50 (M); 0/50, 0/49, 1/49 (F)	NS	
Mouse, B6C3F1 (M, F) 2 yr stop study Bucher *et al.* (1993)	0, 1 000, 5 000, 10 000 ppm for 2 h, then held for 2 yr 60/group	Malignant lymphomas: 7/59, 8/58, 8/58, 10/58 (M); 13/57, 19/56, 18/57, 13/58 (F)	NS	Purity NR
		Forestomach (squamous neoplasms): 0/59, 1/58, 1/58, 3/58 (M); 0/57, 1/56, 0/57, 0/58 (F)	NS	
		Mammary gland (acinar cell neoplasms): 0/59, 0/58, 0/58, 1/58 (M); 2/57, 1/56, 3/57, 4/58 (F)	NS	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 2 yr Miller *et al.* (1989), Melnick *et al.* (1990a, b, 1993), Melnick & Huff (1993), NTP (1993), Melnick & Sills (2001), Kim *et al.* (2005)	0, 6.25, 20, 62.5, 200, 625 ppm, 6 h/d, 5 d/wk for 103 wk 70/group for dosages 0–200 ppm, 90/group for 625 ppm	Lymphoma (all lymphomas): 4/50, 2/50, 4/50, 6/50, 2/50, 51/73 (M); 6/50, 12/50, 11/50, 7/50, 9/50, 32/80 (F)	$P < 0.001$ (trend), $P = 0.302$N, $P = 0.528$, $P = 0.238$, $P = 0.627$, $P < 0.001$ (M); $P < 0.001$, $P = 0.068$, $P = 0.029$, $P = 0.055$, $P < 0.001$, $P < 0.001$(F)	> 99% pure Two-yr survival was decreased for M and F exposed to ≥ 20 ppm due to chemical-related tumours. No F exposed to 200 or 625 ppm or M exposed to 625 ppm survived till the end of the experiments.
		Lymphocytic lymphomas: 2/50, 0/50, 2/50, 4/50, 2/50, 49/73 (M); 1/50, 3/50, 6/50, 3/50, 8/50, 31/80 (F)	$P < 0.001$, $P = 0.227$N, $P = 0.671$, $P = 0.253$, $P = 0.529$, $P < 0.001$ (M); $P < 0.001$, $P = 0.278$, $P = 0.026$, $P = 0.160$, $P < 0.001$, $P < 0.001$ (F)	
		Histiocytic sarcomas: 0/50, 0/50, 4/50, 5/50, 7/50, 4/73 (M); 3/50, 2/50, 7/50, 4/50, 7/50, 4/80 (F)	$P < 0.001$, –, $P = 0.051$, $P = 0.021$, $P < 0.001$, $P = 0.043$ (M); $P < 0.001$, $P = 0.518$N, $P = 0.077$, $P = 0.195$, $P = 0.002$, $P = 0.038$ (F)	
		Heart (haemangiosarcomas): 0/50, 0/49, 1/50, 5/48, 20/48, 4/73 (M); 0/50, 0/50, 0/50, 1/49, 21/50, 23/80 (F)	$P < 0.001$, –, $P = 0.451$, $P = 0.011$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, –, –, $P = 0.392$, $P < 0.001$, $P < 0.001$ (F)	
		Lung (alveolar/bronchiolar adenomas): 18/50, 20/50, 10/50, 25/49, 21/50, 3/73 (M); 4/50, 11/50, 12/50, 17/50, 14/50, 17/78 (F)	$P < 0.001$, $P = 0.579$N, $P = 0.269$N, $P = 0.004$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.031$, $P = 0.003$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 2 yr Miller *et al.* (1989), Melnick *et al.* (1990a, b, 1993), Melnick & Huff (1993), NTP (1993), Melnick & Sills (2001), Kim *et al.* (2005) Contd.		Lung (alveolar/bronchiolar adenocarcinomas or carcinomas): 5/50, 6/50, 11,50, 12/49, 22/50, 3/73 (M);0/50, 5/50, 11/50, 9/50, 19/50, 8/78 (F)	$P < 0.001$, $P = 0.577$, $P = 0.017$, $P = 0.006$, $P < 0.001$, $P < 0.001$ (M), $P < 0.001$, $P = 0.029$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Lung (combined alveolar/ bronchiolar adenomas, adenocarcinomas, or carcinomas): 21/50, 23/50, 19/50, 31/49, 35/50, 3/73 (M); 4/50, 15/50, 19/50, 24/50, 25/50, 22/78 (F)	$P < 0.001$, $P = 0.552$N, $P = 0.276$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.004$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Fore-stomach (squamous cell papillomas): 1/50, 0/50, 0/50, 1/50, 7/50, 2/73 (M); 0/50, 0/50, 2/50, 1/50, 3/50, 16/80 (F)	$P < 0.001$, $P = 0.481$N, $P = 0.545$N, $P = 0.679$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, –, $P = 0.149$, $P = 0.260$, $P = 0.004$, $P < 0.001$ (F)	
		Fore-stomach (squamous cell carcinomas): 0/50, 0/50, 0/50, 0/50, 1/50, 2/73 (M); 0/50, 0/50, 1/50, 1/50, 1/50, 6/80 (F)	$P < 0.001$, –, –, –, $P = 0.325$, $P = 0.018$ (M); $P < 0.001$, –, $P = 0.414$, $P = 0.277$, $P = 0.374$, $P < 0.001$ (F)	
		Fore-stomach (squamous cell papillomas or squamous cell carcinomas): 1/50, 0/50, 0/50, 1/50, 8/50, 4/73 (M); 0/50, 0/50, 3/50, 2/50, 4/50, 22/80 (F)	$P < 0.001$, $P = 0.481$N, $P = 0.545$N, $P = 0.679$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$,–, $P = 0.056$, $P = 0.044$, $P = 0.001$, $P < 0.001$ (F)	
		Liver (hepatocellular adenomas): 13/50, 13/50, 19/50, 16/48, 24/48, 5/72 (M); 11/49, 10/49, 9/50, 14/50, 12/50, 1/80	$P < 0.001$, $P = 0.480$, $P = 0.036$, $P = 0.056$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.590$, $P = 0.419$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 2 yr Miller *et al.* (1989), Melnick *et al.* (1990a, b, 1993), Melnick & Huff (1993), NTP (1993), Melnick & Sills (2001), Kim *et al.* (2005) Contd.		Liver (hepatocellular carcinomas): 11/50, 16/50, 16/50, 17/48, 26/48, 1/72 (M); 4/49, 6/49, 8/50, 9/50, 8/50, 1/80 (F)	$P < 0.001$, $P = 0.289$, $P = 0.071$, $P = 0.020$, $P < 0.001$, $P = 0.009$ (M); $P < 0.001$, $P = 0.330$, $P = 0.064$, $P = 0.003$, $P < 0.001$, $P = 0.150$ (F)	
		Liver (hepatocellular adenomas or carcinomas): 21/50, 23/50, 30/50, 25/48, 33/48, 5/72 (M); 15/49, 14/49, 15/50, 19/50, 16/50, 2/80 (F)	$P < 0.001$, $P = 0.562$N, $P = 0.011$, $P = 0.022$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.555$, $P = 0.162$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Harderian gland (adenomas): 6/50, 7/50, 8/50, 19/50, 30/50, 6/73 (M); 8/50, 10/50, 6/50, 15/50, 20/50, 9/80 (F)	$P < 0.001$, $P = 0.575$, $P = 0.218$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.304$, $P = 0.544$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Harderian gland (carcinomas): 0/50, 1/50, 1/50, 3/50, 2/50, 0/73 (M); 0/50, 1/50, 1/50, 0/50,1/50, 0/80 (F)	$P = 0.080$, $P = 0.522$, $P = 0.425$, $P = 0.067$, $P = 0.166$, – (M); logistic regression test $P = 0.873$N, $P = 0.493$, $P = 0.631$, –, $P = 0.085$, – (F)	
		Harderian gland (adenomas or carcinomas): 6/50, 7/50, 9/50, 20/50, 31/50, 6/73 (M); 8/50, 10/50, 7/50, 15/50, 20/50, 9/80 (F)	$P < 0.001$, $P = 0.575$, $P = 0.141$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.304$, $P = 0.426$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Preputial gland (carcinomas): 0/50, 0/50, 0/50, 0/50, 5/50, 0/73 (M)	$P < 0.001$, –, –, –, $P < 0.001$, – (M)	
		Ovary (benign granulosa cell tumours): 1/49, 0/49, 1/48, 6/50, 6/50,6/79	$P < 0.001$, $P = 0.517$N, $P = 0.680$, $P = 0.003$, $P < 0.001$, $P < 0.001$ (F)	
		Ovary (malignant granulosa cell tumours): 0/49, 0/49, 0/48, 3/50, 2/50, 0/79 (F)	$P < 0.001$, –, –, $P = 0.018$, $P = 0.003$, –, (F)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 2 yr Miller *et al.* (1989), Melnick *et al.* (1990a, b, 1993), Melnick & Huff (1993), NTP (1993), Melnick & Sills (2001), Kim *et al.* (2005) Contd.		Ovary (benign or malignant granulosa cell tumours): 1/49, 0/49, 1/48, 9/50, 8/50, 6/79 (F)	$P < 0.001$, $P = 0.517$N, $P = 0.680$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Mammary gland (adenoacanthomas): 0/50, 1/50, 2/50, 6/50, 4/50, 0/80 (F)	$P = 0.025$, $P = 0.489$, $P = 0.152$, $P < 0.001$, $P = 0.021$, $P =$ – (F)	
		Mammary gland (carcinomas): 0/50, 2/50, 2/50, 6/50, 11/50,12/80 (F)	$P < 0.001$, $P = 0.221$, $P = 0.192$, $P = 0.008$, $P < 0.001$, $P < 0.001$ (F)	
		Mammary gland (malignant mixed tumours): 0/50,0/50, 0/50,0/50,0/50, 4/80 (F)	$P =$ < 0.001, –, –, –, –, $P = 0.003$ (F)	
		Mammary gland (adenoacanthomas, carcinomas, or malignant mixed tumours): 0/50, 2/50, 4/50, 12/50, 15/50, 16/80 (F)	Logistic regression test: $P = 0.026$, $P = 0.228$, $P = 0.056$, $P < 0.001$, $P = 0.004$, $P < 0.001$ (F)	
		Kidney (renal tubule adenomas): 0/50, 1/50, 0/50, 3/48, 1/49, 0/73 (M); 0/49, 0/49, 0/48, 0/50, 2/50, 0/80 (F)	Logistic regression test: $P = 0.630$, $P = 0.522$, –, $P = 0.053$, $P = 0.580$, – (M); $P = 0.816$, –, –, –, $P = 0.276$, – (F)	
		Small intestine (adenomas or carcinomas): 0/50, 1/50, 1/50, 1/50, 2/50, 0/73 (M); 0/50, 3/50, 0/50, 1/50, 0/50, 0/80 (F)	$P = 1.000$, $P = 0.101$, –, $P = 0.375$, –, – (F)	
		Skin, subcutaneous tissue (neurofibrosarcomas or sarcomas): 1/50, 2/50, 3/50, 5/50, 3/50, 3/80 (F)	$P < 0.001$, $P = 0.476$, $P = 0.238$, $P = 0.017$, $P = 0.002$, $P = 0.013$ (F)	
		Zymbal gland (adenomas): 0/50, 0/50, 0/50, 0/50, 0/50, 1/80 (F)	NS	
		Zymbal gland: carcinoma: 0/50, 0/50, 0/50, 0/50, 0/50, 1/80 (F)	NS	
		Zymbal gland (adenomas or carcinomas): 0/50, 0/50, 0/50, 0/50, 2/80	NS	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M) 2 yr stop study Melnick *et al.* (1993), Melnick & Huff (1993), NTP (1993), Melnick & Sills (2001), Kim *et al.* (2005)	0, 200 ppm for 40 wk, 625 ppm for 13 wk, 312 ppm for 52 wk, or 625 ppm for 26 wk 50/group	Lymphoma (all lymphomas): 4/50, 8/50, 22/50, 8/50, 33/50 (M)	–, $P = 0.023$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	> 99% pure Survival of all stop-exposure groups was markedly lower than that of controls due to development of malignant tumours, particularly malignant lymphoma and haemangiosarcoma of the heart. Neoplasms were induced usually after only 13 wk of exposure.
		Lymphocytic lymphomas: 2/50, 6/50, 17/50, 4/50, 30/50 (M)	–, $P = 0.033$, $P < 0.001$, $P = 0.034$, $P < 0.001$ (M)	
		Histiocytic sarcomas: 0/50, 5/50, 2/50, 7/50, 2/50 (M)	–, $P = 0.006$, $P < 0.011$, $P < 0.001$, $P = 0.036$ (M)	
		Heart (haemangiosarcomas): 0/50, 15/50, 7/50, 33/50, 13/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	
		Lung (alveolar/bronchiolar adenomas): 18/50, 24/50, 17/50, 26/50, 12/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, (M)	
		Lung (alveolar/bronchiolar adenocarcinomas or carcinomas): 5/50, 22/50, 18/50, 16/50, 11/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	
		Lung (alveolar/bronchiolar adenomas, adenocarcinomas, or carcinomas): 21/50, 36/50, 28/50, 32/50, 17/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	
		Liver (hepatocellular adenomas): 13/50, 27/49, 19/49, 19/50, 11/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	
		Fore-stomach (squamous cell carcinomas): 0/50, 0/50, 4/50, 5/50, 6/50 (M)	–, –, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	
		Harderian gland (adenomas): 6/50, 26/50, 20/50, 28/50, 13/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, (M)	
		Harderian gland (carcinomas): 0/50, 2/50, 4/50, 2/50, 0/50 (M)	–, $P = 0.182$, $P < 0.001$, $P = 0.028$, – (M)	
		Harderian gland (adenomas or carcinomas): 6/50, 27/50, 23/50, 30/50, 13/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	
		Preputial gland (adenomas): 0/50, 0/50, 1/50, 0/50, 0/50 (M)	NS	
		Preputial gland (carcinomas): 0/50, 1/50, 4/50, 4/50, 3/50 (M)	–, $P = 0.247$, $P = 0.012$, $P < 0.001$, $P < 0.001$ (M)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M) 2 yr stop study Melnick *et al.* (1993), Melnick & Huff (1993), NTP (1993), Melnick & Sills (2001), Kim *et al.* (2005) Contd.		Preputial gland (adenomas or carcinomas): 0/50, 1/50, 5/50, 4/50, 3/50 (M)	–, $P = 0.247$, $P = 0.003$, $P < 0.001$, $P < 0.001$ (M)	
		Kidney: renal tubule adenoma: 0/50, 4/48, 1/50, 3/49, 1/50 (M)	–, $P = 0.016$, $P = 0.181$, $P = 0.007$, $P = 0.278$ (M)	
		Brain (malignant gliomas): 0/50, 0/50, 2/50, 0/50, 1/50 (M)	NS	
		Brain (neuroblastomas): 0/50, 0/50, 2/50, 0/50, 0/50 (M)	NS	
		Zymbal gland (adenomas): 1/50, 0/50, 0/50, 0/50, 0/50 (M)	NS	
		Zymbal gland (carcinomas): 0/50, 1/50, 2/50, 0/50, 2/50 (M)	NS	
		Zymbal gland (adenomas or carcinomas): 1/50, 1/50, 2/50, 0/50, 2/50	–. $P = 0.531$, $P = 0.178$, $P = 0.998$, $P = 0.009$ (M)	
Diepoxybutane				
Mouse, B6C3F1 (F) 18 mo Henderson *et al.* (1999, 2000)	0, 2.5, 5.0 ppm diepoxybutane 6 h/d, 5 d/wk for 6 wk 50/group	Harderian gland (adenomas): 0/40, 2/42, 5/36	$P < 0.05$ (high-dose F)	Purity > 99%
Rat, Sprague-Dawley (F) 18 mo Henderson *et al.* (1999, 2000)	0, 2.5, 5.0 ppm diepoxybutane 6 h/d, 5 d/wk for 6 wk 50/group	Nose (papillomas): 0/47, 0/48, 2/48 (F)	[NS]	Purity > 99%
		Nose (squamous cell carcinomas): 0/47, 11/48, 21/48 (F)	–, [$P < 0.001$], [$P < 0.001$]	
		Nose (adenocarcinomas): 0/47, 0/48, 2/48 (F)	[NS]	
		Nose (sarcomas): 0/47, 2/48, 2/48 (F)	[NS]	

d, day or days; F, female; h, hour or hours; M, male; mo, month or months; NR, not reported; NS, not significant; wk, week or weeks; yr, year or years

Table 3.2 Carcinogenicity studies in experimental animals exposed to diepoxybutane by intra-peritoneal or subcutaneous injection

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
D,L-Diepoxybutane Rat, Eastern Sprague-Dawley (F) 550 d Van Duuren *et al.* (1966)	**Subcutaneous injection** Tricaprylin vehicle (0.1 ml) control, untreated control, or 1 mg D,L-diepoxybutane injected into the axillary region once/wk 50/group	Fibrosarcomas at injection site: 0/50, 0/50, 9/50	[$P < 0.01$]	
		Adenocarcinomas at injection site: 1/50, 0/50/, 1/50	[NS]	
		Fibroadenomas: 0/50, 1/50, 0/50	[NS]	
Diepoxybutane Mouse, A/J (M, F) 39 wk Shimkin *et al.* (1966)	**Intraperitoneal injection** Total dose: 0, 19.4, 78.1, 314, 1 255, 2 232 µmol/kg bw in water, 12 injections given over a 4-wk period 165 M and 195 F vehicle control at start; 30, 30, 45, 30, 30 (total M, F)/group at start	Lung tumours (M, F combined): 107/339 (32%), 6/28 (21%), 12/30 (40%), 17/31 (55%), 18/28 (64%), 21/27 (78%)	NR	
	Total dose: 0, 34.8, 139, 558, 2 232 µmol/kg bw in tricaprylin, 12 injections given over a 4-wk period 60 M and 60 F tricaprylin vehicle control at start; 30, 30, 30, 30 (total M, F)/group at start	Lung tumours (M, F combined): 37/108 (34%), 12/30 (33%), 13/30 (43%), 12/25 (48%), 12/24 (50%)	NR	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
D,L-Diepoxybutane Mouse, ICR/Ha Swiss (F) 401 d Van Duuren *et al.* (1966)	**Subcutaneous injection** Tricaprylin vehicle (0.05 ml) control, untreated control, or subcutaneous injection of 1.1 mg D,L-diepoxy-butane into the axillary region once/wk 30/group	Fibrosarcomas at injection site 0/30, 0/30, 5/30 Adenocarcinomas at injection site: 0/30, 0/30, 0/30	[$P < 0.05$] [NS]	
D,L-Diepoxybutane Mouse, ICR/Ha Swiss (F) 589 d Van Duuren *et al.* (1966)	**Subcutaneous injection** Tricaprylin-vehicle (0.05 ml) control, untreated control, or subcutaneous injection of 0.1 mg D,L-diepoxy-butane into the axillary region once/wk 50/group	Fibrosarcomas at injection site: 0/50, 0/50, 5/50 Adenocarcinomas at injection site: 0/50, 0/50, 2/50	[NS] [NS]	

bw, body weight; d, day or days; F, female; M, male; NR, not reported; NS, not significant; wk, week or weeks

Table 3.3 Carcinogenicity study in rats exposed intragastrically to diepoxybutane

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
D,L-Diepoxybutane Rat, Eastern Sprague-Dawley (F) 363 d Van Duuren *et al.* (1966)	Tricaprylin vehicle (0.5 ml) 0 or 5 mg D,L-diepoxybutane once/wk 5/group	0/5, 0/5	[NS]	

d, day or days; F, female; NS, not significant; wk, week or weeks

Table 3.4 Carcinogenicity studies in mice exposed to diepoxybutane by skin application

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
D,L-Diepoxybutane Mouse, Swiss-Millerton (M) 78 d (median survival, treated group) Van Duuren *et al.* (1963)	Acetone-vehicle control, untreated control, or 100 mg D,L-1,2,3,4-diepoxybutane in acetone per application on the back 3 × /wk 120 acetone-vehicle controls, 276 untreated controls, 30/group for treated	Skin papillomas: 8/120, 13/267, 1/30 Squamous dermoid carcinomas: 0/120, 1/267, 1/30	[NS] [NS]	
meso-Diepoxybutane Mouse, Swiss-Millerton (M) 154 d (median survival, treated group) Van Duuren *et al.* (1963)	Acetone-vehicle control, untreated Control, or 100 mg *meso*-diepoxybutane in acetone per application on the back 3 × /wk 120 acetone-vehicle controls, 276 untreated controls, 30/group for treated	Skin papillomas: 8/120, 13/267, 2/30 Squamous dermoid carcinomas: 0/120, 1/267, 4/30	[NS] [$P < 0.005$]	
D,L-Diepoxybutane Mouse, Swiss-Millerton (F) Lifetime Van Duuren *et al.* (1965)	Acetone-vehicle control, untreated control, 30 mg, or 100 mg D,L-diepoxybutane painted on the back 3 × /wk 120 acetone-vehicle controls, 60 untreated controls, 30/group for treated	Skin papillomas: 0/120, 0/60, 10/30, 1/30 Squamous dermoid carcinomas: 0/120, 0/60, 6/30, 0/30	[$P < 0.0001$, 30 mg-treated group] [$P < 0.0001$, 30 mg-treated group]	Median survival of 472, 441, 475 and 165 d, respectively
meso-Diepoxybutane Mouse, Swiss-Millerton (F) Lifetime Van Duuren *et al.* (1965)	Acetone-vehicle control, untreated control, 30 mg, or 100 mg *meso*-diepoxybutane painted on the back 3 × /wk 120 acetone-vehicle controls, 60 untreated controls, 30/group for treated	Skin papillomas: 0/120, 0/60, 1/30, 5/30 Squamous dermoid carcinomas: 0/120, 0/60, 0/30, 4/30	[$P < 0.0005$, 100 mg-treated group] [$P < 0.005$, 100 mg-treated group]	Median survival of 472, 441, 491 and 357 d, respectively

d, day or days; F, female; M, male; NS, not significant; wk, week or weeks

4. Other Relevant Data

Experimental studies on butadiene have been evaluated in previous *IARC Monographs* (IARC, 1999, 2008). There is an extensive body of data on the mechanism of butadiene-induced carcinogenicity, encompassing toxicokinetics, metabolism, biomarkers, genotoxicity, and molecular biology. The carcinogenicity of butadiene is mediated by its metabolites. This view is based largely on the observations that butadiene-induced mutagenicity requires metabolic activation (Jackson *et al.*, 2000) and that the DNA-reactive epoxides formed during butadiene bio-transformation are direct-acting mutagens (IARC, 1999, 2008). Thus, butadiene metabolism, formation of reactive epoxides, interaction of these epoxides with DNA, and resultant mutagenicity are likely key steps in the mechanism of carcinogenicity for this agent.

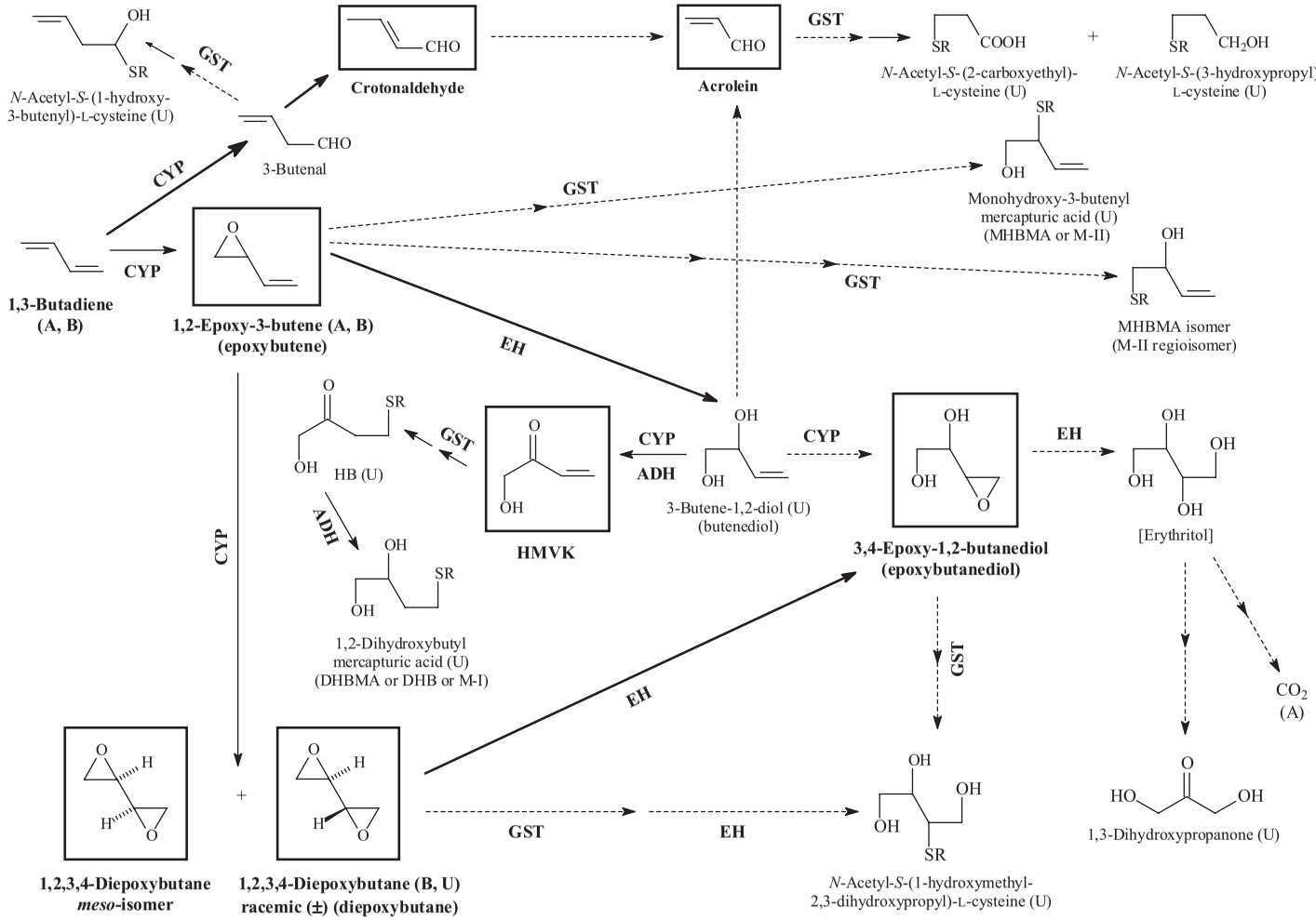
4.1 Metabolism of butadiene

The detailed pathways in the metabolism of butadiene have been described previously (Himmelstein *et al.*, 1997; IARC 1999, 2008) and are outlined in Fig. 4.1. Briefly, the first step in butadiene metabolism involves cytochrome P450 (CYP)-mediated oxidation to epoxybutene (Himmelstein *et al.*, 1997). At low concentrations of butadiene, metabolism via CYP2E1 predominates (IARC, 1999, 2008). Epoxybutene may be metabolized by conjugation with glutathione (GSH) mediated by glutathione *S*-transferase (GST), or by hydrolysis catalysed by epoxide hydrolase (EH) (Csanády *et al.*, 1992; Himmelstein *et al.*, 1997). Epoxybutene may also be oxidized to multiple diastereomers of diepoxybutane (Seaton *et al.*, 1995; Krause & Elfarra, 1997), while dihydroxybutene formed by hydrolysis of epoxybutene may be oxidized to epoxybutanediol. The latter epoxides are also detoxified by GST or EH (Boogaard *et al.*, 1996a, b). Partial hydrolysis of diepoxybutane also produces epoxybutanediol.

Each of the epoxide intermediates may contribute to the mutagenicity and carcinogenicity of butadiene. Factors that impact their relative contributions include concentration in tissues, reactivity with DNA, and repair of the ensuing DNA adducts. Variability in the expression of key enzymes involved in the biotransformation of butadiene may have an effect on metabolite concentrations in tissues, and on the subsequent mutagenic response (IARC, 2008). For example, genetically modified mice that are deficient in microsomal epoxide hydrolase (mEH) activity are more susceptible than wild-type mice to the mutagenic effects of butadiene and diepoxybutane, presumably because tissue concentrations of the epoxides are higher in the mEH-deficient mice (Wickliffe *et al.*, 2003). The detection of metabolites derived from hydroxymethylvinylketone and crotonaldehyde in the urine of rats or mice treated with butenediol suggests that these compounds may also be formed during the metabolism of butadiene (Sprague & Elfarra, 2003, 2004). The potential contribution of these DNA-alkylating agents (hydroxymethylvinylketone and crotonaldehyde) to the mutagenicity and carcinogenicity of butadiene is not known.

The enzymes that catalyse epoxide formation and elimination are polymorphic in human populations and some may be induced by a variety of environmental and pharmaceutical agents. While some reports indicate that genetic polymorphisms in GST and mEH affect the in-vitro mutagenicity of butadiene-derived epoxides or the in-vivo mutagenicity of butadiene in occupationally exposed workers (Wiencke *et al.*, 1995; Abdel-Rahman *et al.*, 2003), the extent to which these enzyme polymorphisms influence the carcinogenicity of butadiene is not known. Rates of butadiene metabolism have been reported for human tissues cultured *in vitro*. However, the range of observed rates is limited by the extent of the inter-individual variability in CYP, EH

Fig. 4.1 Metabolic pathways of butadiene deduced from findings in mammalian in-vitro systems and in mammals *in vivo*



A, B, U, metabolites in exhaled air, blood, urine, respectively; ADH, alcohol dehydrogenase; CYP, cytochrome P450; DHB, 4-(*N*-acetyl-L-cystein-*S*-yl)-1,2-dihydroxybutane; EH, epoxide hydrolase; GST, glutathione-*S*-transferase; HB, 4-(*N*-acetyl-L-cystein-*S*-yl)-1-hydroxy-2-butanone; HMVK, hydroxymethylvinyl ketone
Solid frame, electrophilic metabolites that can form DNA or haemoglobin adducts; dashed lines, assumed pathways
From IARC (2008)

and GST activities of the tissues sampled, and by the fact that only small numbers of human liver and lung samples were analysed (Csanády, *et al.*, 1992; Seaton, *et al.*, 1995; Boogaard, *et al.* 1996 a, b; Bolt *et al.*, 2003; Thier *et al.*, 2003; Norppa, 2004; Schlade-Bartusiak *et al.*, 2004). Thus, the actual kinetic range of the metabolism of butadiene in the human population is unknown.

The metabolism of butadiene in mice and rats shows linear elimination kinetics at exposures of up to about 1000 ppm [2210 mg/m^3] (Kreiling *et al.*, 1986b). Responses that increase proportionally above the levels of metabolic saturation probably represent effects of the parent compound. In the range of linear kinetics, mice metabolize butadiene about twice as rapidly as do rats. Although epoxybutene is formed primarily through CYP-mediated oxidation of butadiene, the formation of this alkylating agent by a myeloperoxidase-catalysed reaction in bone-marrow cells (Maniglier-Poulet *et al.*, 1995) may be relevant to the induction of haematopoietic cancers in mice and humans.

Data on urinary metabolites indicate that the elimination of epoxybutene in mice occurs to a greater extent by conjugation with GSH than by hydrolysis (IARC, 1999, 2008). Although no studies have been reported that characterize the full profile of urinary metabolites of butadiene in humans, the high ratio of 1,2-dihydroxybutyl-mercapturic acid (DHBMA) *vs* monohydroxy-3-butenyl-mercapturic acid (MHBMA) in exposed workers indicates that epoxybutene is preferentially metabolized by hydrolysis in humans (IARC, 2008). In rats, metabolic elimination of epoxybutene formed from butadiene occurs to a similar extent by hydrolysis or GSH conjugation. In molecular epidemiological studies of occupational exposure to butadiene, the ratio of MHBMA to MHBMA + DHBMA was lower in workers who were homozygous for *GSTM1*-null and *GSTT1*-null (Albertini *et al.*, 2001, 2003).

The formation of epoxybutanediol or diepoxybutane requires a second oxidation of either butenediol or epoxybutene, respectively. At increasing exposure concentrations of butadiene, competition between butadiene and butenediol or epoxybutene for CYP may limit the extent to which the second oxidation reaction may occur. Consequently, concentration of epoxybutanediol in blood is greater in rats exposed to 200 ppm [442 mg/m3] butadiene than in those exposed to 1000 ppm [2210 mg/m^3] or higher (Filser *et al.*, 2007). Competitive inhibition by butadiene of the second oxidation (Filser *et al.*, 2001) may account for the greater *Hprt* mutation efficiency in rats exposed to 62.5 ppm [138 mg/m^3] or mice exposed to 3 ppm [6.63 mg/m^3] compared with exposure of either species to 625 or 1250 ppm [1381 or 2762.5 mg/m^3] (Meng *et al.*, 2007). Thus, high-dose studies of butadiene (> 625 ppm) in animals may not adequately reveal the full mutagenic or carcinogenic potential of this substance at lower levels of exposure.

4.2 Haemoglobin adducts

While haemoglobin adducts are not causally related to mutagenic events, they offer an effective measure of exposure to reactive intermediates of chemicals. Haemoglobin adducts accumulate over the life-span of the erythrocyte, which is approximately 120 days in humans. Three adducts have been identified following reaction of butadiene epoxides with haemoglobin: *N*-(2-hydroxy-3-butenyl)valine (MHbVal), *N*,*N*-(2,3-dihydroxy-1,4-butadiyl) valine (PyrVal) and *N*-(2,3,4-trihydroxybutyl)valine (THbVal). These adducts are considered to reflect blood concentrations of epoxybutene, diepoxybutane and epoxybutanediol, respectively. Each of these adducts has been measured in rats and mice exposed to butadiene at concentrations as low as 3 ppm [6.63 mg/m3]. At equivalent exposures to butadiene, the levels of MHbVal and PyrVal were higher in mice than in rats, while levels of the major adduct, THbVal, were similar in these species (Boysen *et al.*, 2004, 2007). The formation

of each of these adducts in mice and rats was more efficient at 3 ppm than at higher exposure concentrations of butadiene.

MHbVal and THbVal have also been measured in workers exposed to butadiene (mean 8-hour TWA exposures, 0.3–0.8 ppm [0.66–1.76 mg/m^3]), while PyrVal could not be detected in workers exposed to mean concentrations of 0.37 ppm [0.82 mg/m^3]. In all samples, the amount of the PyrVal adduct was below the limit of quantification for the assay (Albertini *et al.*, 2003, 2007).

Species-specific differences in the amount of these haemoglobin adducts reflect differences in exposure to butadiene, blood concentrations of the epoxide intermediates, reactivity of the epoxide with the *N*-terminal valine and other reactive sites in haemoglobin, and the half-life of the red blood cell. When the amounts of adducts are normalized per gram of haemoglobin per ppm of butadiene, the levels of MHbVal adducts in workers are slightly lower than those in rats exposed to 3 ppm [6.63 mg/m^3] butadiene, while the levels of THbVal adducts are higher in workers than in rats or mice exposed to 3 ppm butadiene. These data demonstrate the systemic availability of epoxybutene and epoxybutanediol in workers exposed to occupational levels of butadiene. In these workers, the THbVal-adduct levels are influenced by the combined polymorphisms for *CYP2E1*, *GSTM1* and *GSTT1* genes (Fustinoni *et al.*, 2002).

4.3 DNA adducts

The major DNA adducts formed in the liver, lung and kidney of rats and mice exposed to butadiene are at the N7 position of guanine. These adducts are: N7-(2-hydroxy-3-butenyl) guanine (G1); N7-(1-(hydroxymethyl)-2-propenyl)guanine (G2); N7-(1-(hydroxymethyl)-2,3-dihydroxypropyl)guanine (G3); N7-(2,3,4-trihydroxybut-1-yl)guanine (G4). The G4 adducts are much more abundant than the G1 and G2 adducts, which are derived from epoxybutene (Koc *et al.*, 1999). The G4 adducts reach a plateau in rats after exposure to about 62 ppm [137 mg/m^3] butadiene, while G1 and G2 adducts increase nearly linearly with exposures to butadiene of up to 625 ppm [1381 mg/m^3]. Powley *et al.* (2005) have proposed that the similarity in the shape of the dose–response curves for THbVal-adduct formation in haemoglobin, G4-adduct formation in DNA, and *Hprt* mutation induction in splenic T-cells from mice and rats exposed to butenediol, suggests that epoxybutanediol may play a role in the mutagenicity and carcinogenicity of butadiene.

N7-Guanine adducts can undergo spontaneous depurination, which leaves an apurinic site in the DNA. Epoxide metabolites of butadiene can also react at sites involved in base-pairing and form adducts at N3 of cytosine, N1 of adenine, N^6 of adenine, N1 of guanine and N^2 of guanine (Selzer & Elfarra, 1996a, b, 1997; Zhao *et al.*, 1998; Zhang & Elfarra, 2004). An increase in N1-trihydroxybutyladenine adducts was detected in lymphocytes of workers exposed to butadiene (Zhao *et al.*, 2000). Alkylation of N1-adenine by epoxybutene followed by hydrolytic deamination under formation of deoxyinosine is a highly mutagenic event (Rodriguez *et al.*, 2001): deoxy-inosine forms a base-pair with cytosine during DNA replication, which leads to the generation of A→G mutations.

Diepoxybutane is a bi-functional alkylating agent that can form DNA–DNA crosslinks. Diepoxybutane first alkylates the N7 position of guanine in DNA and forms N7-(2′-hydroxy-3′,4′-epoxybut-1′-yl)-guanine mono-adducts (Tretyakova *et al.*, 1997). The epoxide group of this adduct can then undergo hydrolysis to yield N7-(2′,3′,4′-trihydroxybut-1′-yl)-guanine, or, less frequently, react with another site in DNA, such as the N7 of another guanine or the N1 of an adenine. The latter reactions result in formation of 1,4-bis-(guan-7-yl)-2,3-butanediol and 1-(guan-7-yl)-4-(aden-1-yl)-2,3-butanediol

crosslinks (Goggin *et al.*, 2009). These two diepoxybutane-specific DNA–DNA crosslinks have been identified in mice and rats exposed to 625 ppm butadiene, with much higher amounts of both crosslinks occurring in mice compared with rats (Goggin *et al.*, 2009). Depurination of these inter-strand or intra-strand lesions can induce point mutations and large deletion mutations. When diepoxybutane alkylates DNA at the N^6-position of adenine, an exocyclic adenine adduct is formed preferentially to DNA–DNA crosslinked products (Antsypovich *et al.*, 2007). Diepoxybutane is considered to be the most potent genotoxic metabolite of butadiene due to its strong genotoxicity and mutagenicity attributed to its ability to form DNA–DNA crosslinks.

4.4 Mutagenicity of butadiene and butadiene metabolites

Butadiene and its epoxide metabolites are genotoxic at multiple tissue sites in mice and rats, and in a variety of other test systems. In-vitro studies demonstrate that diepoxybutane is more potent than epoxybutene or epoxybutanediol in inducing micronuclei and gene mutations in mammalian cells. To investigate the role of two 1,3-butadiene (BD) metabolites, viz. 1,2-epoxybutene (EB) and 1,2,3,4-diepoxybutane (DEB) in the mutagenicity of the parent compound, in-vivo and in-vitro mutational spectra of BD, EB and DEB were analysed in *lac-i*-transgenic mice and in cultured human and rodent cells. A mutation that was consistently found across all biological systems examined was the AT→TA transversion, which was increased in the spleen and bone marrow of BD-exposed B6C3F1 *lac-i*-transgenic mice, in Rat2 *lac-i* cells exposed to EB, in the lungs of EB-exposed B6C3F1 *lac-i*-transgenic mice and at the *HPRT* locus in human TK6 lymphoblasts exposed to either EB or DEB (Recio *et al.*, 2001).

The mutation frequencies and mutation spectra induced by 3,4-epoxy-1,2-butanediol (EBD) and 1,2,3,4-diepoxybutane (DEB) were investigated at the *Hprt* locus in Chinese hamster ovary-K1 cells (CHO-K1). EBD was mutagenic at levels that were approximately 100 times higher than mutagenic concentrations of DEB. Among 41 EBD-induced mutants, there were 16 exon deletions, 11 GC→AT transitions, and five AT→GC transitions. Among 39 DEB-induced mutants, 15 exon deletions, 11 GC→AT transitions and five AT→TA transversions were found. In this study, the most common base substitution induced by both substances was the GC→AT transition. The sites of the single base substitutions that were induced by EBD and DEB were guanine and adenine, which is consistent with the DNA-adduct profiles (Lee *et al.*, 2002).

Fernandes & Lloyd (2007) have shown that replication of DNAs containing specific butadiene-derived 2′-deoxyuridine adducts in mammalian COS-7 cells resulted in predominantly C→T transitions. They also showed that replicative DNA polymerases were blocked by these lesions *in vitro*.

The genotoxic effects of butadiene can be modulated by alterations in key determinants of its metabolism, which suggests that markers of individual susceptibility can be identified. For example, mice that lack a functional *mEH* gene were more susceptible than wild-type mice to the mutagenic effects of butadiene or diepoxybutane (Wickliffe *et al.*, 2003). Epoxide hydrolase (EH) activity varies considerably among humans. Butadiene-exposed workers with the genotype for low-activity EH were reported to be more susceptible to butadiene-induced genotoxicity (assessed by *HPRT* mutant-variant frequency in lymphocytes) than individuals with the more common *EH* genotype (Abdel-Rahman *et al.*, 2001, 2003). No significant effects were observed for induction of *HPRT* mutations or sister chromatid exchange (SCE) in individuals with *GSTM1* or *GSTT1* polymorphisms (Abdel-Rahman *et al.*,

2001). These differences in response are consistent with the known important role of EH in the detoxification of butadiene epoxides in tissues in which these intermediates are produced.

In contrast, several other molecular epidemiological studies report no effect of butadiene – at occupational exposure levels – on *HPRT* mutation frequency or chromosomal changes, and no significant associations with genotype (Zhang *et al.*, 2004; Albertini *et al.*, 2001, 2007; Lovreglio *et al.*, 2006; Wickliffe *et al.*, 2009). Discrepancies among these studies may be related to differences in levels of exposure to butadiene at the workplace, the influence of exposures to butadiene or other genotoxic agents from other sources (e.g. cigarette smoke, automobile exhaust), the group size and the level of enzyme activity associated with a particular genotype.

The induction of SCE in human lymphocytes exposed *in vitro* to diepoxybutane was significantly higher in cells from *GSTT1*-null individuals than from *GSTT1*-positive individuals (Wiencke *et al.*, 1995), which indicates that the GST pathway may be important in the detoxification of diepoxybutane released into the blood. Epoxybutene can induce SCE and chromosomal aberrations in human peripheral lymphocytes treated *in vitro*; the lack of induction of these effects in G0 lymphocytes appears to be due to effective excision repair of DNA lesions (Kligerman *et al.*, 1999). Other studies also demonstrate the importance of DNA repair in the genotoxicity of butadiene-derived epoxides. For example, mice deficient in nucleotide excision-repair are more susceptible than wild-type mice to the mutagenic effects of butadiene and diepoxybutane (Wickliffe *et al.*, 2007).

The mechanistic link between animal and human neoplasia induced by butadiene is supported by the identification in mice of genetic alterations in butadiene-induced tumours that are frequently involved in the development of a variety of human cancers as well. The K-*Ras*, H-*Ras*, *p53*, *p16*/*p15* and *β-catenin* mutations detected in tumours in mice probably occurred as a result of the DNA-reactive properties and the genotoxic effects of butadiene-derived epoxides. A consistent pattern of K-*Ras* mutations (G→C transversion at codon 13) was observed in butadiene-induced cardiac haemangiosarcomas, neoplasms of the lung and fore-stomach, and lymphomas (Hong *et al.*, 2000; Sills *et al.*, 2001; Ton *et al.*, 2007). Alterations in the *p53* gene in mouse-brain tumours were mostly G→A transition mutations (Kim *et al.*, 2005). Inactivation of the tumour-suppressor genes *p16* and *p15* may also be important in the development of butadiene-induced lymphomas (Zhuang *et al.*, 2000). Mammary gland adenocarcinomas induced by butadiene in mice frequently had mutations in the *p53*, H-*Ras* and *β-catenin* genes (Zhuang *et al.*, 2002). These observations point to a genotoxic mechanism that underlies the development of butadiene-induced cancers. Although genotoxicity data indicate that diepoxybutane is the most genotoxic of the butadiene epoxides, the relative contribution of these metabolic intermediates to the mutagenicity and carcinogenicity of butadiene is not known. A comparison of the weight of evidence on metabolism, haemoglobin-adduct formation and genetic changes in rodents and humans exposed to butadiene is summarized in Table 4.1.

4.5 Synthesis

The numerous studies that have been conducted on butadiene toxicokinetics, metabolism, and genotoxicity provide strong evidence that the carcinogenicity of butadiene involves a genotoxic mechanism of action mediated by reactive epoxide metabolites. The metabolic pathways for butadiene in experimental animals have also been demonstrated in humans. This mechanism of action is based on the observations that butadiene-induced mutagenicity requires metabolic activation, and that the DNA-reactive epoxides formed during butadiene biotransformation

Table 4.1 Comparison of the degree of evidence on metabolism, haemoglobin-adduct formation and genetic changes in rodents and humans exposed to butadiene

Parameter	Rats	Mice	Humans
In-vitro metabolism of butadiene to epoxybutene	Strong	Strong	Strong
In-vitro metabolism of epoxybutene to diepoxybutane	Strong	Strong	Strong
In-vivo measure of epoxybutene in blood	Strong	Strong	NR
In-vivo measure of diepoxybutane in blood	Strong	Strong	NR
N-(2,3,4-Trihydroxybutyl)valine-haemoglobin adducts	Strong	Strong	Strong
N-(2-Hydroxy-3-butenyl)valine-haemoglobin adducts	Strong	Strong	Strong
N,*N*-(2,3-Dihydroxy-1,4-butadiyl)valine-haemoglobin adduct	Strong	Strong	Weak[a]
Urinary excretion of butadiene-derived mercapturic acid metabolites	Strong	Strong	Strong
DNA adducts	Strong	Strong	Strong
Mutations in reporter genes in somatic cells	Strong	Strong	Inconsistent[b]
Chromosomal aberrations or micronuclei	No evidence	Strong	Weak[a]

NR, not reported
[a] Possibly due to a lack of adequate studies
[b] One positive and three negative studies
From IARC (2008)

are direct-acting mutagens (IARC, 1999, 2008). Thus, butadiene metabolism, formation of reactive epoxides, interaction of these epoxides with DNA, and resultant mutagenicity are key steps in the mechanism that underlies the carcinogenicity of this agent.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of 1,3-butadiene. 1,3-Butadiene causes cancer of the haematolymphatic organs.

There is *sufficient evidence* for the carcinogenicity of 1,3-butadiene in experimental animals.

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

There is strong evidence that the carcinogenicity of 1,3-butadiene in humans operates by a genotoxic mechanism that involves formation of reactive epoxides, interaction of these direct-acting mutagenic epoxides with DNA, and resultant mutagenicity. The metabolic pathways for 1,3-butadiene in experimental animals have also been demonstrated in humans.

1,3-Butadiene is *carcinogenic to humans (Group 1).*

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