

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/m³ = 2.21 × 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/m³ to less than 2 mg/m³ 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/m³, while current exposure measurements in modern facilities in North America and western Europe generally show values below 2 mg/m³. Concentrations of butadiene-in-air reported from the People's Republic of China are somewhat higher (~4 mg/m³) ([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/m³), 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 ($\mu\text{g}/\text{m}^3$) 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 $0.1 \mu\text{g}/\text{m}^3$ (maximum, $1.7 \mu\text{g}/\text{m}^3$) ([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 $0.02 \mu\text{g}/\text{m}^3$ between 1995 and 2004; mean UB levels decreased from 0.64 to $0.15 \mu\text{g}/\text{m}^3$ in 1993–2004; mean UI levels came down from 0.85 to $0.35 \mu\text{g}/\text{m}^3$ in 1995–2000; and mean BR levels went from 3.3 to $0.57 \mu\text{g}/\text{m}^3$ 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/m³], and in multiple organs in mice at exposure concentrations ranging from 6.25 to 1250 ppm [13.8–2760 mg/m³]. 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/m³] (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 intra-peritoneal 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 <i>et al.</i> (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) Uterus (sarcomas): 1/100, 4/100, 5/100 (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) Mammary gland (benign): 0/100, 2/100, 0/100 (M); 32/100, 64/100, 55/100 (F) Mammary gland (malignant): 1/100, 0/100, 0/100 (M); 18/100, 15/100, 26/100 (F) Mammary gland (total combined benign and malignant mammary tumours): 1/100, 2/100, 0/100 (M); 50/100, 79/100, 81/100 (F) Thyroid (follicular cell adenomas): 3/100, 5/100, 1/100 (M); 0/100, 2/100, 10/100 (F) Thyroid (carcinomas): 1/100, 0/100, 0/100 (M); 0/100, 2/100, 1/100 (F) Testis (leydig cell tumours): 0/100, 3/100, 8/100 (M) Brain (glial cell tumours (malignant)): 1/100, 4/100, 5/100 (M)	$P \leq 0.001$ (high-dose M) $P \leq 0.001$ (trend M) $P \leq 0.005$ (trend F) Carcinoma: $P \leq 0.05$ (trend F) NS NS $P \leq 0.001$ (trend F) NS $P \leq 0.01$ (trend F) NS $P \leq 0.001$ (trend M) $P \leq 0.05$ (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.

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) Lung (alveolar/bronchiolar carcinomas): 0/50, 2/49, 5/49 (M); 0/49, 6/48, 8/49 (F) Lung (alveolar/bronchiolar adenomas or carcinomas): 2/50, 14/49, 15/49 (M); 3/49, 12/48, 23/49 (F) Lymphoma (all lymphomas): 0/50, 23/50, 29/50 (M); 1/50, 10/49, 10/49 (F) Heart (haemangiosarcomas): 0/50, 16/49, 7/49 (M); 0/50, 11/48, 18/49 (F) Fore-stomach (all papillomas): 0/49, 5/40, 0/44; (M) 0/49, 4/42, 10/49 (F) Fore-stomach (squamous cell carcinomas): 0/49, 2/40, 1/44 (M); 0/49, 1/42, 1/49 (F) Fore-stomach (all papillomas or carcinomas): 0/49, 7/40, 1/44 (M); 0/49, 5/42, 10/49 (F) Liver (hepatocellular adenomas): 0/50, 1/47, 4/49 (F)	$P < 0.001$ (trend), $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P < 0.001$, $P < 0.001$ (F) $P < 0.001$, $P = 0.018$, $P < 0.001$ (M); $P = 0.001$, $P < 0.001$ (F) $P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P < 0.001$, $P < 0.001$ (F) $P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P = 0.006$, $P < 0.001$, $P = 0.003$ (F) $P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P < 0.001$, $P < 0.001$ (F) $P = 0.036$, $P < 0.001$, – (M); $P < 0.001$, $P = 0.001$, $P < 0.001$ (F) NS $P = 0.006$, $P < 0.001$, $P = 0.248$ (M); $P < 0.001$, $P < 0.001$, $P < 0.001$ (F) $P = 0.015$, $P = 0.278$, $P = 0.030$ (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.

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) Liver (hepatocellular adenomas or carcinomas): 0/50, 2/47, 5/49 (F) Mammary gland (acinar cell carcinomas): 0/50, 2/49, 6/49 (F) Mammary gland (adenosquamous carcinomas): 0/50, 4/49, 0/49 (F) Ovary (benign granulosa cell tumours): 0/49, 6/45, 12/48 (F) Preputial gland (carcinomas): 0/50, 3/50, 2/50 (M) Brain (gliomas): 0/50, 2/50, 1/50 (M) Zymbal gland (carcinomas): 0/50, 0/50, 2/50 (M); 0/50, 0/49, 1/49 (F)	NS $P = 0.009$, $P = 0.048$, $P = 0.015$ (F) $P = 0.004$, $P = 0.048$, $P = 0.007$ (F) $P = 0.575$, $P = 0.030$, – (F) $P < 0.001$, $P < 0.001$, $P < 0.001$ (F) NS NS 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) Forestomach (squamous neoplasms): 0/59, 1/58, 1/58, 3/58 (M); 0/57, 1/56, 0/57, 0/58 (F) Mammary gland (acinar cell neoplasms): 0/59, 0/58, 0/58, 1/58 (M); 2/57, 1/56, 3/57, 4/58 (F)	NS NS NS	Purity NR

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) 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)	$P < 0.001$ (trend), $P = 0.302N$, $P = 0.528$, $P = 0.238$, $P = 0.627$, $P < 0.001$ (M); $P < 0.001$, $P = 0.068$, $P = 0.029$, $P = 0.055$, $P < 0.001$, $P < 0.001$ (F) $P < 0.001$, $P = 0.227N$, $P = 0.671$, $P = 0.253$, $P = 0.529$, $P < 0.001$ (M); $P < 0.001$, $P = 0.278$, $P = 0.026$, $P = 0.160$, $P < 0.001$, $P < 0.001$ (F)	> 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.
		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); $P < 0.001$, $P = 0.518N$, $P = 0.077$, $P = 0.195$, $P = 0.002$, $P = 0.038$ (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)	$P < 0.001$, $P = 0.579N$, $P = 0.269N$, $P = 0.004$, $P < 0.001$, $P < 0.001$ (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) 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) 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) 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, 6/80 (F) Fore-stomach (squamous cell papillomas or squamous cell carcinomas): 1/50, 0/50, 1/50, 8/50, 4/73 (M); 0/50, 0/50, 3/50, 2/50, 4/50, 22/80 (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	$P < 0.001$, $P = 0.577$, $P = 0.017$, $P = 0.006$, $P < 0.001$, $P < 0.001$ (M), $P < 0.001$, $P = 0.029$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F) $P < 0.001$, $P = 0.552$ 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) $P < 0.001$, $P = 0.481$ 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) $P < 0.001$, $P = 0.325$, $P = 0.018$ (M); $P < 0.001$, $P = 0.414$, $P = 0.277$, $P = 0.374$, $P < 0.001$ (F) $P < 0.001$, $P = 0.481$ 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) $P < 0.001$, $P = 0.480$, $P = 0.036$, $P = 0.056$, $P < 0.001$, $P < 0.001$ (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) 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) 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) 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) 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) Preputial gland (carcinomas): 0/50, 0/50, 0/50, 0/50, 5/50, 0/73 (M) Ovary (benign granulosa cell tumours): 1/49, 0/49, 1/48, 6/50, 6/50, 6/79 Ovary (malignant granulosa cell tumours): 0/49, 0/49, 0/48, 3/50, 2/50, 0/79 (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) $P < 0.001$, $P = 0.562$ 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) $P < 0.001$, $P = 0.575$, $P = 0.218$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.304$, $P = 0.544$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F) $P = 0.080$, $P = 0.522$, $P = 0.425$, $P = 0.067$, $P = 0.166$, – (M); logistic regression test $P = 0.873$ N, $P = 0.493$, $P = 0.631$, –, $P = 0.085$, – (F) $P < 0.001$, $P = 0.575$, $P = 0.141$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.304$, $P = 0.426$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F) $P < 0.001$, –, –, –, $P < 0.001$, – (M) $P < 0.001$, $P = 0.517$ N, $P = 0.680$, $P = 0.003$, $P < 0.001$, $P < 0.001$ (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) Mammary gland (adenoacanthomas): 0/50, 1/50, 2/50, 6/50, 4/50, 0/80 (F) Mammary gland (carcinomas): 0/50, 2/50, 2/50, 6/50, 11/50, 12/80 (F) Mammary gland (malignant mixed tumours): 0/50, 0/50, 0/50, 0/50, 4/80 (F) Mammary gland (adenoacanthomas, carcinomas, or malignant mixed tumours): 0/50, 2/50, 4/50, 12/50, 15/50, 16/80 (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) Small intestine (adenomas or carcinomas): 0/50, 1/50, 1/50, 2/50, 0/73 (M); 0/50, 3/50, 0/50, 1/50, 0/50, 0/80 (F) Skin, subcutaneous tissue (neurofibrosarcomas or sarcomas): 1/50, 2/50, 3/50, 5/50, 3/50, 3/80 (F) Zymbal gland (adenomas): 0/50, 0/50, 0/50, 0/50, 1/80 (F) Zymbal gland: carcinoma: 0/50, 0/50, 0/50, 0/50, 1/80 (F) Zymbal gland (adenomas or carcinomas): 0/50, 0/50, 0/50, 2/80	$P < 0.001$, $P = 0.517N$, $P = 0.680$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F) $P = 0.025$, $P = 0.489$, $P = 0.152$, $P < 0.001$, $P = 0.021$, $P = -$ (F) $P < 0.001$, $P = 0.221$, $P = 0.192$, $P = 0.008$, $P < 0.001$, $P < 0.001$ (F) $P = < 0.001$, -, -, -, -, -, $P = 0.003$ (F) Logistic regression test: $P = 0.026$, $P = 0.228$, $P = 0.056$, $P < 0.001$, $P = 0.004$, $P < 0.001$ (F) Logistic regression test: $P = 0.630$, $P = 0.522$, -, $P = 0.053$, $P = 0.580$, - (M); $P = 0.816$, -, -, -, $P = 0.276$, - (F) $P = 1.000$, $P = 0.101$, -, $P = 0.375$, -, - (F) $P < 0.001$, $P = 0.476$, $P = 0.238$, $P = 0.017$, $P = 0.002$, $P = 0.013$ (F) NS NS 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) Lymphocytic lymphomas: 2/50, 6/50, 17/50, 4/50, 30/50 (M) Histiocytic sarcomas: 0/50, 5/50, 2/50, 7/50, 2/50 (M) Heart (haemangiosarcomas): 0/50, 15/50, 7/50, 33/50, 13/50 (M) Lung (alveolar/bronchiolar adenomas): 18/50, 24/50, 17/50, 26/50, 12/50 (M) Lung (alveolar/bronchiolar adenocarcinomas or carcinomas): 5/50, 22/50, 18/50, 16/50, 11/50 (M) Lung (alveolar/bronchiolar adenomas, adenocarcinomas, or carcinomas): 21/50, 36/50, 28/50, 32/50, 17/50 (M) Liver (hepatocellular adenomas): 13/50, 27/49, 19/49, 19/50, 11/50 (M) Fore-stomach (squamous cell carcinomas): 0/50, 0/50, 4/50, 5/50, 6/50 (M) Harderian gland (adenomas): 6/50, 26/50, 20/50, 28/50, 13/50 (M) Harderian gland (carcinomas): 0/50, 2/50, 4/50, 2/50, 0/50 (M) Harderian gland (adenomas or carcinomas): 6/50, 27/50, 23/50, 30/50, 13/50 (M) Preputial gland (adenomas): 0/50, 0/50, 1/50, 0/50, 0/50 (M) Preputial gland (carcinomas): 0/50, 1/50, 4/50, 4/50, 3/50 (M)	-, $P = 0.023$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M) -, $P = 0.033$, $P < 0.001$, $P = 0.034$, $P < 0.001$ (M) -, $P = 0.006$, $P < 0.011$, $P < 0.001$, $P = 0.036$ (M) -, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M) -, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M) -, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M) -, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M) -, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M) -, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M) -, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M) -, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M) -, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M) -, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M) -, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M) NS -, $P = 0.247$, $P = 0.012$, $P < 0.001$, $P < 0.001$ (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.

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) Kidney: renal tubule adenoma: 0/50, 4/48, 1/50, 3/49, 1/50 (M) Brain (malignant gliomas): 0/50, 0/50, 2/50, 0/50, 1/50 (M) Brain (neuroblastomas): 0/50, 0/50, 2/50, 0/50, 0/50 (M) Zymbal gland (adenomas): 1/50, 0/50, 0/50, 0/50 (M) Zymbal gland (carcinomas): 0/50, 1/50, 2/50, 0/50, 2/50 (M) Zymbal gland (adenomas or carcinomas): 1/50, 1/50, 2/50, 0/50, 2/50	-, $P = 0.247$, $P = 0.003$, $P < 0.001$, $P < 0.001$ (M) -, $P = 0.016$, $P = 0.181$, $P = 0.007$, $P = 0.278$ (M) NS NS NS NS -, $P = 0.531$, $P = 0.178$, $P = 0.998$, $P = 0.009$ (M)	
Diepoxybutane				
Mouse, B6C3F1 (F) 18 mo Henderson et al. (1999) , 2000 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 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 Nose (papillomas): 0/47, 0/48, 2/48 (F) Nose (squamous cell carcinomas): 0/47, 11/48, 21/48 (F) Nose (adenocarcinomas): 0/47, 0/48, 2/48 (F) Nose (sarcomas): 0/47, 2/48, 2/48 (F) [NS]	$P < 0.05$ (high-dose F) [NS] -, [$P < 0.001$], [$P < 0.001$] [NS] [NS]	Purity > 99% Purity > 99%

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 <i>et al.</i> (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 Adenocarcinomas at injection site: 1/50, 0/50/1, 1/50 Fibroadenomas: 0/50, 1/50, 0/50	[P < 0.01] [NS] [NS]	
Diepoxybutane Mouse, A/J (M, F) 39 wk Shimkin <i>et al.</i> (1966)	Intra-peritoneal 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 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 (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%) Lung tumours (M, F combined): 37/108 (34%), 12/30 (33%), 13/30 (43%), 12/25 (48%), 12/24 (50%)	NR 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, [P < 0.05] 0/30, 5/30 Adenocarcinomas at injection site: 0/30, 0/30, 0/30	[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 <i>et al.</i> (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]	
<i>meso</i> -Diepoxybutane Mouse, Swiss-Millerton (M) 154 d (median survival, treated group) Van Duuren et al. (1963)	Acetone-vehicle control, untreated Control, or 100 mg <i>meso</i> - 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 < 0.0005]	
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
<i>meso</i> -Diepoxybutane Mouse, Swiss-Millerton (F) Lifetime Van Duuren et al. (1965)	Acetone-vehicle control, untreated control, 30 mg, or 100 mg <i>meso</i> - 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 < 0.0005, 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

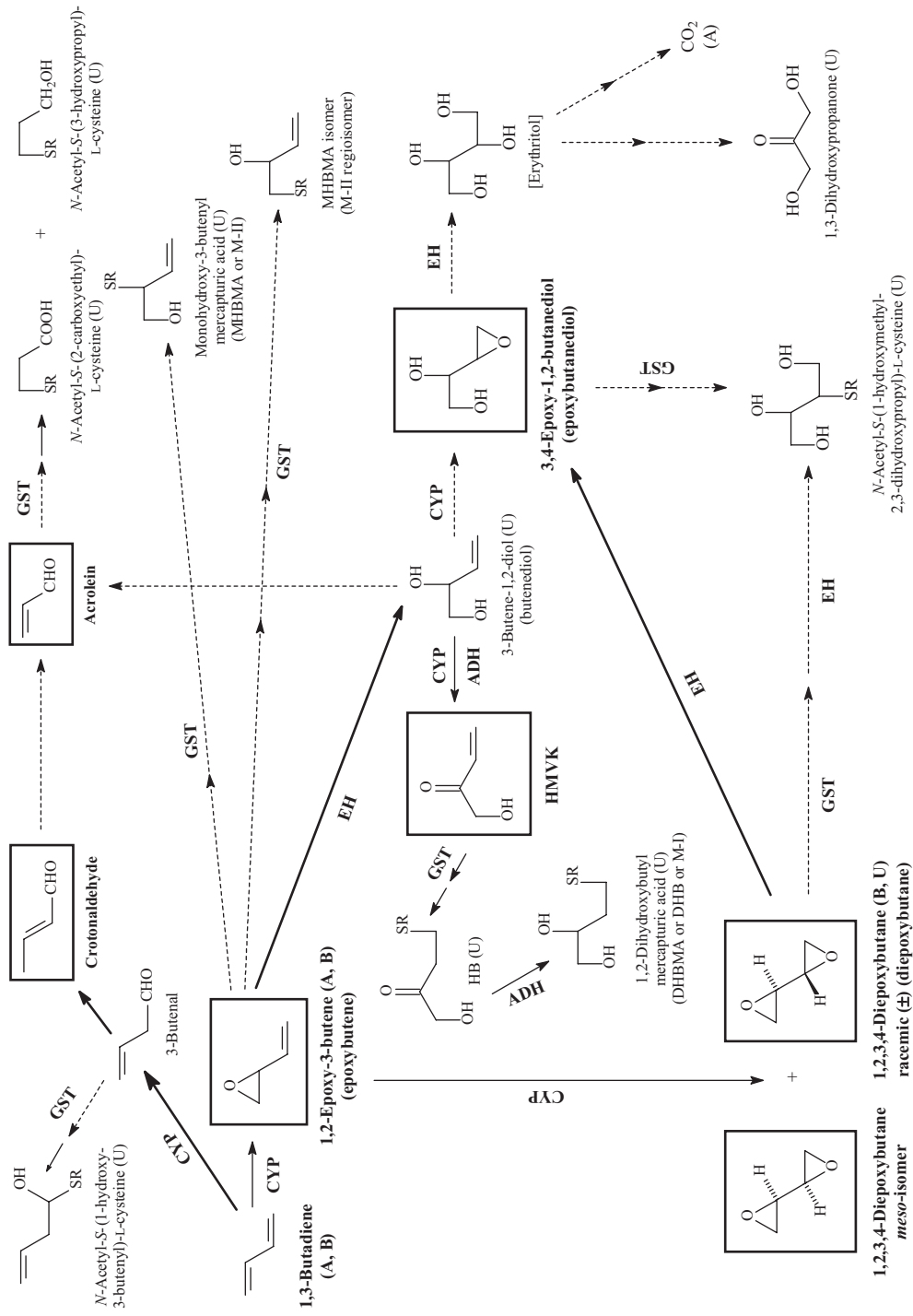
The detailed pathways in the metabolism of butadiene have been described previously ([Himmelstein *et al.*, 1997](#); [IARC 1999, 2008](#)) and are outlined in Fig. 4.1. Briefly, the first step in butadiene metabolism involves cytochrome P450 (CYP)-mediated oxidation to epoxybutene ([Himmelstein *et 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 & Elfarrá, 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 & Elfarrá, 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/m³] ([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/m³] 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/m³] or mice exposed to 3 ppm [6.63 mg/m³] compared with exposure of either species to 625 or 1250 ppm [1381 or 2762.5 mg/m³] ([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/m³]), while PyrVal could not be detected in workers exposed to mean concentrations of 0.37 ppm [0.82 mg/m³]. 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/m³] 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/m³] butadiene, while G1 and G2 adducts increase nearly linearly with exposures to butadiene of up to 625 ppm [1381 mg/m³]. [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, N⁶ of adenine, N1 of guanine and N² 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](#)): deoxyinosine 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 N⁶-position of adenine, an exocyclic adenine adduct is formed preferentially to DNA–DNA crosslinked products ([Antsyovich 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).

References

- Abdel-Rahman SZ, Ammenheuser MM, Ward JB Jr (2001). Human sensitivity to 1,3-butadiene: role of microsomal epoxide hydrolase polymorphisms. *Carcinogenesis*, 22: 415–423. doi:10.1093/carcin/22.3.415 PMID:11238181
- Abdel-Rahman SZ, El-Zein RA, Ammenheuser MM *et al.* (2003). Variability in human sensitivity to 1,3-butadiene: Influence of the allelic variants of the microsomal epoxide hydrolase gene. *Environ Mol Mutagen*, 41: 140–146. doi:10.1002/em.10142 PMID:12605384
- Albertini RJ, Sram RJ, Vacek PM *et al.* (2001). Biomarkers for assessing occupational exposures to 1,3-butadiene. *Chem Biol Interact*, 135-136: 429–453. doi:10.1016/S0009-2797(01)00181-8 PMID:11397405
- Albertini RJ, Sram RJ, Vacek PM *et al.* (2003). Biomarkers in Czech workers exposed to 1,3-butadiene: a transitional epidemiologic study. *Res Rep Health Eff Inst*, 116: 1–141, discussion 143–162. PMID:12931846
- Albertini RJ, Sram RJ, Vacek PM *et al.* (2007). Molecular epidemiological studies in 1,3-butadiene exposed

- Czech workers: female-male comparisons. *Chem Biol Interact*, 166: 63–77. doi:10.1016/j.cbi.2006.07.004 PMID:16949064
- Antsyrovich S, Quirk-Dorr D, Pitts C, Tretyakova N (2007). Site specific N6-(2-hydroxy-3,4-epoxybut-1-yl)adenine oligodeoxynucleotide adducts of 1,2,3,4-diepoxybutane: synthesis and stability at physiological pH. *Chem Res Toxicol*, 20: 641–649. doi:10.1021/tx060178k PMID:17355152
- Bolt HM, Roos PH, Thier R (2003). The cytochrome P-450 isoenzyme CYP2E1 in the biological processing of industrial chemicals: consequences for occupational and environmental medicine. *Int Arch Occup Environ Health*, 76: 174–185. PMID:12690492
- Boogaard PJ, Sumner SCJ, Bond JA (1996a). Glutathione conjugation of 1,2:3,4- diepoxybutane in human liver and rat and mouse liver and lung in vitro. *Toxicol Appl Pharmacol*, 136: 307–316. doi:10.1006/taap.1996.0037 PMID:8619238
- Boogaard PJ, Sumner SCJ, Turner MJ, Bond JA (1996b). Hepatic and pulmonary glutathione conjugation of 1,2:3,4-diepoxybutane in human, rat, and mouse in vitro. *Toxicology*, 113: 297–299. doi:10.1016/0300-483X(96)03460-9 PMID:8901912
- Boysen G, Georgieva NI, Upton PB *et al.* (2004). Analysis of diepoxide-specific cyclic N-terminal globin adducts in mice and rats after inhalation exposure to 1,3-butadiene. *Cancer Res*, 64: 8517–8520. doi:10.1158/0008-5472.CAN-04-3184 PMID:15574756
- Boysen G, Georgieva NI, Upton PB *et al.* (2007). N-terminal globin adducts as biomarkers for formation of butadiene derived epoxides. *Chem Biol Interact*, 166: 84–92. doi:10.1016/j.cbi.2006.10.005 PMID:17084829
- Bucher JR, Melnick RL, Hildebrandt PK (1993). Lack of carcinogenicity in mice exposed once to high concentrations of 1,3-butadiene. *J Natl Cancer Inst*, 85: 1866–1867. doi:10.1093/jnci/85.22.1866 PMID:8230269
- CAREX (1999). Carex: industry specific estimates – Summary. Available at http://www.ttl.fi/en/chemical_safety/carex/Documents/5_exposures_by_agent_and_industry.pdf.
- Cheng H, Sathiakumar N, Graff J *et al.* (2007). 1,3-Butadiene and leukemia among synthetic rubber industry workers: exposure-response relationships. *Chem Biol Interact*, 166: 15–24. doi:10.1016/j.cbi.2006.10.004 PMID:17123495
- Csanády GA, Guengerich FP, Bond JA (1992). Comparison of the biotransformation of 1,3-butadiene and its metabolite, butadiene monoepoxide, by hepatic and pulmonary tissues from humans, rats and mice. *Carcinogenesis*, 13: 1143–1153. doi:10.1093/carcin/13.7.1143 PMID:1638680
- Delzell E, Sathiakumar N, Graff J *et al.* (2006). *An Updated Study of Mortality among North American Synthetic Rubber Industry Workers*. Boston, MA: Health Effects Institute, pp. 1–72.
- Delzell E, Sathiakumar N, Hovinga M *et al.* (1996). A follow-up study of synthetic rubber workers. *Toxicology*, 113: 182–189. doi:10.1016/0300-483X(96)03443-9 PMID:8901897
- Divine BJ & Hartman CM (2001). A cohort mortality study among workers at a 1,3 butadiene facility. *Chem Biol Interact*, 135-136: 535–553. doi:10.1016/S0009-2797(01)00212-5 PMID:11397411
- Dollard GJ, Dumitrean P, Telling S *et al.* (2007). Observed trends in ambient concentrations of C₂-C₈ hydrocarbons in the United Kingdom over the period from 1993 to 2004. *Atmos Environ*, 41: 2559–2569. doi:10.1016/j.atmosenv.2006.11.020
- Fernandes PH & Lloyd RS (2007). Mutagenic bypass of the butadiene-derived 2'-deoxyuridine adducts by polymerases η and ζ. *Mutat Res*, 625: 40–49. doi:10.1016/j.mrfmmm.2007.05.003 PMID:17586533
- Filser JG, Faller TH, Bhowmik S *et al.* (2001). First-pass metabolism of 1,3-butadiene in once-through perfused livers of rats and mice. *Chem Biol Interact*, 135-136: 249–265. doi:10.1016/S0009-2797(01)00194-6 PMID:11397395
- Filser JG, Hutzler C, Meischner V *et al.* (2007). Metabolism of 1,3-butadiene to toxicologically relevant metabolites in single-exposed mice and rats. *Chem Biol Interact*, 166: 93–103. doi:10.1016/j.cbi.2006.03.002 PMID:16616907
- Fustinoni S, Soleo L, Warholm M *et al.* (2002). Influence of metabolic genotypes on biomarkers of exposure to 1,3-butadiene in humans. *Cancer Epidemiol Biomarkers Prev*, 11: 1082–1090. PMID:12376511
- Goggin M, Swenberg JA, Walker VE, Tretyakova N (2009). Molecular dosimetry of 1,2,3,4-diepoxybutane-induced DNA-DNA cross-links in B6C3F1 mice and F344 rats exposed to 1,3-butadiene by inhalation. *Cancer Res*, 69: 2479–2486. doi:10.1158/0008-5472.CAN-08-4152 PMID:19276346
- Graff JJ, Sathiakumar N, Macaluso M *et al.* (2005). Chemical exposures in the synthetic rubber industry and lymphohematopoietic cancer mortality. *J Occup Environ Med*, 47: 916–932. doi:10.1097/01.jom.0000172866.16615.db PMID:16155477
- Grosse Y, Baan R, Straif K *et al.* WHO International Agency for Research on Cancer Monograph Working Group (2007). Carcinogenicity of 1,3-butadiene, ethylene oxide, vinyl chloride, vinyl fluoride, and vinyl bromide. *Lancet Oncol*, 8: 679–680. doi:10.1016/S1470-2045(07)70235-8 PMID:17726789
- Health Canada (2000). *Priority Substances List Assessment Report: 1,3-Butadiene*. 112 pp.
- Henderson RF, Barr EB, Belinsky SA *et al.* (2000). 1,3-butadiene: cancer, mutations, and adducts. Part I: Carcinogenicity of 1,2,3,4-diepoxybutane. *Res Rep Health Eff Inst*, 92: 11–43, discussion 45–48. PMID:10925838
- Henderson RF, Hahn FF, Barr EB *et al.* (1999). Carcinogenicity of inhaled butadiene diepoxide

- in female B6C3F1 mice and Sprague-Dawley rats. *Toxicol Sci*, 52: 33–44. doi:10.1093/toxsci/52.1.33 PMID:10568696
- Himmelstein MW, Acquavella JF, Recio L *et al.* (1997). Toxicology and epidemiology of 1,3-butadiene. *Crit Rev Toxicol*, 27: 1–108. doi:10.3109/10408449709037482 PMID:9115622
- Hong HH, Devereux TR, Melnick RL *et al.* (2000). Mutations of ras protooncogenes and p53 tumor suppressor gene in cardiac hemangiosarcomas from B6C3F1 mice exposed to 1,3-butadiene for 2 years. *Toxicol Pathol*, 28: 529–534. doi:10.1177/019262330002800404 PMID:10930038
- Huff JE, Melnick RL, Solleveld HA *et al.* (1985). Multiple organ carcinogenicity of 1,3-butadiene in B6C3F1 mice after 60 weeks of inhalation exposure. *Science*, 227: 548–549. doi:10.1126/science.3966163 PMID:3966163
- IARC (1992). Occupational exposures to mists and vapours from strong inorganic acids and other industrial chemicals. *IARC Monogr Eval Carcinog Risks Hum*, 54: 1–310. PMID:1345371
- IARC (1999). Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. *IARC Monogr Eval Carcinog Risks Hum*, 71: 1–315. PMID:10507919
- IARC (2008). 1,3-Butadiene, ethylene oxide and vinyl halides (vinyl fluoride, vinyl chloride and vinyl bromide). *IARC Monogr Eval Carcinog Risks Hum*, 97: 1–510. PMID: 20232717.
- Jackson TE, Lilly PD, Recio L *et al.* (2000). Inhibition of cytochrome P450 2E1 decreases, but does not eliminate, genotoxicity mediated by 1,3-butadiene. *Toxicol Sci*, 55: 266–273. doi:10.1093/toxsci/55.2.266 PMID:10828257
- Kauppinen T, Toikkanen J, Pedersen D *et al.* (2000). Occupational exposure to carcinogens in the European Union. *Occup Environ Med*, 57: 10–18. doi:10.1136/oem.57.1.10 PMID:10711264
- Kim Y, Hong HH, Lachat Y *et al.* (2005). Genetic alterations in brain tumors following 1,3-butadiene exposure in B6C3F1 mice. *Toxicol Pathol*, 33: 307–312. doi:10.1080/01926230590922848 PMID:15814359
- Kligerman AD, Doerr CL, Tennant AH (1999). Cell cycle specificity of cytogenetic damage induced by 3,4-epoxy-1-butene. *Mutat Res*, 444: 151–158. PMID:10477349
- Koc H, Tretyakova NY, Walker VE *et al.* (1999). Molecular dosimetry of N-7 guanine adduct formation in mice and rats exposed to 1,3-butadiene. *Chem Res Toxicol*, 12: 566–574. doi:10.1021/tx980265f PMID:10409395
- Krause RJ & Elfarra AA (1997). Oxidation of butadiene monoxide to meso- and (+/-)-diepoxybutane by cDNA-expressed human cytochrome P450s and by mouse, rat, and human liver microsomes: evidence for preferential hydration of meso-diepoxybutane in rat and human liver microsomes. *Arch Biochem Biophys*, 337: 176–184. doi:10.1006/abbi.1996.9781 PMID:9016811
- Kreiling R, Laib RJ, Filser JG, Bolt HM (1986b). Species differences in butadiene metabolism between mice and rats evaluated by inhalation pharmacokinetics. *Arch Toxicol*, 58: 235–238. doi:10.1007/BF00297112 PMID:3718226
- Lee DH, Kim TH, Lee SY *et al.* (2002). Mutations induced by 1,3-butadiene metabolites, butadiene diolepoxide, and 1,2,3,4-diepoxybutane at the Hprt locus in CHO-K1 cells. *Mol Cells*, 14: 411–419. PMID:12521305
- Lide DR, editor (2008). *CRC Handbook of Chemistry and Physics*, 89th ed., Boca Raton, FL: CRC Press, pp. 3–72.
- Lovreglio P, Bukvic N, Fustinoni S *et al.* (2006). Lack of genotoxic effect in workers exposed to very low doses of 1,3-butadiene. *Arch Toxicol*, 80: 378–381. doi:10.1007/s00204-005-0046-0 PMID:16307232
- Macaluso M, Larson R, Lynch J *et al.* (2004). Historical estimation of exposure to 1,3-butadiene, styrene, and dimethyldithiocarbamate among synthetic rubber workers. *J Occup Environ Hyg*, 1: 371–390. doi:10.1080/15459620490452004 PMID:15238328
- Maniglier-Poulet C, Cheng X, Ruth JA, Ross D (1995). Metabolism of 1,3-butadiene to butadiene monoxide in mouse and human bone marrow cells. *Chem Biol Interact*, 97: 119–129. doi:10.1016/0009-2797(95)03612-P PMID:7606811
- Matanoski G, Francis M, Correa-Villaseñor A *et al.* (1993). Cancer epidemiology among styrene-butadiene rubber workers. *IARC Sci Publ*, 127: 363–374. PMID:8070882
- Matanoski GM, Santos-Burgoa C, Schwartz L (1990). Mortality of a cohort of workers in the styrene-butadiene polymer manufacturing industry (1943–1982). *Environ Health Perspect*, 86: 107–117. doi:10.1289/ehp.9086107 PMID:2401250
- Matanoski GM & Schwartz L (1987). Mortality of workers in styrene-butadiene polymer production. *J Occup Med*, 29: 675–680. PMID:3655951
- McMichael AJ, Spirtas R, Gamble JF, Tousey PM (1976). Mortality among rubber workers: Relationship to specific jobs. *J Occup Med*, 18: 178–185. doi:10.1097/00043764-197603000-00012 PMID:1255279
- McMichael AJ, Spirtas R, Kupper LL (1974). An epidemiologic study of mortality within a cohort of rubber workers, 1964–72. *J Occup Med*, 16: 458–464. PMID:4842655
- Meinhardt TJ, Lemen RA, Crandall MS, Young RJ (1982). Environmental epidemiologic investigation of the styrene-butadiene rubber industry. Mortality patterns with discussion of the hematopoietic and lymphatic malignancies. *Scand J Work Environ Health*, 8: 250–259. doi:10.5271/sjweh.2469 PMID:7170621
- Melnick RL, Huff J, Chou BJ, Miller RA (1990b). Carcinogenicity of 1,3-butadiene in C57BL/6 x C3H F1 mice at low exposure concentrations. *Cancer Res*, 50: 6592–6599. PMID:2208121
- Melnick RL & Huff JE (1993). 1,3-Butadiene induces cancer in experimental animals at all concentrations

- from 6.25 to 8000 parts per million. *IARC Sci Publ*, 127: 309–322. PMID:8070878
- Melnick RL, Huff JE, Roycroft JH *et al.* (1990a). Inhalation toxicology and carcinogenicity of 1,3-butadiene in B6C3F1 mice following 65 weeks of exposure. *Environ Health Perspect*, 86: 27–36. doi:10.2307/3430929 PMID:2401263
- Melnick RL, Shackelford CC, Huff J (1993). Carcinogenicity of 1,3-butadiene. *Environ Health Perspect*, 100: 227–236. doi:10.1289/ehp.93100227 PMID:8354171
- Melnick RL & Sills RC (2001). Comparative carcinogenicity of 1,3-butadiene, isoprene, and chloroprene in rats and mice. *Chem Biol Interact*, 135-136: 27–42. doi:10.1016/S0009-2797(01)00213-7 PMID:11397379
- Meng Q, Walker DM, McDonald JD *et al.* (2007). Age-, gender-, and species-dependent mutagenicity in T cells of mice and rats exposed by inhalation to 1,3-butadiene. *Chem Biol Interact*, 166: 121–131. doi:10.1016/j.cbi.2006.07.005 PMID:16945358
- Miller RA, Melnick RL, Boorman GA (1989). Neoplastic lesions induced by 1,3-butadiene in B6C3F1 mice. *Exp Pathol*, 37: 136–146. doi:10.1016/S0232-1513(89)80033-7 PMID:2637144
- NIOSH (1990). *National Occupational Exposure Survey (1981–83)*. Cincinnati, OH: US Department of Health and Human Services, Public Health Service, National Institute for Occupational Safety and Health. Available at <http://www.cdc.gov/noes/noes4/13410sco.html>
- Norppa H (2004). Cytogenetic biomarkers and genetic polymorphisms. *Toxicol Lett*, 149: 309–334. doi:10.1016/j.toxlet.2003.12.042 PMID:15093278
- National Toxicology Program (1984). NTP toxicology and carcinogenesis studies of 1,3-Butadiene (CAS NO. 106-99-0) in B6C3F1 mice (inhalation studies). *Natl Toxicol Program Tech Rep Ser*, 288: 1–111. PMID:12748715
- National Toxicology Program (1993). NTP toxicology and carcinogenesis studies of 1,3-Butadiene (CAS NO. 106-99-0) in B6C3F1 mice (inhalation studies). *Natl Toxicol Program Tech Rep Ser*, 434: 1–389. PMID:12616297
- O’Neil MJ, editor (2006). *Merck Index*, 14th ed., Whitehouse Station, NJ: Merck & Co., Inc., p. 248.
- Owen PE & Glaister JR (1990). Inhalation toxicity and carcinogenicity of 1,3-butadiene in Sprague-Dawley rats. *Environ Health Perspect*, 86: 19–25. doi:10.1289/ehp.908619 PMID:2401255
- Owen PE, Glaister JR, Gaunt IF, Pullinger DH (1987). Inhalation toxicity studies with 1,3-butadiene. 3. Two year toxicity/carcinogenicity study in rats. *Am Ind Hyg Assoc J*, 48: 407–413. doi:10.1080/15298668791384959 PMID:3591659
- Powley MW, Li Y, Upton PB *et al.* (2005). Quantification of DNA and hemoglobin adducts of 3,4-epoxy-1,2-butanediol in rodents exposed to 3-butene-1,2-diol. *Carcinogenesis*, 26: 1573–1580. doi:10.1093/carcin/bgi119 PMID:15888494
- Recio L, Steen AM, Pluta LJ *et al.* (2001). Mutational spectrum of 1,3-butadiene and metabolites 1,2-epoxybutene and 1,2,3,4-diepoxybutane to assess mutagenic mechanisms. *Chem Biol Interact*, 135-136: 325–341. doi:10.1016/S0009-2797(01)00220-4 PMID:11397399
- Rodriguez DA, Kowalczyk A, Ward JB Jr *et al.* (2001). Point mutations induced by 1,2-epoxy-3-butene N1 deoxyinosine adducts. *Environ Mol Mutagen*, 38: 292–296. doi:10.1002/em.10026 PMID:11774359
- Sathiakumar N & Delzell E (2007). A follow-up study of women in the synthetic rubber industry: study methods. *Chem Biol Interact*, 166: 25–28. doi:10.1016/j.cbi.2006.11.005 PMID:17229413
- Sathiakumar N & Delzell E (2009). A follow-up study of mortality among women in the North American synthetic rubber industry. *J Occup Environ Med*, 51: 1314–1325. doi:10.1097/JOM.0b013e3181bd8972 PMID:19858743
- Sathiakumar N, Graff J, Macaluso M *et al.* (2005). An updated study of mortality among North American synthetic rubber industry workers. *Occup Environ Med*, 62: 822–829. doi:10.1136/oem.2004.018176 PMID:16299089
- Schlade-Bartusiak K, Rozik K, Laczmanska I *et al.* (2004). Influence of GSTT1, mEH, CYP2E1 and RAD51 polymorphisms on diepoxybutane-induced SCE frequency in cultured human lymphocytes. *Mutat Res*, 558: 121–130. PMID:15036125
- Seaton MJ, Follansbee MH, Bond JA (1995). Oxidation of 1,2-epoxy-3-butene to 1,2,3,4-diepoxybutane by cDNA-expressed human cytochromes P450 2E1 and 3A4 and human, mouse and rat liver microsomes. *Carcinogenesis*, 16: 2287–2293. doi:10.1093/carcin/16.10.2287 PMID:7586124
- Selzer RR & Elfarra AA (1996a). Characterization of N1- and N6-adenosine adducts and N1-inosine adducts formed by the reaction of butadiene monoxide with adenosine: evidence for the N1-adenosine adducts as major initial products. *Chem Res Toxicol*, 9: 875–881. doi:10.1021/tx960039a PMID:8828924
- Selzer RR & Elfarra AA (1996b). Synthesis and biochemical characterization of N1-, N2-, and N7-guanosine adducts of butadiene monoxide. *Chem Res Toxicol*, 9: 126–132. doi:10.1021/tx950101o PMID:8924581
- Selzer RR & Elfarra AA (1997). Chemical modification of deoxycytidine at different sites yields adducts of different stabilities: characterization of N3- and O2-deoxycytidine and N3-deoxyuridine adducts of butadiene monoxide. *Arch Biochem Biophys*, 343: 63–72. doi:10.1006/abbi.1997.0164 PMID:9210647
- Shimkin MB, Weisburger JH, Weisburger E *et al.* (1966). Bioassay of 29 alkylating chemicals by the pulmonary-tumor response in strain A mice. *Journal of the National Cancer Institute*, 36: 915–935.

- Sills RC, Hong HL, Boorman GA *et al.* (2001). Point mutations of K-ras and H-ras genes in forestomach neoplasms from control B6C3F1 mice and following exposure to 1,3-butadiene, isoprene or chloroprene for up to 2-years. *Chem Biol Interact*, 135-136: 373–386. doi:10.1016/S0009-2797(01)00179-X PMID:11397402
- Sprague CL & Elfarra AA (2003). Detection of carboxylic acids and inhibition of hippuric acid formation in rats treated with 3-butene-1,2-diol, a major metabolite of 1,3-butadiene. *Drug Metab Dispos*, 31: 986–992. doi:10.1124/dmd.31.8.986 PMID:12867486
- Sprague CL & Elfarra AA (2004). Mercapturic acid urinary metabolites of 3-butene-1,2-diol as in vivo evidence for the formation of hydroxymethylvinyl ketone in mice and rats. *Chem Res Toxicol*, 17: 819–826. doi:10.1021/tx049949f PMID:15206903
- Thier R, Brüning T, Roos PH *et al.* (2003). Markers of genetic susceptibility in human environmental hygiene and toxicology: the role of selected CYP, NAT and GST genes. *Int J Hyg Environ Health*, 206: 149–171. doi:10.1078/1438-4639-00209 PMID:12872524
- Ton TV, Hong HH, Devereux TR *et al.* (2007). Evaluation of genetic alterations in cancer-related genes in lung and brain tumors from B6C3F1 mice exposed to 1,3-butadiene or chloroprene. *Chem Biol Interact*, 166: 112–120. doi:10.1016/j.cbi.2006.04.015 PMID:16860786
- Tretyakova NYu, Sangaiah R, Yen TY, Swenberg JA (1997). Synthesis, characterization, and in vitro quantitation of N-7-guanine adducts of diepoxybutane. *Chem Res Toxicol*, 10: 779–785. doi:10.1021/tx970004q PMID:9250412
- Tsai SP, Wendt JK, Ransdell JD (2001). A mortality, morbidity, and hematology study of petrochemical employees potentially exposed to 1,3-butadiene monomer. *Chem Biol Interact*, 135-136: 555–567. doi:10.1016/S0009-2797(01)00186-7 PMID:11397412
- Van Duuren BL, Langseth L, Orris L *et al.* (1966). Carcinogenicity of epoxides, lactones, and peroxy compounds. IV. Tumor response in epithelial and connective tissue in mice and rats. *J Natl Cancer Inst*, 37: 825–838. PMID:5955045
- Van Duuren BL, Nelson N, Orris L *et al.* (1963). Carcinogenicity of epoxides, lactones, and peroxy compounds. *J Natl Cancer Inst*, 31: 41–55. PMID:14043038
- Van Duuren BL, Orris L, Nelson N (1965). Carcinogenicity of epoxides, lactones, and peroxy compounds. II. *J Natl Cancer Inst*, 35: 707–717. PMID:5841060
- Ward EM, Fajen JM, Ruder AM *et al.* (1995). Mortality study of workers in 1,3-butadiene production units identified from a chemical workers cohort. *Environ Health Perspect*, 103: 598–603. doi:10.1289/ehp.95103598 PMID:7556014
- Ward EM, Fajen JM, Ruder AM *et al.* (1996). Mortality study of workers employed in 1,3-butadiene production units identified from a large chemical workers cohort. *Toxicology*, 113: 157–168. doi:10.1016/0300-483X(96)03441-5 PMID:8901895
- White WC (2007). Butadiene production process overview. *Chem Biol Interact*, 166: 10–14. doi:10.1016/j.cbi.2007.01.009 PMID:17324391
- Whitworth KW, Symanski E, Coker AL (2008). Childhood lymphohematopoietic cancer incidence and hazardous air pollutants in southeast Texas, 1995–2004. *Environ Health Perspect*, 116: 1576–1580. doi:10.1289/ehp.11593 PMID:19057714
- Wickliffe JK, Ammenheuser MM, Adler PJ *et al.* (2009). Evaluation of frequencies of HPRT mutant lymphocytes in butadiene polymer workers in a Southeast Texas facility. *Environ Mol Mutagen*, 50: 82–87. doi:10.1002/em.20437 PMID:19107895
- Wickliffe JK, Ammenheuser MM, Salazar JJ *et al.* (2003). A model of sensitivity: 1,3-butadiene increases mutant frequencies and genomic damage in mice lacking a functional microsomal epoxide hydrolase gene. *Environ Mol Mutagen*, 42: 106–110. doi:10.1002/em.10181 PMID:12929123
- Wickliffe JK, Herring SM, Hallberg LM *et al.* (2007). Detoxification of olefinic epoxides and nucleotide excision repair of epoxide-mediated DNA damage: Insights from animal models examining human sensitivity to 1,3-butadiene. *Chem Biol Interact*, 166: 226–231. doi:10.1016/j.cbi.2006.04.017 PMID:16730686
- Wiencke JK, Pemble S, Ketterer B, Kelsey KT (1995). Gene deletion of glutathione S-transferase theta: correlation with induced genetic damage and potential role in endogenous mutagenesis. *Cancer Epidemiol Biomarkers Prev*, 4: 253–259. PMID:7606200
- Zhang L, Hayes RB, Guo W *et al.* (2004). Lack of increased genetic damage in 1,3-butadiene-exposed Chinese workers studied in relation to EPHX1 and GST genotypes. *Mutat Res*, 558: 63–74. PMID:15036120
- Zhang XY & Elfarra AA (2004). Characterization of the reaction products of 2'-deoxyguanosine and 1,2,3,4-diepoxybutane after acid hydrolysis: formation of novel guanine and pyrimidine adducts. *Chem Res Toxicol*, 17: 521–528. doi:10.1021/tx034243r PMID:15089094
- Zhao C, Koskinen M, Hemminki K (1998). 32P-postlabelling of N6-adenine adducts of epoxybutanediol in vivo after 1,3-butadiene exposure. *Toxicol Lett*, 102-103: 591–594. doi:10.1016/S0378-4274(98)00253-7 PMID:10022318
- Zhao C, Vodicka P, Srám RJ 1, Hemminki K (2000). Human DNA adducts of 1,3-butadiene, an important environmental carcinogen. *Carcinogenesis*, 21: 107–111. doi:10.1093/carcin/21.1.107 PMID:10607741
- Zhuang SM, Wiseman RW, Söderkvist P (2000). Mutation analysis of the pRb pathway in 2',3'-dideoxycytidine- and 1,3-butadiene-induced mouse lymphomas. *Cancer Lett*, 152: 129–134. doi:10.1016/S0304-3835(99)00447-4 PMID:10773403

Zhuang SM, Wiseman RW, Söderkvist P (2002). Frequent mutations of the Trp53, Hras1 and beta-catenin (Catnb) genes in 1,3-butadiene-induced mammary adenocarcinomas in B6C3F1 mice. *Oncogene*, 21: 5643–5648. doi:10.1038/sj.onc.1205649 PMID:12165863