1,3-BUTADIENE

This substance was considered by previous Working Groups in June 1985 (IARC, 1986; see also correction, IARC, 1987a), March 1987 (IARC, 1987b), October 1991 (IARC, 1992) and February 1998 (IARC, 1999). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

One of the metabolites of 1,3-butadiene, 1,2:3,4-diepoxybutane, was also evaluated previously by an IARC Working Group (IARC, 1976), and its re-evaluation by the present Working Group is included in this monograph.

1. Exposure Data

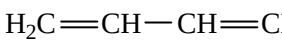
1.1 Chemical and physical data

Butadiene

1.1.1 *Nomenclature* (IARC, 1999; IPCS-CEC, 2000; O'Neil, 2006)

Chem. Abstr. Serv. Reg. No.: 106-99-0
Chem. Abstr. Name: 1,3-Butadiene
IUPAC Systematic Name: 1,3-Butadiene
RTECS No.: EI9275000
UN TDG No.: 1010 (stabilized)
EC No.: 601-013-00-X
Synonyms: Biethylene; bivinyl; butadiene; buta-1,3-diene; α,γ-butadiene; *trans*-butadiene; divinyl; erythrene; pyrrolylene; vinylethylene

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_4H_6 Relative molecular mass: 54.09

1.1.3 *Chemical and physical properties of the pure substance*

From IARC (1999), IPCS-CEC (2000), Lide (2005) and O'Neil (2006) unless otherwise specified

(a) *Description*: Colourless gas
(b) *Boiling-point*: –4.4 °C
(c) *Melting-point*: –108.9 °C
(d) *Density*: d_4^{20} 0.6149
(e) *Spectroscopy data*: Ultraviolet (Grasselli & Ritchey, 1975), infrared (Sadtler Research Laboratories, 1995; prism [893a], grating [36758]), nuclear magnetic resonance and mass spectral data (NIH/EPA Chemical Information System, 1983) have been reported.
(f) *Solubility*: Slightly soluble in water (1 g/L at 20 °C); soluble in ethanol, diethyl ether, benzene and organic solvents; very soluble in acetone (see also Verschueren, 1996)
(g) *Vapour pressure*: 120 kPa at 0 °C; 273 kPa at 25 °C (Grub & Löser, 2005)
(h) *Relative vapour density (air = 1)*: 1.87 (Verschueren, 1996)
(i) *Stability*: As a result of flow and agitation, electrostatic charges can be generated. The vapours are uninhibited and may form polymers in vents or flame arresters of storage tanks, and result in the blockage of vents. On exposure to air, the substance can form peroxides and initiate explosive polymerization. It may also polymerize due to warming by fire or an explosion. It decomposes explosively on rapid heating under pressure and may react vigorously with oxidants and many other substances, causing fire and explosion hazards (IPCS-CEC, 2000).
(j) *Flash-point*: –76 °C (IPCS-CEC, 2000)
(k) *Auto-ignition temperature*: 414 °C (IPCS-CEC, 2000)
(l) *Explosive limits*: Lower, 1.1%; upper, 12.3% (IPCS-CEC, 2000)
(m) *Octanol/water partition coefficient*: log P_{ow}, 1.99 (IPCS-CEC, 2000)
(n) *Odour threshold*: 1–1.6 ppm [2.2–3.5 mg/m^3] (recognition) (ACGIH, 2001)
(o) *Henry's law constant (calculated at 25 °C and 101.325 kPa)*: 7460 Pa × m^3/mol (Health Canada, 1999)
(p) *Organic carbon partition coefficient*: log K_{oc}, 1.86–2.36 (Health Canada, 1999)
(q) *Conversion factor*: mg/m^3 = 2.21 × ppm[1]

Diepoxybutane

Diepoxybutane is the racemic mixture of four different isomers, with the following Chem. Abstr. Serv. Reg. Nos: 1464-53-5, diepoxybutane; 298-18-0, (±)-diepoxybutane;

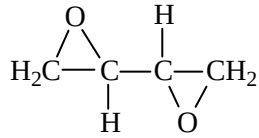
[1] Calculated from: mg/m^3 = (molecular weight/24.47) × ppm, assuming normal temperature (25 °C) and pressure (101.3 kPa)

564-00-1, *meso*-diepoxybutane; 30419-67-1, D-diepoxybutane; 30031-64-2, L-diepoxybutane.

1.1.1 *Nomenclature*

Chem. Abstr. Name: 2,2′-Bioxirane
IUPAC Systematic Name: 1,2:3,4-Diepoxybutane
Synonyms: Butadiene dioxide (diepoxybutane); 1,3-butadiene diepoxide ((±)-diepoxybutane); D-1,2:3,4-diepoxybutane (D-diepoxybutane): L-1,2:3,4-diepoxybutane; (5,5)-1,2:3,4-diepoxybutane (L-diepoxybutane)

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_6O_2$ Relative molecular mass: 86.10

1.1.3 *Chemical and physical properties*

From O'Neil (2006)
(a) *Description*: Colourless liquid
(b) *Boiling-point*: 138 °C
(c) *Melting-point*: –19 °C
(d) *Solubility*: Miscible with water (hydrolyses)
(e) *Vapour pressure:* 918 Pa at 25 °C

1.1.4 *Technical products and impurities*

In the production of polymers such as styrene–butadiene copolymer resins, the polymerization catalysts used are sensitive to some impurities such as oxygen and moisture. Butadiene that is used for polymerization is 99.9% pure. Up to 22 different volatile components of light molecular mass were detected as impurities with the ASTM method D2593-93 (reapproved in 2004; ASTM, 2004).

1.1.5 *Analysis*

Selected methods for the analysis of butadiene in various matrices are listed in Table 1. Those for the analysis of butadiene in air have been evaluated; there appears to be no single preferred method, but more recent ones give a higher performance. Thermal desorption provides high levels of accuracy and precision (Bianchi *et al*., 1997).

Several gas detector tubes are used in conjunction with common colorimetric reactions to detect butadiene. The reactions include the reduction of chromate or dichromate to chromous ion and the reduction of ammonium molybdate and palladium sulfate to molybdenum blue (Saltzman & Harman, 1989).

Passive dosimeters that use different techniques (thermal desorption and gas chromatography, colorimetric reactions) are also available for the detection of butadiene.

Table 1. Selected methods for the analysis of butadiene in various matrices

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Adsorb (charcoal); extract (carbon disulfide)	GC/FID	200 µg/m^3	Occupational Safety and Health Administration (1990a)
	Adsorb (charcoal); extract (dichloromethane)	GC/FID	0.2 µg/sample	Eller (1994)
	Adsorb on Perkin-Elmer ATD 400 packed with polymeric or synthetic adsorbent material; thermal desorption	GC/FID	200 µg/m^3	Health and Safety Executive (1992)
	3M passive monitoring	GC/FID	0.029 mg/m^3 for a 20.5-L sample	Anttinen-Klemetti *et al.* (2004)
Foods and plastic food-packaging material	Dissolve (*N*,*N*-dimethylacetamide) or melt; inject headspace sample	GC/MS–SIM	~1 µg/kg	Startin & Gilbert (1984)
Plastics, liquid foods	Dissolve in *ortho*-dichlorobenzene; inject headspace sample	GC/FID	2–20 µg/kg	Food and Drug Administration (1987)
Solid foods	Cut or mash; inject headspace sample	GC/FID	2–20 µg/kg	Food and Drug Administration (1987)

GC/FID, gas chromatography/flame ionization detection; GC/MS–SIM, gas chromatography/mass spectrometry with single-ion monitoring

1.2 Production and use

1.2.1 *Production*

Butadiene was first produced in the late nineteenth century by pyrolysis of various organic materials. Commercial production began in the 1930s (Sun & Wristers, 2002).

(a) Manufacturing processes

(i) *Ethylene co-production*

Butadiene is manufactured primarily as a co-product of the steam cracking of hydrocarbon streams to produce ethylene. This process accounts for over 95% of global butadiene production (White, 2007).

Steam cracking is a complex, highly endothermic pyrolysis reaction, during which a hydrocarbon feedstock is heated to approximately 800 °C and 34 kPa for less than 1 sec and the carbon–carbon and carbon–hydrogen bonds are broken. As a result, a mixture of olefins, aromatic compounds, tar and gases is formed. These products are cooled and separated into specific boiling-range cuts of C_1, C_2, C_3 and C_4 compounds. The C_4 fraction contains butadiene, isobutylene, 1-butene, 2-butene and some other minor hydrocarbons. The overall yields of butadiene during the process depend on both the parameters of the process and the composition of feedstocks. Generally, heavier steam-cracking feedstocks produce greater amounts of butadiene. Separation and purification of butadiene from other components is carried out mainly by an extractive distillation process. The most commonly used solvents are acetonitrile and dimethylformamide; dimethylacetamide, furfural and *N*-methyl-2-pyrrolidinone have also been used to this end (Sun & Wristers, 2002; Walther, 2003).

(ii) *Dehydrogenation*

The intentional dehydrogenation of *n*-butane or *n*-butenes also yields butadiene. This is achieved by the Houdry process for dehydrogenation of *n*-butane or by oxidative dehydrogenation of *n*-butenes (Walther, 2003).

(iii) *Ethanol-based production*

A plant in India produces butadiene in a two-step process from ethanol. Initial dehydrogenation is achieved through a copper catalyst, and the resulting mixture is then dehydrated at atmospheric pressure in the presence of a zirconium oxide or tantalum oxide–silica gel catalyst at 300–350 °C. Overall yields of butadiene in the second reaction are about 70%. This process is very similar to the adol condensation of acetaldehyde (Walther, 2003).

(b) Butadiene extraction processes

Regardless of the production process, final purification of butadiene requires removal of any butane, butene or acetylene impurities. Currently, seven different commercial

processes exist for the extraction of butadiene that employ different extraction solvents. The processes, identified by the licencer and type of solvent, are: BASF Aktiengesellshaft — *N*-methylpyrrolidone; Lyondell Petrochemical Company — acetonitrile; Zeon Corporation — dimethylformamide; ConocoPhillips — furfural; Shell Chemical Company — acetonitrile; Solutia — β-methoxyproprionitrile with 15% furfural; Dow (formerly Union Carbide Corporation) — dimethylacetamide; and (no licencer) — cuprous ammonium acetate solution (Walther, 2003).

(c) Production volume

An estimated 9.3 million tonnes of butadiene were produced worldwide in 2005 (CMAI, 2006). Production volumes for different regions for the years 2004 and 2006 are given in Table 2.

World capacity grew by 3.5% per year between 1997 and 2002. During that period, most of the increase in capacity occurred in Asia, South America and the Middle East. Asia is now the largest producer of butadiene, and accounts for one-third of the world capacity (Walther, 2003).

Diepoxybutane is not believed to be produced commercially except in small quantities for research purposes (National Library of Medicine, 2008).

Table 2. Butadiene production (in tonnes) by world region from 1981 to 2006

Region	1981	1990	1996	2004	2006
North America	1480	1593	1956	2862	2878
South America	–	–	–	377	377
Western Europe	636[a]	1256	1017[b]	1902	2232
Eastern Europe	–	–	–	1170	736
Middle East/Africa	–	–	–	180	340
Asia/Pacific	518[c]	1253	1755[d]	3104	4405

From IARC (1999), CMAI (2004, 2006)
[a] No data available for Germany
[b] No data available for the United Kingdom or Italy
[c] Value for Japan only
[d] No data available for China

1.2.2 *Use*

Butadiene is used primarily in the production of synthetic rubbers and polymers. These polymers are used in a wide variety of industrial and consumer products, to improve their functionality, performance and safety and lower their costs. Butadiene-based products are important components of automobiles, construction materials, appliance

parts, computers and telecommunications equipment, clothing, protective clothing, packaging and household articles (White, 2007).

The synthetic rubbers that are produced from butadiene include styrene–butadiene rubber, polybutadiene 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 polybutadiene; polymers that consist of acrylonitrile, butadiene and styrene; and a copolymer of methyl methacrylate, butadiene and styrene, which is used as a modifier for poly(vinyl)chloride. Butadiene is also used as an intermediate in the production of chloroprene, adiponitrile and other basic petrochemicals (White, 2007).

Diepoxybutane has been proposed for use in curing polymers and cross-linking textile fibres (National Library of Medicine, 2008).

1.3 Occurrence

1.3.1 *Natural occurrence*

Butadiene is not known to occur as a natural product.

1.3.2 *Occupational exposure*

According to the 1990–93 CAREX database (see General Remarks) for 15 countries of the European Union (Kauppinen *et al.*, 2000) and the 1981–83 US National Occupational Exposure Survey (NOES, 1997), approximately 31 500 workers in Europe and 50 000 workers in the USA were potentially exposed to butadiene.

Based on data from CAREX, the major categories of industrial exposure to butadiene in 15 European countries are the manufacture of industrial chemicals (8000 persons), rubber products (7000 persons), plastic products (7000 persons), petroleum refining (2200 persons) and building construction (1600 persons) (Kauppinen *et al.*, 2000).

In the studies presented below, the accuracy of the levels of exposure to butadiene measured with the methods used until the mid-1980s may have been affected by the inability to distinguish between butadiene and other C_4 compounds, low desorption efficiency at low concentrations, possible sample breakthrough in charcoal tubes and possible loss during storage (Lunsford *et al.*, 1990; Bianchi *et al.*, 1997).

(a) Petroleum refining and butadiene monomer production

Detailed industrial hygiene surveys were conducted in the USA by the National Institute for Occupational Safety and Health in 1985 in four of 10 facilities where butadiene was produced by solvent extraction of C_4 fractions that originated from ethylene co-product streams (Krishnan *et al.*, 1987). Levels of butadiene to which workers in various job categories were exposed are summarized in Table 3. Jobs that required workers to handle or transport containers, such as emptying sample cylinders or

loading and unloading tank trucks or rail cars, presented the greatest potential exposure. Geometric means of full-shift exposure levels for other job categories were below 1 ppm [2.2 mg/m^3]. Short-term samples showed that activities such as open-loop sampling and emptying cylinders were associated with peak exposures of 100 ppm [220 mg/m^3]. Full-shift area samples indicated that ambient concentrations of butadiene were greatest in the rail car terminals (geometric mean, 1.8 ppm [3.9 mg/m^3]) and in the tank storage farm (2.1 ppm [4.7 mg/m^3]).

Table 3. Eight-hour time-weighted average exposure levels in personal breathing zone samples at four butadiene monomer production facilities in the USA, 1985

Job category	No. of samples	Exposure level (ppm [mg/m^3])		
		Arithmetic mean	Geometric mean	Range
Process technician				
Control room	10	0.45 [1.0]	0.09 [0.2]	< 0.02–1.87 [< 0.04–4.1]
Process area	28	2.23 [4.9]	0.64 [1.4]	< 0.08–34.9 [< 0.18–77]
Loading area				
Rail car	9	14.6 [32.4]	1.00 [2.2]	0.12–124 [0.27–273]
Tank truck	3	2.65 [5.9]	1.02 [2.3]	0.08–5.46 [0.18–12.1]
Tank farm	5	0.44 [0.97]	0.20 [0.44]	< 0.04–1.53 [< 0.09–3.4]
Laboratory technician				
Analysis	29	1.06 [2.3]	0.40 [0.88]	0.03–6.31 [0.07–14.0]
Cylinder emptying	3	126 [277]	7.46 [16.5]	0.42–374 [0.93–826]

From Krishnan *et al*. (1987)

Monitoring in a plant in Finland generally indicated ambient air levels of butadiene of less than 10 ppm [22 mg/m^3] at different sites (33 samples; mean sampling time, 5.3 h). In personal samples for 16 process workers, the concentrations ranged from < 0.1 to 477 ppm [< 0.22–1050 mg/m^3] (mean, 11.5 ppm [25 mg/m^3]; median, < 0.1 ppm [< 0.22 mg/m^3]; 46 samples; mean sampling time, 2.5 h). The highest concentrations were measured during the collection of samples, for which protective clothing and respirators were used (Work Environment Fund, 1991).

A study of biological monitoring for the mutagenic effects of exposure to butadiene reported estimated average exposures of 1 ppm [2.2 mg/m^3] for workers in a butadiene monomer plant. Ambient air concentrations in production areas averaged 3.5 ppm [7.7 mg/m^3], while average concentrations of 0.03 ppm [0.07 mg/m^3] were reported for the control area (Ward, J.B. *et al*., 1996).

Levels of exposure to butadiene of workers in various job groups in the production and distribution of gasoline (see IARC, 1989) are shown in Table 4. Table 5 shows exposures in 1984–87 of workers in different areas of petroleum refineries and

Table 4. Personal exposures to butadiene associated with gasoline in 1984–85 in 13 European countries[a] (540 measurements)

Activity	Exposure level (mg/m^3)		
	Arithmetic mean	Range	Duration (TWA)
Production on-site (refining)	0.3	ND–11.4	8 h
Production off-site (refining)	0.1	ND–1.6	8 h
Loading ships (closed system)	6.4	ND–21.0	8 h
Loading ships (open system)	1.1	ND–4.2	8 h
Loading barges	2.6	ND–15.2	8 h
Jetty man	2.6	ND–15.9	8 h
Bulk loading road tankers			
Top loading < 1 h	1.4	ND–32.3	< 1 h
Top loading > 1 h	0.4	ND–4.7	8 h
Bottom loading < 1 h	0.2	ND–3.0	< 1 h
Bottom loading > 1 h	0.4	ND–14.1	8 h
Road tanker delivery (bulk plant to service station)	ND		
Rail car top loading	0.6	ND–6.2	8 h
Drumming	ND		
Service station attendant (dispensing fuel)	0.3	ND–1.1	8 h
Self-service station (filling tank)	1.6	ND–10.6	2 min

From CONCAWE (1987)
ND, not detected; TWA, time-weighted average
[a] Countries included not reported

Table 5. Eight-hour time-weighted average concentrations of butadiene to which workers in different jobs in petroleum refineries and petrochemical facilities were exposed in the USA, 1984–87

Job area	No. of facilities	Arithmetic mean[a]		Range	
		ppm	mg/m^3	ppm	mg/m^3
Production	7	0.24	0.53	0.008–2.0	0.02–4.4
Maintenance	6	0.11	0.24	0.02–0.37	0.04–0.82
Distribution	1	2.90	6.41	–	–
Laboratory	4	0.18	0.40	0.07–0.4	0.16–0.88

From Heiden Associates (1987)
[a] Weighted by number of exposed workers

petrochemical facilities where crude butadiene was produced (usually a C_4 stream obtained as a by-product of ethylene production). Table 6 shows more recent data on crackers at butadiene production plants for the years 1986–93 (ECETOC, 1997).

Exposure data from 15 monomer extraction sites for the years 1984–93 showed that less than 10% of the measured concentrations exceeded 5 ppm [11 mg/m^3] (Table 7); in 1995 (Table 8), personal exposure levels in general were below 5 ppm [11 mg/m^3] (ECETOC, 1997).

In 1998, personal exposure to butadiene was measured for 24 workers in a monomer production facility in the Czech Republic. The mean (± standard deviation [SD]) concentration of butadiene, calculated from 217 individual time-weighted average (TWA) measurements, was 0.6 ± 2.1 mg/m^3 [0.27 ± 0.95 ppm]. The personal TWA measurements from all monomer production workers ranged from undetectable to 19.9 mg/m^3. The mean concentration for the control group was 0.03 ± 0.03 mg/m^3 [0.01 ± 0.01 ppm], calculated from 28 personal TWA exposure measurements (Albertini *et al*., 2003a).

Personal exposure to butadiene of 10 workers who held different jobs in a petrochemical plant in Finland was assessed using passive monitors shortly after the threshold limit value (TLV) of 1 ppm had come into force. A total of 119 personal breathing zone samples were taken and 117 were analysed. Of these, 32 (27%) samples were under the limit of quantification (0.029 mg/m^3 [0.013 ppm] in a 20.5-L sample), 81 samples (69%) were between the limit of quantification and 1 ppm [2.2 mg/m^3] and four samples (3%) were over the Finnish occupational exposure limit of 1 ppm. The mean value of all samples was 0.17 ppm [0.38 mg/m^3] and the mean value of the samples that exceeded the Finnish occupational exposure limit was 1.75 ppm [3.87 mg/m^3]. The mean level of exposure varied significantly ($p = 0.03$) between the 10 workers. Smoking did not significantly affect the values, but the seasonal effect was significant ($p = 0.02$) (Anttinen-Klemetti *et al*., 2004).

The occupational exposure of 42 workers in a petrochemical plant in Italy where butadiene was produced and used to prepare polymers was assessed by biomonitoring. The control group originated from the same industrial complex and included 43 workers who had no significant occupational exposure to butadiene. Active sampling from the breathing zone of the workers was performed during a full shift. Each exposed worker was assessed three to four times over a period of 6 weeks during different shifts. The mean exposure level of the control group was 0.9 μg/m^3 [0.4 ppb] (SD, 1.0) and the lowest and highest values were < 0.1 and 3.8 μg/m^3 [< 0.05 and 1.7 ppb], respectively. The mean exposure level of the exposed group was 11.5 μg/m^3 [5.2 ppb] (SD, 35.8) and the lowest and highest values were < 0.1 and 220.6 μg/m^3 [< 0.04 and 99.8 ppb], respectively (Fustinoni *et al*., 2004).

An exposure assessment was carried out in southern Taiwan, China, on a 120-acre [486 000 m^2] petrochemical complex that comprised 11 different manufacturing plants. Butadiene was produced in two of the plants, which had an annual production of about 156 000 tonnes per year. Using the Fourier transform infrared spectroscopy technique, data were collected on 77 days during the period 1997–99. The relative number of samples that

Table 6. Personal exposures to butadiene of crackers in butadiene production plants in the European Union

Job category	Year of measurement	No. of workers	No. of samples	Exposure level (ppm)							
				< 1	1–2	2–3	3–4	4–5	5–10	10–25	≥ 25
Unloading, loading, storage	1986–92	210	92	82	3	3	2	0	0	1	0
Distillation (hot)	1986–93	394	92	382	0	3	1	2	0	2	2
Laboratory, sampling	1986–93	132	184	178	2	1	2	1	0	0	0
Maintenance	1986–92	282	371	364	5	0	1	0	0	1	0
Other	1990–92	467	509	487	18	2	1	1	ND	0	0
Total	1986–93	1485	1548	1493	28	9	8	4	0	4	2

From ECETOC (1997)

ND, not detected [limit of detection not stated]

Table 7. Personal exposures to butadiene in extraction units[a] of butadiene production plants in the European Union

Job category	Year of measurement	No. of workers	No. of samples	Exposure level (ppm)							
				< 1	1–2	2–3	3–4	4–5	5–10	10–25	≥ 25
Unloading, loading, storage	1986–93	392	224	178	9	8	7	2	11	22	7
Distillation (hot)	1985–93	256	626	535	20	19	6	11	8	12	15
Laboratory, sampling	1985–93	45	48	29	4	2	2	2	3	5	1
Maintenance	1986–93	248	127	93	14	3	2	1	3	4	7
Other	1984–92	45	10	8	2	0	0	0	0	0	0
Total	1984–93	986	1035	843	49	32	17	16	25	23	30

From ECETOC (1997)

[a] Isolation of butadiene from C_4 stream

Table 8. Personal exposures to butadiene at 15 monomer extraction sites in the European Union in 1995

Job category	Exposure level (ppm)	
	Time-weighted averages	Range of values
Production		
Extraction	< 0.01–2	0–14
Derivation[a]	1.4–3.4	0.07–60
Storage and filling	< 0.02–5	0–18.1
Transport	< 0.1–0.7	0.02–1.2
Laboratory	0.03–1	0–13.1

From ECETOC (1997)
[a] Integrated monomer extraction and styrene–butadiene production on same site

were above the detection limit was 15.2% and the mean value of the measurements was 10.5 ± 36.7 ppb [23.2 ± 81.1 μg/m^3]. The maximum concentration measured was 3.1 ppm [6.8 mg/m^3] (Chan *et al.*, 2006). [Measurements were area samples and may underestimate exposure of the workers.]

In the monomer industry, potential exposure to compounds other than butadiene includes exposure to extraction solvents and components of the C_4 feedstock. Extraction solvents differ between facilities: common solvents include dimethylformamide, dimethylacetamide, acetonitrile, β-methoxypropionitrile (Fajen, 1985a), furfural and aqueous cuprous ammonium acetate (Occupational Safety and Health Administration, 1990b). Stabilizers are commonly used to prevent the formation of peroxides in air and during polymerization. No information was available on these exposures or on exposure to chemicals other than butadiene that are produced in some facilities such as butylenes, ethylene, propylene, polyethylene and polypropylene resins, methyl-*tert*-butyl ether and aromatic hydrocarbons (Fajen, 1985b,c).

(b) Production of polymers and derivatives

In samples taken at a styrene–butadiene rubber plant in the USA in 1976 (Table 9), levels of butadiene above 100 ppm [220 mg/m^3] were encountered by technical services personnel (115 ppm [253 mg/m^3]) and an instrument man (174 ppm [385 mg/m^3]; Meinhardt *et al.*, 1978). At another styrene–butadiene rubber manufacturing plant in the USA in 1979, the only two departments in which levels were greater than 10 ppm [22 mg/m^3] were the tank farm (53.4 ppm [118 mg/m^3]) and maintenance (20.7 ppm [46 mg/m^3]; Checkoway & Williams, 1982). Overall mean 8-h TWA exposure levels differed

Table 9. Eight-hour time-weighted average exposure levels of butadiene measured in two styrene–butadiene rubber manufacturing plants in the USA

Reference	Year of sampling	Job classification or department	No. of samples	Exposure level	
				ppm	mg/m^3
Meinhardt *et al.* (1978)	1976	Instrument man	3	58.6	130
		Technical services personnel	12	19.9	43.9
		Head production operator	5	15.5	34.3
		Carpenter	4	7.80	17.2
		Production operator	24	3.30	7.29
		Maintenance mechanic	17	3.15	6.96
		Common labourer	17	1.52	3.36
		Production foreman	1	1.16	2.56
		Operator helper	3	0.79	1.75
		Pipe fitter	8	0.74	1.64
		Electrician	5	0.22	0.49
Checkoway & Williams (1982)	1979	Tank farm	8	20.0	44.3
		Maintenance	52	0.97	2.1
		Reactor recovery	28	0.77	1.7
		Solution	12	0.59	1.3
		Factory service	56	0.37	0.82
		Shipping and receiving	2	0.08	0.18
		Storeroom	1	0.08	0.18

considerably between the two plants: 13.5 ppm [30 mg/m^3] and 1.24 ppm [2.7mg/m^3], respectively (Meinhardt *et al*., 1982).

Detailed industrial hygiene surveys were conducted in 1986 in five of 17 facilities in the USA where butadiene was used to produce styrene–butadiene rubber, nitrile–butadiene rubber, polybutadiene rubber, neoprene and adiponitrile (Fajen, 1988). Levels of butadiene to which workers in various job categories were exposed are summarized in Table 10. Process technicians in unloading, in the tank farm and in the purification, polymerization and reaction areas, laboratory technicians and maintenance technicians were exposed to the highest levels. Short-term sampling showed that activities such as sampling a barge and laboratory work were associated with peak exposures of more than 100 ppm [220 mg/m^3]. Full-shift area sampling indicated that geometric mean ambient concentrations of butadiene were less than 0.5 ppm [1.1 mg/m^3] and usually less than 0.1 ppm [0.22 mg/m^3] in all locations measured at the five plants.

A biological monitoring study that used personal sampling reported average levels of butadiene of 0.30, 0.21 and 0.12 ppm [0.66, 0.46 and 0.27 mg/m^3] for the high-, intermediate- and low-exposure groups, respectively, in a styrene–butadiene rubber plant in Texas, USA (Ward, J.B. *et al*., 1996).

Table 10. Eight-hour time-weighted average exposure levels in personal breathing zone samples at five plants that produced butadiene-based polymers and derivatives in the USA, 1986

Job category	No. of samples	Exposure level (ppm [mg/m^3])		
		Arithmetic mean	Geometric mean	Range
Process technician				
Unloading area	2	14.6 [32.27]	4.69 [10.37]	0.770–28.5 [1.7–63.0]
Tank farm	31	2.08 [4.60]	0.270 [0.60]	< 0.006–23.7 [< 0.01–2.4]
Purification	18	7.80 [17.24]	6.10 [13.48]	1.33–24.1 [3.0–53.3]
Polymerization or reaction	81	0.414 [0.92]	0.062 [0.14]	< 0.006–11.3 [< 0.01–5.0]
Solutions and coagulation	33	0.048 [0.11]	0.029 [0.06]	< 0.005–0.169 [< 0.01–4]
Crumbing and drying	35	0.033 [0.07]	0.023 [0.05]	< 0.005–0.116 [< 0.01–0.26]
Packaging	79	0.036 [0.08]	0.022 [0.05]	< 0.005–0.154 [< 0.01–0.34]
Warehouse	20	0.020 [0.04]	0.010 [0.02]	< 0.005–0.068 [< 0.01–0.15]
Control room	6	0.030 [0.07]	0.019 [0.04]	< 0.012–0.070 [< 0.03–0.16]
Laboratory technician	54	2.27 [5.02]	0.213 [0.47]	< 0.006–37.4 [< 0.01–82.65]
Maintenance technician	72	1.37 [3.02]	0.122 [0.27]	< 0.006–43.2 [< 0.01–95.47]
Utilities operator	6	0.118 [0.26]	0.054 [0.12]	< 0.006–0.304 [< 0.01–0.67]

From Fajen (1988)

In 13 of 27 European sites where styrene–butadiene rubber and styrene–butadiene latex were produced, less than 10% of the concentrations measured exceeded 5 ppm (Table 11; ECETOC, 1997).

Data from the Netherlands are available from 1976 onwards, but the measurement methods used in the early surveys are unknown (Kwekkeboom, 1996; Dubbeld, 1998). No clear trend can be seen for the years 1990–97, but average exposures were relatively low (arithmetic mean < 3 ppm [6.6 mg/m^3]) (Table 12).

Exposure of 38 workers was measured in a butadiene polymer production facility in China. Personal full-shift measurements established that workers in butadiene operations were exposed to a median level of 2.0 ppm [4.4 mg/m^3]. Short-term breathing zone measurements of butadiene showed great extremes in exposure; DMF [dimethylformamide] analysts had a median exposure of 54 ppm [119 mg/m^3] (range, below detection to 3090 ppm [6829 mg/m^3]; 50 samples), polymer analysts had a median exposure of 6.5 ppm [14.4 mg/m^3] (range, below detection to 1078 ppm [2382 mg/m^3]; 41 samples) and maintenance-recovery workers had a median exposure of 7.0 ppm [15.5 mg/m^3] (range, below detection to > 12 000 ppm [> 26 520 mg/m^3]; 24 samples) (Hayes *et al*., 2001).

A biomonitoring study carried out in a styrene–butadiene rubber plant in Southeast Texas, in which 37 workers were monitored during their entire work shift using passive samplers, demonstrated that levels in the tank area exceeded the current Occupational

Table 11. Eight-hour time-weighted average personal exposures to butadiene in styrene–butadiene rubber plants in the European Union (1984–93)

Job category	No. of workers	No. of samples	Exposure level (ppm)								
			< 0.5	0.51–1	1.01–2	2.01–3	3.01–4	4.01–5	5.01–10	10.01–25	≥ 25
Unloading, loading and storage	132	77	47	1	8	6	3	0	5	5	2
Polymerization	324	147	61	23	25	18	6	4	7	3	0
Recovery	103	165	113	9	9	14	7	4	5	4	0
Finishing	247	120	90	16	3	4	5	1	1	0	0
Laboratory sampling	115	113	68	13	12	6	4	2	3	5	0
Maintenance	141	39	28	1	2	1	1	2	1	2	1
Total	1062	661	407	63	59	49	26	13	22	19	3

From ECETOC (1997)

Table 12. Eight-hour time-weighted average exposure levels of butadiene in personal breathing zone samples at a plant that produced styrene–butadiene polymer in the Netherlands, 1990–97

Year	No. of samples	Exposure level (mg/m^3 [ppm])		
		Arithmetic mean	Range	Method[a]
1990	27	5.45 [2.47]	0.35–69.06 [0.16–31.24]	3M 3500
1991	19	1.11 [0.50]	0.09–2.88 [0.04–1.30]	NIOSH 1024
1992	23	2.79 [1.26]	0.13–11.78 [0.06–5.33]	3M 3520
1993	38	2.87 [1.30]	0.15–13.13 [0.07–5.94]	3M 3520/ NIOSH 1024
1996/97 process operators	20	2.77 [1.25]	0.13–46.62 [0.06–21.10]	3M 3520
1996/97 maintenance workers	14	0.54 [0.24]	0.12–9.89 [0.05–4.48]	3M 3520

From Kwekkeboom (1996); Dubbeld (1998)

[a] Analytical methods used are described by Bianchi *et al*. (1997). Methods 3M 3500 and 3M 3520 involve absorption onto butadiene-specific activated charcoal, followed by desorption with carbon disulfide or with dichloromethane, respectively, and analysis by direct-injection gas chromatography with flame ionization detection.

Safety and Health Administation permissible exposure limit for butadiene. However, the workers wore protective equipment on this particular job. TWA values in various work areas are summarized in Table 13 (Ward *et al*., 2001).

In 1998, 319 personal workshift TWA measurements of exposure to butadiene were obtained for 34 workers in a polymer production plant in the Czech Republic. The mean (± SD) concentration of butadiene was 1.8 ± 4.7 mg/m^3 [0.8 ± 2.1 ppm]. The individual TWA measurements from all polymer production workers ranged from 0.002 to 39.0 mg/m^3 [0.001–17.6 ppm]. The level of exposure of the control group was 0.03 ± 0.03 mg/m^3 [0.01 ± 0.01 ppm], calculated from 28 personal TWA measurements (Albertini *et al*., 2003a).

A Finnish study assessed personal exposure to butadiene in three plants that manufactured styrene–butadiene latex. Full-shift air samples were collected from the breathing zone of 28 workers using passive samplers over 4 months. A total of 885 samples were collected and the number of samples per participant ranged from 19 to 39. Samples were collected at the same time in all three plants. The data showed that 624 (70.5%) of the samples were below the limit of quantification; 240 (27.1%) samples were between the limit of quantification and 1 ppm [2.2 mg/m^3] and 21 (2.4%) were over the Finnish occupational exposure limit of 1 ppm [2.2 mg/m^3]. Mean butadiene concentrations in the three plants were 0.068, 0.125 and 0.302 ppm [0.15, 0.28 and 0.67 mg/m^3], respectively.

Table 13. Time-weighted average exposures to butadiene in a styrene–butadiene rubber plant in the USA, 1998

Work area	Subjects	Detectable samples	Samples below the LOD[a]	Exposure level (mean ± SD) (ppm [mg/m^3])
Tank farm	6	17	0	4.04 ± 3.45 [8.9 ± 7.6]
Recovery	6	17	0	1.09 ± 2.35 [2.41 ± 5.19]
Reactor	9	17	3	0.64 ± 1.26 [1.41 ± 2.78]
Low areas[b]	14	22	19	0.05 ± 0.06 [0.11 ± 0.13]
Laboratory	1	2	0	0.29 ± 0.33 [0.64 ± 0.73]
Blending	1	3	0	0.49 ± 0.24 [1.08 ± 0.53]

From Ward *et al.* (2001)
LOD, limit of detection; SD, standard deviation
[a] Half of the 0.002 ppm detection limit was used to calculate exposure to butadiene for the samples
[b] Coagulation, baling, packing, water paint, shipping, warehouse and control room

Statistical analysis of the data did not indicate any significant difference between the plants when all results were considered (Anttinen-Klemetti *et al*., 2006).

In a Czech study that included 26 female control workers, 23 female butadiene-exposed workers, 25 male control workers and 30 male butadiene-exposed workers, 10 personal full-shift (8-h) measurements per worker over a 4-month period showed mean 8-h TWA exposure levels of 0.008 mg/m^3 and 0.4 mg/m^3 [0.004 and 0.18 ppm] for control and exposed women, respectively. The highest single 8-h TWA value among exposed women was 9.8 mg/m^3 [4.5 ppm]. Mean 8-h TWA exposure levels were 0.007 mg/m^3 and 0.8 mg/m^3 [0.003 and 0.36 ppm] for control and exposed men, respectively; personal single 8-h TWA values of up to 12.6 mg/m^3 [5.7 ppm] were measured in the exposed group. The concentrations for butadiene-exposed workers were significantly higher than those for the controls for both men and women; the concentrations for butadiene-exposed workers were significantly higher for men than for women (Albertini *et al*., 2007). [The difference in exposure levels may be due to differences in tasks performed by men and women.]

Data from a Canadian styrene–butadiene rubber plant indicate a clear decrease in exposure from 1977 to 1991 (Sathiakumar & Delzell, 2007; Table 14). The data were used to validate the estimates of historical exposure to butadiene (Macaluso *et al.*, 1996, 2004; Sathiakumar *et al*., 2007).

The manufacture of butadiene-based polymers and butadiene derivatives implies potential exposure to a number of other chemical agents that vary according to product and process and include other monomers (styrene, acrylonitrile, chloroprene), solvents, additives (e.g. activators, antioxidants, modifiers), catalysts, mineral oils, carbon black, chlorine, inorganic acids and caustic solutions (Fajen, 1986a,b; Roberts, 1986). Styrene, benzene and toluene levels were measured in 1979 in various departments of a plant that

manufactured styrene–butadiene rubber in the USA: mean 8-h TWA levels of styrene were below 2 ppm [8.4 mg/m^3], except for tank-farm workers (13.7 ppm [57.5 mg/m^3], eight samples); mean benzene levels did not exceed 0.1 ppm [0.3 mg/m^3], and those of toluene did not exceed 0.9 ppm [3.4 mg/m^3] (Checkoway & Williams, 1982). Meinhardt *et al*. (1982) reported that the mean 8-h TWA levels of styrene in two styrene–butadiene rubber manufacturing plants were 0.94 ppm [3.9 mg/m^3] (55 samples) and 1.99 ppm [8.4 mg/m^3] (35 samples) in 1977; the average level of benzene measured in one of the plants was 0.1 ppm [0.3 mg/m^3] (three samples). Average levels of styrene, toluene, benzene, vinyl cyclohexene and cyclooctadiene were reported to be below 1 ppm in another styrene–butadiene rubber plant in 1977 (Burroughs, 1977). Dimethyldithiocarbamate has been used in some plants and dermal exposure to this compound potentially exists (Delzell *et al.*, 2001).

Table 14. Exposure levels of butadiene in a styrene–butadiene rubber plant in Canada

Year	No. of jobs monitored	No. of measurements	Exposure level (mean[a] ± SD) (ppm [mg/m^3])
1977	3	56	24.8 ± 69.9 [54.8 ± 154.5]
1978	11	527	16.0 ± 166.6 [35.4 ± 368.2]
1979	13	274	10.6 ± 153.2 [23.4 ± 338.6]
1980	13	301	14.5 ± 137.8 [32.0 ± 304.5]
1981	15	307	4.8 ± 38.4 [10.6 ± 84.9]
1982	21	406	3.8 ± 28.2 [8.4 ± 62.3]
1983	13	113	3.9 ± 19.4 [8.6 ± 42.9]
1984	27	658	2.5 ± 20.3 [5.5 ± 44.9]
1985	27	482	2.6 ± 18.4 [5.7 ± 40.7]
1986	30	504	2.3 ± 16.2 [5.08 ± 35.8]
1987	26	310	0.85 ± 6.3 [1.9 ± 13.9]
1988	28	417	1.0 ± 5.2 [2.2 ± 11.5]
1989	27	238	1.5 ± 5.5 [3.3 ± 12.2]
1990	27	223	0.63 ± 3.3 [1.4 ± 7.3]
1991	25	162	0.34 ± 0.61 [0.75 ± 1.35]

From Sathiakumar *et al*. (2007)
SD, standard deviation
[a] Weighted by the number of measurements for job/year combinations in a year

(c) Manufacture of rubber and plastics products

In a tyre and tube manufacturing plant in the USA in 1975, a cutter man/Banbury operator was reported to have been exposed to 2.1 ppm [4.6 mg/m^3] butadiene (personal 6-h sample) (Ropert, 1976).

Personal 8-h TWA measurements taken in 1978 and 1979 in companies where acrylonitrile–butadiene–styrene moulding operations were conducted showed levels of

< 0.05–1.9 mg/m^3 [< 0.11–4.2 ppm] (Burroughs, 1979; Belanger & Elesh, 1980; Ruhe & Jannerfeldt, 1980).

In a polybutadiene rubber warehouse, levels of 0.003 ppm [0.007 mg/m^3] butadiene were found in area samples; area and personal samples taken in tyre plants revealed levels of 0.007–0.05 ppm [0.016–0.11 mg/m^3] butadiene (Rubber Manufacturers' Association, 1984).

Unreacted butadiene was detected as a trace (0.04–0.2 mg/kg) in 15 of 37 bulk samples of polymers and other chemicals synthesized from butadiene and analysed in 1985–86. Only two samples contained measurable amounts of butadiene: tetrahydrophthalic anhydride (53 mg/kg) and vinylpyridine latex (16.5 mg/kg) (JACA Corp., 1987).

Detailed industrial hygiene surveys were conducted in 1984–87 in the USA at a rubber tyre plant and an industrial hose plant where styrene–butadiene rubber, polybutadiene and acrylonitrile–butadiene rubber were processed. No butadiene was detected in any of 124 personal full-shift samples from workers in the following job categories that were identified as involving potential exposure to butadiene: Banbury operators, mill operators, extruder operators, curing operators, conveyer operators, calendering operators, wire winders, tube machine operators, tyre builders and tyre repair and buffer workers (Fajen *et al*., 1990).

Occupational exposures to many other agents in the rubber goods manufacturing industry have been reviewed previously (IARC, 1982).

(d) Comparison of exposure levels in monomer and styrene–butadiene rubber production facilities

Exposures measured in monomer production facilities in the USA demonstrated overall mean levels of 3.5 ppm [7.7 mg/m^3] (measured in 1979–92; number not reported; stationary sampling; Cowles *et al*., 1994) and 7.1 ppm [15.7 mg/m^3] (measured in 1985; 87 samples; personal sampling; Krishnan *et al*., 1987). Recently reported values from the Czech Republic and Finland were 0.64 ppm [1.41 mg/m^3] (measured in 1998; 217 samples; personal sampling) and 0.17 ppm [0.38 mg/m^3] (measured in 2002; 117 samples; personal sampling) (Albertini *et al*., 2003a; Anttinen-Klemetti *et al*., 2004).

Measurement of butadiene concentrations in a styrene–butadiene rubber plant in Canada demonstrated a decrease in exposure during the 14 years of monitoring. The levels dropped from 24.8 ppm in 1977 [54.8 mg/m^3] to 0.34 ppm [0.75 mg/m^3] in 1991 (Table 14) (Sathiakumar *et al*., 2007).

The decreasing trend of exposure was apparent in both monomer and styrene–butadiene rubber production; however, the lack of data from the 1940s to the 1970s does not allow comparison between the two processes.

1.3.3 *Environmental occurrence*

According to the Environmental Protection Agency Toxic Chemical Release Inventory in the USA, industrial releases of butadiene to the atmosphere from industrial

facilities in the USA were 4425 tonnes in 1987, 2360 tonnes in 1990 and 1385 tonnes in 1995. According to the same database, fugitive air emissions were 157 973 kg and point source air emissions were 450 926 kg in 2005 (Environmental Protection Agency Toxic Release Inventory, 2005; National Library of Medicine, 2008).

Under laboratory conditions, non-catalyst vehicles emitted butadiene at a rate of 20.7 ± 9.2 mg/kg. Vehicles that had a functioning catalyst–emission control device had an average emission rate of 2.1 ± 1.5 mg/km. Based on these numbers, the authors concluded that vehicle emissions of butadiene have been substantially underestimated (Ye *et al.*, 1997). Based on an average of 20 000 km per year per car and approximately 243 million registered cars in the USA in 2004, and considering the average emission rates estimated by Ye *et al*. (1997), emissions of butadiene from automobile exhausts can be estimated to amount to approximately 106 770 tonnes per year.

Butadiene is also released to the atmosphere from the smoke of bush fires, the thermal breakdown or burning of plastics and by volatization from gasoline (Agency for Toxic Substances and Disease Registry, 1992; see IARC, 1992).

Kim *et al*. (2001, 2002) measured the concentrations of 15 volatile organic compounds, including butadiene, in a wide range of urban micro-environments in the United Kingdom (Table 15) and estimated the personal exposure of 12 urban dwellers directly and indirectly via static monitoring combined with a personal activities diary (Table 16).

Table 15. Mean concentrations of butadiene in micro-environments in the United Kingdom

Environment	No. of samples	Concentration (mean ± SD) (μg/m^3)
Home	64	1.1 ± 1.9
Office	12	0.3 ± 0.2
Restaurant	6	1.5 ± 0.8
Public house	6	3.0 ± 2.0
Department store	8	0.6 ± 0.4
Cinema	6	0.6 ± 0.3
Perfume store	3	0.9 ± 0.1
Library	6	0.4 ± 0.2
Laboratory	8	0.2 ± 0.1
Train station	12	2.2 ± 1.7
Coach station	12	0.9 ± 0.7
Road with traffic	12	1.8 ± 0.9
Car	35	7.9 ± 4.7
Train	18	1.0 ± 0.6
Bus	18	1.7 ± 0.9
Smoking home	32	1.7 ± 2.5
Nonsmoking home	32	0.5 ± 0.3

From Kim *et al*. (2001)
SD, standard deviation

Table 16. Daytime and night-time concentrations (μg/m^3) of butadiene recorded during personal exposure monitoring in the United Kingdom, 1999–2000

Period	No. of samples	Mean	Standard deviation	Minimum	Maximum
Daytime	473	1.1	0.4	ND	26.3
Night-time	99	0.8	0.4	ND	7.9

From Kim *et al*. (2002)
ND, not detected

Environmental exposure to emissions, including butadiene, was compared between bus and cycling commuters on a route in Dublin. Samples were collected during both morning and afternoon rush-hour periods using continuous sampling. The average concentrations experienced by the cyclist and the bus passenger for all journeys were 0.47 ppb [103 μg/m^3] (SD, 0.19; min., 0.24; max., 0.81) and 0.78 ppb [1.7 μg/m^3] (SD, 0.34; min., 0.34; max., 1.49), respectively (O'Donoghue *et al.*, 2007).

In the United Kingdom, the estimated emission of butadiene in 1996 was 10.6 thousand tonnes. Road vehicle exhaust emissions dominated and comprised 68% of the total emissions, while emissions from off-road vehicles and machinery accounted for 14%. The remaining emissions arose from the chemical industry, during the manufacture of butadiene and its use in the production of various rubber compounds. These two processes accounted for 8 and 10%, respectively, of total emissions in the United Kingdom in 1996 (Dollard *et al.*, 2001).

Municipal structural fires are a source of butadiene, and the mean level of butadiene from nine fires ranged from 0.03 to 4.84 ppm [0.07–9.9 mg/m^3] (Austin *et al.*, 2001). Domestic wood burning also has an impact on levels of butadiene in homes. Wood burners had a significantly higher personal exposure to butadiene (median, 0.18 μg/m^3) than the reference group. Similarly, significantly higher indoor levels were reported (median, 0.23 μg/m^3) in homes of wood burners than in the homes of the reference group (Gustafson *et al*., 2007).

The intake of butadiene that results from exposure to environmental tobacco smoke for a person who lives with one or more smokers in homes where smoking is permitted was estimated to be in the range of 16–37 μg per day (Nazaroff & Singer, 2004). The levels of butadiene in public houses in Dublin were assessed before and after the smoking ban in 2004. The average level before the ban was 4.15 μg/m^3 [1.87 ppb]. The levels of butadiene recorded in the same establishments when cigarettes were no longer being smoked dropped significantly to 0.22 μg/m^3 [0.1 ppb], which is still higher than the average ambient level (0.12 μg/m^3 [0.05 ppb]) (McNabola *et al*., 2006).

In the metropolitan area of Mexico City, three persons who were simultaneously monitored for butadiene inside the home and outdoors had median levels of 2.1 μg/m^3 [1 ppb] (max., 11.5 μg/m^3 [5.2 ppb]), 2.0 μg/m^3 [0.9 ppb] (max., 8.3 μg/m^3 [3.7 ppb]) and

0.8 μg/m^3 [0.4 ppb] (max., 4.6 μg/m^3 [2.1 ppb]) for personal, indoor and outdoor exposure, respectively (Serrano-Trespalacios *et al*., 2004).

Ambient concentrations of butadiene were measured in Japan during the years 1997–2003 at general environmental stations, roadside stations and industrial vicinity stations. The mean levels in 1998 were 0.28, 0.56 and 0.37 μg/m^3 [0.13, 0.25 and 0.17 ppb] for the general environment, roadside and industrial vicinity, respectively. The overall level was 0.36 μg/m^3 [0.16 ppb]. In 2003, corresponding levels were 0.22, 0.42 and 0.31 μg/m^3 [0.10, 0.19 and 0.14 ppb], with an overall level of 0.29 μg/m^3 [0.13 ppb] (Higashino *et al*., 2006).

Mainstream and sidestream cigarette smoke contain approximately 20–40 μg and 80–130 μg butadiene per cigarette, respectively; levels of butadiene in smoky indoor environments are typically 10–20 μg/m^3 [5–9 ppb] (IARC, 2004).

Based on its physical and chemical properties, butadiene is unlikely to be detected in water or in soil (Agency for Toxic Substances and Disease Registry, 1992).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for butadiene in several countries, regions or organizations are given in Table 17.

The government of the United Kingdom has imposed an air quality standard for butadiene of 2.25 μg/m^3 [1.00 ppb] to be achieved by December 2003 (running annual mean) (AEA Energy & Environment, 2002).

Table 17. Occupational exposure limits and guidelines for butadiene in several countries/regions or organizations

Country/region or organization	TWA (ppm)[a]	STEL (ppm)[a]	Carcinogenicity[b]	Notes
Belgium	2		Ca	
Brazil	780			
Canada				
Alberta	2			Schedule 2
British Columbia	2		2	K2
Ontario	5			
Quebec	2		A2	
China (mg/m^3)	5	12.5		STEL based on the 'ultra limit coefficient'
China, Hong Kong SAR	2		A2	
Czech Republic (mg/m^3)	10	20		
Finland	1			
Germany-MAK			1	
Ireland	1		Ca2	
Japan-JSOH			1	
Malaysia	2			

Table 17 (contd)

Country/region or organization	TWA (ppm)[a]	STEL (ppm)[a]	Carcinogenicity[b]	Notes
Mexico	1000	1250	A2	
Netherlands	21		Ca	
New Zealand	10		A2	
Norway	1		Ca	
Poland-MAC (mg/m^3)	10	40		
South Africa-DOL CL	10			
Spain	2		Ca1	
Sweden	0.5	5	Ca	
United Kingdom	10		R45	
USA				
ACGIH (TLV)	2		A2	Cancer
NIOSH IDLH (ceiling)		2000	Ca	
OSHA PEL	1	5		

From ACGIH® Worldwide (2005)
ACGIH, American Conference of Governmental Industrial Hygienists; DOL CL, Department of Labour – ceiling limits; IDLH, immediately dangerous to life or health; JSOH, Japanese Society of Occupational Health; MAC, maximum acceptable concentration; MAK, maximum allowed concentration; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; STEL, short-term exposure limit; TLV, threshold limit value; TWA, time-weighted average
[a] Unless otherwise specified
[b] Ca (Belgium, Netherlands, Sweden, NIOSH), carcinogen/substance is carcinogenic; Ca (Norway), potential cancer-causing agent; 2, considered to be carcinogenic to humans; A2, suspected human carcinogen/carcinogenicity suspected in humans; 1, substance which causes cancer in man/carcinogenic to humans; Ca2, suspected human carcinogen; Ca1, known or presumed human carcinogen; R45, may cause cancer

2. Studies of Cancer in Humans

2.1 Background

Over the last 30 years, the relationship between exposure to butadiene and cancer in human populations has been investigated in numerous studies. The most relevant investigations focused on working populations who were employed in butadiene monomer and styrene–butadiene rubber production.

Three independent cohorts of monomer production workers in the USA have been studied: at two Union Carbide plants in West Virginia (Ward *et al*., 1995), at a Texaco plant in Texas (Divine & Hartman, 2001) and at a Shell plant in Texas (Tsai *et al*., 2001).

Two independent groups of styrene–butadiene rubber production workers have been studied. One was studied by the National Institute of Occupational Safety and Health (NIOSH) in a two-plant complex in Ohio, USA (McMichael *et al*., 1974, 1976; Meinhardt *et al.*, 1982), and the other comprised workers from eight facilities in the USA and Canada who were studied by researchers from the Johns Hopkins' University (Matanoski & Schwartz, 1987; Matanoski *et al.*, 1990, 1993).

Subsequently, researchers from the University of Alabama at Birmingham (Delzell *et al*. 1996) studied the two-plant complex originally investigated by NIOSH plus seven of the eight plants studied by the Johns Hopkins' University. The Johns Hopkins' researchers also conducted nested case–control studies within this working population (Santos-Burgoa *et al.*, 1992; Matanoski *et al.*, 1997). The University of Alabama at Birmingham group recently updated the follow-up of the cohort and revised and refined their assessment of exposures both to butadiene and to possible confounding co-exposures (Macaluso *et al*., 2004). A number of largely overlapping publications from these groups have been reviewed. The most recent results were published by Graff *et al*. (2005), Sathiakumar *et al*. (2005), and Cheng *et al*. (2007).

In addition to industry-based studies, a population-based case–control study in Canada (Parent *et al*., 2000) and a cohort study of students at a high school adjacent to a styrene–butadiene rubber production plant in the USA (Loughlin *et al*., 1999) are also reviewed here.

Overall, the available studies focused consistently on a possible increased risk for neoplasms of the lymphatic and haematopoietic system from exposure to butadiene.

Epidemiological studies of cancer and exposure to butadiene are summarized in Table 18.

2.2 Industry-based studies

2.2.1 *Monomer production*

A cohort mortality study included men who were assigned to any of three butadiene production units located within several chemical plants in the Kanawha Valley of West Virginia, USA. Of the 364 men included in the study, 277 (76%) were employed in a 'Rubber Reserve' plant that operated during the Second World War (Ward *et al*., 1995). The plants produced butadiene from ethanol or from olefin cracking. The butadiene production units included in this study were selected from an index of chemical departments that was developed by the Union Carbide Corporation and included only departments where butadiene was a primary product and neither benzene nor ethylene oxide was present. The cohort studied was part of a large cohort of 29 139 chemical workers whose mortality experience had been reported earlier, although without regard to specific exposures (Rinsky *et al*., 1988). Three subjects were lost to follow-up (0.8%). Mortality from all cancers was not increased (48 deaths; standardized mortality ratio [SMR], 1.1; 95% confidence interval [CI], 0. 8–1.4). Seven deaths from lymphatic and

Table 18. Epidemiological studies of exposure to 1,3-butadiene and the risk for lympho-haematopoietic neoplasms

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Butadiene monomer production								
Ward, E.H. *et al*. (1995, 1996), USA	364 male workers in three units	Employment in butadiene departments; no benzene or ethylene oxide present				**SMR**	Age, time period; county reference rates	All 4 cases of lympho/reticulo-sarcomas had been employed ≥ 2 years (SMR, 8.3; 95% CI, 1.6–14.8), as had those of stomach cancer (SMR, 6.6; 95% CI, 2.1–15.3); all occurred in the rubber reserve plant.
			All (140–208)		48	1.1 (0.8–1.4)		
			Lymphatic and haematopoietic		7	1.8 (0.7–3.6)		
			Lymphosarcoma and reticulosarcoma (200)		4	5.8 (1.6–14.8)		
			Leukaemia (204–208)		2	1.2 (0.2–4.4)		
Divine & Hartman (2001), USA	2800 male workers employed ≥ 6 months in 1943–96	Industrial hygiene sampling data				**SMR**	Age, time period, age at hire	No increasing trend by duration of employment; no increasing trend by exposure group; lymphatic haematopoietic cancers and lymphosarcoma significantly increased in the highest exposure category; elevations were found in workers employed < 1950, and were highest in short-term workers.
			All cancers (140–209)	Employed	333	0.9 (0.8–1.0)		
				< 5 years	170	1.0 (0.8–1.1)		
				5–19 years	55	0.8 (0.6–1.1)		
				≥ 20 years	108	0.8 (0.7–1.0)		
			Lymphohaematopoietic (200–209)	Employed	50	1.4 (1.1–1.9)		
				< 5 years	26	1.6 (1.0–2.3)		
				5–19 years	8	1.2 (0.5–2.4)		
				≥ 20 years	16	1.3 (0.8–2.2)		
				High exposure				
				< 5 years	20	1.8 (1.1–2.8)		
				≥ 5 years	14	1.5 (0.8–2.5)		
				First employed				
				1942–49	46	1.5 (1.1–2.1)		
				≥ 1950	4	0.7 (0.2–1.8)		

Table 18 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Divine & Hartman (2001) (contd)			Non-Hodgkin lymphoma (200, 202)	Employed	19	1.5 (0.9–2.3)		
				< 5 years	12	1.3 (0.3–3.7)		
				5–19 years	3	0.9 (0.3–2.3)		
				≥ 20 years	4	2.0 (0.9–3.9)		
				High exposure				
				< 5 years	8	1.1 (0.3–2.9)		
				≥ 5 years	4	1.6 (0.9–2.6)		
				First employed				
				1942–49	17	1.6 (0.9–2.6)		
				≥ 1950	2	0.9 (0.1–3.2)		
			Leukaemia (204–207)	Employed	18	1.3 (0.8–2.0)		
				< 5 years	9	1.4 (0.6–2.6)		
				5–19 years	2	0.7 (0.1–2.6)		
				≥ 20 years	7	1.5 (0.6–3.1)		
				High exposure				
				< 5 years	8	1.9 (0.8–3.7)		
				≥ 5 years	5	1.4 (0.4–3.2)		
				First employed				
				1942–49	18	1.5 (0.9–2.4)		
				≥ 1950	0	0 (0–178)		
Tsai *et al.* (2001), USA	614 male workers	Employed ≥ 5 years in butadiene production; most 8-h TWAs for butadiene < 10 ppm				**SMR**	Age, race, calendar year; reference county-specific rates	A concurrent morbidity study failed to show differences in haematological values between butadiene-exposed and unexposed workers within the complex.
			All cancers		16	0.6 (0.3–0.9)		
			Lymphatic and haemopoietic (200–209)		3	1.1 (0.3–1.5)		

Table 18 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Styrene–butadiene rubber (SBR) production								
McMichael *et al.* (1976), USA	Case–cohort of 6678 male rubber workers	Employment for > 2 years in SBR production based on work histories	All lymphatic and haematopoietic (200–9)	≥ 5 years in synthetic plant	51	6.2 (4.1–12.5)[a]	Age	No information on exposure to specific agents
			Lymphatic leukaemia (204)		14	3.9 (2.6–8.0)[a]		
Meinhardt *et al.* (1982), USA (overlapping with Delzell *et al.*, 1996)	2756 white men employed for at least 6 months (Plant A, 1662 men; Plant B, 1094 men)	Duration and time of employment				**SMR**	Age, time period, race	
			Lymphatic and haematopoietic (200–5)	Plant A	9	1.6		
			Lymphosarcoma and reticulosarcoma	Plant A, total	3	1.8		
				Plant A, working 1943–45	3	2.1		
				Plant B, total	1	1.3		
			Leukaemia (204)	Plant A, total	5	2.0		
				Plant A, working 1943–45	5	2.8		
				Plant B, total	1	1.0		

Table 18 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Delzell *et al.* (1996), USA and Canada (includes data from Meinhardt *et al.* (1982); Matanoski & Schwartz, 1987; Lemen *et al.*, 1990; Matanoski *et al.*, 1990, Santos-Burgoa *et al.*, 1992; Matanoski *et al.*, 1993, 1997)	15 649 workers employed for at least 1 year in eight production plants in 1943–91	8281 unique combinations of work area/job title, grouped in 308 work areas with similar exposure	All cancers (140–208) Lymphosarcoma (200) Other lymphopoietic (202) Leukaemia (204–208)	Five main process groups and seven sub-groups Polymerization Maintenance Labour Laboratories	950 11 42 48 15 13 10	0.93 (0.87–0.99) 0.8 (0.4–1.4) 1.0 (0.7–1.5) 1.3 (1.0–1.7) 2.5 (1.4–4.1) 2.7 (1.4–4.5) 4.3 (2.1–7.9)	Age, race, calendar time	Among ‘ever hourly paid’ workers, 45 leukaemia deaths (SMR, 1.4; 95% CI, 1.0–1.9); SMR for hourly workers having worked for > 10 years and hired ≥ 20 years ago, 2.2 (95% CI, 1.5–3.2) based on 28 leukaemia deaths
Macaluso *et al.* (1996), USA and Canada (over-lapping with Delzell *et al.*, 1996)	12 412 subjects	Retrospective quantitative estimates of exposure to butadiene, styrene and benzene by work area	Leukaemia (204–208)	*ppm–years* 0 < 1 1–19 20–79 ≥ 80 *p*-trend 0 < 1 1–19 20–79 ≥ 80 *p*-trend	 8 4 12 16 18	**SMR** 0.8 (0.3–1.5) 0.4 (0.4–1.1) 1.3 (0.7–2.3) 1.7 (1.0–2.7) 2.6 (1.6–4.1) = 0.01 **Mantel-Haenszel** 1.0 2.0 (NR) 2.1 (NR) 2.4 (NR) 4.5 (NR) 0.01	Age, race, co-exposure to styrene and benzene; Mantel-Haenszel rate ratios adjusted by race, cumulative exposure to styrene	Including 7 decedents for whom leukaemia was listed as contributory cause of death

Table 18 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Matanoski *et al.* (1997), USA and Canada (over-lapping with Delzell *et al.*, 1996)	Nested case–control study from a cohort of 12 113 employees at SBR plant	Estimated cumulative exposure and average intensity of exposure to butadiene	Hodgkin lymphoma (201) Leukaemia	Average intensity of exposure to butadiene, 1 ppm compared with 0 ppm	8 26	1.7 (0.99–3.0) 1.5 (1.1–2.1)	Birth year, age at hire before 1950, race, length of employ-ment	Additional results from the same cohort are presented in the text (Matanoski & Schwartz, 1987; Matanoski *et al.*, 1990; Santos-Burgoa *et al.*, 1992); non-Hodgkin lymphoma and multiple myeloma were not associated with exposure to butadiene.
Sathiakumar *et al*. (1998), USA and Canada (same as Delzell *et al*., 1996)	12 412 subjects	See Macaluso *et al.* (1996)	Non-Hodgkin lymphoma (202)	Hourly workers ≥ 10 years worked and ≥ 20 years since hire	15	**SMR** 1.4 (0.8–2.3)	Age, race, calendar time	No pattern by duration of employment, time since hire, period of hire or process group

Table 18 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Delzell *et al.* (2001), USA and Canada	13 130 men employed for at least 1 year during 1943–91 at 6 SBR plants	Quantitative estimates	Leukaemia (204–208)	*Butadiene ppm–years*		**Poisson regression**	Age, years since hire	The association of risk for leukaemia with butadiene was stronger for ppm–years due to exposure intensities > 100 ppm.
				0	7	1.0		
				> 0–< 86.3	17	1.2 (0.5–3.0)		
				86.3–< 362.2	18	2.0 (0.8–4.8)		
				≥ 362.2	17	3.8 (1.6–9.1)		
				p-trend		< 0.001		
				Butadiene ppm–years			Age, years since hire, co-exposure to other agents	
				0	7	1.0		
				> 0–< 86.3	17	1.3 (0.4–4.3)		
				86.3–< 362.2	18	1.3 (0.4–4.6)		
				≥ 362.2	17	2.3 (0.6–8.3)		
				p-trend		= 0.250		
				Butadiene ppm–years exposure intensity < 100 ppm			Age, years since hire	
				0	7	1.0		
				> 0–< 37.8	17	1.1 (0.5-2.7)		
				37.8–< 96.3	17	2.8 (1.2-6.8)		
				≥ 96.3	18	3.0 (1.2-7.1)		
				p-trend		= 0.25		
				Butadiene ppm–years exposure intensity > 100 ppm			Age, years since hire	
				0	7	1.0		
				> 0–< 46.5	17	2.1 (0.9–5.1)		
				46.5–< 234.3	17	2.8 (1.2–6.7)		
				≥ 234.3	18	5.8 (2.4–13.8)		
				p-trend		= 0.01		

Table 18 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Graff *et al*. (2005), USA and Canada	16 579 men working at 6 plants ≥ 1 year by 1991 and followed up through to 1998	Same as Delzell *et al*. (2001); cumulative exposure estimates for butadiene, styrene and DMDTC	Leukaemia (204–208)	*Butadiene ppm–years*		**Poisson regression**	Age, years since hire	SMR analyses with external reference rates (national and state-specific) also conducted and results for leukaemia consistent with those of internal analysis using Poisson regression models.
				0	10	1.0		
				> 0–< 33.7	7	1.4 (0.7–3.1)		
				33.7–< 184.7	18	1.2 (0.6–2.7)		
				184.7–< 425.0	18	2.9 (1.4–6.4)		
				≥ 425.0	18	3.7 (1.7–8.0)		
				p-trend		< 0.001		
				Butadiene ppm–years			Age, years since hire, other agents	
			Leukaemia (204–208)	0	10	1.0		
				> 0–< 33.7	17	1.4 (0.5–3.9)		
				33.7–< 184.7	18	0.9 (0.3–2.6)		
				184.7–< 425.0	18	2.1 (0.7–6.2)		
				≥ 425.0	18	3.0 (1.0–9.2)		
				p-trend		= 0.028		
			Chronic lymphocytic leukaemia (204.1)	< 33.7	7	1.0		
				33.7–< 425.0	11	1.5 (0.6–4.0)		
				≥ 425.0	7	3.9 (1.3–11.0)		
				p-trend		= 0.014		
			Chronic myelogenous leukaemia (205.1)	< 33.7	3	1.0		
				33.7–< 425.0	8	2.7 (0.7–10.4)		
				≥ 425.0	5	7.2 (1.7–30.5)		
				p-trend		= 0.007		
			Other leukaemia	< 33.7	5	1.0		
				33.7–< 425.0	5	1.1 (0.3–3.9)		
				≥ 425.0	4	4.0 (0.3–15.0)		
				p-trend		= 0.060		

Table 18 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Sathiakumar *et al*. (2005), USA and Canada	17 924 male workers employed ≥ 1 year before 1992 followed through to 1998	Same as Delzell *et al.* (1996)				**SMR**	Age, race, calendar period	Leukaemia excesses in production mainly due to chronic lymphatic leukaemia: polymerization (8 cases; SMR, 4.97; 95% CI, 2.15–9.80), coagulation (5 cases; SMR, 6.07; 95% CI, 1.97–14.17) and finishing (7 cases; SMR, 3.44; 95% CI, 1.38–7.09); myelogenous leukaemia particularly high in maintenance labour (acute, 5 cases; SMR, 2.95; 95% CI, 0.96–6.88) and laboratory (total, 6 cases; SMR, 3.31; 95% CI, 1.22–7.20; chronic, 3 cases; SMR, 5.22; 95% CI, 1.08–15.26)
			Non-Hodgkin lymphoma (200, 202)	All workers	53	1.0 (0.8–1.3)		
				Hourly workers	49	1.1 (0.8–1.5)		
			All cancer		1608	0.92 (0.88–0.97)		
			Lymphohaematopoietic (200–208)		162	1.06 (0.9–1.2)		
			Hodgkin lymphoma		12	1.1 (0.6–2.0)		
			Multiple myeloma (203)		26	0.95 (0.62–1.4)		
			Leukaemia (204–208)	All workers	71	1.2 (0.9–1.5)		
				Hourly workers	63	1.2 (0.9–1.6)		
				Hourly workers *≥ 20 years since hire –10 years worked*	19	2.6 (1.6–4.0)		
				Production				
				Polymerization	18	2.0 (1.2–3.2)		
				Coagulation	10	2.3 (1.1–4.3)		
				Finishing	19	1.6 (0.9–2.4)		
				Labour maintenance	15	2.0 (1.1–3.4)		
				Laboratories	14	3.3 (1.8–5.5)		
			Chronic lymphocytic leukaemia (204.1)	All workers	16	1.5 (0.9–2.5)		
				Hourly workers	15	1.7 (0.96–2.8)		

Table 18 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Delzell *et al.* (2006), USA and Canada				*Butadiene ppm–years*			Age, years since hire, other agents	
			Non-Hodgkin lymphoma (200, 202)	0	11	1.0		
				> 0–< 33.7	16	1.0 (0.4–2.6)		
				33.7–< 184.7	10	0.4 (0.1–1.2)		
				184.7–< 425.0	12	0.9 (0.3–2.7)		
				≥ 425.0	9	0.7 (0.2–2.3)		
			Non-Hodgkin lymphoma and chronic lymphocytic leukaemia combined (200, 202, 204.1)	0	12	1.0		
				> 0–< 33.7	18	0.9 (0.4–2.1)		
				33.7–< 184.7	14	0.4 (0.2–1.1)		
				184.7–< 425.0	17	1.0 (0.4–2.7)		
				≥ 425.0	14	0.9 (0.3–2.7)		
			Lymphoid neoplasms (200–204)	0	24	1.0		
				> 0–< 33.7	28	0.9 (0.5–2.0)		
				33.7–< 184.7	25	0.7 (0.3–1.6)		
				184.7–< 425.0	21	1.3 (0.6–3.1)		
				≥ 425.0	22	1.5 (0.6–3.8)		
			Myeloid neoplasms (205, 206), (erythroleukaemia, myelofibrosis, myelosdysplasia, polycythemia vera, myeloproliferative disease)	< 33.7	19	1.0		
				33.7–< 184.7	15	0.8 (0.3–1.7)		
				184.7–< 425.0	11	1.6 (0.6–4.1)		
				≥ 425.0	11	2.4 (0.9–6.8)		

Table 18 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Adjustment for potential confounders	Comments
Cheng *et al*. (2007), USA and Canada	Same as Sathiakumar *et al*. (2005)	Same as Delzell *et al.* (2001)	Leukaemia (204–208)	*Cumulative ppm–years* Continuous Mean scored deciles *Total number of peaks* Continuous Mean scored deciles *Average intensity* Continuous Mean scored deciles	81	**Cox regression coefficient (β) for exposure–response SE, *p*-value** $\beta = 3.0*10^{-4}$ SE $1.4*10^{-4}$, $p = 0.04$ $(0.1*10^{-4}–5.8*10^{-4})$ $\beta = 5.8*10^{-4}$ SE $2.7*10^{-4}$, $p = 0.03$ $(0.5*10^{-4}–11.1*10^{-4})$ $\beta = 5.6*10^{-5}$ SE $2.4*10^{-5}$, $p = 0.02$ $(0.8*10^{-5}–10.4*10^{-5})$ $\beta = 7.5*10^{-5}$ SE $3.7*10^{-5}$, $p = 0.04$ $(0.3*10^{-5}–14.7*10^{-5})$ $\beta = 3.6*10^{-3}$ SE $2.1*10^{-3}$, $p = 0.09$ $(–0.5*10^{-3}–7.7*10^{-3})$ $\beta = 3.8*10^{-3}$ SE $3.7*10^{-3}$, $p = 0.40$ $(–3.5*10^{-3}–11.0*10^{-3})$	Age, year of birth, race, plant, years since hire, DMDTC	Lymphoid neoplasms associated with butadiene ppm–years and myeloid neoplasms with butadiene peaks; neither trend significant after adjusting for covariates; DMDTC as a continuous variable not associated with leukaemia; risk estimates for quartiles of exposure to DMDTC significantly increased without monotonic trend.

CI, confidence interval; DMDTC, dimethyldithiocarbamate; ICD, International Classification of Diseases; NR, not reported; SE, standard error; SMR, standardized mortality ratio; TWA, time-weighted average

[a] 99.9% confidence interval

haematopoietic cancers occurred (SMR, 1.8; 95% CI, 0.7–3.6), including four cases of lymphosarcoma and reticulosarcoma (SMR, 5.8; 95% CI, 1.6–14.8). Three cases had a duration of employment of 2 years or more (SMR, 8.3; $p < 0.05$). Two cases of leukaemia (SMR, 1.2; 95% CI, 0.2–4.4) also occurred. A non-significant excess of mortality from stomach cancer was observed (SMR, 2.4; 95% CI, 0.8–5.7). All five cases of stomach cancer occurred among the subset of workers who had been employed in the 'Rubber Reserve' plant for 2 years or more (SMR, 6.6; 95% CI, 2.1–15.3).

The mortality of a cohort of workers who manufactured butadiene monomer in Texas, USA (Downs *et al*., 1987), has been investigated repeatedly with updated and extended follow-up (Divine, 1990; Divine *et al*., 1993; Divine & Hartman, 1996). The latest available update, that included 5 additional years of follow-up up to 31 December 1999, was reported by Divine and Hartman (2001). The cohort at that time included 2800 male workers (of whom 216 were non-white and 10 were of unknown race) who had been employed for at least 6 months between 1943 and 1996. Exposure assessment was based on job history and industrial hygiene sampling data for the years after 1981. Each job was assigned a score for exposure to butadiene that took into account calendar period and type of operation. No information was reported on exposure to chemicals other than butadiene. The number of workers lost to follow-up was 192 (6.7%), all but 17 (< 1%) of whom were known to be alive at the end of 1998. A total of 1422 deaths were identified through to 1999, and death certificates were obtained for all but 19 (1.3%) of the deaths. SMRs were calculated using mortality rates for the US population as the reference. The SMR for all causes of death was 0.9 (95% CI, 0.8–0.9) and that for all cancers (333 deaths) was 0.9 (95% CI, 0.8–1.0). Fifty deaths from lymphatic and haematopoietic cancers (International Classification of Diseases [ICD]-8, 200–209; SMR, 1.4; 95% CI, 1.1–1.9), nine deaths from lymphosarcoma and reticulosarcoma (ICD-8, 200; SMR, 2.0; 95% CI, 0.9–3.9), 19 deaths from non-Hodgkin lymphoma (ICD-8, 200, 202; SMR, 1.5; 95% CI, 0.9–2.3), four deaths from Hodgkin lymphoma (ICD-8, 201; SMR, 1.6; 95% CI, 0.4–4.1), 18 deaths from leukaemia (ICD-8, 204–207; SMR, 1.3; 95% CI, 0.8–2.0), seven deaths from multiple myeloma (ICD-8, 203; SMR, 1.3; 95% CI, 0.5–2.6) and 18 deaths from cancer of other lymphatic tissue (ICD-8, 202, 203, 208; SMR, 1.3; 95% CI, 0.8–2.1) were observed. However, the latter category overlapped with non-Hodgkin lymphoma and multiple myeloma. The SMRs for the lymphatic and haematopoietic cancers did not increase with length of employment. Analysis by date of employment showed an increased risk for lymphatic and haematopoietic cancers among those first employed between 1942 and 1949. A separate mortality analysis for non-whites showed lower than expected mortality for all malignant neoplasms (17 observed, 19 expected) and a single death from lymphatic and haematopoietic cancer. Subcohort analyses were made for groups that were classified as having background, low and varied exposure. The background-exposure group included persons in offices, transportation, utilities and warehouses; the low-exposure group had spent some time in operating units; and the varied-exposure group included those with greatest potential exposure in operating units, laboratories and maintenance. In the background-exposure group, four deaths from lymphatic and haematopoietic cancers

(ICD-8, 200–209) were observed among those employed for < 5 years (SMR, 1.9; 95% CI, 0.5–4.7) and four among those exposed for > 5 years (SMR, 1.7; 95% CI, 0.5–4.3). Eleven deaths from lymphatic and haematopoietic cancers (ICD-8, 200–209) were observed in the low-exposure group, seven of which were among those with < 5 years of employment (SMR, 0.9; 95% CI, 0.4–1.9) and four among those employed for > 5 years (SMR, 0.6; 95% CI, 0.2–1.6). In the varied-exposure group, with the highest potential for exposure to butadiene, 34 deaths from lymphatic and haematopoietic cancers (ICD-8, 200–209) were observed, 20 of which were among those employed for < 5 years (SMR, 1.8; 95% CI, 1.1–2.8) and 14 among those exposed for > 5 years (SMR, 1.5; 95% CI, 0.8–2.5). In all groups, the SMRs for lymphatic and haematopoietic cancer decreased with duration of employment. For lymphosarcoma and reticulosarcoma, two deaths occurred in the low-exposure group (one among those employed < 5 years and one among those employed > 5 years) and seven deaths were observed in the varied-exposure group, five of which were among those employed for < 5 years (SMR, 3.7; 95% CI, 1.2–8.7) and two among those employed for > 5 years (SMR, 1.87; 95% CI, 0.23–6.76). Three deaths from leukaemia occurred (SMR, 0.7; 95% CI, 0.1–2.0) in the low-exposure subgroup and 13 cases were observed in the varied-exposure group, eight of which were among those employed for < 5 years (SMR, 1.9; 95% CI, 0.8–3.7) and five among those employed > 5 years (SMR, 1.4; 95% CI, 0.4–3.2). Six deaths from non-Hodgkin lymphoma were observed in the low-exposure group, four among short-term employees (SMR, 1.5; 95% CI, 0.4–3.8) and two among those employed for > 5 years (SMR, 0.9; 95% CI, 0.1–3.2), and 12 deaths occurred in the varied-exposure group, eight of which were among those employed for < 5 years (SMR, 2.0; 95% CI, 0.9–3.9) and four among long-term employees (SMR, 1.1; 95% CI, 0.3–2.9). The 'varied-exposure' group with high potential for exposure to butadiene showed elevated SMR estimates for all subcategories of lymphatic and haematopoietic cancers, but the increase was statistically significant only for lympho/reticulosarcoma among those employed for < 5 years. Slightly elevated SMRs were also found in the low-exposure group for cancer of the kidney (three cases; SMR, 1.6; 95% CI, 0.3–4.6; and three cases; SMR, 1.9; 95% CI, 0.4–5.4; among short- and long-term employees, respectively). In the varied-exposure group, a suggestive excess incidence of kidney cancer was only present among those employed for > 5 years (four cases; SMR, 1.65; 95% CI, 0.45–4.22). Survival analysis by Cox regression was carried out using a cumulative exposure score as a time-dependent explanatory variable for all lymphohaematopoietic neoplasms, non-Hodgkin lymphoma and leukaemia. None of these cancers was significantly associated with the cumulative exposure score. The elevated risk for all the lymphohaematopoietic cancers and their subcategories occurred among persons who were first employed before 1950. [The Working Group noted that although there was no evidence of an exposure–response relationship, it is probable that many workers during the years of the Second World War would have had short but relatively intense exposures, and thus duration of exposure may not be the most relevant dose metric.]

Another relatively small retrospective mortality study, together with a prospective morbidity survey, was performed on male employees at the Shell Deer Park Manufacturing Complex in the USA (Cowles *et al*., 1994) and was updated with a 9-year extension of the follow-up (Tsai *et al*., 2001). Butadiene monomer production took place in the facility between 1941 and 1948 and from 1970 onwards. The cohort comprised 614 eligible male employees who had worked for 5 years or more in jobs that entailed potential exposure to butadiene. Also eligible were employees who had worked for at least half of their total duration of employment during 1948–89 in a job that entailed potential exposure to butadiene (with a minimum 3-month period in such jobs). Female employees were excluded because of the small number (35) who met the eligibility criteria. Industrial hygiene data from 1979 to 1992 showed that few exposures to butadiene exceeded 10 ppm [22 mg/m^3] as an 8-h TWA and that most were below 1 ppm [2.2 mg/m^3]; the arithmetic mean exposure was 3.5 ppm [7.7 mg/m^3]. Only one study member had unknown vital status at the end of the follow-up. Person–years were accrued after 1 April 1948 from the time that a person first met the eligibility criteria. Death certificates were obtained for all known decedents. SMRs adjusted for age, race and calendar year were calculated using county-specific mortality rates as the reference. Six hundred and fourteen cohort members contributed a total of 12 391 person–years during the expanded study period, during which 61 deaths were identified. The SMR for all causes of death was 0.55 (95% CI, 0.42–0.70) and that for all malignant neoplasms was 0.6 (16 deaths; 95% CI, 0.3–0.9). Eight deaths were due to lung cancer (SMR, 0.7; 95% CI, 0.2–3.1) and three to cancer of the lymphatic and haematopoietic tissues (SMR, 1.1; 95% CI, 0.3–1.5). No deaths from leukaemia were observed, whereas one death was expected. The morbidity study included 289 of the 614 cohort members who were actively employed at some time between 1 January 1992 and 31 December 1998. The morbidity experience of this group was compared with that of an internal comparison group of 1386 active employees during the same period who had had no exposure to butadiene. A morbidity event was defined as an absence from work of > 5 days during 1992–98 that resulted from a specific diagnosed disorder. No meaningful differences in morbidity events between the butadiene-exposed and unexposed employees in the rest of the Shell Deer Park Manufacturing Complex were observed. [The Working Group noted that one criteria for inclusion in the cohort (at least half of total employment during 1948–89 in a potentially exposed job) was a potential source of bias, and that the SMR for all causes was unusually low.]

2.2.2 *Styrene–butadiene rubber production*

The 9-year mortality experience of a cohort of 6678 male rubber workers from a single, large tyre manufacturing plant in Ohio, USA, approximately 4% of whom worked in the manufacture of synthetic rubber, was investigated during 1964–72 (McMichael *et al*., 1974, 1976). Death rates from various specific causes were increased and included lymphatic and haematopoietic cancers in general (43 observed deaths; SMR, 1.36),

lymphosarcoma and Hodgkin lymphoma (15 observed; SMR, 1.64) and leukaemia (17 observed; SMR, 1.26). A case–cohort study was nested within the cohort to investigate the association of excesses of mortality with specific jobs within the rubber industry to compare workers who died from cancers in the 10-year period 1964–73 with a sample of members of the whole cohort and to elucidate differences in work histories (McMichael *et al*., 1976). A 6.2-fold increase in risk for lymphatic and haematopoietic cancers (99.9% CI, 4.1–12.5) and a 3.9-fold increase in risk for lymphatic leukaemia (99.9% CI, 2.6–8.0) were found in association with more than 5 years of work in manufacturing units that produced mainly styrene–butadiene rubber during 1940–60. [The Working Group noted that no information was provided on exposure to specific substances including potentially confounding chemicals such as benzene.]

Meinhardt *et al*. (1982) studied the mortality experience of white male workers who had been employed for at least 6 months in a two-plant complex styrene–butadiene rubber facility in the USA. A total of 1662 workers employed in Plant A between 1943 and 1976 and 1094 workers employed in Plant B between 1950 and 1976 were followed up through to 31 March 1976. Nine deaths from cancer of the lymphatic and haematopoietic tissues (ICD-7, 200–205) were seen in workers in Plant A (SMR, 1.6). The SMR among those from Plant A who were first employed between January 1943 and December 1945 was 2.1. Five deaths from leukaemia (ICD-7, 204) were observed in Plant A among workers employed between 1943 and 1945 (SMR, 2.8). In Plant B, two deaths from lymphatic and haematopoietic neoplasms (one lymphosarcoma/reticulosarcoma and one leukaemia) were observed.

Matanoski *et al.* (1990) investigated mortality patterns from 1943 (synthetic rubber production began in 1942) through to 1982 among 12 113 employees at styrene–butadiene rubber plants in Canada and the USA who had previously been followed up through to 1979 by Matanoski and Schwartz (1987). Overall, there were no increases in mortality from lymphatic and haematopoietic cancers. When employees were classified according to their longest-held job, production workers (presumed by the authors to be those with highest exposures to butadiene) had a significant excess of 'other lymphatic cancer' (nine deaths; SMR, 2.60; 95% CI, 1.19–4.94). When mortality among production workers was examined by race, a significant excess for leukaemia was seen in blacks (three deaths; SMR, 6.56; 95% CI, 1.35–19.06). Of 92 deaths among black production workers, six were from all lymphohaemopoietic cancers (SMR, 5.07; 95% CI, 1.87–11.07).

Nested case–control studies were conducted within the styrene–butadiene rubber cohort in the USA and Canada (Santos-Burgoa *et al.*, 1992; Matanoski *et al*., 1997). In the study by Santos-Burgoa *et al*. (1992), 59 cases of lymphatic and haematopoietic cancer in male workers (1943–82) were matched to 193 controls by plant, age, year of hire, duration of employment and survival to time of death of the case. Each job was assigned an estimated rank of exposure to butadiene and styrene, and cumulated exposure for each worker was calculated using employment histories. A strong association was identified for both butadiene (odds ratio, 9.4; 95% CI, 2.1–22.9) and styrene (odds ratio, 3.1; 95% CI, 0.8–11.2). After controlling for the other exposure, the odds ratio for

exposure to butadiene remained high and significant (odds ratio, 7.4; 95% CI, 1.3–41.3) whereas the relative risk estimate for styrene was approximately unity (odds ratio, 1.1; 95% CI, 0.23–4.95).

Matanoski *et al.* (1997) conducted a second case–control study that was nested in the styrene–butadiene cohort and included as cases most of the same lymphatic and haematopoietic cancer decedents studied by Santos-Burgoa *et al.* (1992). In this study, hospital records obtained for 55 of the 59 cases studied by Santos-Burgoa *et al.* (1992) were reviewed to confirm death certificate reports of lymphatic and haematopoietic cancer. The review confirmed all leukaemias, eliminated two cases and added one case of non-Hodgkin lymphoma and confirmed all cases of Hodgkin lymphoma and multiple myeloma. The final case groups included 58 total lymphatic and haematopoietic cancers, 12 non-Hodgkin lymphomas (seven lymphosarcomas and five other non-Hodgkin lymphomas), eight Hodgkin lymphomas, 26 leukaemias and 10 multiple myelomas. Controls were 1242 employees who were chosen to reflect the distribution of the cohort by plant and age, who had to have lived at least as long as cases and who represented approximately 1% of the cohort, but were not matched individually to cases. Quantitative exposure estimates for butadiene and styrene were developed by using exposure measurements for work areas and jobs, when available, and a modelling procedure to obtain estimates for jobs that had no measurements. Plant- and work area-specific exposure estimates were linked to work histories to obtain indices of cumulative exposure (ppm–months) and average intensity of exposure (ppm). Odds ratios for an average intensity of exposure of 1 ppm compared with 0 ppm and for ppm–months as a continuous variable were estimated using logarithmically transformed exposure data in unconditional logistic regression models that controlled for year of birth, period of hire, age at hire, race and length of employment. Leukaemia and Hodgkin lymphoma were associated positively with average intensity of exposure to butadiene (odds ratio at 1 ppm: leukaemia, 1.50; 95% CI, 1.07–2.10; Hodgkin lymphoma, 1.7; 95% CI, 0.99–3.02) and with ppm–months of exposure to butadiene. Non-Hodgkin lymphoma and multiple myeloma were associated positively with average intensity of exposure and cumulative exposure to styrene but not with indices of exposure to butadiene. Further analyses indicated that, in models that included both average intensity of exposure to butadiene and an indicator for longest employment in service, labour and laboratory work areas, both variables were statistically significantly, positively associated with leukaemia. Separate analyses of lymphoid leukaemia (10 cases) and myeloid leukaemia (15 cases) found that the average intensity of exposure to butadiene, but not work area, was significantly associated with lymphoid leukaemia. Average intensity of exposure to butadiene and work area were both associated positively with myeloid leukaemia, but the association was significant only for work area. Matanoski *et al.* (1997) suggested that misclassification of quantitative indices of exposure to butadiene could explain the latter results.

Delzell *et al*. (1996) and Sathiakumar *et al*. (1998) evaluated the mortality experience of 15 649 men (87% white and 13% black) who had been employed for at least 1 year at any of eight styrene–butadiene rubber plants in the USA and Canada and who had

worked in styrene–butadiene rubber-related operations in these plants. Seven of the plants had been studied previously by Matanoski and Schwartz (1987), Matanoski *et al*. (1990), Santos-Burgoa *et al.* (1992) and Matanoski *et al*.(1993, 1997); the two-plant complex studied earlier by Meinhardt *et al*. (1982) and Lemen *et al.* (1990) was also included. Complete work histories were available for 97% of the subjects: about 75% was exposed to butadiene and 83% was exposed to styrene. A list was developed to identify every combination of work area and job title, for a total of 8281 unique combinations. Using information from the plant on processes and operations and on jobs and tasks within each type of operation, 308 groups of work area were specified, and comprised processes and jobs that were considered to be similar. For analysis, these were further classified into five main process groups and seven process subgroups. During 1943–91, the cohort had a total of 386 172 person–years of follow-up and 734 individuals were lost to follow-up (5%). A total of 3976 deaths were observed compared with 4553 deaths expected on the basis of general population mortality rates for the USA or (for the Canadian subcohort) Ontario (SMR, 0.87; 95% CI, 0.85–0.90). Mortality from cancer was slightly lower than expected (950 deaths; SMR, 0.93; 95% CI, 0.87–0.99). Eleven lymphosarcomas were observed (SMR, 0.80; 95% CI, 0.40–1.40) and 42 'other lymphopoietic cancers' (SMR, 0.97; 95% CI, 0.70–1.52) which included 17 non-Hodgkin lymphomas, eight Hodgkin lymphomas, 14 multiple myelomas, one polycythaemia vera and two myelofibroses. In addition, 48 deaths from leukaemia were observed in the full cohort (SMR, 1.31; 95% CI, 0.97–1.74), including 45 among 'ever hourly paid' (86% of the cohort) subjects (SMR, 1.43; 95% CI, 1.04–1.91). The excess was fairly consistent across plants and was concentrated among 'ever hourly paid' subjects with 10 or more years of employment and 20 or more years since hire (28 deaths; SMR, 2.24; 95% CI, 1.49–3.23) and among polymerization workers (15 deaths; SMR, 2.51; 95% CI, 1.40–4.14), maintenance labourers (13 deaths; SMR, 2.65; 95% CI, 1.41–4.53) and laboratory workers (10 deaths; SMR, 4.31; 95% CI, 2.07–7.93). Polymerization workers and maintenance labourers had potentially high exposure to butadiene but only low-to-moderate exposure to styrene. Sathiakumar *et al*. (1998) reported that, among subjects with ≥ 10 years of employment and ≥ 20 years since hire, moderately non-significantly increased mortality from non-Hodgkin lymphoma was also apparent (15 deaths; SMR, 1.37; 95% CI, 0.77–2.26), but without any consistent pattern by duration of employment, time since hire, period of hire or process group. Mortality from other types/sites of cancer was not significantly elevated in this cohort.

Macaluso *et al*. (1996) reported an additional analysis, with more detailed exposure assessment, of mortality from leukaemia among 16 610 subjects (12 412 exposed to butadiene) employed at six of the eight North American styrene–butadiene rubber manufacturing plants investigated by Delzell *et al.* (1996) [14 295 workers were included but a further 2350 workers from the same plants who were not employed in styrene–butadiene rubber operations at those plants were not included in Delzell *et al*. (1996)]. A total of 418 846 person–years of follow-up through 1991 and 58 leukaemia deaths, seven of which were reported to be the contributory ('underlying') cause of death, were included only in analyses that used internal comparisons. Retrospective quantitative estimates of

exposure to butadiene, styrene and benzene were developed and the estimation procedure identified work areas within each manufacturing process, historical changes in exposure potential and specific tasks that involved exposure. Mathematical models were then used to calculate job- and time period-specific average exposures. The resulting estimates were linked with work histories to obtain cumulative exposure estimates that were employed in stratified and Poisson regression analyses of mortality rates. Mantel-Haenszel rate ratios adjusted by race, age and cumulative exposure to styrene increased with cumulative exposure to butadiene from 1.0 in the unexposed category through to 2.0, 2.1, 2.4 and 4.5 in the estimated exposure categories 0, < 1, 1–19, 20–79 and ≥ 80 ppm–years, respectively (p for trend = 0.01). The trend of increasing risk with exposure to butadiene was still significant after exclusion of the unexposed category ($p = 0.03$). The risk pattern was less clear and statistically non-significant for exposure to styrene (rate ratios, 0.9, 5.4, 3.4 and 2.7 in the estimated exposure categories of < 5, 5–9, 10–39 and ≥ 40 ppm–years, respectively; p for trend = 0.14) and the association with benzene disappeared after controlling for exposure to butadiene and styrene.

Irons and Pyatt (1998) suggested that apparently different patterns of risk for leukaemia between workers employed in butadiene monomer production and those involved in styrene–butadiene rubber production might be linked to a class of chemicals with haematotoxic and immunotoxic potential (dithiocarbamates) that were present in styrene–butadiene rubber but not butadiene monomer production. In particular, they suggested that dimethyldithiocarbamate, which was used between the early 1950s and 1965 in the majority of styrene–butadiene rubber plants as a stopping agent in the cold polymerization reaction, might confound an association between butadiene and leukaemia in exposed workers.

A further analysis was then conducted among the North American synthetic rubber industry workers to evaluate the relative relevance of butadiene, styrene and dimethyldithiocarbamate in the statistically significantly increased risk for mortality from leukaemia (Delzell *et al*., 2001). The analysis included 13 130 men who had been employed for at least 1 year during 1943–91 at any of six synthetic rubber plants (of the eight previously studied by Delzell *et al*. (1996) and Sathiakumar *et al*. (1998)) that had sufficient information for quantitative exposure estimation. Revised exposure estimates for butadiene and styrene and new quantitative estimates for dimethyldithiocarbamate were developed (Macaluso *et al*., 2004). Quantitative estimates of cumulative exposure were obtained by (*a*) identifying work area/job groups ('jobs') that consisted of homogeneous work activities; (*b*) identifying the tasks that comprised each job; (*c*) identifying historical changes in exposure potential for each task; (*d*) computing time period-specific average exposure concentrations in parts per million for tasks and jobs; (*e*) compiling the job-specific estimates into a job–exposure matrix for each plant; and (*f*) linking the resulting job–exposure matrices with work histories to obtain cumulative exposure estimates. The job–exposure matrix contained plant-specific estimates of 8-h TWA exposure concentrations for butadiene and other chemicals for each job and for each year from 1943 through to 1991. Mathematical models were used to calculate plant-, task-,

work area/job group- and time period-specific average exposures. The revised TWA exposure estimates, compared with the original estimates, were about four to six times higher for butadiene and two times higher for styrene. Estimates of the annual number of peaks of exposure in each job (i.e. for each job, the number of component tasks in which intensity exceeded 100 ppm for butadiene and 50 ppm for styrene) were also computed. Vital status at the end of 1991 was known for over 99% of subjects. A total of 234 416 person–years of observation (average, 18 person–years per subject) were accrued during the follow-up period and included in the analysis. Information from death certificates was available for 3813 (98%) of 3892 decedents; the death certificates of 58 decedents mentioned leukaemia as the underlying or contributing cause of death. For 48 of these, medical records confirmed that they had had leukaemia; for 10, medical records were not available and these were retained in the case group. An additional decedent had medical records that indicated leukaemia but the death certificate mentioned myelodysplasia: he was added to the case group (total, 59 decedents). Poisson regression analysis was used to estimate relative rates of mortality from leukaemia and their 95% CIs for a particular agent/exposure category compared with the category of workers who were unexposed or had low exposure; regression models for each agent took into account the level of exposure, age, years since hire and, in some models, co-exposure to one or both of the other chemicals. In some analyses, exposure was lagged under the assumption that exposures that occurred within 5 or 10 years before death were etiologically irrelevant. Mortality from leukaemia showed a consistently positive association with increasing exposure to butadiene (relative rate, 1.0; 1.2; 95% CI, 0.5–3.0; 2.0; 95% CI 0.8–4.8; and 3.8; 95% CI, 1.6–9.1; for exposure to 0, 0–86.3, 86.3–362.2 and ≥ 362.2 ppm–years, respectively) [p for trend < 0.001] and to styrene (relative rate, 1.0; 1.2; 95% CI, 0.5–3.3; 2.3; 95% CI, 0.9–6.2; and 3.2; 95% CI, 1.2–8.8; for exposure to 0, 0–20.6, 20.6–60.4 and ≥ 60.4 ppm–years, respectively) [p for trend = 0.001]. Exposure to dimethyldithiocarbamate also showed consistently increased relative rates, but with no monotonic trend. After controlling for exposure to styrene and dimethyldithiocarbamate as well as for age and years since hire, exposure to butadiene remained consistently but not statistically significantly associated with increasing mortality from leukaemia (relative rate, 1.0; 1.3; 95% CI, 0.4–4.3; 1.3; 95% CI, 0.4–4.6; and 2.3; 95% CI, 0.6–8.3) [p for trend = 0.250].

When exposure to total peaks (> 100 ppm for butadiene and > 50 ppm for styrene) was used in the analysis, a positive association of exposure to butadiene and styrene with mortality from leukaemia was again apparent; adjustments for other agents made the associations irregular and imprecise. Lagging for a 5- or 10-year exposure period did not alter the results materially. Models were also run to estimate relative rates for leukaemia for exposures to butadiene calculated at intensities of < 100 ppm and > 100 ppm. In the former analysis, a statistically non-significant trend of increasing relative rate with increasing number of butadiene ppm–years was found (relative rate, 1.0; 1.1; 95% CI, 0.5–2.7; 2.8; 95% CI, 1.2–6.8; and 3.0; 95% CI, 1.2–7.1; for exposure to 0, 0–37.8, 37.8–96.3 and ≥ 96.3 ppm–years, respectively; p for trend = 0.25), whereas the trend for exposure to > 100 ppm was statistically significant (relative rate, 1.0; 2.1; 95% CI, 0.9–

5.1; 2.8; 95% CI, 1.2–6.7; and 5.8; 95% CI, 2.4–13.8; for exposure to 0, 0–46.5, 46.5–234.3 and ≥ 234.3 ppm–years, respectively; *p* for trend = 0.01). Analyses based on quartiles or quintiles of exposure yielded patterns of results similar to those seen in the analyses based on tertiles shown above. Exposure to butadiene and dimethyldithiocarbamate was further categorized to study interaction, but no clear interaction between the two agents was apparent. The analysis confirmed a significant, although weaker, association with exposure to butadiene even after controlling for dimethyldithiocarbamate: the relative rate for leukaemia for exposure to 0–38.7, 38.7–287.3 and ≥ 287.3 ppm–years of butadiene was, respectively, 1.0, 1.5 (95% CI, 0.8–2.9) and 3.4 (95% CI, 1.8–6.4) when unadjusted for exposure to dimethyldithiocarbamate (*p* for trend = 0.0001) and 1.0, 1.0 (95% CI, 0.5–2.1) and 2.0 (95% CI, 0.9–4.3) when adjusted for exposure to dimethyldithiocarbamate (*p* for trend = 0.007). Dimethyldithiocarbamate was associated with a non-monotonically increasing risk and the trend was statistically non-significant after controlling for butadiene. The relative rate for exposure to 0–342.4, 342.4–1222.6 and ≥ 1222.6 mg–years/cm of dimethyldithiocarbamate was, respectively, 1.0, 3.6 (95% CI, 1.9–6.7) and 2.9 (95% CI, 1.5–5.3) when unadjusted for exposure to butadiene (*p* for trend = 0.003) and 1.0, 3.2 (95% CI, 1.6–6.3) and 2.1 (95% CI, 1.0–4.4) when adjusted for exposure to butadiene (*p* for trend = 0.196). A similar analysis was conducted for co-exposure to butadiene and styrene. The marginal trend was consistent and statistically significant for exposure to butadiene adjusted for styrene (*p* for trend = 0.006), but not for styrene adjusted for butadiene (*p* for trend = 0.763). Analyses by specific subgroups of leukaemia were conducted but sparse data rendered the results largely uninformative. [The Working Group noted the difficulties in estimating exposures to dimethyldithiocarbamate, which are primarily dermal, and that substantial misclassification of exposure to this chemical was possible. Furthermore, the assessment of exposure to dimethyldithiocarbamate was performed with the knowledge of which departments had excess mortality from leukaemia and could conceivably have been biased by this knowledge.]

Mortality from lymphatic and haematopoietic cancer in the updated North American cohort was studied in relation to exposure to butadiene, styrene and dimethyldithiocarbamate (Graff *et al*., 2005). Two of the US plants were not included in this analysis because information on work area/job group was not sufficient for quantitative exposure estimation for all substances. Included were 16 579 men who had worked at any of the six study plants for at least 1 year by the end of 1991 and who were followed up between 1944 and 1998. All work histories and exposure data came from the previous study of Delzell *et al*. (2001), and exposure estimation procedures were those described by Macaluso *et al*. (2004). Information on vital status through to 1998 was established for 97% of the study group. Cause of death was ascertained through death certificates, the national death index and a search of medical records. Most analyses used Poisson regression models to obtain maximum likelihood estimates of the relative rate for the contrast between categories of one agent, adjusting for other agents and for additional potential confounders. SMR analyses by level of exposure to the agent were also made

using state-specific US and Canadian male mortality rates as the comparison group and data from death certificates and the national death index only to determine causes of death. During the observation period, 500 174 person–years were accrued. Based on a review of medical records where possible, 81 deaths from leukaemia, 58 from non-Hodgkin lymphoma, 27 from multiple myeloma and 13 from Hodgkin disease were ascertained. Single-agent analyses, adjusting for age and years since hire, indicated a positive association between exposure to butadiene and leukaemia (relative risk, 1.0; 1.4; 95% CI, 0.7–3.1; 1.2; 95% CI, 0.6–2.7; 2.9; 95% CI, 1.4–6.4; and 3.7; 95% CI, 1.7–8.0; for exposure to 0, 0–33.7, 33.7–184.7, 184.7–425.0 and ≥ 425.0 ppm–years, respectively) [*p* for trend < 0.001] and between exposure to styrene and leukaemia (relative risk, 1.0, 1.3; 95% CI, 0.6–3.2; 1.6; 95% CI, 0.7–3.9; 3.0; 95% CI, 1.2–7.1; and 2.7; 95% CI, 1.1–6.4; for exposure to 0, 0–8.3, 8.3–31.8, 31.8–61.1 and ≥ 61.1 ppm–years, respectively) [*p* for trend = 0.001]. Exposure to dimethyldithiocarbamate was also positively associated, but with no monotonic increase (relative risk, 1.0; 2.5; 95% CI, 1.2–5.0; 3.0; 95% CI, 1.5–5.9; 4.9; 95% CI, 2.5–9.7; and 2.7; 95% CI, 1.4–5.4; for exposure to 0, 0–185.3, 185.3–739.4, 739.4–1610.3 and ≥ 1610.3 mg–years/cm, respectively) [*p* for trend = 0.001]. Similar results were obtained for butadiene and styrene when total peaks were used as exposure metrics. Exposures to butadiene, styrene and dimethyldithiocarbamate were found to be highly correlated. In the models that also controlled for other agents, the estimated relative rates for the increasing categories of butadiene ppm–years were 1.0, 1.4 (95% CI, 0.5–3.9), 0.9 (95% CI, 0.3–2.6), 2.1 (95% CI, 0.7–6.2) and 3.0 (95% CI, 1.0–9.2) [*p* for trend = 0.028]; for styrene, the association appeared to be negative [*p* for trend = 0.639]; and for dimethyldithiocarbamate, a positive association was still apparent with no monotonic exposure–response pattern (relative risk, 1.0, 2.6; 95% CI, 1.2–5.8; 3.1; 95% CI, 1.4–7.1; 4.4; 95% CI, 1.9–10.2; and 2.0; 95% CI, 0.8–4.8) [*p* for trend = 0.066]. Individual adjustment for one or both of the other main exposure factors yielded similar results: the positive increasing association between exposure to butadiene and leukaemia was slightly reduced; no association remained for styrene; dimethyldithiocarbamate still exhibited a positive association with leukaemia but with no dose–response. Leukaemia was positively associated with butadiene ppm–years accrued at exposure intensities > 100 ppm and < 100 ppm. There was no evidence of an interaction between butadiene and dimethyldithiocarbamate or between butadiene and styrene. Analyses by histological type clearly showed an association of butadiene ppm–years with chronic lymphocytic leukaemia (relative risk, 1.0; 1.5; 95% CI, 0.6–4.0; 3.9; 95% CI, 1.3–11.0) [*p* for trend = 0.014], chronic myelogenous leukaemia (relative risk, 1.0; 2.7; 95% CI, 0.7–10.4; 7.2; 95% CI, 1.7–30.5) [*p* for trend = 0.007] and other leukaemia (relative risk, 1.0; 1.1; 95% CI, 0.3–3.9; 4.0; 95% CI, 1.0–15.0) [*p* for trend = 0.060] for exposures to 0–33.7, 33.7–425.0 and ≥ 425.0 ppm–years, respectively. No associations were found for non-Hodgkin lymphoma, multiple myeloma or acute myelogenous leukaemia. Results for a case series comprised of chronic lymphocytic leukaemia and non-Hodgkin lymphoma combined were similar to those for non-Hodgkin lymphoma alone (Delzell *et al*., 2006). External analyses that compared mortality rates of workers in each cumulative exposure category

to the rate in the general population and controlled for age, race and calendar year provided consistent results.

The mortality follow-up of North American synthetic rubber industry workers who had been employed for at least 1 year before 1 January 1992 at any of seven US and one Canadian styrene–butadiene rubber plants was later extended for a further 7 years from 1991 through to the end of 1998 (Sathiakumar et al., 2005) and included 17 924 subjects (15 583 white and 2341 non-white). Work histories provided information on each job held, a description of the work area and job and payroll classification. Analyses of data by work area were restricted to 15 612 subjects who had been employed in styrene–butadiene rubber-related operations in the eight plants and classified into five major work areas: production, maintenance, labour, laboratories and other operations. For six of the eight plants (a total of 14 273 subjects), work histories were sufficiently detailed to permit further specification of three subgroups of work area in production, two in maintenance and two in labour. Vital status was updated through to 1998 and ascertained for 97% of the study group. In total, 570 (3%) subjects were lost to follow-up. A total of 540 586 person–years of observation were accrued. SMRs were calculated using mortality rates of the male population in Ontario (Canada) and in the three US states where the plants were located. The update added 83 401 person–years (18% increase), 1578 deaths (34%), 492 deaths from cancer (44%) and 20 deaths from leukaemia (39%) to those of the previous study. Mortality from all causes was lower than that expected (6237 deaths; SMR, 0.86; 95% CI, 0.84–0.88) as was mortality from all cancers (1608 deaths; SMR, 0.92; 95% CI, 0.88–0.97). Mortality from lymphatic and haematopoietic cancer was slightly elevated (162 deaths; SMR, 1.06; 95% CI, 0.90–1.23); 12 deaths from Hodgkin lymphoma yielded an SMR of 1.11 (95% CI, 0.58–1.95) and 71 deaths from leukaemia represented a modest excess above expectation (SMR, 1.16; 95% CI, 0.91–1.47). Mortality from non-Hodgkin lymphoma was as expected (53 deaths; SMR, 1.00; 95% CI, 0.75–1.30) and that from multiple myeloma was lower than expected (26 deaths; SMR, 0.95; 95% CI, 0.62–1.40). No consistent patterns were observed when mortality was analysed by ever/never hourly paid, by years since hire or by years worked. In the overall study group, the excess mortality from leukaemia was concentrated among men who had been hired in the 1950s (31 deaths; SMR, 1.50; 95% CI, 1.01–2.11). The excess mortality from all leukaemias was highest among hourly paid workers (63 deaths; SMR, 1.23; 95% CI, 0.94–1.57), especially among those who had 20–29 years since hire and ≥ 10 years worked (19 deaths; SMR, 2.58; 95% CI, 1.56–4.03). Analysis by specific histological type was possible for 65 of the 71 leukaemias and for the 1968–98 time period only. For all subjects included in this analysis, the SMR for all forms of leukaemia (65 deaths) was 1.26 (95% CI, 0.97–1.61), that for lymphocytic leukaemia (19 deaths) was 1.28 (95% CI, 0.77–2.00) and that for myelogenous leukaemia (28 deaths) was 1.27 (95% CI, 0.84–1.83). In the SMR analysis restricted to 1968–98, hourly paid workers had an overall SMR for leukaemia (65 deaths) of 1.35 (95% CI, 1.03–1.75), and the excess mortality was particularly marked among those who had 20–29 years since hire and ≥ 10 years worked (SMR, 2.84; 95% CI, 1.68–4.49); a non-significant increase was seen for

lymphocytic leukaemia (four deaths; SMR, 2.33; 95% CI, 0.65–5.97), whereas the excess for myelogenous leukaemia was significant (nine deaths; SMR, 3.20; 95% CI, 1.46–6.07) and was stronger for the chronic myelogenous form (six deaths; SMR, 6.55; 95% CI, 2.40–14.26). Analysis by production work areas in the whole cohort, with follow-up between 1944 and 1998, showed an association between mortality from leukaemia and working in the following areas: polymerization (18 deaths; SMR, 2.04; 95% CI, 1.21–3.22), coagulation (10 deaths; SMR, 2.31; 95% CI, 1.11–4.25) and finishing (19 deaths; SMR, 1.56; 95% CI, 0.94–2.44); in maintenance labour (15 deaths; SMR, 2.03; 95% CI, 1.14–3.35); and in laboratories (14 deaths; SMR, 3.26; 95% CI, 1.78–5.46). The excesses of mortality in production were mainly due to chronic lymphatic leukaemia: polymerization (eight deaths; SMR, 4.97; 95% CI, 2.15–9.80), coagulation (five deaths; SMR, 6.07; 95% CI, 1.97–14.17) and finishing (seven deaths; SMR, 3.44; 95% CI, 1.38–7.09), whereas mortality from myelogenous leukaemia was particularly high in maintenance labour (acute, five deaths; SMR, 2.95; 95% CI, 0.96–6.88) and in laboratory workers (total, six deaths; SMR, 3.31; 95% CI, 1.22–7.20; chronic, three deaths; SMR, 5.22; 95% CI, 1.08–15.26).

Cheng *et al.* (2007) used Cox proportional hazard models on the set of data analysed by Graff *et al*. (2005) to examine further the exposure–response relations between several butadiene exposure indices and leukaemia (81 decedents), and to assess exposure–response relations between butadiene and all lymphoid neoplasms (120 decedents from lymphatic leukaemia, non-Hodgkin lymphoma, Hodgkin lymphoma and multiple myeloma) and all myeloid neoplasms (56 decedents from myeloid and monocytic leukaemia, myelofibrosis, myelodysplasia, myeloproliferative disorders and polycythemia vera). Cox regression techniques were considered to permit estimation of the exposure–response relations throughout the exposure range, to provide optimal control of confounding by age and to be less affected by intercorrelations among exposure variables. A subset of 488 subjects was excluded because they dropped out from follow-up at ages below that of the youngest leukaemia decedent. Potential confounders for which the analyses controlled included dimethyldithiocarbamate, race, plant, years since hire and year of birth. The butadiene exposure indices used in these analyses were: cumulative exposure in ppm–years, total number of exposures to peaks (> 100 ppm) and average intensities of exposure in parts per million. All three exposure indices were associated positively with the risk for leukaemia. Penalized spline regression indicated that the natural logarithm of the hazard ratio for leukaemia increased in a fairly linear fashion in the exposure range below the 95th percentile for all three indices. Analysis by decile of exposure to butadiene showed an irregular pattern of estimated rate ratios. After controlling for all co-variates, estimated relative rates by decile range of values exhibited a non-monotonic trend that was of borderline statistical significance for ppm–years [p for trend = 0.049], but not for exposure to peaks [p for trend = 0.071] or average intensity of exposure [p for trend = 0.433]. Models that used continuous exposure variables indicated that, for butadiene ppm–years, the exposure–response relation with leukaemia was positive and statistically significant in all but two of the eight models evaluated. After

adjustment for all co-variates, the regression coefficient β that estimated the slope of the exposure–response relation was 3.0×10^{-4} (95% CI, 0.1×10^{-4}–5.8×10^{-4}; $p = 0.04$) in models that used continuous, untransformed ppm–years, 5.8×10^{-4} (95% CI, 0.5×10^{-4}–11.1×10^{-4}; $p = 0.03$) in models that used mean scored deciles and 6.1×10^{-4} ($p = 0.04$) in models that used mean scored quintiles. The exposure–response trend for butadiene peaks and leukaemia was positive and statistically significant in all eight models. The regression coefficient β was 5.6×10^{-5} (95% CI, 0.8×10^{-5}–10.4×10^{-5}; $p = 0.02$) in models that used continuous, untransformed butadiene peaks and 7.5×10^{-5} (95% CI, 0.3×10^{-5}–14.7×10^{-5}; $p = 0.04$) in models that used mean scored deciles of butadiene peaks, after controlling for all covariates. The association of average intensity of exposure to butadiene with leukaemia was statistically significant only in the model that used the square-root transformation of parts per million butadiene. Lagging of exposure had a small impact on the value of the coefficients for the three exposure variables. Lymphoid neoplasms were associated with the ppm–years exposure index and myeloid neoplasms with exposure to peaks. However, neither of the relations was statistically significant after control for covariates. Cumulative exposure to dimethyldithiocarbamate, when treated as a continuous variable, was not associated with leukaemia in any of the models. The risk estimates for each quartile of cumulative exposure to dimethyldithiocarbamate were significantly increased, even after adjustment for cumulative exposure to butadiene, without, however, a monotonic trend. Relative rate estimates were, respectively, 2.4 (95% CI, 1.2–5.0), 2.9 (95% CI, 1.4–5.8), 4.5 (95% CI, 2.2–8.9) and 2.1 (95% CI, 1.0–4.4) [p for trend = 0.458].

[The Working Group noted that, given the strong correlation between original and revised estimates (Spearman's $r = 0.9$) reported, the validation of the exposure estimates in one plant during 1977–91 (Sathiakumar & Delzell, 2007) showed that estimates from the most recent measurements were in general very close to measured exposures, especially for the styrene–butadiene rubber-related job titles. The ranking of jobs had hardly changed from the first estimates (Macaluso *et al.*, 1996) to the adjusted estimates (Macaluso *et al.*, 2004). However, it is uncertain to what extent this validation can be extrapolated to the other plants.]

2.2.3 *Other rubber production*

Bond *et al.* (1992) reported a mortality study of 2904 male workers engaged in the development and manufacture of styrene-based products, including styrene–butadiene latex, who were potentially exposed to styrene and related chemicals [including butadiene] for at least 1 year between 1937 and 1971. The number of person–years of follow-up during 1970–86 for workers in this production was 11 754. In comparison with USA mortality rates, the SMR for all causes of death among styrene–butadiene latex workers was 0.9, based on 82 deaths. A total of 13 cancers were observed (22.0 expected; SMR, 0.6), and no site had an SMR that exceeded unity. One death from lymphatic and haematopoietic cancers occurred, which was due to leukaemia (ICD-8 204–207) versus

0.9 expected. [The Working Group noted the limited information relating to exposure to butadiene.]

2.3 Population-based studies

The risk for lymphatic and haematopoietic cancers was evaluated among students of a high school in eastern Texas, USA, that was bound at the rear by facilities that had produced synthetic styrene–butadiene rubber since 1943 (Loughlin *et al.*, 1999). A cohort of 15 043 students who had attended the school for at least 3 consecutive months during a school year between 1963 and 1993 was constructed. In total, 338 graduates (241 men and 97 women) had died during the follow-up period of 1963–95, which were fewer than expected. The SMR for all lymphatic and haematopoietic cancer was 0.84 (95% CI, 0.74–0.85) for men (12 observed deaths) and 0.47 (95% CI, 0.06–1.70) for women (two observed deaths). The SMR was higher for 1530 men who had attended the school for ≤ 2 years than for the 6352 who had attended for ≥ 2 years. For the former group, the SMR for all lymphatic and haematopoietic cancer was 3.20 (95% CI, 0.87–8.20).

A population-based case–control study in the Montréal area, Canada (Parent *et al.*, 2000), assessed the association between renal-cell carcinoma and a large number of occupational exposures among men aged 35–70 years between 1979 and 1985. Cases were identified at all large hospitals in the area and were histologically confirmed; case ascertainment was 95% complete. Comparison was carried out with two sets of controls: subjects with other types of cancer and people selected from the general population. Questionnaires on cancer risk factors that included lifetime occupational history were administered. Relative risks were estimated by odds ratios from unconditional logistic regression models. In the analysis, 142 cases, 533 population controls and 1900 other cancer controls were available. The odds ratio for exposure to 'styrene–butadiene rubber' was 2.1 (10 exposed cases; 95% CI, 1.1–4.2) after controlling for age, family income, tobacco smoke and body mass index and 1.8 (95% CI, 0.9–3.7) after controlling for other occupational exposures. [The Working Group noted that it was unclear what was meant by exposure to styrene–butadiene rubber.]

3. Studies of Cancer in Experimental Animals

3.1 Inhalation exposure

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F$_1$ mice, 8–9 weeks of age, were exposed by whole-body inhalation to 625 or 1250 ppm [1380 or 2760 mg/m^3] butadiene (minimum purity, > 98.9%) for 6 h per day on 5 days per week for 60 weeks (males) or 61 weeks (females). Equal numbers of animals were sham-exposed and served as controls. The

study was terminated after 61 weeks because of a high incidence of lethal neoplasms in the exposed animals. Deaths were mainly due to malignant lymphomas. The numbers of survivors at 61 weeks were: males — 49/50 control, 11/50 low-dose and 7/50 high-dose; females — 46/50 control, 15/50 low-dose and 30/50 high-dose. As shown in Table 19, butadiene induced haemangiosarcomas that originated in the heart and metastasized to various organs. The incidence of haemangiosarcomas of the heart in historical controls was extremely low (1/2372 males, 1/2443 females). Other types of neoplasm for which the incidence was significantly increased (Fisher's exact test) in animals of each sex were malignant lymphomas, alveolar/bronchiolar adenomas or carcinomas of the lung and papillomas or carcinomas of the forestomach. Tumours that occurred with significantly increased incidence in females only included hepatocellular adenoma or carcinoma of the liver: 0/50 control, 2/47 ($p = 0.232$) low-dose and 5/49 ($p = 0.027$) high-dose; acinar-cell carcinoma of the mammary gland: 0/50 control, 2/49 low-dose and 6/49 ($p = 0.012$) high-dose; and granulosa-cell tumours of the ovary: 0/49 control, 6/45 ($p = 0.01$) low-dose and 12/48 ($p < 0.001$) high-dose. Gliomas were observed in the brain of one male mouse exposed to 1250 ppm and in two male mice exposed to 625 ppm butadiene (National Toxicology Program, 1984; Huff *et al*., 1985).

Table 19. Incidence of tumours in B6C3F$_1$ mice exposed to butadiene by inhalation at 625 and 1250 ppm for 61 weeks

	Male			Female		
	0	625 ppm	1250 ppm	0	625 ppm	1250 ppm
Haemangiosarcoma of heart (with metastases)	0/50	16/49 ($p < 0.001$)[a]	7/49 ($p = 0.006$)	0/50	11/48 ($p < 0.001$)	18/49 ($p < 0.001$)
Malignant lymphoma	0/50	23/50 ($p < 0.001$)	29/50 ($p < 0.001$)	1/50	10/49 ($p = 0.003$)	10/49 ($p = 0.003$)
Lung alveolar/bronchiolar adenoma or carcinoma	2/50	14/49 ($p < 0.001$)	15/49 ($p < 0.001$)	3/49	12/48 ($p = 0.01$)	23/49 ($p < 0.001$)
Forestomach papilloma or carcinoma	0/49	7/40 ($p = 0.003$)	1/44 ($p = 0.47$)	0/49	5/42 ($p = 0.018$)	10/49 ($p < 0.001$)

From National Toxicology Program (1984); Huff *et al.* (1985)
[a] *p* values from Fisher's exact test

Because of the reduced survival of mice in the initial study, further studies were conducted at lower exposure concentrations. In one study, groups of 70–90 male and 70–90 female B6C3F$_1$ mice, 6.5 weeks of age, were exposed by whole-body inhalation to 0, 6.25, 20, 62.5, 200 or 625 ppm [0, 14, 44, 138, 440 or 1380 mg/m^3] butadiene (purity, > 99%) for 6 h per day on 5 days per week for up to 2 years. Up to 10 animals per group were killed and evaluated after 40 and 65 weeks of exposure. Survival was significantly reduced ($p < 0.05$) in all groups of mice exposed to 20 ppm or higher; terminal survivors

were: males — 35/70 control, 39/70 at 6.25 ppm, 24/70 at 20 ppm, 22/70 at 62.5 ppm, 3/70 at 200 ppm and 0/90 at 625 ppm; females — 37/70 control, 33/70 at 6.25 ppm, 24/70 at 20 ppm, 11/70 at 62.5 ppm, 0/70 at 200 ppm and 0/90 at 625 ppm. As shown in Table 20, exposure to butadiene produced increases in both sexes in the incidence of lymphomas, heart haemangiosarcomas, lung alveolar/bronchiolar adenomas and carcinomas, forestomach papillomas and carcinomas, Harderian gland adenomas and adenocarcinomas and hepatocellular adenomas and carcinomas. The incidence of mammary gland adenocarcinomas and benign and malignant ovarian granulosa-cell tumours was increased in females. Lymphocytic lymphomas were seen as early as after 23 weeks of exposure and were the principal cause of death in male and female mice exposed to 625 ppm butadiene (Melnick *et al*., 1990; National Toxicology Program, 1993).

In the same study, a stop-exposure experiment was conducted in which groups of 50 male B6C3F$_1$ mice, 6.5 weeks of age, were exposed to butadiene (purity, > 99%) by whole-body inhalation for 6 h per day on 5 days per week at concentrations of 200 ppm [440 mg/m^3] for 40 weeks, 312 ppm [690 mg/m^3] for 52 weeks, 625 ppm [1380 mg/m^3] for 13 weeks or 625 ppm [1380 mg/m^3] for 26 weeks. The multiple of the exposure concentration and duration of exposure (ppm–weeks) was approximately 8000 ppm–weeks or 16 000 ppm–weeks for the exposure groups. After the exposures were terminated, the animals were placed in control chambers for up to 104 weeks after the beginning of treatment. A group of 70 males served as chamber controls (0 ppm). [The Working Group noted that this was the same control group as that used in the experiment described in the above paragraph.] Survival was reduced ($p < 0.05$) in all exposed groups; the numbers of survivors at the end of the study were 35 controls, nine exposed to 200 ppm, one exposed to 312 ppm, five exposed to 625 ppm for 13 weeks and none exposed to 625 ppm for 26 weeks. As shown in Table 21, exposure to butadiene produced increases in the incidence of lymphomas, heart haemangiosarcomas, lung alveolar/bronchiolar adenomas and carcinomas, forestomach papillomas and carcinomas, Harderian gland adenomas and adenocarcinomas, preputial gland carcinomas and kidney tubular adenomas. This exposure protocol revealed additional tumour sites in males (preputial gland and renal cortex). The incidence of lymphocytic lymphoma was greater after exposure to higher concentrations of butadiene for a short time than after exposure to lower concentrations for an extended period (Melnick *et al*., 1990; National Toxicology Program, 1993). Brain neoplasms including two neuroblastomas and three gliomas were observed in mice exposed to 625 ppm for 13 or 26 weeks in the stop-exposure study (National Toxicology Program, 1993). Brain neoplasms are rare and had never been seen in historical National Toxicology Program controls at the time of the study. [The Working Group noted that brain tumours are rare in mice and were observed, although at a low incidence, in both National Toxicology Program bioassays (National Toxicology Program, 1984, 1993).]

Follow-up studies were completed to test the hypothesis that the high incidence of lymphocytic lymphomas in mice exposed to concentrations of 200 ppm butadiene or higher was at least partially dependent on the activation of an endogenous retrovirus in

Table 20. Survival and incidence of tumours in mice exposed to butadiene by inhalation for up to 2 years

	Exposure concentration (ppm)					
	0	6.25	20	62.5	200	625
Males						
Initial number[a]	70	70	70	70	70	90
Number of survivors	35	39	24	22	3	0
Lymphoma	4 (8%)[b]	3 (6%)	8 (19%)	11 (25%)[c]	9 (27%)[c]	69 (97%)[c]
Heart haemangiosarcoma	0 (0%)	0 (0%)	1 (2%)	5 (13%)[c]	20 (57%)[c]	6 (53%)[c]
Lung alveolar/bronchiolar adenoma and carcinoma	22 (46%)	23 (48%)	20 (45%)	33 (72%)[c]	42 (87%)[c]	12 (73%)[c]
Forestomach papilloma and carcinoma	1 (2%)	0 (0%)	1 (2%)	5 (13%)	12 (36%)[c]	13 (75%)[c]
Harderian gland adenoma and adenocarcinoma	6 (13%)	7 (15%)	11 (25%)	24 (53%)[c]	33 (77%)[c]	7 (58%)[c]
Hepatocellular adenoma and carcinoma	31 (55%)	27 (54%)	35 (68%)	32 (69%)	40 (87%)[c]	12 (75%)
Preputial gland adenoma or carcinoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (17%)[c]	0 (0%)
Females						
Initial number[a]	70	70	70	70	70	90
Number of survivors	37	33	24	11	0	0
Lymphoma	10 (20%)	14 (30%)	18 (41%)[c]	10 (26%)	19 (58%)[c]	43 (89%)[c]
Heart haemangiosarcoma	0 (0%)	0 (0%)	0 (0%)	1 (3%)	20 (64%)[c]	26 (84%)[c]
Lung alveolar/bronchiolar adenoma and carcinoma	4 (8%)	15 (32%)[c]	19 (44%)[c]	27 (61%)[c]	32 (81%)[c]	25 (83%)[c]
Forestomach papilloma and carcinoma	2 (4%)	2 (4%)	3 (8%)	4 (12%)	7 (31%)[c]	28 (85%)[c]
Harderian gland adenoma and adenocarcinoma	9 (18%)	10 (21%)	7 (17%)	16 (40%)[c]	22 (67%)[c]	7 (48%)[c]
Hepatocellular adenoma and carcinoma	17 (35%)	20 (41%)	23 (52%)[c]	24 (60%)[c]	20 (68%)[c]	3 (28%)
Mammary gland adenocarcinoma	0 (0%)	2 (4%)	2 (5%)	6 (16%)[c]	13 (47%)[c]	13 (66%)[c]
Ovarian benign and malignant granulosa-cell tumour	1 (2%)	0 (0%)	0 (0%)	9 (24%)[c]	11 (44%)[c]	6 (44%)

From Melnick *et al*. (1990); National Toxicology Program (1993)

[a] Initial numbers include animals removed from the study for interim sacrifices at 40 and 65 weeks of exposure.

[b] Mortality-adjusted tumour rates are given in parentheses.

[c] $p < 0.05$, based on logistic regression analysis with adjustment for intercurrent mortality

Table 21. Survival and incidence of tumours in male mice exposed to butadiene in stop-exposure studies[a]

	Exposure				
	0 ppm	200 ppm, 40 weeks	312 ppm, 52 weeks	625 ppm, 13 weeks	625 ppm, 26 weeks
Initial number	70	50	50	50	50
Number of survivors	35	9	1	5	0
Lymphoma	4 (8%)[b]	12 (35%)[c]	15 (55%)[c]	24 (61%)[c]	37 (90%)[c]
Heart haemangiosarcoma	0 (0%)	15 (47%)[c]	33 (87%)[c]	7 (31%)[c]	13 (76%)[c]
Lung alveolar/bronchiolar adenoma and carcinoma	22 (46%)	35 (88%)[c]	32 (88%)[c]	27 (87%)[c]	18 (89%)[c]
Forestomach squamous-cell papilloma and carcinoma	1 (2%)	6 (20%)[c]	13 (52%)[c]	8 (33%)[c]	11 (63%)[c]
Harderian gland adenoma and adenocarcinoma	6 (13%)	27 (72%)[c]	28 (86%)[c]	23 (82%)[c]	11 (70%)[c]
Preputial gland adenoma and carcinoma	0 (0%)	1 (3%)	4 (21%)[c]	5 (21%)[c]	3 (31%)[c]
Renal tubular adenoma	0 (0%)	5 (16%)[c]	3 (15%)[c]	1 (5%)	1 (11%)

From Melnick *et al*. (1990); National Toxicology Program (1993)

[a] After exposures were terminated, animals were placed in control chambers until the end of the study at 104 weeks.

[b] Mortality-adjusted tumour rates are given in parentheses.

[c] $p < 0.05$, based on logistic regression analysis with adjustment for intercurrent mortality

the strain of mouse used in the study. Groups of 60 male B6C3F$_1$ and 60 male NIH Swiss mice, 4–6 weeks of age, were exposed to 0 or 1250 ppm [2760 mg/m^3] butadiene (> 99.5% pure) by whole-body inhalation for 6 h per day on 5 days per week for 52 weeks. An additional group of 50 male B6C3F$_1$ mice was exposed similarly to butadiene for 12 weeks and held until termination of the experiment at 52 weeks. The incidence of thymic lymphomas was 1/60 control, 10/48 exposed for 12 weeks and 34/60 exposed for 52 weeks in B6C3F$_1$ mice and 8/57 in NIH Swiss mice exposed for 52 weeks. Haemangiosarcomas of the heart were observed in 5/60 B6C3F$_1$ mice and 1/57 NIH Swiss mice exposed for 52 weeks. Exposure of B6C3F$_1$ mice to 1250 ppm of butadiene for 6 h per day on 5 days per week for 3–21 weeks greatly increased the quantity of ecotropic retrovirus recovered from bone marrow, thymus and spleen. This was not the case for NIH Swiss mice in which proviral ecotropic sequences are truncated and the virus is not expressed. The authors suggested that the lack of retroviruses provides resistance to the induction of the lymphomas by butadiene (Irons *et al*., 1987a, 1989; Irons, 1990). [The Working Group noted that Swiss mice are less sensitive to the induction of haemangiosarcomas of the heart and lymphomas and that genetic factors may contribute to the development of these tumours.]

One study addressed the question of whether a single, high level of exposure to butadiene was sufficient to induce neoplasia. Groups of 60 male and 60 female B6C3F$_1$ mice, 8–10 weeks of age, were exposed by whole-body inhalation for a single 2-h period to 0, 1000, 5000 or 10 000 ppm [0, 2200, 11 000 or 22 000 mg/m^3] butadiene [purity unspecified]. The mice were then held for 2 years, at which time all survivors were killed and tissues and organs were examined histopathologically. Survival, weight gains and tumour incidence in exposed mice were not affected by exposure to butadiene (survival: males — 28/60 control, 34/60 low-dose, 44/60 mid-dose and 34/60 high-dose; females — 45/60, 36/60, 38/60 and 48/60, respectively) (Bucher *et al*., 1993).

3.1.2 *Rat*

Groups of 100 male and 100 female Sprague-Dawley rats, 4–5 weeks of age, were exposed by whole-body inhalation to 0, 1000 or 8000 ppm [0, 2200 or 17 600 mg/m^3] butadiene (minimal purity, 99.2%) for 6 h per day on 5 days per week for 111 weeks (males) or 105 weeks (females). Survival was reduced in low- ($p < 0.05$) and high-dose ($p < 0.01$) females and in high-dose males ($p < 0.05$); the numbers of survivors were: males — 45 control, 50 low-dose and 32 high-dose; females — 46 control, 32 low-dose and 24 high-dose. Tumours that occurred at a significantly increased incidence in males were pancreatic exocrine adenomas (control, 3/100; low-dose, 1/100; and high-dose, 10/100, $p \leq 0.001$) and interstitial-cell tumours of the testis (control, 0/100; low-dose, 3/100; and high-dose, 8/100; p for trend ≤ 0.001). Those that occurred at a significantly increased incidence in females were follicular-cell adenomas of the thyroid gland (control, 0/100; low-dose, 2/100; and high-dose, 10/100; p for trend ≤ 0.01), sarcomas of the uterus (control, 1/100; low-dose, 4/100; and high-dose, 5/100; p for trend ≤ 0.05),

carcinomas of the Zymbal gland (control, 0/100; low-dose, 0/100; and high-dose, 4/100; *p* for trend ≤ 0.01) and benign and malignant mammary tumours (control, 50/100; low-dose, 79/100; and high-dose, 81/100; *p* for trend ≤ 0.001). Mammary adenocarcinomas were found in 18/100 control, 15/100 low-dose and 26/100 high-dose female rats (Owen *et al*., 1987; Owen & Glaister, 1990).

3.2 Carcinogenicity of metabolites

3.2.1 *1,2-Epoxy-3-butene (epoxybutene)*

Mouse

A group of 30 male Swiss mice, 8 weeks of age, received dermal applications of 100 mg undiluted epoxybutene, the initial monoepoxide metabolite of butadiene, three times per week for life. The median survival time was 237 days; three skin papillomas and one squamous-cell carcinoma were observed (Van Duuren *et al*., 1963). [The Working Group noted that this incidence was similar to that in control groups that were left untreated.]

3.2.2 *1,2:3,4-Diepoxybutane (diepoxybutane)*

(a) Mouse

Two groups of 30 male Swiss mice, 8 weeks of age, received dermal applications of 100 mg D,L-diepoxybutane (10% in acetone) or 100 mg *meso*-diepoxybutane (10% in acetone) three times a week for life. The median survival times were 78 and 154 weeks, respectively. Two skin papillomas and one squamous-cell carcinoma were observed following treatment with D,L-diepoxybutane and six skin papillomas and four squamous-cell carcinomas were observed following treatment with *meso*-diepoxybutane. Eight skin papillomas and no carcinomas were observed in 120 acetone-treated controls (Van Duuren *et al*., 1963).

D,L-Diepoxybutane induced one skin papilloma or 10 skin papillomas and six squamous-cell carcinomas when applied to the skin of two groups of 30 female Swiss mice, 8 weeks of age, at respective doses of 10 or 3 mg in 100 mg acetone three times per week for life. *meso*-Diepoxybutane induced five skin papillomas and four squamous-cell carcinomas or one skin papilloma when applied to the skin of two groups of 30 female Swiss mice at respective doses of 10 or 3 mg in 100 mg acetone three times per week for life. No tumours were observed in 60 acetone-treated control mice (Van Duuren *et al*., 1965).

Groups of 15 male and 15 female strain A mice, 4–6 weeks of age, received 12 thrice weekly intraperitoneal injections of L-diepoxybutane at total doses ranging from 1.7 to 192 mg/kg bw (35–2200 µmol/kg bw) in water or tricaprylin. The experiment was terminated 39 weeks after the first injection. L-Diepoxybutane slightly increased the

incidence (40–78% versus 27–37% in controls) and multiplicity (0.5–1.5 tumour/mouse versus 0.29–0.48 tumours/mouse in controls) of lung tumours (Shimkin *et al*., 1966).

Groups of male Swiss mice, 6 weeks of age, received subcutaneous injections of 0.1 or 1.1 mg D,L-diepoxybutane in 0.05 mL tricaprylin once a week for 401–589 days. Five local fibrosarcomas and two adenocarcinomas of breast origin were observed in a group of 50 mice (0.1-mg dose) and five local sarcomas in a group of 30 mice (1.1-mg dose). No tumours were observed in three tricaprylin-treated control groups (total of 110 animals) (Van Duuren *et al*., 1966).

Groups of 56 female B6C3F$_1$ mice [it is unclear whether the animals were 6 or 10–11 weeks of age] were exposed by inhalation to 0, 2.5 or 5.0 ppm D,L-diepoxybutane for 6 h per day on 5 days per week for 6 weeks. Eight animals per group were examined for acute toxicity at the end of the exposures and most of the 48 remaining animals in each group were held for 18 months to observe tumour development (four animals were actually held for 6 months and four others for 12 months). The exposures resulted in nasal lesions that led to reduced survival. At the end of the experiment, neoplastic lesions were observed in the nasal mucosa, reproductive organs, lymph nodes, bone, liver, Harderian gland, pancreas and lung, but the only statistically significant increase was in the incidence of Harderian gland lesions. The incidence of total Harderian gland tumours was 0/40 control, 2/42 low-dose and 5/36 high-dose animals ($p < 0.05$, χ^2 test) (Henderson *et al*., 1999, 2000).

(b) Rat

Subcutaneous injection of 1 mg D,L-diepoxybutane in 0.1 mL tricaprylin once a week for 550 days induced nine local fibrosarcomas and one adenocarcinoma of breast origin in 50 female Sprague-Dawley rats that were 6 weeks of age at the beginning of the experiment. One adenocarcinoma of breast origin was observed in 50 control animals (Van Duuren *et al*., 1966).

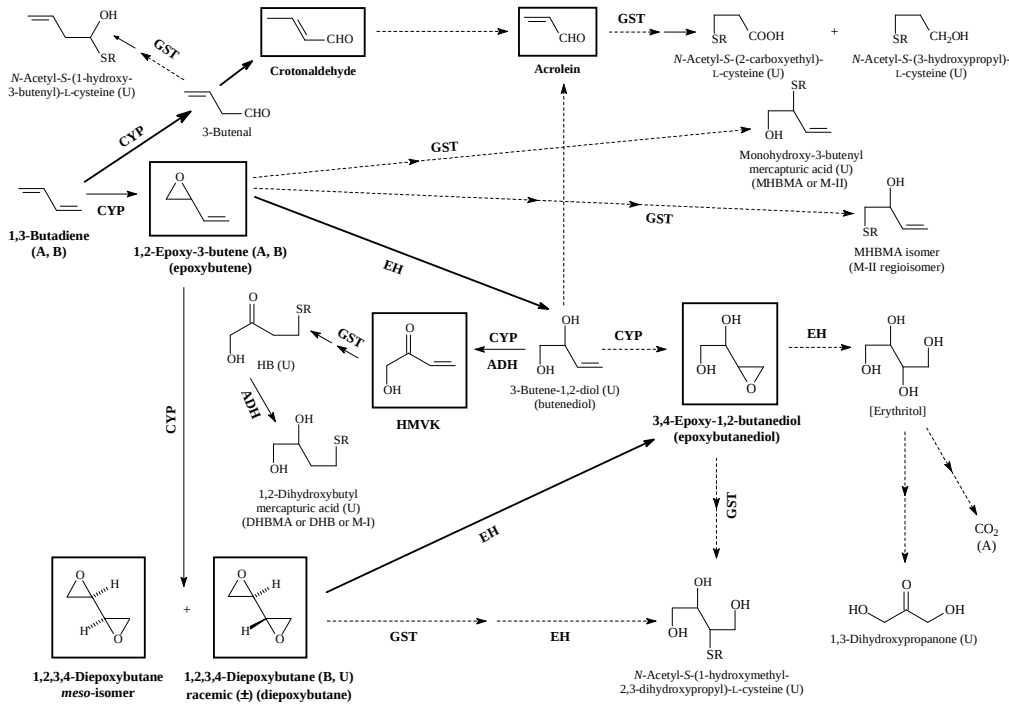
A 5-mg/mL dose of diepoxybutane dissolved in 0.5 mL tricaprylin was administered once a week by a gastric feeding tube to five female Sprague-Dawley rats, 6 weeks of age, for 363 days. No gastric tumours were observed (Van Duuren *et al*., 1966).

Groups of 56 female Sprague-Dawley rats [it is unclear whether the animals were 6 or 10–11 weeks of age] were exposed by inhalation to 0, 2.5 or 5.0 ppm D,L-diepoxybutane for 6 h per day on 5 days per week for 6 weeks. Eight animals per group were examined for acute toxicity at the end of the exposures and most of the 48 remaining animals in each group were held for 18 months to observe tumour development (four animals were actually held for 6 months and four others for 12 months). The exposures resulted in nasal lesions that led to reduced survival. At the end of the experiment, the only significant increase was in the incidence of neoplasms of the nasal mucosa. The incidence of these tumours, principally squamous-cell carcinomas, was 0/47 control, 12/48 low-dose and 24/48 high-dose animals. Three high-dose rats had multiple tumours (Henderson *et al*., 1999, 2000).

4. Mechanistic and Other Relevant Data

The toxicokinetics and toxicology of butadiene have been comprehensively reviewed (IARC, 1999). No measured data on the pharmacokinetics of butadiene in exposed humans were available at that time. Data on a limited number of metabolites and haemoglobin adducts of butadiene in exposed humans and data on butadiene and its metabolites in experimental systems using human tissues were included. However, considerably more information is currently available regarding the metabolic pathways of butadiene and its major metabolites (see Figure 1).

Figure 1. Metabolic pathways of butadiene deduced from findings in mammals *in vitro* and *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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

(a) Butadiene

Perbellini *et al*. (2003) determined the concentrations of butadiene in alveolar air, blood and urine in humans after non-occupational exposure. Breath, blood and urine samples were taken from 61 men who lived in small mountain villages of Northeast Italy. The mean age of the subjects was 44.5 years (range, 26–64 years); 15 were smokers and 46 were nonsmokers (11 of these were exposed to secondhand tobacco smoke). Samples were collected after overnight rest and analysed by headspace and GC–MS methods. The median concentrations of butadiene were 1.2 ng/L (range, < 0.8–13.2 ng/L) in alveolar air, 2.2 ng/L (range, < 0.5–50.2 ng) in blood and 1.1 ng/L (range, < 1–8.9 ng/L) in urine. Concentrations were significantly higher (two to three times) in smokers compared with nonsmokers in all biological media. The ratio of the median butadiene concentration in blood to alveolar air was 1.8, which is consistent with published values for measured blood:air partition coefficients.

Lin *et al*. (2001) conducted an inhalation study to identify influential physiological factors in the respiratory uptake of butadiene in humans. Healthy volunteers (71 men and 62 women) were exposed to 2 ppm [4.42 mg/m^3] butadiene for 20 min followed by purified air for 40 min. Exhaled breath samples were collected during exposure to determine the uptake of butadiene (micrograms per kilogram of body weight (bw) butadiene absorbed), which varied from 0.6 to 4.9 μg/kg bw. The blood:air partition coefficient and alveolar ventilation were most significant in determining uptake. Women had a slightly higher uptake than men; increasing age and cigarette smoking resulted in decreased uptake. The mean percentage of total inhaled butadiene that was absorbed was 45.6% for men and 43.4% for women.

Lin *et al*. (2002) developed an automated exposure system to study the toxicokinetics of inhaled gases in humans. Butadiene was used for system validation at three levels (0.08, 0.4 and 2.0 ppm [0.18, 0.88 and 4.42 mg/m^3]) and the system was tested in three subjects who were exposed to 2.0 ppm butadiene for 20 min. Steady-state levels of butadiene in alveolar air were obtained before the end of the 20-min exposure. Levels ranged between approximately 1 and 1.9 ppm. By the end of the 40-min post-exposure period, levels of butadiene in alveolar air had fallen by at least 10-fold.

Smith *et al*. (2001) assessed genetic and dietary factors that affect human metabolism of butadiene by monitoring exhaled breath during and after a 20-min exposure of 71 male and 62 female volunteers to 2 ppm [4.42 mg/m^3] butadiene. Chlorzoxazone was administered following exposure and urine was collected to measure the cytochrome P450 (CYP) 2E1 phenotype. A physiologically based pharmacokinetic model was fit to the exhaled breath measurements of each volunteer to estimate model parameters, including metabolic rate. No correlation was found between total butadiene uptake and any of the parameters (metabolic rate, oxidation rate and CYP2E1 phenotype or genotype).

Gordon *et al*. (2002) used real-time breath measurement methods to investigate the suitability of volatile organic compounds, including butadiene, as breath biomarkers for active and passive smoking. Five adult smoker/nonsmoker pairs were recruited into the study. During cigarette smoking, smokers exhaled into the breath analyser throughout a 2–2.5-h smoking period. After smoking, the long-term decay in the breath of the smokers was recorded continuously for 15 min. Exhaled breath in nonsmokers, who were in the room with the smokers, was also monitored. The maximum breath concentration of butadiene was relatively constant among smokers and averaged 373 μg/m^3 [169 ppm]. Following cessation of smoking, the mean residence time was 0.47 min. The average room air concentration of butadiene was 18.9 μg/m^3 [42 ppm]. Many of the post-exposure measurements of the breath of nonsmokers were below the limit of quantitation. The average increase in breath concentration of butadiene in nonsmokers and smokers, respectively, was 14.4 and 353 μg/m^3 [6.5 and 160 ppm].

(b) Metabolites

Several investigators have quantified the presence of metabolites derived from butadiene in the urine of humans in controlled laboratory, environmental or workplace settings. A scheme that describes metabolic pathways of butadiene deduced from in-vitro and in-vivo findings in mammals is presented in Figure 1. Two urinary metabolites have been identified: 1,2-dihydroxybutyl mercapturic acid (DHBMA; also referred to as DHB, MI, M-I or M1) and monohydroxy-3-butenyl mercapturic acid (MHBMA; also referred to as MII, M-II or M2). Both metabolites are mercapturic acids and are derived from the glutathione (GSH) conjugates of electrophilic butadiene metabolites (Figure 1). MHBMA results from the enzyme-mediated reaction of 1,2-epoxy-3-butene (epoxybutene) with GSH. Two isomeric forms of MHBMA have been quantified in the urine of rats and mice (Elfarra *et al.*, 1995). It should be noted, however, that some of the early studies may have not quantified both of these isomers. DHBMA results from the hydrolysis of epoxybutene by epoxide hydrolase (EH) followed by further enzymatic reaction by CYP or alcohol dehydrogenase (ADH) to yield hydroxymethylvinyl ketone (HMVK) before eventual conjugation with GSH (Sprague & Elfarra, 2004). The relative proportions of these metabolites that were measured depended on the species. Due to the higher concentrations of DHBMA in control subjects, DHBMA appears to be a less specific biomarker for exposure than MHBMA, for which background levels are relatively low. However, both metabolites appear to be elevated in humans exposed to butadiene compared with unexposed controls.

Sapkota *et al*. (2006) collected personal air and urine samples from individuals in traffic-dense environments in order to characterize exposure to butadiene in this setting. Urine samples were analysed for MHBMA and DHBMA. Exposure to butadiene differed among the groups; median values were 2.38, 1.62 and 0.88 μg/m^3 [5, 3.6 and 1.9 ppm] for toll collectors (nine individuals), urban–weekday (seven individuals) and suburban–weekend (seven individuals) groups, respectively. These groups represented high,

medium and low levels of exposure intensity. All individuals were nonsmokers in non-smoking households. For the three groups, mean levels of MHBMA were 9.7, 6.0 and 6.8 ng/mL and those of DHBMA were 378, 258, and 306 ng/mL, respectively.

Urban *et al*. (2003) applied a tandem liquid chromatography (LC)–MS method to determine levels of MHBMA and DHBMA in the urine of humans. Exposure to tobacco smoke had a significant effect on the urinary excretion of MHBMA and the metabolite ratio DHBMA/(DHBMA + MHBMA). Urine samples were collected over a 24-h period from 10 smokers and 10 nonsmokers. Mean MHBMA levels were 12.5 and 86.4 μg/24 h for nonsmokers and smokers, respectively. Mean DHBMA levels were 459 and 644 μg/24 h for nonsmokers and smokers, respectively. The metabolic ratio was 0.970 and 0.859 for the two groups, respectively.

Fustinoni *et al*. (2004) assessed exposure to butadiene in two groups who worked in a butadiene plant: 42 occupationally exposed workers and 43 unexposed controls. Personal exposure to butadiene was assessed by collecting air samples during the 8-h workshift (three to four times over a period of 6 weeks for the exposed and once for the control group). Urine and exhaled air samples were collected at the beginning and end of the workshift and blood samples were taken at the end of the workshift for the exposed; all samples were collected at the end of the workshift for the controls. Concentrations of DHBMA and MHBMA were determined in the urine samples. Mean airborne butadiene concentrations were 11.5 and 0.9 μg/m^3 [5.2 and 0.4 ppm] for exposed and control groups, respectively. Mean concentrations of butadiene metabolites in blood and urine were higher in exposed workers: 8.3 ng/L and 5.9 ng/L in blood and 4.3 ng/L and 3.1 ng/L in urine of exposed and control groups before the workshift, respectively. Concentrations of butadiene metabolites in urine at the end of the workshift were 605 μg/L and 602 μg/L DHBMA and 10.5 μg/L and 7.5 μg/L MHBMA in exposed and controls, respectively.

(c) Haemoglobin adducts

While haemoglobin adducts are not causally related to mutagenic events, they provide an effective measure of exposure to reactive intermediates of chemicals. Haemoglobin adducts accumulate during the life of red cells which is approximately 120 days in humans. The butadiene metabolite, epoxybutene, has been shown to react with haemoglobin to form *N*-(2-hydroxy-3-butenyl)valine (MHbVal) adducts. Another haemoglobin adduct of butadiene is *N*-(2,3,4-trihydroxybutyl)valine (THbVal), which can be formed in humans by the reaction of 3,4-epoxy-1,2-butanediol (epoxybutanediol) with haemoglobin or by the reaction of 1,2:3,4-diepoxybutane (diepoxybutane) with haemoglobin followed by hydrolysis of the haemoglobin adduct (Swenberg *et al.*, 2000a). Swenberg *et al*. (2001) reviewed data on various biomarkers for butadiene and noted that those on both DNA and haemoglobin adducts strongly support the conclusion that epoxybutanediol is the major electrophile that binds to these macromolecules.

Boogaard (2002) reviewed the use of haemoglobin adducts to monitor exposure and drew several important conclusions. In theory, the removal of haemoglobin adducts is a

zero-order process and is only determined by the life-span of the erythrocyte. Poor correlations were found between concentrations of adducts in a spot blood sample and air concentrations on a limited number of days (i.e. 1–3 days during the shift). In contrast, good correlations were found when very frequent air monitoring was carried out, such as 10 full-day shift measurements over a 2-month period. Good correlations were also found between an increase in adduct concentration over a short period of time and the cumulative exposure during this time, which was determined by continuous monitoring. The author noted that, when specific adducts are determined, information is also obtained regarding the chemical nature of the reactive intermediates. Butadiene forms at least two other reactive intermediates other than epoxybutene: diepoxybutane and epoxybutanediol, both of which form the same THbVal adducts. One of the major data gaps in the assessment of exposure to butadiene is a sensitive biomarker that is specific for diepoxybutane. One potential biomarker is the cyclic adduct, *N,N*-(2,3-dihydroxy-1,4-butadiyl)valine (PyrVal), which has recently been quantified in rats and mice exposed to levels as low as 1 ppm [2.21 mg/m^3] butadiene for 10 days, but its presence has yet to be quantified in human haemoglobin samples (Boysen *et al.*, 2004; Swenberg *et al.*, 2007).

The utility of haemoglobin adducts as biomarkers for 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.

Van Sittert *et al*. (2000) and Boogaard *et al*. (2001a) reported on the assessment of urinary metabolites and haemoglobin adducts. One study involved 44 male workers in a butadiene monomer production facility in the Netherlands who were exposed to low levels of butadiene and 28 male administrative workers with no occupational exposure to butadiene. A second study conducted in a butadiene monomer and styrene–butadiene rubber production facility in Prague, Czech Republic, involved 24 male workers from the monomer unit, 34 from the polymer unit and 25 administrative workers. This study is described in detail by Albertini *et al*. (2001, 2003a). In the two studies, airborne levels of butadiene, MHBMA, DHBMA and MHbVal were determined. MHBMA was more sensitive than DHBMA for monitoring recent exposures and could be detected at an 8-h TWA exposure as low as 0.13 ppm [0.29 mg/m^3]. The sensitivity of DHBMA was restricted by high background levels, of which the origin is unknown. This study confirmed the higher hydrolytic activity in humans compared with rodents, as shown in other studies, which was reflected in the much higher ratio of DHBMA/(MHBMA + DHBMA).

In the study in the Netherlands, airborne levels of butadiene ranged from 0.2 to 9.5 ppm [0.09–4.3 mg/m^3] (8-h TWA) and levels of MHbVal ranged from 0.6 to 3.8 pmol/g haemoglobin in exposed workers and from 0.1 to 1.2 pmol/g haemoglobin in controls. In the study in Prague, airborne levels of butadiene ranged from 0 to 0.038 ppm [0–0.02 mg/m^3] for the controls, 0.02 to 1.6 ppm [0.009–0.72 mg/m^3] for the monomer workers and 0.02 to 4.2 ppm [0.009–1.9 mg/m^3] for the polymer workers. There was a strong correlation between the 60-day average airborne concentration of butadiene and

MHbVal, which the authors concluded was a sensitive method for monitoring cumulative exposures to butadiene above 0.35 ppm [0.16 mg/m^3]. In controls, urinary levels of MHBMA ranged from 0.1 to 7.3 µg/L and those of DHBMA from 197 to 747 µg/L. In the monomer and polymer workers, levels of MHBMA ranged from < 0.1 to 44 µg/L and from 1.7 to 962 µg/L, respectively, in end-of-shift urine samples. Levels of DHBMA ranged from 52 to 3522 µg/L in the monomer and from 190 to 26 207 µg/L in the polymer workers. For each metabolite there was a strong correlation between the urinary concentration and 8-h TWA levels of airborne butadiene.

Albertini *et al*. (2001, 2003a) conducted a molecular epidemiological study of humans exposed to butadiene in an occupational setting in Prague, Czech Republic (24 butadiene monomer production workers, 34 polymerization workers and 25 controls). Personal 8-h TWA measurements of exposure to butadiene were made on several occasions over a 60-day period, and biological samples were obtained for the measurement of biomarkers of butadiene metabolism: urinary metabolites of butadiene, MHBMA and DHBMA and the haemoglobin adducts, MHbVal and THbVal. Mean 8-h TWA levels of exposure to butadiene were 0.642 mg/m^3 [1.42 ppm] for the monomer workers, 1.794 mg/m^3 [3.96 ppm] for the polymerization workers and 0.023 mg/m^3 [0.05 ppm] for the controls. All four biomarkers were significantly correlated with levels of exposure to butadiene; the haemoglobin adducts were the most highly correlated. After a workshift, mean concentrations of DHBMA were 764, 4647 and 353 µg/L for the monomer production, polymerization and administration workers, respectively, and mean concentrations of MHBMA were 9.44, 120.17 and 1.70 µg/L, respectively. The proportion of MHBMA to the total urinary metabolites (MHBMA + DHBMA) was analysed relative to the glutathione-*S*-transferase (GST) genotypes, *GSTM1* and *GSTT1*, for all three exposure groups. The mean ratios for workers with the homozygous null genotypes in all groups were always lower than those for workers with positive genotypes. Thus, although MHBMA represents a minor pathway in butadiene metabolism, there is a decrease in the proportion of this metabolite formed by GST-null individuals. Concentrations of MHbVal were 0.47, 2.23 and 0.22 pmol/g haemoglobin for the monomer production, polymerization and administration workers, respectively. The group means were correlated with the measured mean levels of exposure to butadiene. Mean concentrations of the THbVal adduct were 178.73, 716.70 and 94.77 pmol/g haemoglobin for the three groups, respectively. Thus, the THbVal adduct is present at concentrations that are increased approximately fivefold over background. There was no effect of *GSTT1* or *GSTM1* genotype on the formation of haemoglobin adducts.

Albertini *et al*. (2007) reported a second study in Czech workers that included both men and women: 23 female workers exposed to butadiene, 26 female control workers, 30 male workers exposed to butadiene and 25 male control workers. Multiple external exposure measurements were made over a 4-month period before biological samples were collected. Mean 8-h TWA exposure levels were 0.008 mg/m^3 [0.0035 ppm] and 0.397 mg/m^3 [0.180 ppm] for female controls and exposed workers, respectively. Exposure levels for men were 0.007 mg/m^3 [0.0032 ppm] and 0.808 mg/m^3 [0.370 ppm]

for controls and exposed, respectively. Urinary concentrations of MHBMA and DHBMA were elevated in exposed male and female workers compared with controls. Mean levels of DHBMA were 331.6 μg/L and 508.1 μg /L for control and exposed women, respectively, and 512.8 μg/L and 854.1 μg/L for control and exposed men, respectively. Mean levels of MHBMA were 8.3 μg/L and 19.2 μg /L for control and exposed women, respectively, and 14.9 μg/L and 47.9 μg/L for control and exposed men, respectively. As part of this study, assays were conducted to determine the presence of the haemoglobin adduct, PyrVal. This adduct is specific for the highly genotoxic metabolite of butadiene, diepoxybutane. All samples were below the limit of quantitation for the assay (0.3 pmol/g haemoglobin).

Swenberg *et al*. (2007) quantified PyrVal in the blood of mice and rats exposed to 1 ppm [2.21 mg/m^3] butadiene. For these comparative studies, 50 mg haemoglobin from workers, rats and mice were analysed by the method of Boysen *et al*. (2007). Whereas all rat and mouse samples had quantifiable amounts of PyrVal, none of the human samples did. The authors reported that, although the TWA exposures to butadiene in the Czech study were below 1 ppm [2.21 mg/m^3] for both men and women, the duration of exposure was much greater for humans than for rodents, which made the cumulative exposures comparable. Since haemoglobin adducts form over the lifespan of the erythrocyte (humans, 120 days; rats, 63 days; and mice, 43 days), human haemoglobin had a cumulative exposure similar to that of both rats and mice (women: 0.18 ppm butadiene × 17.1 weeks = 3.1 ppm–weeks; men: 0.37 ppm butadiene × 17.1 weeks = 6.3 ppm–weeks; rodents: 1 ppm butadiene × 4 weeks = 4.0 ppm–weeks). They also reported that the analytical peaks in samples from rats exposed to 1 ppm [2.21 mg/m^3] butadiene were sufficiently large that quantitative measurements could have been made with one-third of the haemoglobin, which suggests that humans form at least three times less PyrVal than similarly exposed rats and 100 times less than similarly exposed mice.

Fustinoni *et al*. (2002) investigated the role of genetic polymorphisms in the metabolism of butadiene in 40 Italian subjects. Thirty were occupationally exposed during butadiene monomer production or polymerization processes and 10 were unexposed administrative clerks. Air samples were collected from butadiene-exposed workers during the workshift using personal samplers. Personal exposure to airborne butadiene ranged from 4 to 201 μg/m^3 [1.8–90.5 ppm]. At the end of the workshift, blood and urine samples were collected from all subjects. Concentrations of the urinary metabolite DHBMA and the haemoglobin adduct THbVal were assessed. Median urinary DHBMA concentrations were 17.1 mg/g creatinine in exposed workers and 1.42 mg/g creatinine in unexposed workers; THBVal levels were 37 pmol/g haemoglobin and 35.3 pmol/g haemoglobin in exposed and unexposed groups, respectively. Smoking influenced the formation of haemoglobin adducts and higher THbVal levels were found in subjects with *GSTM1* null and *GSTT1* null genotypes.

Hayes *et al*. (2001) examined a spectrum of outcomes in 41 butadiene polymer production workers and 38 controls in China. Smokers accounted for 86.7% of the exposed group and 78.6% of the controls. Median butadiene concentrations (6-h TWA)

were 2 ppm [0.90 mg/m^3] for the exposed group and 0 ppm for the controls, as determined during a 6-h workshift using personal samplers. A post-shift blood sample and during-shift urine samples were also collected. Median urinary concentrations of DHBMA were 1.3 μg/mg creatinine in the exposed group and 0.6 μg/mg creatinine in the controls. THbVal haemoglobin adducts were significantly more common [numerical data not provided] in exposed workers than in the unexposed ($p < 0.0001$) and correlated well with air measurement (Spearman's $\rho = 0.40$, $p = 0.03$) and weakly with urinary butadiene (Spearman's $\rho = 0.37$, $p = 0.24$).

(d) Physiologically based toxicokinetic models

Physiologically based models founded on informative prior distributions for population parameters as well as previously published data on exhaled breath concentrations of butadiene in exposed humans have been designed to facilitate a global sensitivity analysis of the kinetics of butadiene and its metabolites in humans; the most recent was published by Brochot *et al.* (2007) and its purpose was to guide the design of new human experiments intended to collect critically useful kinetic data on butadiene and its metabolites.

4.1.2 *Experimental systems*

At the beginning of each of the following sections, brief highlights on the disposition of butadiene that were summarized previously (IARC, 1999) are presented. The reader should refer to that monograph for citations and more complete descriptions of the relevant studies. These highlights are followed by information that has been published since that time. For this and other sections, diepoxybutane refers to the (±) racemic mixture of diastereomers, unless otherwise specified.

(a) Butadiene

Inhalation pharmacokinetic studies conducted in Sprague-Dawley rats and B6C3F$_1$ mice demonstrated linear metabolic elimination kinetics at exposures of up to about 1000 ppm [2200 mg/m^3]; maximal rates of butadiene metabolism were higher in mice (400 μmol/h/kg) than in rats (220 μmol/h/kg) (IARC, 1999). In the linear range, metabolism is limited by the uptake of this gas. At equivalent, non-saturating exposure levels, steady-state blood concentrations of butadiene are about twofold higher in mice than in rats. Numerous metabolites have been identified in the urine of rats and mice exposed to butadiene (Figure 1); the major urinary metabolites are mercapturic acids (DHBMA and MHBMA) that result from conjugation of epoxybutene or HMVK, a metabolite of butenediol, with GSH.

Exhaled epoxybutene was measured in chamber atmospheres in which male B6C3F$_1$ mice or Sprague-Dawley rats were exposed for up to 8 h to constant concentrations of butadiene that ranged from 1 ppm to 6000 ppm [2.21–13 260 mg/m^3] (mice) or 10 000 ppm [22 100 mg/m^3] (rats) (Filser *et al*., 2007). In additional experiments, blood

levels of diepoxybutane, butenediol and epoxybutanediol were measured at the end of 6-h exposures that ranged from 60 ppm [132.6 mg/m^3] up to about 1200 ppm [2652 mg/m^3]. Epoxybutanediol is formed by partial hydrolysis of diepoxybutane or by oxidation of butenediol (Figure 1). Blood concentrations of epoxybutene were estimated from the product of its atmospheric concentration at steady state and its blood:air partition coefficient. Mouse:rat ratios of blood concentrations of epoxybutene were 2.0–2.6 at concentrations of butadiene below 10 ppm [22.1 mg/m^3], 3.8 at 100 ppm [221 mg/m^3], 4.9 at 625 ppm [1381 mg/m^3] and 8.0 at 1250 ppm [2762.5 mg/m^3]. Blood concentrations of diepoxybutane in mice were 0.30, 2.2 and 3.2 μmol/L at 67, 630 and 1270 ppm [148, 1392.3 and 2806.7 mg/m^3] butadiene, respectively. Diepoxybutane was not detected in the blood of rats exposed to up to 900 ppm [1989 mg/m^3] butadiene (detection limit, 10 nmol/L) (Filser *et al.*, 2007). However, previous studies (IARC, 1999) reported diepoxybutane levels of 2.4–5 nmol/L in male and 11 nmol/L in female Sprague-Dawley rats exposed to 62.5 ppm [138 mg/m^3] butadiene. Butenediol levels reached 60 μmol/L in both species exposed to 1200 ppm [2652 mg/m^3] butadiene, whereas maximum blood levels of epoxybutanediol were 42 μmol/L in mice exposed to 300 ppm [663 mg/m^3] butadiene and 9.5 μmol/L in rats exposed to 150 ppm [331.5 mg/m^3] butadiene. The limited epoxybutanediol formation was suggested to be due to competition by butadiene for the CYP-mediated oxidation of epoxybutene to diepoxybutane and of butenediol to epoxybutanediol. In rats, the total blood concentrations of epoxybutene and epoxybutanediol were greater after exposure to 200 ppm [442 mg/m^3] butadiene than after exposure to 1000 or 8000 ppm [2210 or 17 680 mg/m^3]; the latter values are the concentrations of butadiene that were used in a rat carcinogenicity study.

First-pass metabolism of butadiene to epoxybutene, diepoxybutane, butenediol, epoxybutanediol and crotonaldehyde was quantified in the livers of male Sprague-Dawley rats and B6C3F$_1$ mice perfused in a gas-tight all-glass system (Filser *et al.*, 2001). Concentrations of butadiene in the perfusate and perfusion rates were 330 nmol/mL at 3–4 mL/min for mouse liver and 240 nmol/mL at 17–20 mL/min for rat liver. Concentrations of butadiene and its metabolites in the perfusate that entered and left the liver were measured during 100-min perfusions. The perfusate consisted of Krebs-Henseleit buffer that contained bovine erythrocytes, bovine serum albumin and a constant concentration of butadiene. The rate of single-pass butadiene metabolism was estimated to be 0.014 and 0.055 mmol/h per liver of mouse and rat, respectively. The mean concentration of epoxybutene was 1.1 nmol/mL in the rat liver effluent and 9.4 nmol/mL in the mouse liver effluent. Butenediol concentrations in the perfusates that left the liver were similar in rats and mice (approximately 7 and 8 nmol/mL, respectively). Levels of diepoxybutane and epoxybutanediol were below the limit of detection in the effluent from the rat liver and low in the mouse liver effluent (approximately 0.06 and 0.07 nmol/mL, respectively). Concentrations of crotonaldehyde were below the limit of detection (60 nmol/L) in both rat and mouse liver.

Filser *et al.* (2001) also studied butadiene metabolism in perfused livers obtained from rats that were depleted of GSH by pretreatment with diethylmaleate. Under these

conditions, the rate of metabolism was not altered; however, concentrations of epoxybutene and butenediol were increased 25- and 10-fold, respectively, and concentrations of diepoxybutane and crotonaldehyde in the effluent perfusates were quantifiable (0.15 and 0.10 nmol/mL, respectively). The authors concluded that GST activity is important in controlling the production of diepoxybutane and crotonaldehyde in rats.

(b) Metabolites in vitro (see Figure 1)

Butadiene is oxidized initially to epoxybutene by nicotinamide adenine dinucleotide phosphate (NADPH)-dependent metabolism that primarily involves CYP2E1 and CYP2A6. This metabolic pathway has been measured in microsomal fractions obtained from the livers and lungs of mice, rats and humans and from the kidney and testis of rats and mice (IARC, 1999). At low concentrations of butadiene, metabolism via CYP2E1 predominates. Liver microsomes from mice, rats and humans can also oxidize epoxybutene to diepoxybutane. Kinetic parameters (maximum velocity [V_{max}] and Michaelis-Menten constant [K_m]) indicated faster rates of oxidative metabolism of butadiene and epoxybutene in microsomes from mice than in those from rats, while human liver microsomes showed a wide range of activites (Csanady *et al*., 1992; Duescher & Elfarra, 1994), but only a small number of human liver and lung samples were analysed. Mouse, rat and human microsomes can convert epoxybutene to both (±)-diepoxybutane and *meso*-diepoxybutane. The latter isomer is preferentially hydrolysed in rat and human liver microsomes (Krause & Elfarra, 1997). Epoxybutene and diepoxybutane are eliminated by microsomal EH (mEH) and by cytosolic GST activities. In-vitro kinetics of EH- and GST-catalysed metabolism of epoxybutene and diepoxybutane in microsomal and cytosolic fractions from livers and lungs of mice, rats and humans have also been reported (IARC, 1999). GST activities were highest in mouse and lowest in human cytosol; EH activities were highest in human and lowest in mouse microsomes. Bone-marrow cells of mice and humans can also oxidize butadiene to epoxybutene by a myeloperoxidase-catalysed reaction. A third epoxide intermediate of butadiene metabolism, epoxybutanediol, can be formed by the oxidation of butenediol (the product of epoxybutene hydrolysis) or by partial hydrolysis of diepoxybutane.

Hepatocytes isolated from male $B6C3F_1$ mice and Sprague-Dawley rats were incubated with epoxybutene and evaluated for the formation of diepoxybutane, butenediol and GSH conjugates at 5-min intervals during incubations of up to 45 min (Kemper *et al*., 2001). The results are shown in Table 22. Cumulative levels of combined racemic and *meso*-diepoxybutane (oxidation of epoxybutene) were higher in mouse than in rat hepatocyte cultures, whereas the cumulative levels of butenediol (hydrolysis of epoxybutene) were higher in the rat hepatocyte cultures. GSH conjugates were present at similar levels in both rat and mouse hepatocyte cultures.

Hepatic microsomes pooled from cynomolgus monkeys or humans exposed to butadiene (monkey microsomes: 46 ppm, 290 ppm or 28 000 ppm [102, 641 or 61 880 mg/m^3]; human microsomes: 45 ppm, 450 ppm or 36 000 ppm [99, 995 or

79 560 mg/m^3]) produced epoxybutene at similar rates (Dahl & Henderson, 2000). Oxidation of epoxybutene to diepoxybutane, which is competitively inhibited by butadiene, was slightly faster in monkey than in human microsomes.

Table 22. Area under the concentration versus time curve (nmol·min/10^6 cells) for epoxybutene-derived metabolites in isolated mouse and rat hepatocytes during 45-min incubations with epoxybutene

Concentration of epoxybutene (μM)	Diepoxybutane	Butenediol	GSH conjugates
Mouse hepatocytes			
5	63 ± 23	9 ± 1	22 ± 14
25	197 ± 28	133 ± 24	117 ± 32
250	616 ± 53	908 ± 172	1101 ± 322
Rat hepatocytes			
5	5 ± 1	78 ± 20	27 ± 1
25	13 ± 3	435 ± 98	153 ± 9
250	80 ± 3	3403 ± 755	1552 ± 206

From Kemper *et al*. (2001)
GSH, glutathione

(c) Metabolites in vivo (see Figure 1)

The identification of epoxybutene in exhaled breath, blood and multiple organs of rats or mice exposed to butadiene indicates the systemic availability of this metabolic intermediate. At equivalent exposure concentrations of butadiene, tissue levels of epoxybutene and diepoxybutane were higher in mice than in rats (IARC, 1999).

^{14}C-Labelled epoxybutene was administered as a single intraperitoneal injection to male Sprague-Dawley rats and B6C3F$_1$ mice at doses of 1, 5, 20 and 50 mg/kg bw; urine and faeces were collected up to 48 h after treatment (Richardson *et al*., 1998). Approximately 50% of the administered radioactivity was excreted in the urine and 2–5% in the faeces. Urinary metabolites identified in this study are listed in Table 23. Because the percentage of urinary radioactivity recovered for each metabolite did not differ in relation to the administered dose, mean values across the dose groups are shown. Both species preferentially metabolized epoxybutene by direct conjugation with GSH. In rats, 48–64% of the urinary radioactivity was derived from the direct reaction of epoxybutene with GSH and 14–25% was derived from hydrolysis of epoxybutene. In mice, 60–72% of the urinary radioactivity was derived from the direct reaction of epoxybutene with GSH and 6–10% was derived from hydrolysis of epoxybutene. No metabolites of diepoxybutane were detected in the urine of either species.

Table 23. Urinary metabolites in rats and mice given a single intraperitoneal injection of 1, 5, 20 or 50 mg/kg bw [4-^{14}C]epoxybutene

Metabolite[a]	% of recovered urinary radioactivity	
	Rat	Mouse
Polar fraction[b]	22 ± 2	23 ± 1
*4-(*N-*Acetyl-*L*-cystein-*S*-yl)-1,2-dihydroxybutane and 4-(*N*-acetyl-*L*-cystein-*S*-yl)-2-hydroxybutanoic acid*	14 ± 3	10 ± 2
*3-(*N-*Acetyl-*L*-cystein-*S*-yl)propan-1-ol and 3-(*N*-acetyl-*L*-cystein-*S*-yl)propanoic acid*	6 ± 2	–
(*R*)-2-(*N*-Acetyl-L-cystein-*S*-yl)-1-hydroxybut-3-ene	13 ± 1	11 ± 1
1-(*N*-Acetyl-L-cystein-*S*-yl)-2-(*S*)-hydroxybut-3-ene and 1-(*N*-acetyl-L-cystein-*S*-yl)-2-(*R*)-hydroxybut-3-ene	19 ± 5	26 ± 6
(*S*)-2-(*N*-Acetyl-L-cystein-*S*-yl)-1-hydroxybut-3-ene	25 ± 2	11 ± 2
4-(*N*-Acetyl-L-cystein-*S*-yl)-1-hydroxybut-2-ene	1 ± 0.4	–
S-(1-Hydroxybut-3-en-2-yl)mercaptoacetic acid	–	10 ± 2
S-(2-Hydroxybut-3-en-1-yl)mercaptoacetic acid	–	10 ± 2

From Richardson *et al*. (1998)

[a] Italicized metabolites are produced subsequent to hydrolysis of epoxybutene; the other metabolites listed are produced by direct reaction of epoxybutene with glutathione.

[b] Individual metabolites in the polar fraction were less than 1% of the recovered urinary radioactivity.

Male Sprague-Dawley rats and B6C3F$_1$ mice were exposed by nose-only inhalation to 200 ppm [442 mg/m^3] [2,3-^{14}C]butadiene for 6 h; radioactivity in urine, faeces, exhaled volatiles and [^{14}C]carbon dioxide was measured during and up to 42 h after exposure (Richardson *et al*., 1999). Total uptake of butadiene was 0.19 mmol/kg bw (10.3 mg/kg) in rats and 0.38 mmol/kg bw (20.5 mg/kg) in mice. In rats, 40% of the recovered radioactivity was exhaled as [^{14}C]carbon dioxide, 42% was excreted in urine, 8% was excreted in faeces and 9% remained in the carcass. In mice, 6% of the recovered radioactivity was exhaled as [^{14}C]carbon dioxide, 71% was excreted in urine, 11% was excreted in faeces and 13% remained in the carcass. Because the position of the radiolabel in this study was on carbons 2 and 3, the formation of [^{14}C]carbon dioxide is a result of the loss of two carbon atoms from butadiene. Urinary metabolites identified in this study are listed in Table 24. Metabolites arising from the direct reaction of epoxybutene with GSH accounted for 8% of the metabolized dose in rats and 16% of the metabolized dose in mice. In contrast to the study of epoxybutene metabolism (Richardson *et al*., 1998), the study on butadiene metabolism identified trihydroxybutyl mercapturic acids in the urine of exposed rats and mice.

Table 24. Urinary metabolites in rats and mice exposed to 200 ppm [^{14}C]butadiene for 6 h

Metabolite[a]	% of total radioactivity recovered	
	Rat	Mouse
Polar fraction	16.6 ± 0.6	33.0 ± 0.6
4-(*N*-Acetyl-L-cystein-*S*-yl)-1,2,3-trihydroxybutane and 3-(*N*-acetyl- L-cystein-*S*-yl)-1,2,4-trihydroxybutane	4.1 ± 0.2	6.7 ± 0.3
*4-(*N-*Acetyl-L-cystein*-S-*yl)-1,2-dihydroxybutane*	7.3 ± 1.2	7.1 ± 0.3
*4-(*N-*Acetyl-L-cystein*-S-*yl)-2-hydroxybutanoic acid*	1.1 ± 0.1	–
*3-(*N-*Acetyl-L-cystein*-S-*yl)propan-1-ol and 3-(*N-*acetyl-L-cystein*-S-*yl)propanoic acid*	0.4 ± 0.1	–
(*R*)-2-(*N*-Acetyl-L-cystein-*S*-yl-1-hydroxybut-3-ene	1.7 ± 0.3	1.9 ± 0.4
1-(*N*-Acetyl-L-cystein-*S*-yl)-2-(*S*)-hydroxybut-3-ene	0.2 ± 0.1	1.3 ± 0.2
1-(*N*-Acetyl-L-cystein-*S*-yl)-2-(*R*)-hydroxybut-3-ene	1.6 ± 0.4	6.1 ± 0.2
(*S*)-2-(*N*-Acetyl-L-cystein-*S*-yl)-1-hydroxybut-3-ene	4.2 ± 0.6	2.5 ± 0.1
4-(*N*-Acetyl-L-cystein-*S*-yl)-1-hydroxybut-2-ene	–	0.1 ± 0.1
S-(1-Hydroxybut-3-en-2-yl)mercaptoacetic acid	–	1.6 ± 0.4
S-(2-Hydroxybut-3-en-1-yl)mercaptoacetic acid	–	2.3 ± 0.1
Unknown	3.9 ± 0.8	–

From Richardson *et al*. (1999)

[a] Italicized metabolites are produced subsequent to hydrolysis of epoxybutene; the other metabolites listed are produced by direct reaction of epoxybutene with glutathione.

Urinary metabolites were also identified in male Sprague-Dawley rats and B6C3F$_1$ mice exposed by nose-only inhalation to 1, 5 or 20 ppm [2.21, 11 or 44 mg/m^3] [2,3-^{14}C]butadiene for 6 h (Booth *et al*., 2004a). This study did not include measurements of radioactivity in faeces, exhaled volatiles, carbon dioxide or the carcass. Urine was collected over an 18-h period after exposure but not during exposure. The profiles of urinary metabolites were somewhat similar to those reported by Richardson *et al*. (1999).

Four mercapturic acids derived from HMVK were identified in the urine from male B6C3F$_1$ mice and Sprague-Dawley rats given a single intraperitoneal injection of 25, 62.5, 125 or 200 mg/kg bw butanediol (Sprague & Elfarra, 2004). The metabolites were *N*-acetyl-L-cystein-*S*-yl conjugates of 1,2-dihydroxybutane, 1-hydroxy-2-butanone, propanol and propionic acid. HMVK may be formed by ADH- or CYP-mediated oxidation of butenediol. These four HMVK-derived metabolites accounted for 7 and 11% of the total radioactivity recovered from rats and mice exposed to butadiene, respectively (Richardson *et al*., 1999).

Trace amounts of 1-hydroxy-2-butanone, crotonic acid, propionic acid and 2-ketobutyric acid were detected in the urine of male B6C3F$_1$ mice and Sprague-Dawley rats administered butanediol by intraperitoneal injection (mice: 125 or 250 mg/kg bw; rats: 62.5, 125, 200 or 250 mg/kg bw) (Sprague & Elfarra, 2003). The combined concentration of these metabolites was less than 1% of the administered dose of butenediol; these low levels may be due to further metabolism of the carboxylic acids prior to their excretion in the urine. The detection of these metabolites suggests the possibility that toxic intermediary metabolites, such as crotonaldehyde, may be formed from butenediol.

(d) Haemoglobin adducts

Epoxybutene can form two diastomeric pairs of adducts at the *N*-terminal valine of haemoglobin termed MHbVal: *N*-(2-hydroxy-3-buten-1-yl)valine and *N*-(1-hydroxy-3-buten-2-yl)valine (IARC, 1999). Levels of these adducts formed in rats and mice are dependent on exposure concentrations of butadiene, duration of exposure and the turnover rates of red blood cells.

Much work has been carried out on the use of haemoglobin adducts as biomarkers of internal levels of the epoxide intermediates of butadiene metabolism. Epoxybutene, diepoxybutane and epoxybutanediol can react with the *N*-terminal valine to form MHbVal, PyrVal and THbVal adducts, respectively (Boysen *et al*., 2007). In addition, THbVal may also be formed by the reaction of haemoglobin with diepoxybutane and subsequent hydrolysis of the remaining epoxide group.

THbVal adduct levels in haemoglobin isolated from erythrocytes of male Sprague-Dawley rats and B6C3F$_1$ mice exposed by nose-only inhalation to 1, 5 or 20 ppm [2.2, 11 or 44 mg/m^3] [2,3-^{14}C]butadiene for 6 h per day for 5 days were 80, 179 and 512 pmol/g haemoglobin for rats, respectively, and 143, 351 and 1100 pmol/g haemoglobin for mice, respectively (Booth *et al*., 2004a). However, the level of radioactivity was approximately 1.3-fold higher in rat than in mouse haemoglobin. The measured levels of THbVal adduct represented only about 1–2% of the total radioactive adducts in the haemoglobin, which indicates that multiple alkylation sites within haemoglobin may be modified by butadiene metabolites. After incubation of epoxybutane with erythrocytes, Moll *et al.* (2000) detected 10 epoxybutane adducts on each of the α- and β-globin chains.

A specific ring-closed adduct, the pyrolidine adduct PyrVal, can be formed by the reaction of haemoglobin with diepoxybutane (Fred *et al*., 2004). This adduct has been measured after in-vitro reactions of rat and mouse erythrocyte haemolysates with diepoxybutane and after intraperitoneal injections of diepoxybutane into Fischer 344 rats and C57/BL mice. The levels of PyrVal adduct per administered dose were similar in rat and mouse haemoglobin after in-vitro or in-vivo exposure to diepoxybutane. In addition, THbVal adducts were measured in rats and mice given intraperitoneal injections of diepoxybutane or epoxybutanediol. Levels of this adduct were similar in mice and rats dosed with epoxybutanediol; however, after treatment with diepoxybutane, levels of THbVal adducts were three to four times higher in rats.

Several haemoglobin adducts were measured in rats and mice after inhalation exposure to butadiene (Boysen *et al*., 2004, 2007). Female B6C3F$_1$ mice were exposed to 3, 62.5 or 1250 ppm [6.6, 138 or 3453 mg/m^3] butadiene for 6 h per day for 2 weeks, while female Fischer 344 rats were exposed to 3 or 62.5 ppm [6.6 or 138 mg/m^3] for 10 days. At equivalent butadiene exposures, the levels of MHbVal and PyrVal were higher in mice than in rats, while levels of THbVal were similar in these species (Table 25). The formation of each of these adducts in mice and rats was more efficient (pmol adduct/g haemoglobin per ppm butadiene) at 3 ppm butadiene than at higher exposure concentrations. The major adduct formed in mice and rats is THbVal. In rats, formation of THbVal reaches a plateau at exposures to butadiene above 62.5 ppm (Boysen *et al*., 2007).

Table 25. *N*-Terminal haemoglobin adducts (pmol/g haemoglobin and adducts/ ppm butadiene) in mice and rats exposed to butadiene (BD) for 10 days

	Exposure (ppm)	MHbVal		PyrVal		THbVal	
		pmol/g	/ppm BD	pmol/g	/ppm BD	pmol/g	/ppm BD
Mice	0	0.91 ± 0.9				54 ± 8	
	3	38 ± 6	12.7	49 ± 3	16.2	339 ± 41	112.8
	62.5	145 ± 21	2.3	130 ± 64	2.1	3202 ± 302	51.2
	1250	7386 ± 227	5.9	2487 ± 426	2.0	14 838 ± 975	11.9
Rats	0	2.6 ± 0.6				60 ± 2	
	3	1.3 ± 0.7	4.3	4 ± 1	1.3	397 ± 12	132
	62.5	86 ± 1.2	1.4	38 ± 1	0.6	2937 ± 39	47
	1250	1682 ± 58	1.4			5555 ± 469	4.4

From Boysen *et al*. (2007)
MHbVal, *N*-(2-hydroxy-3-butenyl)valine; PyrVal, *N*,*N*-(2,3-dihydroxy-1,4-butadiyl)valine; THbVal, *N*-(2,3,4-trihydroxybutyl)valine

The levels of THbVal adducts in female B6C3F$_1$ mice exposed by inhalation to 6, 18 or 36 ppm butanediol for 6 h per day on 5 days per week for 4 weeks were approximately twofold higher than those in female Fischer 344 rats exposed to the same concentrations of this metabolite. The similarity in the shape of the dose–response curves for the formation of these adducts and the induction of hypoxanthine–guanine phosphoribosyl transferase gene (*Hprt*) mutant frequency in splenic T cells from mice and rats exposed to butenediol suggests that epoxybutanediol (the product of butenediol epoxidation) may play a significant role in the mutagenicity of butadiene (Powley *et al*., 2005).

(e) Physiological toxicokinetic models of butadiene disposition

Several physiologically based toxicokinetic models have been developed to characterize the disposition of butadiene and epoxybutene in rats and mice (IARC, 1999).

These models were based on species-specific physiological parameters, in-vitro metabolic parameters, and blood:air, tissue:air and tissue:blood partition coefficients. Adjustable parameters were estimated by fitting the models to data on butadiene and epoxybutene uptake in rats and mice exposed separately to these gases in closed chambers. Although each model was fairly effective in reproducing butadiene uptake in rats and mice, they overpredicted blood levels of epoxybutene measured subsequently in rats and mice exposed to butadiene. This discrepancy was reduced by assuming that epoxybutene formed from butadiene is partially sequestered in an intrahepatic compartment that allows first-pass metabolism of epoxybutene by EH or by assuming that only a small fraction of butadiene (19–24%) is oxidized to epoxybutene. None of the models included the formation or elimination of epoxybutanediol.

Predictions of human blood levels of diepoxybutane are sensitive to parameters that affect the metabolism of butadiene to epoxybutene, the rate at which epoxybutene is oxidized to diepoxybutane and the rates of hydrolysis of epoxybutene and diepoxybutane.

To address the finding that physiological models of butadiene disposition that reproduced the uptake of this gas and epoxybutene from closed chambers but overpredicted blood concentrations of epoxybutene measured subsequently in rats and mice exposed to butadiene, a modified model was proposed in which epoxybutene formed from butadiene has privileged access to EH (Kohn & Melnick, 2000; based on work of Oesch & Daly, 1972). This was then expanded to include equations for the production and metabolism of butenediol and epoxybutanediol (Kohn & Melnick, 2001). The model predicts higher concentrations of epoxybutanediol than either epoxybutene or diepoxybutane in all metabolizing tissues of rats and mice at all butadiene exposures examined.

4.2 Genetic and related effects

The genetic toxicology of butadiene and its major metabolites, epoxybutene and diepoxybutane, has been reviewed (IARC, 1999; Henderson, 2001).

4.2.1 *Humans*

The *HPRT* variant frequency in lymphocytes as well as the concentration of the urinary metabolite of butadiene, DHBMA, was examined in 49 workers in a styrene–butadiene rubber production facility in Texas, USA (Ammenheuser *et al.*, 2001). The study included 24 subjects who had high exposure and 25 who had low exposure for *HPRT* variant frequency analysis, 22 subjects who had high exposure and 24 who had low exposure for dosimeter measurements and 24 who had high exposure and 23 who had low exposure for urine analyses. The mean value of exposure to butadiene was 1.48 ± 0.37 ppm [3.27 ± 0.82 mg/m^3] (mean ± standard error [SE]) for the high-exposure group and 0.15 ± 0.02 ppm [0.33 ± 0.04 mg/m^3] for the low-exposure group. The frequency of *HPRT* variants was 6.66 ± 1.4 × 10^{-6} (mean ± SE) for the high-exposure group and 2.10 ±

0.2×10^{-6} for the low-exposure group ($p < 0.0002$). When smoking status was considered, the frequency of *HPRT* variants was $6.8 \pm 1.2 \times 10^{-6}$ for 19 nonsmokers in the high-exposure group and $1.8 \pm 0.2 \times 10^{-6}$ for 20 nonsmokers in the low-exposure group ($p < 0.0005$). The concentration of DHBMA was 2046 ± 348 ng/mg creatinine in the urine of the high-exposure group compared with 585 ± 98 ng/mg creatinine in the urine of the low-exposure group ($p < 0.0005$).

Another study was conducted in the same styrene–butadiene facility in 1998 (Ward *et al*., 2001). The frequency of *HPRT* variants in lymphocytes as well as the concentration of the urinary metabolite of butadiene, DHBMA, were examined in 37 workers (22 who had high exposure and 15 who had low exposure). The mean value of exposure to butadiene was 1.71 ± 0.54 ppm [3.78 ± 1.2 mg/m^3] (mean ± SE) for the high-exposure group and 0.07 ± 0.03 ppm [0.15 ± 0.07 mg/m^3] for the low-exposure group. The frequency of *HPRT* variants was $10.67 \pm 1.51 \times 10^{-6}$ (mean ± SE) for the high-exposure group and $3.54 \pm 0.61 \times 10^{-6}$ for the low-exposure group ($p < 0.001$). The concentration of the metabolite was 378 ± 53 ng/mg creatinine in the urine of the high-exposure group compared with 271 ± 50 ng/mg creatinine in the urine of the low-exposure group. Unlike the previous study, this difference was not significant ($p > 0.05$).

A subset of the workers from the Texas facility was analysed for their frequency of *HPRT* mutants (Ma *et al*., 2000). *HPRT* mutants were analysed by a multiplex polymerase chain reaction (PCR) and the frequency of large deletions in butadiene-exposed workers (17.5%; 25/143) was significantly higher than that in control subjects (9.7%; 21/217; $p < 0.05$). This increase in large deletions was due primarily to an increase in multiple exon deletions ($p < 0.05$). When the *HPRT* mutants were analysed for cDNA sequence mutations, the majority of the mutations observed in both exposure groups were single-base substitutions. However, the overall distribution of the types of mutation was significantly different between the two groups ($p < 0.05$). A non-significant increase in mutations at AT sites was observed in butadiene-exposed workers (46%) compared with the control group (39%), and the proportion of A:T→T:A transversions was also increased in the butadiene-exposed group (16%) compared with the control group (8%; $p = 0.25$). Three new mutable sites were identified at positions 116, 370 and 410. The frequency of -1 frame shift mutations was significantly higher (11%; $p < 0.05$) in exposed workers than in the controls (2%). Polymorphisms in the *mEH* gene may play a significant role in the sensitivity of humans to the genotoxic effects of butadiene. In workers exposed to > 150 ppb [331.5 μg/m^3] butadiene, individuals who had at least one polymorphic *mEH His* allele had a significant ($p < 0.001$) threefold increase in the frequency of *HPRT* variant (mutant) lymphocytes compared with individuals who had the *Tyr/Tyr* genotype (Abdel-Rahman *et al*., 2001).

Blood samples from 19 exposed (butadiene monomer production unit) and 19 control (heat production unit) workers at a petrochemical company in the Czech Republic were analysed for chromosomal aberrations and sister chromatid exchange (Šrám *et al*., 1998). The median exposure concentration of butadiene was 0.53 mg/m^3 [0.24 ppm] for the exposed group and 0.013 mg/m^3 [0.006 ppm] for the control group. A significant increase

in the percentage of aberrant cells was observed in the exposed group (3.11 ± 1.33 versus 2.03 ± 1.01%; $p < 0.01$). When smoking status was considered in the exposed group, no difference in the percentage of aberrant cells was observed between smokers (3.11 ± 1.50) and nonsmokers (3.10 ± 1.24), although nonsmokers were exposed to nearly three times more butadiene (1.73 mg/m^3) than smokers (0.53 mg/m^3). There was also a significant increase in the frequencies of sister chromatid exchange per cell in the exposed compared with the control groups (6.96 ± 1.51 and 4.87 ± 1.11 [mean ± SD], respectively; $p < 0.001$).

A comparison of conventional cytogenetic analyses and fluorescence in-situ hybridization (FISH) was conducted among 82 workers in a chemical plant in the Czech Republic (Šrám *et al.*, 2004). Twenty-three subjects worked in monomer production and were exposed to 0.642 mg/m^3 [0.3 ppm] butadiene, 34 worked in polymer production (1.794 mg/m^3 [0.812 ppm] butadiene) and 25 matched controls (0.023 mg/m^3 [0.01 ppm] butadiene) worked in administration. Using both methods, no significant differences in chromosomal aberration frequency were detected between any of the groups. When subjects with suspected clonality were excluded, re-analysis of the data by FISH found a significant difference between the polymer production workers (2.73 ± 1.51 genomic frequencies of stable chromosomal exchanges) and the monomer production and control groups (1.72 ± 1.14 and 2.06 ± 1.31 genomic frequencies of stable chromosomal exchanges, respectively).

A study that used several different biomarkers to determine the effects of exposure to butadiene on workers was undertaken in the Czech Republic (Albertini *et al.*, 2001). Eighty-three workers from the same industrial site were divided into three groups: controls (25 from administration), and monomer (24) and polymer (34) workers. Polymer workers typically had levels of exposure (1.76 ± 4.69 mg/m^3 [0.8 ppm]) that were significantly higher than those of the monomer workers (0.64 ± 2.06 mg/m^3 [0.29 ppm]) and controls (0.3 ± 0.03 mg/m^3 [0.14 ppm]). Analysis by autoradiography of *HPRT* mutations in 49 workers showed a significant difference between the control (10.75 ± 6.11 × 10^{-6}) and the monomer (5.73 ± 4.72 × 10^{-6}) and the polymer workers (6.48 ± 4.77 × 10^{-6}). This trend contradicts that expected. Analysis by a cloning assay of *HPRT* mutations in 75 workers showed no significant differences between the groups (controls, 13.00 ± 8.1 × 10^{-6}; monomer workers, 10.69 ± 5.4 × 10^{-6}; polymer workers, 18.83 ± 17.41 × 10^{-6}). Assays for sister chromatid exchange were completed for 73 study subjects. No significant differences were observed between the groups. Chromosomal aberrations were analysed by traditional methods in 82 workers. The mean percentages of cells with aberrations were 1.56, 1.52 and 1.54% for the control, monomer and polymer groups, respectively. The mean number of chromosomal breaks per cell was also similar between the groups. Chromosomal changes analysed by FISH also showed no significant differences. [The Working Group noted the very high levels of butadiene-derived metabolites in the urine of controls.]

A Health Effects Institute Report summarized the study of workers in the Czech Republic (Albertini *et al.*, 2003a). The conclusions on the genotoxicity studies were that

'none of these measures showed positive responses at exposure levels encountered in this study'. *HPRT* mutations analysed by cloning or autoradiographic techniques showed no effect of butadiene, and mutation spectra were not significantly different between exposed and unexposed workers. *HPRT* mutation frequencies were also unrelated to the metabolic genotypes examined. When smoking status was considered, there was no significant effect of smoking and no significant exposure group-by-smoking interaction on *HPRT* mutation frequencies. Chromosomal aberrations analysed by traditional or FISH methods and sister chromatid exchange were also unaffected by exposure to butadiene. The results of the study by Šrám *et al.* (1998) were in conflict with these conclusions and showed increased chromosomal aberrations.

Further molecular epidemiological analysis of butadiene-exposed workers in the Czech Republic was conducted to determine whether any gender differences in the response to butadiene existed (Albertini *et al.*, 2007). The average level of exposure to butadiene (mg/m^3) was 0.397 ± 0.502 (mean ± SD) [0.18 ppm] for the 23 women and 0.808 ± 1.646 [0.37 ppm] for the 30 men. Thus, exposed male workers had a significantly higher level of exposure than female workers in this study. It should be noted that exposed male workers also had higher levels of exposure to styrene than female workers and that exposed female workers had higher levels of exposure to toluene and benzene than the exposed male workers. Although urinary concentrations of the mercapturic acid metabolites of butadiene, DHBMA and MHBMA, were higher in butadiene-exposed women than in female controls, the differences were not significant. The levels of both DHBMA and MHBMA (μg/L) were significantly increased in male butadiene-exposed subjects (854.1 ± 567.0 and 47.9 ± 44.3, respectively) compared with male controls (512.8 ± 272.1 and 14.9 ± 10.3, respectively; $p < 0.05$). Thus, significantly higher concentrations of metabolites occurred in men than in women in both the control and exposed groups. *HPRT* mutations measured by the T-cell assay did not differ significantly between exposed and control groups of either sex. No significant associations between exposure to butadiene and sister chromatid exchange or chromosomal aberrations were detected in either sex. Effects of genotype were also examined. In this study, *GSTT1*-null workers showed a significantly slower rise in the rate of MHBMA excretion ($p < 0.05$). In addition, individuals with the EH genotype that specifies low activity showed a significantly higher rise in urinary MHBMA/(DHBMA + MHBMA) ratios with increasing exposure to butadiene.

Zhang *et al.* (2004) measured chromosomal changes in the peripheral blood lymphocytes of 39 butadiene polymer production workers and 38 unexposed controls in Yanshan, China. The median exposure level for the butadiene-exposed workers as a 6-h TWA was 2 ppm [4.42 mg/m^3] whereas the control group had a median level of 0 ppm. Tobacco use was controlled for by including a similar percentage of smokers with similar pack–years of smoking in both the control and exposed groups. No significant numerical or structural chromosomal changes were detected using FISH with probes for chromosomes 1, 7, 8 or 12. *GSTT1* and *GSTM1* genotypes had no significant effect on the frequency of hyperdiploidy of the above chromosomes or on the frequency of structural

changes of chromosomes 8 and 12 in the butadiene-exposed group. The EH *EPHX1* Y113H polymorphism had no effect on chromosomal damage or *HPRT* mutant frequency in either the exposed or control workers. However, workers with the histidine arginine HR or RR allele of the *EPHX1* H139R polymorphism had increased levels of hyperdiploidy of chromosomes 1, 7 and 8. Overall, predicted EPHX1 activity did not influence genetic damage at low occupational exposures to butadiene. Further analysis at this plant of 41 workers exposed to the same median levels of butadiene (2 ppm) and 38 controls was undertaken (Hayes *et al*., 2001). No differences were observed in the Glycophorin A assay between the exposed workers and the controls. The mutation frequency of *HPRT* measured with the T-cell cloning assay was also not significantly different and no significant increase was detected in sister chromatid exchange frequency.

Another study at a tyre plant in the Slovak Republic examined 110 workers, who were exposed to several xenobiotics of which butadiene was the most prominent, for markers of genotoxicity in relation to several polymorphisms (Vodicka *et al.*, 2004). The workers were divided into three groups: high exposure (butadiene concentration, 2.6 ± 0.2 mg/m^3 [1.18 ppm]), low exposure (butadiene concentration, 2.3 ± 2.2 mg/m^3 [1.04 ppm]) and no exposure (trace amounts). The frequencies of total chromosomal aberrations were significantly lower (1.3 ± 1.3; $p < 0.01$) in the low-exposure group compared with the high- and no-exposure groups (2.2 ± 1.4 and 2.3 ± 1.1, respectively). No significant differences were observed in DNA single-strand breaks or in single-strand break endo III-sensitive site frequencies. A non-significant twofold higher rate of DNA repair was found in the high-exposure group (0.6 ± 0.6 single-strand breaks/10^9 daltons) compared with the low- and no-exposure groups (0.3 ± 0.3 and 0.3 ± 0.4 single-strand breaks/10^9 daltons, respectively). A weak but non-significant association was found between CYP2E1 expression in peripheral blood lymphocytes and the frequency of chromosomal aberrations ($r = 0.298$; $p = 0.097$). In all individuals, assessment of several genetic polymorphisms suggested that individuals who had low *EPHX1*-activity genotypes had the highest level of chromosomal aberrations. Significantly lower frequencies of chromosomal aberration ($p = 0.024$) were detected in individuals who had the variant CC genotype associated with the XPD exon 23.

A study of 27 healthy male Caucasian workers exposed to butadiene and 26 matched controls from an Italian petrochemical plant analysed genotoxic effects (Lovreglio *et al*., 2006). The mean exposure to butadiene was 6.4 ± 14.0 μg/m^3 [0.003 ppm] for the butadiene-exposed workers which was significantly different from that of the controls (0.8 ± 1.1 μg/m^3 [0.0004 ppm]; $p < 0.001$). No significant differences were observed in sister chromatid exchange, the percentage of cells with a high frequency of sister chromatid exchange, chromosomal aberrations or proliferation index in the peripheral blood lymphocytes of these two groups. When subjects were classified according to smoking status, a significant increase was observed in the mean frequency of sister chromatid exchange in smokers (6.6 ± 1.2) compared with nonsmokers (5.5 ± 0.8; $p = 0.001$). Exposure to butadiene was also higher in smokers than in nonsmokers but this was not statistically significant ($p = 0.3$).

4.2.2 *Experimental systems*

(a) Butadiene (see Table 26 for details and references)

The genotoxicity of butadiene has been reviewed previously (IARC, 1999).

The frequency of DNA single-strand breaks was increased in NMRI mice exposed to butadiene *in vivo*.

Decreases in cloning efficiency of T cells and a significant increase in *Hprt* mutation frequency was observed in female B6C3F$_1$ mice exposed to 3 ppm [6.63 mg/m^3] butadiene for 2 weeks and in rats exposed to 62.5 ppm [138.1 mg/m^3] butadiene for 2 weeks; these are the currently reported lowest-observed-effect levels for butadiene that induced *Hprt* mutations in mice and rats, respectively. The *Hprt* mutation frequency was also increased in *Ephx*-1 null and *Xpc* (DNA repair enzyme) null mice exposed to butadiene for 4 weeks compared with normal mice.

Characterization of *Hprt* mutations in cDNA and genomic DNA from splenic T-cell mutants was carried out in male B6C3F$_1$ mice and Fischer 344 rats. The mean *Hprt* mutant frequency in mice was $9.20 \pm 3.25 \times 10^{-6}$ in the butadiene-exposed animals compared with $1.48 \pm 0.84 \times 10^{-6}$ ($p < 0.001$) in the controls. In rats, the mean *Hprt* mutant frequency in the exposed animals was $8.08 \pm 2.82 \times 10^{-6}$ compared with $3.07 \pm 0.98 \times 10^{-6}$ ($p < 0.001$) in the controls. In mice, real time PCR and cDNA sequencing showed a statistically significant difference in the overall proportion of mutation types detected in control versus butadiene-exposed mice ($p = 0.042$), while multiplex PCR of genomic DNA showed that deletion mutations were significantly increased in butadiene-exposed mice ($p = 0.031$). Analysis of the individual mutation types that occurred in both treated and control mice showed that exposure to butadiene significantly increased the frequencies of each type of base substitution, except for A:T→G:C transitions, and frameshifts and deletions. In male rats, no difference in the overall mutational spectra was detected using cDNA sequencing alone or when combined with multiplex PCR. Statistical analyses of the individual mutation types showed that exposure to butadiene significantly increased base substitution at A:T→T:A transversions in both mice and rats, and single-base insertions, deletion mutations and complex mutations as well as G:C→C:G transversions in mice.

In the bone marrow of B6C3F$_1$ *LacI* transgenic mice exposed to butadiene, a significant increase in point mutations was observed at A:T base pairs compared with air-exposed mice. In the spleen of the exposed mice, a significant increase in base substitution mutations (GC→AT transitions and GC→TA transversions at non-CpG sites) was detected, and a significant increase in A:T base pairs occurred, similar to that observed in the bone marrow.

Micronucleus formation was increased in NMRI mice and male and female B6C3F$_1$/CrBR mice exposed to butadiene. Male B6C3F$_1$ mice were exposed to butadiene and the bone marrow was harvested 24 h after onset of exposure (Jackson *et al.*, 2000). The frequencies of micronucleated polychromatic erythrocytes were significantly increased (28.2 ± 3.1/100 cells; $p < 0.05$) compared with controls (9.87 ± 2.1/100 cells) as

Table 26. Genetic and related effects of butadiene

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, reverse mutation	–	+	1080 ppm	Araki *et al*. (1994)
Salmonella typhimurium TA1530, reverse mutation	–	+	86 ppm	de Meester *et al*. (1980)
Salmonella typhimurium TA1535, reverse mutation	–	+	216 ppm	Araki *et al*. (1994)
Salmonella typhimurium TA1537, TA98, reverse mutation	–	–	1080 ppm	Araki *et al*. (1994)
Escherichia coli WP2 *uvrA*, reverse mutation	–	–	1080 ppm	Araki *et al*. (1994)
Drosophila melanogaster, somatic mutation or recombination	–		10 000 ppm inh	Victorin *et al*. (1990)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		500 ppm inh	Foureman *et al*. (1994)
DNA single-strand breaks, NMRI mouse alveolar macrophages *in vitro*	–	NT	40 ppm	Walles *et al*. (1995)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	650 ppm	McGregor *et al*. (1991)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	(+)	1.35	Sasiadek *et al*. (1991a)
Sister chromatid exchange, human lymphocytes *in vitro*	+	+	108	Sasiadek *et al*. (1991b)
Binucleated cells, human bronchial epithelial cells *in vitro*	+	NT	25 μg as soot/mL medium	Catallo *et al*. (2001)
DNA cross-links, B6C3F$_1$ mouse liver *in vivo*	+		450 ppm inh 7 h	Jelitto *et al*. (1989)
DNA cross-links, B6C3F$_1$ mouse liver *in vivo*	–		2070 ppm inh 8 h/d, 7 d	Ristau *et al*. (1990)
DNA cross-links, B6C3F$_1$ mouse lung and liver *in vivo*	+		250 ppm inh 7 h	Vangala *et al*. (1993)
DNA single-strand breaks, B6C3F$_1$ mouse liver *in vivo*	+		2000 ppm inh 7 h/d, 7 d	Vangala *et al*. (1993)
DNA single-strand breaks, NMRI mouse lung and liver *in vivo*	+		200 ppm inh 16 h	Walles *et al*. (1995)
DNA strand breaks, CD-1 mouse liver, bone marrow or testis *in vivo*	–		130 ppm inh 6 h/d, 4 wk	Anderson *et al.* (1997)
DNA damage, CD-1 mouse testicular cells *in vivo*	+		125 ppm inh 6 h	Brinkworth *et al.* (1998)
DNA single-strand breaks and γ-irradiation-specific DNA repair activity, NMRI mice *in vivo*	+		500 mg/m^3 6 h/d, 28 d	Vodicka *et al*. (2006)
DNA cross-links, Sprague-Dawley rat liver *in vivo*	–		550 ppm inh 7 h	Jelitto *et al*. (1989)
DNA cross-links, Sprague-Dawley rat liver *in vivo*	–		1240 ppm inh 8 h/d, 7 d	Ristau *et al.* (1990)
DNA cross-links, Sprague-Dawley rat liver and lung *in vivo*	–		2000 ppm inh 7 h	Vangala *et al*. (1993)
DNA single-strand breaks, Sprague-Dawley rat liver *in vivo*	+		2000 ppm inh 7 h/d, 7 d	Vangala *et al*. (1993)

Table 26 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, *lac*Z mouse bone marrow *in vivo*	+		625 ppm inh 6 h/d, 5 d/wk, 1 wk	Recio *et al.* (1992)
Gene mutation, B6C3F$_1$ mouse T lymphocytes, *Hprt* locus *in vivo*	+		625 ppm inh 6 h/d, 5 d/wk, 4 wk	Cochrane & Skopek (1993)
Gene mutation, B6C3F$_1$ mouse T lymphocytes, *Hprt* locus *in vivo*	+		625 ppm inh 6 h/d, 5 d/wk, 2 wk	Cochrane & Skopek (1994)
Gene mutation, *LacI* mice *in vivo*	+		62.5 ppm inh 6 h/d, 5 d/wk, 4 wk	Sisk *et al.* (1994)
Gene mutation, B6C3F$_1$ mouse T lymphocytes, *Hprt* locus *in vivo*	+		1300 ppm inh 6 h/d, 5 d/wk, 1 wk	Tates *et al.* (1994)
Gene mutation, *LacI* mice *in vivo*	+		1250 ppm inh 6 h/d, 5 d/wk, 4 wk	Recio & Meyer (1995)
Gene mutation, (102/E1 × C3H/E1)F$_1$ mouse splenocytes, *Hprt* locus *in vivo*	+		500 ppm inh 6 h/d, 5 d	Tates *et al.* (1998)
Gene mutation, CD-1 mouse splenocytes, *Hprt* locus *in vivo*	–		1300 ppm inh 6 h/d, 5 d/wk, 4 wk	Tates *et al.* (1998)
Gene mutation, female B6C3F$_1$ mice, *Hprt* locus *in vivo*	+		20 ppm 6 h/d, 5 d/wk, 2 wk	Meng *et al.* (1999a)
Gene mutation, female B6C3F$_1$ mouse thymic and splenic T cells, *Hprt* locus *in vivo*	+		20 ppm 6 h/d, 5 d/wk, 2 wk	Walker & Meng (2000)
Gene mutation, female Fischer 344 rats, *Hprt* locus *in vivo*	(+)		625 ppm 6 h/d, 5 d/wk, 4 wk	Walker & Meng (2000)
Gene mutation, female B6C3F$_1$ mice, *Hprt* locus *in vivo*	+		3 ppm 6 h/d, 5 d/wk, 2 wk	Meng *et al.* (2001)
Gene mutation, male B6C3F$_1$ mice and Fischer 344 rats, *Hprt* locus *in vivo*	+		1250 ppm 6 h/d, 5 d/wk, 2 wk	Meng *et al.* (2004)

Table 26 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, B6C3F$_1$ *LacI* transgenic mice *in vivo*	+		625 ppm 6 h/d, 5 d/wk, 4 wk	Recio *et al.* (2001)
Gene mutation, B6C3F$_1$ mice, *Znfn1a1/Ikaros* gene *in vivo*	(+)		NG	Karlsson *et al*. (2002)
Gene mutation, *Ephx1*-plus mice, *Hprt* locus *in vivo*	(+)		20 ppm 7 h/d, 5 d/wk, 4 wk	Wickliffe *et al.* (2003, 2007)
Gene mutation, *Ephx1*-null mice, *Hprt* locus *in vivo*	+		20 ppm 7 h/d, 5 d/wk, 4 wk	Wickliffe *et al.* (2003, 2007)
Gene mutation, *Xpc*-plus mice, *Hprt* locus *in vivo*	(+)		20 ppm 7 h/d, 5 d/wk, 4 wk	Wickliffe *et al.* (2007)
Gene mutation, *Xpc*-null mice, *Hprt* locus *in vivo*	+		20 ppm 7 h/d, 5 d/wk, 4 wk	Wickliffe *et al.* (2007)
Gene mutation, male B6C3F$_1$ mice and female Fischer 344 rats, *Hprt* locus *in vivo*	+		1250 ppm 6 h/d, 5 d/wk, 2 wk	Meng *et al*. (2007a)
Gene mutation, female Fischer 344 rats, *Hprt* locus *in vivo*	(+)		62.5 ppm 6 h/d, 5 d/wk, 2 wk	Meng *et al.* (2007a)
Mouse spot test, female T-stock mice	+		500 ppm inh 6 h/d, 5 d/wk, 1 wk	Adler *et al*. (1994)
Sister chromatid exchange, B6C3F$_1$ mouse bone marrow *in vivo*	+		116 ppm inh 6 h	Cunningham *et al*. (1986)
Sister chromatid exchange, Sprague-Dawley rat bone marrow *in vivo*	–		4000 ppm inh 6 h	Cunningham *et al*. (1986)
Sister chromatid exchange, B6C3F$_1$ mouse bone marrow *in vivo*	+		7 ppm inh 6 h/d, 5 d/wk, 2 wk	Tice *et al*. (1987)
Micronucleus formation, B6C3F$_1$ mouse bone marrow *in vivo*	+		116 ppm inh 6 h	Cunningham *et al*. (1986)
Micronucleus formation, B6C3F$_1$ mouse peripheral blood *in vivo*	+		70 ppm inh 6 h/d, 5 d/wk, 2 wk	Tice *et al*. (1987)

Table 26 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, B6C3F_1 mouse peripheral blood *in vivo*	+		7 ppm inh 6 h/d, 5 d/wk, 13 wk	Jauhar *et al*. (1988)
Micronucleus formation, NMRI mouse bone marrow *in vivo*	+		35 ppm inh 23 h	Victorin *et al*. (1990)
Micronucleus formation, (102/E1 × C3H/E1)F_1 and CB6F_1 mice *in vivo*	+		50 ppm inh 6 h/d, 5 d/wk	Adler *et al*. (1994); Autio *et al*. (1994)
Micronucleus formation, (102 × C3H) mice *in vivo*	+		200 ppm inh 6 h/d, 5 d/wk	Xiao & Tates (1995)
Micronucleus formation, (102/E1 × C3H/E1)F_1 mouse splenocytes *in vivo*	+		130 ppm inh 6 h/d, 5 d	Stephanou *et al.* (1998)
Micronucleus formation (102/E1 × C3H/E1)F_1 mouse spermatids *in vivo*	+		250 ppm inh 6 h/d, 5 d	Tommasi *et al.* (1998)
Micronucleus formation, male B6C3F_1 mice *in vivo*	+	NT	1100 ppm initial concentration 4 h	Jackson *et al.* (2000)
Micronucleus formation, male and female (102/EI × C3H/EI)F_1 mouse primary lung fibroblasts *in vivo*	+	NT	500 ppm 6 h/d, 5 d	Ranaldi *et al*. (2001)
Micronucleus formation, male and female B6C3F_1/CrBR mice *in vivo*	+	NT	1000 ppm 6 h/d, 2 d	Bevan *et al.* (2001)
Micronucleus formation, NMRI mice *in vivo*	+	NT	500 mg/m^3 6 h/d, 28 d	Vodicka *et al*. (2006)
Micronucleus formation, Sprague-Dawley rat bone marrow *in vivo*	–		4000 ppm inh 6 h/d, 2 d	Cunningham *et al*. (1986)
Micronucleus formation, Sprague-Dawley rats *in vivo*	–		500 ppm 6 h/d, 5 d/wk	Autio *et al*. (1994)
Chromosomal aberrations, B6C3F_1 and NIH mouse bone marrow *in vivo*	+		1500 ppm inh 6 h	Irons *et al*. (1987b)
Chromosomal aberrations, B6C3F_1 mouse bone marrow *in vivo*	+		700 ppm inh 6 h/d, 5 d/wk, 2 wk	Tice *et al*. (1987)
Chromosomal aberrations, (102/E1 × C3H/E1)F_1 mouse embryos *in vivo*	+		130 ppm inh 6 h/d, 5 d	Pachierotti *et al.* (1998)
Aneuploidy, B6C3F_1 and NIH mouse bone marrow *in vivo*	–		1500 ppm inh 6 h	Irons *et al.* (1987b)

Table 26 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Dominant lethal test, male CD-1 mice	+		233 ppm inh 6 h/d, 5 d/wk, 1 wk	Morrissey *et al*. (1990)
Dominant lethal test, CD-1 mice	+		1250 ppm inh 6 h/d, 5 d/wk, 10 wk	Anderson *et al*. (1993)
Dominant lethal test, CD-1 mice	–		6250 ppm inh 6 h	Anderson *et al*. (1993)
Dominant lethal test, (102/E1 × C3H/E1)F_1 mice	+		1300 ppm inh 6 h/d, 5 d/wk, 1 wk	Adler *et al*. (1994)
Dominant lethal test, (102/E1 × C3H/E1)F_1 mice	+		500 ppm inh 6 h/d, 5 d	Adler *et al.* (1998)
Dominant lethal test, CD-1 mice	+		65 ppm inh 6 h/d, 5 d/wk, 4 wk	Anderson *et al*. (1998)
Dominant lethal test, CD-1 mice	+		125 ppm inh 6 h/d, 5 d/wk, 10 wk	Brinkworth *et al*. (1998)
Dominant lethal test, Sprague-Dawley rats	–		1250 ppm inh 6 h/d, 5 d/wk, 10 wk	Anderson *et al.* (1998)
Mouse (C3H/E1) heritable translocation test	+		1300 ppm inh 6 h/d, 5 d/wk, 1 wk	Adler *et al*. (1995)
Mouse (102/E1 × C3H/E1)F_1 heritable translocation test	+		500 ppm inh 6 h/d, 5 d	Adler *et al.* (1998)
Binding to DNA, male B6C3F_1 mouse and male Wistar rat liver *in vivo*	+		13 ppm inh 4–6.6 h	Kreiling *et al*. (1986a)
Binding to DNA at N7 of guanine, male B6C3F_1 mouse liver *in vivo*	+		450 ppm inh 7 h	Jelitto *et al*. (1989)
Binding to DNA at N7 of guanine, male B6C3F_1 mouse liver *in vivo*	+		NG	Bolt & Jelitto (1996)
Binding to DNA at N^6 of adenine, mouse lung *in vivo*	+		200 ppm inh 6 h/d, 5 d/wk, 1 wk	Koivisto *et al*. (1996)

Table 26 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Binding to DNA at N7 of guanine, mouse testis and lung *in vivo*	+		200 ppm 6 h/d, 5 d	Koivisto *et al*. (1998)
Binding to DNA at N7 of guanine, male Wistar rat liver *in vivo*	–		550 ppm inh 7 h	Jelitto *et al*. (1989)
Binding to DNA at N7 of guanine, male Wistar rat liver *in vivo*	–		NG	Bolt & Jelitto (1996)
Binding to DNA at N^6 of adenine, rat lung *in vivo*	+		200 ppm inh 6 h/d, 5 d/wk, 1 wk	Koivisto *et al*. (1996)
Binding to DNA at N7 of guanine, male Sprague-Dawley rat liver *in vivo*	+		200 ppm inh 6 h/d, 5 d/wk, 1 wk	Koivisto *et al*. (1997)
Binding to protein, male B6C3F_1 mouse and male Wistar rat liver *in vivo*	+		13 ppm inh 4–6.6 h	Kreiling *et al*. (1986a)
Sperm morphology, CD-1 mice *in vivo*	+		1165 ppm inh 6 h/d, 5 d/wk, 1 wk	Morrissey *et al*. (1990)

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

[b] LED, lowest effective dose; HID, highest ineffective dose; d, day; inh, inhalation exposure; wk, week

determined by acridine orange staining. Two other methods to determine micronucleus frequency also showed similar results.

The contribution of CYP2E1 to the genotoxicity of butadiene was investigated by pretreating animals with 1,2-*trans*-dichloroethylene, a selective CYP2E1 inhibitor, and 1-aminobenzotriazole, an irreversible inhibitor of several CYPs, before exposure to butadiene (Jackson *et al.*, 2000). Pretreatment with 1,2-*trans*-dichloroethylene significantly lowered the micronucleus frequencies observed (19.8 ± 2.5) but the levels were still elevated over those in unexposed controls (11.5 ± 2.0). Pretreatment with 1-aminobenzotriazole caused the micronucleus frequency to fall to a level similar to that in unexposed animals. The frequency of kinetochore-negative micronuclei was also significantly increased in butadiene-exposed animals (21.3 ± 1.2) compared with controls (5.2 ± 1.7), indicating that butadiene is a clastogen.

(b) Butadiene metabolites

(i) *Epoxybutene* (see Table 27 for details and references)

Epoxybutene is mutagenic in *Salmonella typhimurium* in the presence and absence of a metabolic activation system.

The cII mutant frequency was increased only in Big Blue™ mice but not rat fibroblasts *in vitro*. The in-vitro mutational spectrum induced by epoxybutene (1 mM for 24 h) in Rat2 *LacI* cells was compared with a background spectrum. Significant increases in GC→AT transitions at non-CpG sites as well as GC→TA transversions (32% of the epoxybutene-induced mutations) were observed. At A:T base pairs, a significant increase was observed in AT→CG and AT→TA transversions.

Epoxybutene increased micronucleus formation in both Big Blue rat and mouse fibroblasts.

In a human B-lymphoblastoid cell line that does not express active CYP2E1, the mutational spectrum at *HPRT* induced by epoxybutene showed a significant increase in G:C→A:T and A:T→T:A mutations. The 2*S*-stereoisomer increased the *HPRT* mutation frequency in human lymphoblastoid TK6 cells whereas the 2*R*-isomer caused significant increases at higher doses only.

Mean frequencies of sister chromatid exchange were significantly increased in human whole blood lymphocyte cultures by epoxybutene in both the *GSTM1*-null ($p < 0.001$) and *GSTM1*-positive ($p = 0.03$) genotypes compared with unexposed controls.

The effect of *GSTM1* genotype as well as an adaptive dose of epoxybutene on the induction of sister chromatid exchange was examined. Without an adaptive dose, both genotypes showed a significant increase in sister chromatid exchange over controls. With the adaptive dose, the mean number of sister chromatid exchanges was significantly higher following exposure to epoxybutene in the *GSTM1*-null group (17.42 ± 2.43; $p = 0.01$) than in the control group (7.69 ± 1.00) or the *GSTM1*-positive group (14.07 ± 4.22). The results show an increased sensitivity of *GSTM1*-null subjects to the induction of sister chromatid exchange by epoxybutene.

Table 27. Genetic and related effects of epoxybutene

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA98, TA97a, reverse mutation	+	+	2–10 mM	Himmelstein *et al*. (2001)
Gene mutation, Big Blue™ mouse fibroblasts, cII locus *in vitro*	+		125 µM	Erexson & Tindall (2000a)
Gene mutation, Rat2 *LacI* transgenic fibroblasts, *LacI* locus *in vitro*	+		0.6 mM	Saranko *et al.* (1998); Recio *et al*. (2000)
Gene mutation, Big Blue™ rat fibroblasts, cII locus *in vitro*	(+)		500 µM	Erexson & Tindall (2000a)
Sister chromatid exchange, male CD-1 mouse and male CD rat splenic lymphocytes at G_0 stage in the cell cycle *in vitro*	–		931 µM	Kligerman *et al*. (1999a,b)
Micronucleus formation, Big Blue™ mouse and rat fibroblasts *in vitro*	(+)		125 µM	Erexson & Tindall (2000a)
Micronuclei formation, Chinese hanster V79 cells *in vitro*	+		1 mM	Himmelstein *et al*. (2001)
Gene mutation, human lymphoblastoid TK6 cells, *HPRT* locus *in vitro*	+		400 µM for 24 h	Recio *et al*. (2000)
Gene mutation, human lymphoblastoid TK6 cells, *HPRT* and *TK* loci *in vitro*	+		400 µM 2*S*- or 2*R*-isomer for 24 h	Meng *et al*. (2007b)
Gene mutation, human lymphoblastoid TK6 cells, *HPRT* locus *in vitro*	+		400 µM 2*R*-isomer for 24 h	Meng *et al*. (2007b)
Gene mutation, human lymphoblastoid TK6 cells, *HPRT* locus *in vitro*	+		200 µM 2*S*-isomer for 24 h	Meng *et al*. (2007b)
Sister chromatid exchange, human whole blood at G_0 stage in the cell cycle *in vitro*	–		931 µM	Kligerman *et al*. (1999a,b)
Sister chromatid exchange, human whole blood lymphocytes, *GSTM1–*, *GSTM1+ in vitro*	+		25 µM for 24 h	Sąsiadek *et al*. (1999)
Micronucleus formation, human lymphocytes *in vitro*	–		300 µM	Murg *et al*. (1999a)

Table 27 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, human peripheral blood lymphocytes treated with ara-C *in vitro*	+		931 μM	Kligerman *et al*. (1999b)
Hyperdiploidy and chromosomal breakage (1cen-q12 region), human lymphocytes *in vitro*	–		300 μM	Murg *et al*. (1999a)
Inhibition of IL-2 production, human $CD4^+$ lymphocytes *in vitro*	–		10 μM	Irons *et al*. (2001)
Inhibition of clonogenic activity, human $CD34^+$ bone marrow cells *in vitro*	–		1 mM	Irons *et al*. (2000)
Inhibition of clonogenic activity, human $CD34^+$ bone marrow cells *in vitro*	–		100 μM	Irons *et al*. (2001)
Gene mutation, female B6C3F_1 mouse splenic T cells, *Hprt* locus *in vivo*	+		2.5 ppm 6 h/d, 5 d/wk, 4 wk	Meng *et al*. (1999b)
Gene mutation, female Fischer 344 rats splenic T cells, *Hprt* locus *in vivo*	–		25 ppm 6 h/d, 5 d/wk, 4 wk	Meng *et al*. (1999b)
Gene mutation, female B6C3F_1 *LacI* transgenic mouse spleen and bone marrow *in vivo*	–		29.9 ppm 6 h/d, 5 d/wk, 2 wk	Recio *et al*. (2000, 2001); Saranko *et al*. (2001)
Gene mutation, female B6C3F_1 *LacI* transgenic mouse lung *in vivo*	(+)		29.9 ppm 6 h/d, 5 d/wk, 2 wk	Recio *et al*. (2000, 2001); Saranko *et al*. (2001)
Gene mutation, female Fischer 344 *LacI* transgenic rat spleen *in vivo*	–		29.9 ppm 6 h/d, 5 d/wk, 2 wk	Recio *et al*. (2000)
Gene mutation, female Fischer 344 *LacI* transgenic rat bone marrow *in vivo*	(+)		29.9 ppm 6 h/d, 5 d/wk, 2 wk	Recio *et al*. (2000)
Gene mutation, female B6C3F_1 mouse splenic T cells, *Hprt* locus *in vivo*	+		2.5 ppm 6 h/d, 5 d/wk, 4 wk	Walker & Meng (2000)

Table 27 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, female Fischer 344 rat splenic T cells, *Hprt* locus *in vivo*	–		2.5 ppm 6 h/d, 5 d/wk, 4 wk	Walker & Meng (2000)
Gene mutation, *Ephx1*-plus mice, *Hprt* locus *in vivo*	–		80 mg/kg/48 h × 3	Wickliffe *et al*. (2007)
Gene mutation, *Ephx1*-null mice, *Hprt* locus *in vivo*	+		80 mg/kg/48 h × 3	Wickliffe *et al*. (2007)
Gene mutation, *Xpc*-plus mice, *Hprt* locus *in vivo*	–		100 mg/kg/48 h × 3	Wickliffe *et al*. (2007)
Gene mutation, *Xpc*-null mice, *Hprt* locus *in vivo*	+		100 mg/kg/48 h × 3	Wickliffe *et al*. (2007)
Gene mutation, *Xpc* –/– mice, *Hprt* locus *in vivo*	+		150 mg/kg	Wickliffe *et al*. (2006)
Micronucleus formation, male C57/BL mouse polychromatic erythrocytes *in vivo*	+		250 µmol/kg	Fred *et al*. (2005)
Micronucleus formation, male Sprague-Dawley rat polychromatic erythrocytes *in vivo*	–		1125 µmol/kg	Fred *et al*. (2005)

IL, interleukin

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

[b] LED, lowest effective dose; HID, highest ineffective dose; d, day; wk, week

Epoxybutene did not induce sister chromatid exchange or chromosomal aberrations in human blood lymphocytes when added at the G_0 stage of the cell cycle. However, from further studies in which excision repair was inhibited, it is probable that epoxybutene induces chromosomal aberrations and DNA damage that is repaired by the excision process in G_0 lymphocytes (Kligerman *et al*., 1999a,b).

In vivo, a significant increase in mutation frequency was observed at the *Hprt* locus in splenic T cells from female B6C3F$_1$ mice exposed to epoxybutene. Exposure to epoxybutene by inhalation resulted in an approximately threefold increase in the frequency of *LacI* mutants in the lungs of female B6C3F$_1$ *LacI* transgenic mice ($8.3 \pm 3.0 \times 10^{-5}$ and $9.9 \pm 3.0 \times 10^{-5}$) compared with air-exposed controls ($3.1 \pm 0.7 \times 10^{-5}$ and $3.6 \pm 0.7 \times 10^{-5}$). Significant increases in GC→AT transitions at CpG sites ($p = 0.001$) were detected. A number of other alterations (insertions, deletions and tandem changes) were also increased in the lungs of exposed mice (10/54, 10%) compared with controls (2/59, 4%). When these alterations were considered separately, only the frequency of deletions was significantly increased ($p = 0.005$).

(ii) *Epoxybutanediol* (see also Table 28 for details and references)

Epoxybutanediol has the least mutagenic potency of the butadiene epoxides in traditional mutagenic assays.

Epoxybutanediol increased cII mutant frequency in Big Blue™ mouse but not rat fibroblasts.

Mutational spectra of epoxybutanediol were obtained in the *Hprt* locus in Chinese hamster ovary-K1 cells. Of the 41 mutants analysed, 25 (61%) were base substitutions and 16 (39%) were deletions. The most common base substitutions were GC→AT and AT→GC transitions. Among the deletions, the majority of the mutants showed single exon loss.

Epoxybutanediol increased micronucleus formation in Big Blue™ rat fibroblasts *in vitro*. The same effect was seen in Big Blue™ mouse fibroblasts only at higher concentrations.

Epoxybutanediol weakly suppressed the haematopoietic progenitor clonogenic response in human CD34$^+$ bone-marrow cells.

(iii) *Diepoxybutane* (see also Table 29 for details and references)

Diepoxybutane has been shown to be the most mutagenic of the butadiene epoxides in traditional mutagenicity assays.

Diepoxybutane is mutagenic in *S. typhimurium*, *Escherichia coli* and *Sulfolobus acidocaldarius* in the presence and absence of a metabolic activation system.

Thirty-nine diepoxybutane mutants were analysed in Chinese hamster ovary-K1 cells. Of these, 24 (62%) were base substitutions and 15 (38%) were deletions. The major base substitutions were GC→AT transitions (11/24) and AT→TA (5/24) and GC→CG (6/24) transversions. Among the deletions, the majority of the mutants showed single exon loss.

Table 28. Genetic and related effects of epoxybutanediol

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, Big Blue™ mouse fibroblasts, cII locus *in vitro*	+	NT	1000 µM	Erexson & Tindall (2000a)
Gene mutation, Big Blue™ rat fibroblasts, cII locus *in vitro*	–	NT	1000 µM	Erexson & Tindall (2000a)
Gene mutation, Chinese hamster ovary CHO-K1 cells, *Hprt* locus *in vitro*	+	NT	2 mM	Lee *et al*. (2002)
Micronucleus formation, Big Blue™ mouse fibroblasts *in vitro*	+	NT	500 µM	Erexson & Tindall (2000a)
Micronucleus formation, Big Blue™ rat fibroblasts *in vitro*	+	NT	250 µM	Erexson & Tindall (2000a)
Inhibition of IL-2 production, human $CD4^+$ lymphocytes *in vitro*	–	NT	10 µM	Irons *et al*. (2001)
Inhibition of clonogenic activity, human $CD34^+$ bone-marrow cells *in vitro*	–	NT	10^{-3} M	Irons *et al*. (2000)
Inhibition of clonogenic activity, human $CD34^+$ bone-marrow cells *in vitro*	(+)	NT	100 µM	Irons *et al*. (2001)

IL, interleukin

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

[b] LED, lowest effective dose; HID, highest ineffective dose

Table 29. Genetic and related effects of diepoxybutane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli TRG8, his$^+$ revertants, + human O^6-alkylguanine-DNA alkyltransferase	+	NT	1 mM	Valadez *et al*. (2004)
Salmonella typhimurium YG7108, his$^+$ revertants , + human O^6-alkylguanine-DNA alkyltransferase	+	NT	1 mM	Valadez *et al*. (2004)
Escherichia coli KL185, RC50, G1209, reverse mutation or genetic recombination	+	NT	5–300 µg/mL	Reilly & Grogan (2002)
Escherichia coli MBL50 cells, *supF* mutant frequency	+	NT	40 µM all isomers	Kim *et al*. (2007)
Salmonella typhimurium T100, TA97a, reverse mutation	–	–	10 mM	Himmelstein *et al.* (2001)
Salmonella typhimurium TA1535, TA98, reverse mutation	+	+	0.2 mM	Himmelstein *et al.* (2001)
Sulfolobus acidocaldarius DG29, DG38, DG64, reverse or forward mutation	+	NT	5–300 µg/mL	Reilly & Grogan (2002)
Gene mutation, Big Blue™ mouse and rat fibroblasts, cII locus *in vitro*	+	NT	2.5 µM	Erexson & Tindall (2000a)
Gene mutation, Chinese hamster ovary-K1 cells, *Hprt* locus *in vitro*	+	NT	20 µM	Lee *et al*. (2002)
Gene mutation, Rat2 transgenic fibroblasts, *LacI* locus *in vitro*	(+)	NT	10 µM	Recio *et al*. (2000)
Sister chromatid exchange, male CD rat and CD-1 mouse splenic lymphocytes, whole blood and isolated blood lymphocytes at G_0 stage in the cell cycle *in vitro*	+	NT	2.5 µM	Kligerman *et al*. (1999a)
Sister chromatid exchange, Big Blue™ mouse and rat fibroblasts *in vitro*	+	NT	2 µM	Erexson & Tindall (2000b)
Micronucleus formation, Big Blue™ mouse and rat fibroblasts *in vitro*	+	NT	2.5 µM	Erexson & Tindall (2000a)
Micronucleus formation, Rat2 *LacI* cells *in vitro*	+	NT	2 µM	Recio *et al*. (2000, 2001)
Micronucleus formation, Chinese hamster V79 cells *in vitro*	+	NT	12.5 µM	Himmelstein *et al.* (2001)

Table 29 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, human lymphoblastoid TK6 cells, *HPRT* locus *in vitro*	+	NT	4 μM for 24 h	Recio *et al.* (2000)
Gene mutation, human lymphoblastoid TK6 cells, *HPRT* locus *in vitro*	+	NT	2 μM all isomers for 24 h	Meng *et al.* (2007b)
Gene mutation, human lymphoblastoid TK6 cells, *TK* locus *in vitro*	+	NT	2 μM all isomers for 24 h	Meng *et al.* (2007b)
Sister chromatid exchange, human whole blood and isolated blood lymphocytes *in vitro*	+	NT	2.5 μM	Kligerman *et al*. (1999a)
Sister chromatid exchange, human whole blood lymphocytes *in vitro*	+	NT	5 μM	Schlade-Bartusiak *et al*. (2001, 2004)
Micronucleus formation, human lymphocytes *in vitro*	+	NT	2.5 μM	Murg *et al*. (1999a)
Chromosomal breakage (1cen-q12 region), AZH-1 cells from human lymphoblastoid TK6 cells	+	NT	5 μM	Murg *et al*. (1999b)
Chromosomal breakage (1 cen-q12 region), human lymphocytes *in vitro*	+	NT	2.5 μM	Murg *et al.* (1999a)
Hyperdiploidy (chromosome 1), AZH-1 cells from human lymphoblastoid TK6 cells	+	NT	10 μM	Murg *et al*. (1999b)
Hyperdiploidy, human lymphocytes *in vitro*	+	NT	10 μM	Murg *et al*. (1999a)
Inhibition of IL-2 production, human $CD4^+$ lymphocytes	–	NT	10 μM	Irons *et al*. (2001)
Inhibition of clonogenic activity, human $CD34^+$ bone-marrow cells	+	NT	2 μM *meso* or D,L	Irons *et al*. (2000, 2001)
Inhibition of clonogenic response, human CD34+ bone-marrow cells	+	NT	2 μM	Irons *et al.* (2001)
Cell cycle arrest in G_1/G_2, human embryonic lung fibroblasts	+	NT	100 μM for 1 h	Schmiederer *et al*. (2005)
Increased p53 and $p21^{cip1}$, human embryonic lung fibroblasts	+	NT	100 μM for 1 h	Schmiederer *et al*. (2005)

Table 29 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Comet tail moments, *Ephx1*-plus mice *in vivo*	–	NT	15 mg/kg/24 h × 2	Wickliffe *et al*. (2003)
Comet tail moments, *Ephx1*-null mice *in vivo*	+	NT	1.5 mg/kg/24 h × 2	Wickliffe *et al*. (2003)
Gene mutation, female Fischer 344 rat and female B6C3F$_1$ mouse splenic T cells, *Hprt* locus *in vivo*	+	NT	2 ppm 6 h/d, 5 d/wk, 4 wk	Meng *et al.* (1999b); Walker & Meng (2000)
Gene mutation, female Fischer 344 *LacI* transgenic rat bone marrow *in vivo*	(+)	NT	3.8 ppm 6 h/d, 5 d/wk, 2 wk	Recio *et al*. (2000)
Gene mutation, female Fischer 344 *LacI* transgenic rat spleen and female B6C3F$_1$ *LacI* transgenic mouse bone marrow and spleen *in vivo*	-	NT	3.8 ppm 6 h/d, 5 d/wk, 2 wk	Recio *et al*. (2000)
Gene mutation, *Ephx1*-plus mice, *Hprt* locus *in vivo*	+	NT	15 mg/kg/24 h × 2	Wickliffe *et al*. (2003, 2007)
Gene mutation, *Ephx1*-null mice, *Hprt* locus *in vivo*	+	NT	15 mg/kg/24 h × 2	Wickliffe *et al*. (2003, 2007)

IL, interleukin

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

[b] LED, lowest effective dose; HID, highest ineffective dose; d, day; wk, week

At low concentrations of diepoxybutane, increases in the frequency of sister chromatid exchange were observed in Big Blue™ mouse and rat fibroblasts and of micronucleus formation in Rat2 *LacI* cells *in vitro* (Erexson & Tindall, 2000b; Recio *et al.*, 2000, 2001).

Increases in the *HPRT* and thymidine kinase (*TK*) mutation frequency in human lymphoblastoid TK6 cells were observed with all isomers (or the 2*R*,3*R*-, 2*S*,3*S*- and *meso*-stereoisomers) of diepoxybutane. The mutational spectrum of diepoxybutane at *HPRT* in human TK6 cells after exposure to racemic diepoxybutane was compared with background. Significant increases in A:T→T:A transversions and partial deletions were detected. Diepoxybutane increased the frequencies of sister chromatid exchange and micronucleus formation in human lymphocytes *in vitro*. Other genetic alterations such as chromosomal breakage and hyperdiploidy were observed as the concentration of diepoxybutane increased.

The effect of various polymorphisms on the frequency of sister chromatid exhange in whole blood lymphocytes from human volunteers after in-vitro exposure to diepoxybutane was examined. A significant difference in sister chromatid exchange was observed between the *GSTT1*-negative and *GSTT1*-positive individuals (79.28 ± 23.33 and 58.96 ± 17.44, respectively; $p < 0.01$). Individuals who were heterozygous for the C2 allele in CYP2E1 (C1/C2), which is associated with higher enzyme activity, had higher levels of sister chromatid exchange (70.83 ± 10.85) compared with C1/C1 individuals (56.83 ± 17.64; $p < 0.05$). The *RAD51* polymorphism as well as EH activity had no effect. In individuals with the *GSTT1*-null genotype, a significant difference was observed in individuals with very low or low expected EH activity (72.13 ± 12.41) and in individuals whose activity was expected to be high (102.73 ± 21.22). Similar results were observed in an earlier study in which *GSTT1*-null individuals had a sister chromatid exchange frequency of 84.8 ± 20.3 whereas individuals who were *GSTT1*-positive had a frequency of 67.9 ± 10.8 ($p < 0.001$). In this study, no effect was observed in individuals with different *GSTM1* genotypes (Schlade-Bartusiak *et al.*, 2000, 2004).

After in-vivo exposure of female $B6C3F_1$ mice and Fischer 344 rats to diepoxybutane, dose-related increases in mutation frequency at the *Hprt* locus were observed in the splenic T cells of both species. *LacI* transgenic mice and rats exposed to diepoxybutane showed no or a weak increase in *LacI* mutation frequency in the spleen and bone marrow.

4.2.3 *Mechanism of mutation induction*

(a) DNA adducts

Many adducts with epoxybutene, epoxybutanediol and diepoxybutane have been identified in reactions with nucleosides and DNA *in vitro* (see Table 30 for details and references). The mutagenicity and mutation spectra of several of these adducts have been investigated (see Table 31 for details and references). Many of these adducts can also block replication by many polymerases or can cause misincorporation of proper nucleotides (see Table 32 for details and references). DNA adducts have been identified in humans exposed to butadiene and in animals exposed to butadiene and its metabolites.

Table 30. Reactivity of butadiene metabolites with DNA bases *in vitro*

Targets	Butadiene metabolite	Adducts formed	Kinetics	Analytical methods	Analytical methods
2′-Deoxyadenosine	EB	(*R*)-N^6-(1-Hydroxy-3-buten-2-yl)deoxyadenosine; (*S*)-N^6-(1-hydroxy-3-buten-2-yl)deoxyadenosine		NMR, MS, CD	Nechev *et al.* (2001)
2′-Deoxyguanosine	EB	(*R*)-N^2-(1-Hydroxy-3-buten-2-yl)deoxyguanosine; (*S*)-N^2-(1-hydroxy-3-buten-2-yl)deoxyguanosine		NMR, MS, CD spectra	Nechev *et al.* (2001)
2′-Deoxyguanosine	EB	*N*7-(2-Hydroxy-3-butenyl)guanine (G1) (equal amounts); *N*7-(1-(hydroxymethyl)-2-propenyl)guanine (G2) (equal amounts)	Neutral thermal hydrolysis	LC/MS, NMR	Boogaard *et al.* (2001b, 2004)
Single- and double-stranded calf thymus DNA	EB	*N*7-(2-Hydroxy-3-buten-1-yl)guanine (G1); *N*7-(1-hydroxy-3-buten-2-yl)guanine (G2); diastereomers of *N3*-(2-hydroxy-3-buten-1-yl)deoxyuridine; N^6-(2-hydroxy-3-buten-1-yl)deoxyadenosine; *N3*-(2-hydroxy-3-buten-1-yl)adenine (A1); *N*3-(1-hydroxy-3-buten-2-yl)adenine (A2)	Enzymatic and neutral thermal hydrolysis, all adducts detected at EB ≥ 10 mM in ssDNA and ≥ 100 mM in dsDNA; I, II major adducts, III-V more prominent in ssDNA than dsDNA	HPLC, UV, FAB–MS	Selzer & Elfarra (1999); Elfarra *et al.* (2001)
Calf thymus DNA	EB	*N*7-(2-Hydroxy-3-butenyl)guanine (G1); *N*7-(1-(hydroxymethyl)-2-propenyl)guanine (G2); *N3*-(2-hydroxy-3-butenyl)adenine (A1); *N*3-(1-hydroxymethyl-2-propenyl)adenine (A2)	Neutral thermal hydrolysis, G1 ≥ G2 >> A2 > A1	HPLC, UV	Boogaard *et al.* (2004)
2′-Deoxyguanosine	EBD	*N*7-(1-(Hydroxymethyl)-2,3-dihydroxypropyl)guanine (major) (G3); *N*7-(2,3,4-trihydroxybut-1-yl)guanine (minor) (G4)	Neutral thermal hydrolysis	LC/MS, NMR	Boogaard *et al.* (2001b)
Deoxyadenosine 5′-monophosphate	EBD	N^6-2,3,4-Trihydroxybutyladenine; *N*1-trihydroxybutyladenine	Base hydrolysis at 37 °C		Zhao *et al.* (1998)
2′-Deoxyguanosine-5′-phosphate, calf thymus DNA	EBD	*N*7-(2,3,4-Trihydroxybut-1-yl)guanine (G4)	Half-life, 30 ± 4 h	HPLC, UV	Koivisto *et al.* (1999)
Salmon testis DNA	DEB	N^6-2,3,4-Trihydroxybutyladenine; *N*1-trihydroxybutyladenine	Base hydrolysis at 37 °C		Zhao *et al.* (1998)

Table 30 (contd)

Targets	Butadiene metabolite	Adducts formed	Kinetics	Analytical methods	Analytical methods
2′-Deoxyguanosine	DEB	Diastereomeric pairs of *N*-(2-hydroxy-1-oxiranylethyl)-2′-deoxyguanosine (P4-1 and P4-2); 7,8-dihydroxy-3-(2-deoxy-β-D-erythro-pentofuranosyl)-3,5,6,7,8,9-hexahydro-1,3-diazepino[1,2-*a*]purin-11(11H)one (P6); 1-(2-hydroxy-2-oxiranylethyl)-2′deoxyguanosine (P8 and P9); 1-[3-chloro-2-hydroxy-1-(hydroxymethyl)propyl]-2'-deoxyguanosine (1AP9 and 2AP9); 4,8-dihydroxy-1-(2-deoxy-β-D-erythro-pentofuranosyl)-9-hydroxymethyl-6,7,8,9-tetrahydro-1H-pyrimido[2,1-*b*] purinium ion (1BP4 and 2BP4); 6-oxo-2-amino-9-(2-deoxy-β-D-erythro-pentofuranosyl)-7-(2-hydroxy-2-oxiranylethyl)-6,9-dihydro-1H-purinium ion (P5 and P5′)	Product profile similar although much slower at DEB:dG ratio 10:1 compared to 80:1 at pH 7.4	HPLC, MS, NMR	Zhang & Elfarra (2003)
2′-Deoxyguanosine	DEB	7-Hydroxy-6-hydroxymethyl-5,6,7,8-tetrahydropyrimido[1,2-*a*]purin-10(1H)one (H2); 2-amino-1-(4-chloro-2,3-dihydroxybutyl)1,7-dihydro-6H-purine-6-one (H4); 2-amino-1-(2,3,4-trihydroxybutyl)-1,7-dihydro-6H-purin-6-one (H1′/H5′); 7,8-dihyroxy-1,5,6,7,8,9-hexahydro1,3-diazepino[1,2-*a*]purin-11(11H)one (H2′); 5-(3,4-dihydroxy-1-pyrrolidinyl)-2,6-diamino-4(3H)pyrimidinone (H3′); 2-amino-7-(3-chloro-2,4-dihydroxybutyl)-1,7-dihydro-6H-purin-6-one (H3); 2-amino-7-(2,3,4-trihydroxybutyl)-1,7-dihydro-6H-purin-6-one (H4′)	Acid hydrolysis H4′/H3 - hydrolysis products of P5/P5′; H2 - of P4-1, P4-2; H4, H1′/H5′, hydrolysis of P8/P9	HPLC, MS, NMR	Zhang & Elfarra (2004)
2′-Deoxyguanosine	DEB	Diastereomeric pairs of *N*-(2-hydroxy-1-oxiranylethyl)-2′-deoxyguanosine (P4-1 and P4-2); 7,8-dihydroxy-3-(2-deoxy-β-D-erythro-pentofuranosyl)-3,5,6,7,8,9-hexahydro-1,3-diazepino[1,2-*a*]purin-11(11H)one (P6); 1-(2-hydroxy-2-oxiranylethyl)-2′deoxyguanosine (P8 and P9); 6-oxo-2-amino-9-(2-deoxy-β-D-erythro-pentofuranosyl)-7-(2-hydroxy-2-oxiranylethyl)-6,9-dihydro-1H-purinium ion (P5 and P5')	P5, P5′, P8, P9 half-lives of 2.6, 2.7, 16 and 16 h, respectively; P4-1, P4-2 and P6 are stable at physiological conditions (pH 7.4, 37 °C)	HPLC, UV, MS, NMR	Zhang & Elfarra (2005)

Table 30 (contd)

Targets	Butadiene metabolite	Adducts formed	Kinetics	Analytical methods	Analytical methods
2′-Deoxyguanosine	DEB	7,7′-(2,3-Dihydroxy-1,4-butanediyl)bis[2-amino-1,7-dihydro-6H-purin-6-one] (bis-*N*7G-BD); 2′-deoxy-1-[4-(2-amino-1,7-dihydro-6H-purin-6-on-7-yl)-2,3-dihydroxybutyl]-guanosine (*N*7G-*N*1dG-BD); 2-amino-9-hydroxymethyl-4-(4-acetyloxy-2,3-dihydroxybutyl)-8,9-dihydro-7H-[1,4]oxazepino[4,3,2-gh]purin-8-ol (PA1); 2-amino-9-hydroxymethyl-4-{4-[2-amino-9- or 7-(4-acetyloxy-2,3-dihydroxybutyl)-1,7-dihydro-6H-purin-6-on-7- or 9-yl]-2,3-dihydroxybutyl}-8,9-dihydro-7H-[1,4]-oxazepino[4,3,2-gh]purin-8-ol (PA2); 2-amino-7,9-bis(4-acetyloxy-2,3-dihydroxybutyl)-1,7-dihydro-6H-purin-6-one (PA3); 9,9′-bis(4-acetyloxy-2,3-dihydroxybutyl)-7,7′-(2,3-dihydroxy-1,4-butanediyl)bis[2-amino-1,7-dihydro-6H-purin-6-one] (PA4)	PA1-PA4 formed in the reaction in acetic acid P5D +dG produces bis-*N*7G-BD P8, P9 + dG produces *N*7G-*N*1dG-BD	HPLC, UV, MS, NMR	Zhang & Elfarra (2006)
2′-Deoxyadenosine	DEB	(*R*,*R*)-N^6-(2,3,4-Trihydroxybut-1-yl)deoxyadenosine; (*S*,*S*)-N^6-(2,3,4-trihydroxybut-1-yl)deoxyadenosine		NMR, MS, CD	Nechev *et al*. (2001)
2′-Deoxyguanosine	DEB	(*R*,*R*)-*N2*-(2,3,4-Trihydroxybut-1-yl)deoxyguanosine; (*S*,*S*)-*N2*-(2,3,4-trihydroxybut-1-yl)deoxyguanosine		NMR, MS, CD spectra	Nechev *et al*. (2001)
2′-Deoxyguanosine	DEB	*N*7-(2,3,4-Trihydroxybutyl)guanine (G4) (major); *N*7-(1-(hydroxymethyl)-2,3-dihydroxypropyl)guanine (G3) (minor)	Neutral thermal hydrolysis	LC–MS, NMR	Boogaard *et al*. (2001b, 2004)
Guanosine	(±)-DEB	(±)-*N*7-(2,3,4-Trihydroxybutyl)guanine	Acid hydrolysis	LC–MS/MS	Oe *et al*. (1999)
Guanosine	*meso*-DEB	*meso*-*N*7-(2,3,4-Trihydroxybutyl)guanine (G4)	Acid hydrolysis	LC–MS/MS	Oe *et al*. (1999)
2′-Deoxyguanosine-5′-phosphate, calf thymus DNA	RR/SS DEB	*N*7-(2-Hydroxy-3,4-epoxy-1-yl)-5′dGMP	Half-life, 31 ± 3 h	HPLC, UV	Koivisto *et al*. (1999)

Table 30 (contd)

Targets	Butadiene metabolite	Adducts formed	Kinetics	Analytical methods	Analytical methods
Calf thymus DNA	Racemic DEB	1-(Aden-1-yl)-4-(guan-7-yl)-2,3-butanediol (*N*1A-*N*7G-BD; 1); 1-(aden-3-yl)-4-(guan-7-yl)-2,3-butanediol (*N*3A-*N*7G-BD; 2); 1-(aden-7-yl)-4-(guan-7-yl)-2,3-butanediol (*N*7A-*N*7G-BD; 3); 1-(aden-N^6-yl)-4-(guan-7-yl)-2,3-butanediol (N^6A-*N*7G-BD; 4)	Acid hydrolysis; half-lives in dsDNA: 2, 31 h; 3, 17 h; 1 and 4 not released	MS/MS, HPLC, UV	Park *et al.* (2004)
Guanosine; calf thymus DNA	DEB	1,4-bis-(Guan-7-yl)-2,3-butanediol (bis-*N*7G-BD); *N*7-(2′,3′,4′)trihydroxybutylguanine (*N*7-THBG)	Neutral thermal hydrolysis; half-life of bis-*N*7G-BD, 81.5 h; half-life of *N*7-THBG, 48.5 h	UV, MS, NMR	Park & Tretyakova (2004)
Guanosine	*meso*-DEB	*meso*-1,4-bis-(Guan-7-yl)-2,3-butanediol		UV, MS, NMR	Park *et al.* (2005)
2′-Deoxyguanosine; calf thymus DNA	HMVK	Diasteromeric pair of HMVK-derived 1,N^2-propanodeoxyguanosine C-6 adducts; as well as a diastereomeric pair of C-8 HMVK-derived 1,N^2-propanodeoxyguanosine adducts	2′-Deoxyguanosine reaction run at pH 11, calf thymus DNA experiment at pH 7.4	UV, MS, NMR	Powley *et al.* (2003)

BD, butadiene; CD, circular dichroism; DEB, diepoxybutane; dG, deoxyguanine; dGMP, desoxyguanosine monophosphate; dsDNA, double-stranded DNA; EB, epoxybutene; EBD, epoxybutane diol; FAB, positive ion fast atom bombardment; G, guanosine; HMVK, hydroxymethylvinyl ketone; HPLC, high-performance liquid chromatography; LC, liquid chromatography; LC–MS/MS, liquid chromatography in combination with tandem mass spectrometry; MS, mass spectrometry; NMR, nuclear magnetic resonance; ssDNA, single-stranded DNA; THBG, trihydroxybutylguanine; UV, ultraviolet

Table 31. Genetic and related effects of the DNA adducts of butadiene

Adduct	Test system	Result[a] (total mutation %)	Common mutations	References
R-EB-N^2-guanine	*E. coli* AB 2480	(±)	–	Carmical *et al.* (2000a)
S-EB-N^2-guanine	*E. coli* AB 2480	(+) (< 1)	G→T transversions (45%) G→A transitions (32%)	Carmical *et al.* (2000a)
R-EB-deoxyinosine	COS-7 cells	+ (59)	A→G (48%) A→C (7%)	Kanuri *et al.* (2002)
S-EB-deoxyinosine	COS-7 cells	+ (94.5)	A→G (79%) A→C (10%)	Kanuri *et al.* (2002)
R-EB-deoxyinosine	*E. coli* AB 2480	+ (53)	A→G (43%)	Kanuri *et al.* (2002)
S-EB-deoxyinosine	*E. coli* AB 2480	+ (96.5)	A→G (87%)	Kanuri *et al.* (2002)
R-EB-deoxyinosine	*E. coli* AB 2480	+ (90)	A→G (65%) A→T (29%)	Rodriguez *et al.* (2001)
S-EB-deoxyinosine	*E. coli* AB 2480	+ (91)	A→G (63%) A→C (32%)	Rodriguez *et al.* (2001)
R-EB-N^6-adenine	*E. coli* AB 2480	–	–	Carmical *et al.* (2000b)
S-EB-N^6-adenine	*E. coli* AB 2480	–	–	Carmical *et al.* (2000b)
R,R-EBD-N^6-adenine	*E. coli* AB 2480	(+) (0.13)	A→G	Carmical *et al.* (2000b)
S,S-EBD-N^6-adenine	*E. coli* AB 2480	(+) (0.25)	A→C	Carmical *et al.* (2000b)
EB-*N3*-2'-deoxyuridine	COS-7 cells	+ (97)	C→T transitions (53.4%) C→A transversions (32.5%)	Fernandes *et al.* (2006)
R,R-EBD-N^2-guanine	*E. coli* AB 2480	(+) (< 1)	G→A, G→T, G→C (nearly equal)	Carmical *et al.* (2000a)
S,S-EBD-N^2-guanine	*E. coli* AB 2480	(+) (< 1)	G→A, G→T, G→C (nearly equal)	Carmical *et al.* (2000a)
R,R-DEB-N^2-N^2-guanine cross-link	*E. coli* AB 2480	+	G→T	Carmical *et al.* (2000c)
S,S-DEB-N^2-^{N2}-guanine cross-link	*E. coli* AB 2480	+	G→A	Carmical *et al.* (2000c)
R,R-DEB-N^6-N^6-deoxyadenosine cross-link	*E. coli* AB 2480	(+) (8)	A→G (7.5%)	Kanuri *et al.* (2002)

Table 31 (contd)

Adduct	Test system	Result[a] (total mutation %)	Common mutations	References
S,S-DEB-N^6-N^6-deoxyadenosine cross-link	*E. coli* AB 2480	(+) (2.8)	A→G (2.3%)	Kanuri *et al.* (2002)
R,R-DEB-N^6-N^6-deoxyadenosine cross-link	COS-7 cells	+ (54)	A→G transitions(40%) A→C transversions (9%)	Kanuri *et al.* (2002)
S,S-DEB-N^6-N^6-deoxyadenosine cross-link	COS-7 cells	(+) (19.4)	A→G (13%) A→T (5.6%)	Kanuri *et al.* (2002)

[a]+, positive; (+), weakly positive; –, negative
DEB, diepoxybutane; EB, epoxybutene; EBD, epoxybutanediol

Table 32. Effect of the butadiene-derived DNA adducts on replication/repair

Adduct[a]	Test system	Blockage[b]	Single nucleotide incorporation[c]	Comment	References
R-EB-N^6-adenine	*E. coli* DNA polymerases Pol I, II and III	–			Carmical *et al.* (2000b)
S-EB-N^6-adenine	*E. coli* DNA polymerases Pol I, II and III	–			Carmical *et al.* (2000b)
R-EB-N^2-guanine	*E. coli* DNA polymerases Pol I, II and III	+			Carmical *et al.* (2000a)
S-EB-N^2-guanine	*E. coli* DNA polymerases Pol I, II and III	+			Carmical *et al.* (2000a)
R-EB N^2-guanine	Yeast DNA polymerase η	–	Mostly C		Minko *et al.* (2001)
S-EB N^2-guanine	Yeast DNA polymerase η	–	C	More efficient than *R*-stereoisomer	Minko *et al.* (2001)
R-EB N^2-guanine	*E. coli* DNA polymerase Pol I	+			Minko *et al.* (2001)
S-EB N^2-guanine	*E. coli* DNA polymerase Pol I	+			Minko *et al.* (2001)
EB-*N*3-2′-deoxyuridine	Bacterial Klenow (Kf)	+			Fernandes *et al.* (2006)
EB-*N*3-2′-deoxyuridine	Mammalian polymerase δ	(+)			Fernandes *et al.* (2006)
EB-*N*3-2′-deoxyuridine	Yeast polymerase δ	+			Fernandes *et al.* (2006)
EB-*N*3-2′-deoxyuridine	Mammalian polymerase ε	+			Fernandes *et al.* (2006)
R,*R*-EBD-N^2-guanine	Yeast DNA polymerase η	–	C		Minko *et al.* (2001)
S,*S*-EBD-N^2-guanine	Yeast DNA polymerase η	–	C	More efficient than *R*-stereoisomer	Minko *et al.* (2001)
R,*R*-EBD-N^2-guanine	*E. coli* DNA polymerase Pol I	+			Minko *et al.* (2001)
S,S-EBD-N^2-guanine	*E. coli* DNA polymerase Pol I	+			Minko *et al.* (2001)
R,*R*-EBD-N^6-adenine	*E. coli* DNA polymerases Pol I, II and III	–			Carmical *et al.* (2000b)

Table 32 (contd)

Adduct[a]	Test system	Blockage[b]	Single nucleotide incorporation[c]	Comment	References
S,*S*-EBD-N^6-adenine	*E. coli* DNA polymerases Pol I, II and III	–			Carmical *et al.* (2000b)
R,*R*-EBD-N^2-guanine	*E. coli* DNA polymerases Pol I, II and III	+			Carmical *et al.* (2000a)
S,*S*-EBD-N^2-guanine	*E. coli* DNA polymerases Pol I, II and III	+			Carmical *et al.* (2000a)
R,*R*-EBD-N^2-guanine	Bacteriophage T7$^-$ DNA polymerase	(+)	dTTP		Zang *et al.* (2005)
S,*S*-EBD-N^2-guanine	Bacteriophage T7$^-$ DNA polymerase	(+)	dTTP	Misinsertion frequency 40-fold higher than the *R*,*R*-isomer	Zang *et al.* (2005)
R,*R*-EBD-N^2-guanine	HIV-1 reverse transcriptase	+			Zang *et al.* (2005)
S,*S*-EBD-N^2-guanine	HIV-1 reverse transcriptase	+			Zang *et al.* (2005)
R,*R*-DEB-N^2-N^2-guanine cross-link	*E. coli* AB2840 plaque-forming efficiency *in vivo*	+			Carmical *et al.* (2000c)
S,*S*-DEB-N^2-N^2-guanine cross-link	*E. coli* AB2840 plaque-forming efficiency *in vivo*	+			Carmical *et al.* (2000c)
R,*R*-DEB-N^2-N^2-guanine cross-link	*E. coli* DNA polymerases Pol I, II and III	+			Carmical *et al.* (2000c)
S,*S*-DEB-N^2-N^2-guanine cross-link	*E. coli* DNA polymerases Pol I, II and III	+			Carmical *et al.* (2000c)
R,*R*-DEB-N^2-N^2-guanine cross-link	*E. coli* UvrABC nuclease	+			Carmical *et al.* (2000c)

Table 32 (contd)

Adduct[a]	Test system	Blockage[b]	Single nucleotide incorporation[c]	Comment	References
S,*S*-DEB-N^2-N^2-guanine cross-link	*E. coli* UvrABC nuclease	+			Carmical *et al.* (2000a)
R,*R*-DEB-N^2-guanine-N^2-guanine cross-link	Yeast DNA polymerase η	+			Minko *et al.* (2001)
S,*S*-DEB-N^2-guanine-N^2-guanine cross-link	Yeast DNA polymerase η	+			Minko *et al.* (2001)

DEB, diepoxybutane; EB, epoxybutene; EBD, epoxybutanediol; HIV, human immunodeficiency virus

[a] Most were tested in an oligonucleotide

[b] +, highly blocked; (+), partially blocked; –, no blockage

[c] C, cytidine; dTTP, deoxythymidine triphosphate

(i) *Butadiene*

Humans

The levels of the DNA adduct, *N*-1-(2,3,4-trihydroxybutyl)adenine, was determined in 15 male butadiene-exposed (monomer unit) and 11 male control workers from a butadiene monomer production plant in the Czech Republic (Zhao *et al.*, 2000). The median exposure concentration of butadiene for the exposed group was 0.53 mg/m^3 [0.24 ppm] whereas that for the control group was 0.013 mg/m^3 [0.006 ppm]. Because of interfering background peaks, the *N*-1-adenine adducts were converted to N^6-adenine adducts. This adduct was detected in 14 of 15 exposed workers and five of 11 controls. The difference in the levels of adducts between the butadiene-exposed workers (4.5 ± 7.7 adducts/10^9 nucleotides) and the control workers (0.8 ± 1.2 adducts/10^9 nucleotides) was significant ($p = 0.038$). When controls were subdivided into smokers and nonsmokers, the adduct levels were 1.5 ± 1.7 adducts/10^9 nucleotides for four smokers and 0.3 ± 0.6 adducts/10^9 nucleotides for seven nonsmokers but the difference was not significant. A significant correlation between the levels of the *N*-1-(2,3,4-trihydroxybutyl)adenine adduct in lymphocyte DNA and individual exposures to butadiene was found in the exposed group ($r = 0.707$; $p = 0.005$), control ($r = 0.733$; $p = 0.01$) and both groups combined ($r = 0.723$; $p < 0.001$; Zhao *et al.*, 2001). However, no significant correlations were found with other genotoxic effects such as DNA single-strand breaks or micronucleus formation.

Experimental systems

1,4-Bis(guan-7-yl)-2,3-butanediol is the *N*7-guanine–*N*7-guanine crosslink formed from the reaction of DNA with diepoxybutane. This compound has been identified in the livers and lungs of C57BL/6 mice that were exposed to 625 ppm [1381 mg/m^3] butadiene by inhalation for 7 h per day for 5 days (Goggin *et al.*, 2007). The DNA from livers and lungs contained 3.2 ± 0.4 and 1.8 ± 0.5 adducts/10^6 guanines from racemic diepoxybutane but no adducts from *meso*-diepoxybutane were detected.

Male B6C3F$_1$ mice and Sprague-Dawley rats were exposed for 6 h to 200 ppm [442 mg/m^3] [2,3-^{14}C]butadiene by nose-only inhalation and were killed 48 h after treatment; the livers and lungs were analysed for DNA adducts (Boogaard *et al.*, 2001b). In the livers and lungs of both rats and mice, *N*7-(2,3,4-trihydroxybut-1-yl)guanine (G4) was the major adduct detected. Mice had 102 ± 4 and 80 ± 4 G4 adducts/10^8 nucleotides (means ± SE) and rats had 10 ± 2 and 13 ± 0.2 G4 adducts/10^8 nucleotides in the liver and lung, respectively. Smaller amounts of *N*7-(2-hydroxy-3-buten-1-yl)guanine (G1) and *N*7-(1-hydroxy-3-buten-2-yl)guanine (G2) were detected. Mouse liver and lung contained 21 ± 9 and 3.4 ± 1.3 adducts/10^8 nucleotides, respectively, and rat liver and lung contained 1.9 ± 0.2 and 3.6 ± 1.2 adducts/10^8 nucleotides, respectively. In rats, no *N*7-(1,3,4-trihydroxybut-2-yl)guanine (G3) was detected whereas mouse liver and lung contained 25 ± 2 and 4.3 ± 0.1 adducts/10^8 nucleotides, respectively. A similar profile was

also obtained in animals that were killed immediately after cessation of exposure (Boogaard *et al.*, 2004).

In another study, male B6C3F$_1$ mice and Sprague-Dawley rats were exposed to 20 ppm [44.2 mg/m^3] [2,3-^{14}C]butadiene for either 6 h or for 6 h per day for 5 days by nose-only inhalation, and the livers, lungs and testes were analysed for DNA adducts (Booth *et al*., 2004b). Following the single 20-ppm exposure, G4 was the major adduct detected in all tissues in rats and mice. Mice had 13.91 ± 3.64, 13.67 ± 0.97 and 6.04 ± 0.8 G4 adducts/10^8 nucleotides and rats had 5.75 ± 1.32, 3.31 ± 1.74 and 1.39 ± 0.50 G4 adducts/10^8 nucleotides in liver, lung and testis, respectively. Small amounts of G3 (the exact identity was not determined) were detected consistently in mouse liver and in only one rat liver sample and was not detected in any other tissue. Following the 20-ppm 5-day exposure, G4 was again the main adduct detected and levels in mouse tissues were higher than those in corresponding rat tissues. G1 and G2 were detected in the liver and lung of mice and rats but not in mouse testis; rats had detectable levels in the testis. G3 was detected in mouse tissues and in the liver of rats. The amounts of these other metabolites were much lower than that of G4.

Female Sprague-Dawley rats were exposed to 1000 ppm [2210 mg/m^3] butadiene by inhalation for 6 h per day on 5 days per week for 13 weeks. DNA was isolated from the liver and was analysed for the presence of the α-regioisomer of HMVK-derived 1,*N*^{2}-propanodeoxyguanosine by LC–MS/MS (Powley *et al*., 2007). No adducts were detected.

Female B6C3F$_1$ mice and Fischer 344 rats were exposed by inhalation to butadiene for 6 h per day on 5 days per week for 2 weeks (Oe *et al*., 1999; Blair *et al*., 2000). The mean daily concentration of butadiene was approximately 1250 ppm [2762.5 mg/m^3]. DNA was isolated and analysed for the presence of (±)-G4 and *meso*-G4. On exposure day 10, mouse liver had 3.9 and 2.2 (±)-G4 and *meso*-G4 adducts/10^6 normal bases, respectively. On the same exposure day, rat liver had 1.6 and 0.8 (±)-G4 and *meso*-G4 adducts/10^6 normal bases, respectively. The in-vivo half-lives were 4.1 and 5.5 days for (±)-G4 and *meso*-G4, respectively, in mouse liver DNA. Half-lives for (±)-G4 and *meso*-G4 in rat liver DNA were 3.6 and 4.0 days, respectively.

DNA adducts were measured in the livers, lungs and kidneys of Fischer 344 rats and B6C3F$_1$ mice after exposure to 0, 20, 62.5 or 625 ppm [0, 44.2, 138 or 1381 mg/m^3] butadiene for 6 h per day or 5 days per week for 4 weeks (Swenberg *et al.*, 2000b). Adducts corresponding to the *N*7-guanine adducts of epoxybutene (G1 and G2) and the 2,3,4-trihydroxybutane–guanine adduct (G4) that can be formed from either epoxybutanediol or diepoxybutane were analysed. More G4 was detected in mice and rats than G1 and G2. In rats, adduct levels in the different tissues were similar. In general, mouse tissues contained higher levels of DNA adducts.

DNA adducts were measured in the livers, lungs and kidneys of Fischer 344 rats and B6C3F$_1$ mice after exposure to 0, 20, 62.5 or 625 ppm [0, 44.2, 138 or 1381 mg/m^3] butadiene for 6 h per day on 5 days per week for 4 weeks (Koc *et al.*, 1999). Both racemic and *meso*-G4 and G1 and G2 were analysed by LC–MS/MS. At 625 ppm [1381 mg/m^3] butadiene, mouse liver had 31.9 ± 6.5, 32.2 ± 4.2, 3.0 ± 0.1 and 2.4 ± 0.3 racemic G4,

meso- G4, G1 and G2 adducts/10^6guanine bases, respectively. At 625 ppm butadiene, rat liver had 7.7 ± 4.5, 4.2 ± 2.5, 1.2 ± 0.5 and 0.9 ± 0.5 racemic G4, *meso*- G4, G1 and G2 adducts/10^6guanine bases, respectively. The number of adducts for both G4 and G1 and G2 was similar for all three tissues at all doses examined in both rats and mice. Mice had significantly higher amounts of G4 adducts than rats in all three tissues after exposure to 625 ppm. This difference was also significant in lung and kidney at 62.5 ppm but not at 20 ppm. Overall, the amounts of G1 and G2 adducts were lower than those of G4 adducts in both species.

In the lungs of mice exposed to 500 ppm [1105 mg/m^3] butadiene for 6 h per day for 5 days, *N*7-guanine DNA adducts arising from epoxybutene and epoxybutanediol were analysed (Koivisto & Peltonen, 2001). All four epoxybutene-derived adducts were detected, most of which arose from *S*-epoxybutene. For epoxybutanediol, 75% of the total adducts originated from the 2*R*-diol-3*S*-epoxybutene isomer and the reaction occurred almost exclusively at the terminal carbon.

Rats were exposed to 300 ppm [663 mg/m^3] butadiene for 6 h per day for 5 days and their liver DNA was analysed for the N^6-(2,3,4-trihydroxy-but-1-yl)adenine adduct (Zhao *et al*., 1998). The average level of adduct detected in treated rats was 4.5 adducts/10^9 nucleotides whereas none was detected in control rat liver.

(ii) *Butadiene metabolites in experimental systems*

Epoxybutene

Male B6C3F_1 mice and Sprague-Dawley rats received a single intraperitoneal injection of 1–50 mg/kg bw [^{14}C]epoxybutene and were killed 48 h later (Boogaard *et al*., 2004). DNA was isolated from liver and lung and analysed for the presence of adducts. No adducts were detected in the lungs in either rats or mice. Adduct levels in the liver were below the limit of detection in rats treated with 1 mg/kg bw and in mice treated with 1 and 5 mg/kg bw epoxybutene. Overall, the adduct profiles were similar in rats and mice but rats had much higher levels of adducts than mice. In mice treated with 21 mg/kg bw epoxybutene, the average concentrations of G1 and G2, G3 and G4 were 368, 28 and 50 adducts/10^8 nucleotides, respectively. In rats treated with 18 mg/kg bw epoxybutene, the concentrations of G1 and G2, G3 and G4 were 857 ± 291, 21 ± 12 and 101 ± 25 adducts/10^8 nucleotides, respectively.

The livers and lungs of male B6C3F_1 mice and Sprague-Dawley rats that received an intraperitoneal injection of 20 mg/kg bw [4-^{14}C]epoxybutene and were killed 48 h later were analysed for DNA adducts. In rats, 857 G1 and G2 adducts/10^8 nucleotides and 101 G4 adducts/10^8 nucleotides were detected whereas 368 G1 and G2 adducts/10^8 nucleotides and 50 G4 adducts/10^8 nucleotides were detected in mice (Boogaard *et al*., 2001b). No DNA adducts were detected in the lung.

Butanediol

Female B6C3F_1 mice and Fischer 344 rats were exposed by inhalation to 0–36 ppm [0–129.6 mg/m^3] butanediol. DNA was isolated from liver and lung and analysed for the

presence of G4 (Powley *et al*., 2005). Both racemic and *meso*-isomers of this compound can be formed and values were reported as total G4. Mice had significantly greater amounts of adduct than rats in both liver and lung at 6 and 18 ppm [21.6 and 64.8 mg/m^3] but there was little difference between the tissue levels. At 6 ppm, mice had 6030 ± 1740 and 5570 ± 540 fmol adducts/mg DNA whereas rats had 2560 ± 180 and 2320 ± 640 fmol adducts/mg DNA in the liver and lung, respectively. Similarily shaped dose–response curves were observed for G4 and the *Hprt* mutant frequency in splenic T cells in rodents exposed to butanediol.

However, when female Fischer 344 or Sprague-Dawley rats were exposed to higher concentrations of butanediol (36 ppm or 1000 ppm, respectively, for 6 h per day on 5 days per week for 4 weeks), no adducts were detectable in the DNA of the liver of these animals (Powley *et al.*, 2007).

Diepoxybutane

Diepoxybutane induces the formation of DNA–protein cross-links with the DNA repair protein, O^6-alkylguanine–DNA alkyltransferase (AGT) (Loeber *et al.*, 2006). The product of initial DNA alkylation by diepoxybutane, *N*7-(2'-hydroxy-3′,4′-epoxybut-1′-yl)–deoxyguanosine, was incubated with recombinant human AGT and analysed by HPLC–electrospray ionization MS. Analysis of the whole protein showed the presence of a monoalkylated protein and a protein that contained two butanediol cross-links. Peptide mapping revealed that the DNA–AGT cross-link involved the sulfhydryls of Cys^{145} or Cys^{150} within the human AGT active site and the *N*7 position of guanine in duplex DNA. No cross-linking was detected with Cys^5, Cys^{24} or Cys^{62}. The resulting structure was 1-(*S*-cysteinyl)-4-(guan-7-yl)-2,3-butanediol.

The effect of the stereochemistry of the isomers of diepoxybutane and their abilities to form cross-links with calf thymus DNA was investigated (Park *et al*., 2005). Comparable amounts of total 1,4-bis-(guan-7-yl)-2,3-butanediol (bis-*N*7-guanine–butadiene) cross-links and G4 adducts were observed. However, the types of cross-link (either interstrand or intrastrand) varied depending on the stereoisomer used. *S,S*-Diepoxybutane produced the highest amount of 1,3 interstrand cross-links (96%) followed closely by racemic diepoxybutane (90%). *meso*-Diepoxybutane produced almost equal amounts of 1,3 interstrand (49%) and 1,2 intrastrand cross-links (51%). *R,R*-Diepoxybutane produced 19% 1,2 intrastrand cross-links and 68% 1,3 interstrand cross-links but also produced a large quantity of 1,2 interstrand cross-links (13%) that were not detected with the other stereoisomers.

N^6-(2-Hydroxy-3,4-epoxybut-1-yl)adenine can potentially be produced from the reaction of diepoxybutane with DNA. This compound as well as its corresponding DNA oligomer have been synthesized (Antsypovich *et al*., 2007). Yields of the compounds were lower than expected and both readily cyclized to an unidentified exocyclic diepoxybutane–deoxyadenosine side-product. Formation of cross-links by this compound was minimal. The half-life of N^6-(2-hydroxy-3,4-epoxybut-1-yl)adenine in single-stranded DNA was < 2 h at physiological conditions.

Calf thymus DNA incubated with a 40-fold molar excess of epoxybutene resulted in the detection of equimolar amounts of the two *N*7-guanine adducts of epoxybutene (G1 and G2) (Blair *et al*., 2000); when diepoxybutane was used, the (±)-G4 adduct was detected as the major product. When human TK6 cells were exposed to 400 μM epoxybutene for 24 h, the concentration of G1 and G2 adducts was 4.3 ± 0.9 and 4.1 ± 1.0 adducts/10^6normal cells, respectively. Urine samples from Fischer 344 rats and $B6C3F_1$ mice exposed to 1250 ppm [3453 mg/m^3] butadiene were analysed for the presence of *N*7-guanine adducts. For all 3 days on which the adducts were analysed, rats excreted significantly more G1 and G2 and (±)-G4; G1 was the major adduct excreted. In mice, a small amount of *meso*-G4 was detected but none was found in rat urine.

DNA adducts were analysed in MCF-7 cells after a 6-h exposure to epoxybutanediol and diepoxybutane at concentrations of 100–1000 μmol/mg DNA (Koivisto *et al.*, 1999). At all concentrations tested, more diepoxybutane–*N*7-guanine adducts were detected. In the lungs of mice exposed to 50–1300 ppm [110.5–2873 mg/m^3] butadiene for 6 h per day for 5 days, large amounts of epoxybutanediol–*N*7-guanine (G4) adducts were detected. A small peak for the adduct between diepoxybutane and guanine at *N*7 was detected; however, this peak also elutes closely with an epoxybutene-derived adduct.

(b) Structural effects of the adducts on DNA

The (2*R*,3*R*)-N^6-(2,3,4-trihydroxybutyl)-2'-deoxyadenosyl (BDT) DNA adduct of epoxybutanediol causes low levels of A→G mutations and the (2*S*,3*S*)-BDT DNA adduct causes low levels of A→C mutations. These adducts were incorporated at the X^6 position in the *ras61* oligodeoxynucleotide that was then used to examine structural perturbations in duplex DNA (Merritt *et al.*, 2004; Scholdberg *et al*., 2004). Both adducts were orientated in the major groove of the DNA, which resulted in minimal structural perturbation and allowed the Watson-Crick binding to remain intact. However, the major difference between the two stereoisomers was the orientation of the BDT moiety in the major groove. For the *R*,*R*-BDT adduct, the BDT moiety was orientated in plane with the modified base-pair $X^6.T^{17}$ whereas the *S*,*S*-BDT adduct was tilted out of the base-pairing plane. This difference is due to differential interactions of $T^{17}O^4$ with the hydroxyl groups of the BDT moieties. To determine if a structural basis existed for the low levels of A→C mutations observed with the (2*S*,3*S*)-BDT DNA adduct, it was incorporated site-specifically into the *ras61* oligodeoxynucleotide opposite a mismatched deoxyguanine in the complementary strand opposite the adducted deoxyadenine (Scholdberg *et al*., 2005a). Nuclear magnetic resonance studies revealed two conformations of the adducted mismatched duplex. In the major conformation, the presence of the trihydroxy adduct allowed formation of an A·G mismatched base pair in which the adduct was in the major groove of DNA and both mismatched bases were intrahelical. Thus, if this adduct is not repaired, the subsequent mismatch would result in the observed A→C mutations.

*N*1-[1-Hydroxy-3-buten-2(*R*)-yl]-2′-deoxyinosine, a DNA adduct of epoxybutene, is highly mutagenic in several systems and causes a large portion of A→G mutations. By

incorporating this adduct into the *ras61* oligodeoxynucleotide at the second position of codon 61, positioning of the adduct in DNA could be determined (Merritt *et al.*, 2005a). This adduct caused a significant structural perturbation and showed the rotation of the adduct into a syn conformation, which placed the butadiene moiety into the major groove of the DNA duplex. This positioning disrupted Watson-Crick hydrogen bonding and some altered base stacking was observed. This syn conformation may also facilitate incorporation of desoxy cytosine triphosphate via Hoogsteen-type templating with deoxyinosine and result in A→G mutations.

*N*1-[1-Hydroxy-3-buten-2(*S*)-yl]-2′-deoxyinosine has been synthesized into an oligonucleotide that contained the epoxybutene adduct at the second position of codon 61 of the human N-*ras* proto-oncogene (Scholdberg *et al.*, 2005b). The adducted deoxyinosine was rotated into a high syn conformation, which allowed the adduct to be accommodated into the major groove of the DNA. This conformation positions the adduct to form the protonated Hoogsteen-pairing interaction with desoxy cytosine triphosphate during DNA replication thus generating A→G mutations. Some base–base stacking interactions were also perturbed.

The effect of the 1,4-bis(2′-deoxyadenosin-N^6-yl)–2*R*,3*R*-butanediol cross-link in an oligonucleotide that contains the cross-link between the second and third adenines of codon 61 in the human N-*ras* proto-oncogene in duplex DNA was studied (Merritt *et al.*, 2005b). The adduct was orientated in the DNA major groove. Watson-Crick base-pairing was disrupted at $X^6 \cdot T^{17}$. At the cross-link site, an opening of base-pair $X^6 \cdot T^{17}$ altered base stacking patterns and caused slight unwinding of the DNA duplex.

Examination of the effect of the 1,4-bis(2'-deoxyadenosin-N^6-yl)-2*S*,3*S*-butanediol cross-link was also undertaken in a manner similar to that for the *R,R* isomer (Xu *et al.*, 2007). The adduct orientation was similar to that of the *R,R* adduct and it also disrupted Watson-Crick hydrogen bonding at the same base pair. The largest difference between the *S,S* and *R,R* adducts was in the conformation of the butadiene chain. Because of the anti-conformation of the two hydroxyl groups on the *S,S* adduct, a greater structural perturbation to the DNA duplex occurred, and resulted in a 10° bending of the cross-linked duplex.

Biochemical data suggest that both stereoisomers of N^6,N^6-deoxyadenosine intrastrand cross-linked adducts are by-passed by a variety of DNA polymerases, yet can be significantly mutagenic and lead to A→G transitions (Kanuri *et al.*, 2002).

4.2.4 *Alterations in oncogenes and suppressor genes in tumours*

A specific codon 13 mutation in K-*ras* has been described previously (see IARC, 1999).

Lymphomas induced in $B6C3F_1$ mice by exposure to butadiene were analysed for gross structural alterations and point mutations in several proto-oncogenes that are implicated in the *ras*, *p53* or *pRb* pathways (Zhuang & Söderkvist, 2000). Using southern blotting, no structural alterations or amplifications were detected in *Raf1*, *Mdm2*, *c-Myc*,

Cdc25a or *Cdc25b*. Ten tumours exhibited four identical silent base substitutions (GAC522AAC, GTG531GTC, TCG533TCC, GCT543ACT) and allelotypic analysis showed loss of heterozygosity of the *Raf1* locus in six of 31 butadiene-induced lymphomas. No changes were detected in lymphomas induced by long-term inhalation of 20–625 ppm [44.2–1381 mg/m^3] butadiene in B6C3F$_1$ mice that were analysed for genetic alterations in *Rb1, Ccnd1* and *Cdk4* genes. These results suggest that, if the inactivation of other tumour-suppressor genes may be involved in the development of a subset of butadiene-induced lymphomas, the genetic alterations in the above proto-oncogenes do not play an important role in the development of these tumours.

Studies using the *supF* gene and the three diepoxybutane isomers showed that *S,S*-diepoxybutane was the most potent mutagen followed by *R,R*-diepoxybutane and *meso*-diepoxybutane (Kim *et al.*, 2007). The major form of mutation was A:T→T:A transversion following treatment with all three stereoisomers. However, *S,S*-diepoxybutane induced larger numbers of G:C→A:T transitions while *R,R*-diepoxybutane resulted in a higher frequency of G:C→T:A transversions.

Rarely is the nervous system a target in chemical carcinogenesis but, in B6C3F$_1$ mice exposed to 625 ppm [1381 mg/m^3] butadiene, six malignant gliomas and two neuroblastomas were observed. Only one tumour has been reported in more than 500 historical control mice. Morphologically, the characteristics of the malignant gliomas and neuroblastomas were consistent with those reported for humans. Tumours were also evaluated for genetic alterations in *p53*, K- and H-*ras* genes (Kim *et al.*, 2005). One neuroblastoma had a mutation in codon 61 of the H-*ras* gene. Missense mutations in *p53* exons (exons 5–8) were detected in two neuroblastomas and three of six malignant gliomas and consisted mostly of G→A transitions (5/6). Three of five malignant gliomas and both neuroblastomas showed nuclear accumulation of p53 protein. Loss of heterozygosity at the *p53* gene locus was also observed in four of five malignant gliomas and both neuroblastomas. All of these tumours displayed loss of the C57 (B) allele at the *Ink4a/Arf* gene locus, which codes for the $p16^{Ink4a}$ that may play a key role in the development of mouse brain tumours.

Male and female B6C3F$_1$ mice were exposed to a total of 8100 ppm [17 901 mg/m^3]–weeks and 16 200 ppm [35 802 mg/m^3]–weeks butadiene (Ton *et al.*, 2007). Of 51 lung tumours, 34 had a GGC→CGC transversion mutation in the codon 13 of the K-*ras* gene. A loss of heterozygosity on chromosome 6 was observed in 14 of 51 of the lung tumours and all were in the region of K-*ras*. There is mounting evidence that K-*ras*, which is a known oncogene, functions as a tumour-suppressor gene and its loss may play a major role in mouse lung carcinogenesis.

Point mutations in *ras* genes were analysed in forestomach tumours from male and female B6C3F$_1$ mice that had been exposed to 6.25–625 ppm [13.8–1381 mg/m^3] butadiene by inhalation for 6 h per day on 5 days per week for 1–2 years (Sills *et al.*, 2001). Among the butadiene-induced tumours, 20 of 24 contained *ras* gene mutations compared with four of 11 spontaneous forestomach neoplasms. In butadiene-induced

forestomach tumours, the most common transversions were GGC→CGC in codon 13 in K-*ras*, and CAA→CTA in codon 61 in H-*ras*.

In male and female B6C3F$_1$ mice exposed to 6.25–625 ppm [13.8–1381 mg/m^3] butadiene by inhalation for 6 h per day on 5 days per week for 2 years, an increase in the incidence of cardiac haemangiosarcomas was observed. Eleven of these haemangiosarcomas were analysed for alterations in the *p53* gene and *ras* proto-oncogenes (Hong *et al.*, 2000). Most of the butadiene-induced haemangiosarcomas (9/11) had K-*ras* mutations and all nine had G→C transversions in codon 13. Of the nine haemangiosarcomas with the K-*ras* mutations, five also had an H-*ras* codon 61 CGA mutation. Mutations in exons 5–8 of the *p53* gene were identified in five of 11 haemangiosarcomas.

Butadiene-induced mammary tumours (17) were collected from female B6C3F$_1$ mice that were exposed to 6.25–625 ppm [13.8–1381 mg/m^3] butadiene by inhalation for up to 2 years (Zhuang *et al.*, 2002). Genetic alterations in the *p53* gene were found in seven of 17 tumours. All of these tumours also showed loss of the wild-type *p53* allele. Missense mutations in codons 12, 13 or 61 of the H-*ras* gene were found in nine of 17 tumours. Seven of these nine H-*ras* mutations were G→C transversions in the first base of codon 13. Missense mutations in the β-catenin gene were identified in three of 17 tumours. No mutations were identified in the *Apc* or *Axin* genes.

4.3 Mechanistic considerations

The carcinogenicity of butadiene is most probably mediated by its metabolic intermediates. This view is based largely on the fact that butadiene-induced mutagenicity requires metabolic activation, and the DNA-reactive epoxides formed during butadiene biotransformation are direct-acting mutagens (IARC, 1999; Melnick & Kohn, 1995). The first step in butadiene metabolism primarily involves CYP-mediated oxidation to epoxybutene. At low concentrations of butadiene, metabolism via CYP2E1 predominates (IARC, 1999). Epoxybutene may be metabolized by conjugation with GSH via GST or by hydrolysis via EH. Epoxybutene may also be oxidized to multiple diastereomers of diepoxybutane (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. Partial hydrolysis of diepoxybutane also produces epoxybutanediol. Each of these epoxide intermediates may contribute to the mutagenicity and carcinogenicity of butadiene. Factors that impact their relative contributions include tissues levels, reactivity with DNA and repair of covalent DNA adducts. For example, genetically modified mice that are deficient in mEH activity are more susceptible than wild-type mice to the mutagenic effects of butadiene and diepoxybutane (Wickliffe *et al.*, 2003). The detection of metabolites derived from HMVK 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 contributions of these DNA alkylating agents (crotonaldehyde and HMVK) to the mutagenicity and carcinogenicity of butadiene are not fully known.

The enzymes that regulate epoxide formation and elimination are polymorphic in human populations. While there are reports that indicate that genetic polymorphisms in GST and mEH affect the in-vitro mutagenicity of butadiene-derived epoxides or the mutagenicity of butadiene in occupationally exposed workers (Wiencke *et al.*, 1995; Abdel-Rahman *et al.*, 2003), the extent to which these enzyme variabilities impact the carcinogenicity of butadiene is not known. In addition, CYP is inducible by a variety of environmental and pharmaceutical agents. The reported range and distribution of butadiene or epoxybutene oxidation kinetics in human tissues *in vitro* is limited by the high interindividual variability in CYP, EH and GST activities and the fact that small numbers of human liver and lung samples have been analysed (Bolt *et al.*, 2003; Thier *et al.*, 2003; Norppa, 2004; Schlade-Bartusiak *et al.*, 2004).

The metabolism of butadiene in mice and rats demonstrates linear metabolic elimination kinetics at exposures of up to about 1000 ppm [2210 mg/m^3] (Kreiling *et al.*, 1986b). Toxicity studies conducted with much higher exposures add little additional information on the health effects of butadiene metabolites due to saturation of butadiene metabolism. Responses that increase proportionally with exposures above 1000 ppm butadiene probably represent effects of the parent compound. In the range of linear kinetics, mice metabolize butadiene at about twice the rate observed in rats. Species differences in metabolic clearance of butadiene at low exposure concentrations are largely due to differences in blood:air partition coefficients and physiological parameters that include alveolar ventilation rate, cardiac output and blood flow to metabolizing tissues (Kohn & Melnick, 1993; Sweeney *et al.*, 2001).

Although the formation of epoxybutene occurs primarily by CYP-mediated oxidation of butadiene, 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). Although no studies have been reported that characterize the full profile of urinary metabolites for butadiene in humans, the high ratio of DHBMA to MHBMA in exposed workers indicates that epoxybutene is preferentially metabolized by hydrolysis before GSH conjugation in humans. 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 exposures to butadiene, the ratio of MHBMA to MHBMA + DHBMA was lower in workers who had homozygous null genotypes for *GSTM1* and *GSTT1* (Albertini *et al.*, 2001, 2003a).

The formation of epoxybutanediol or diepoxybutane requires a second oxidation step on 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, the blood concentration of epoxybutanediol is greater in rats exposed to 200 ppm [442 mg/m^3] butadiene than in those exposed to levels of 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*., 2007a). Thus, high-dose studies of butadiene in animals (≥ 625 ppm) may not adequately reveal the full carcinogenic potential of this gas at lower levels of exposure.

Haemoglobin adducts have been measured as biomarkers of internal levels of the epoxide intermediates of butadiene metabolism. Levels of MHbVal, PyrVal and THbVal adducts are reflective of 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/m^3]. 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-h TWA exposures of 0.3–0.8 ppm [0.66–1.76 mg/m^3]), while levels of PyrVal in workers exposed to mean concentrations of 0.37 ppm [0.82 mg/m^3] were below the limit of detection (Albertini *et al*., 2003a, 2007). Species differences in the levels of these haemoglobin adducts reflect differences in exposure to butadiene, blood levels of the epoxide intermediates, reactivity of the epoxide with the *N*-terminal valine and other reactive sites of haemoglobin and the half-life of red blood cells. When adduct levels are normalized per gram of haemoglobin per part per million 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 at occupational exposure levels of butadiene. In workers exposed to butadiene, THbVal levels are affected by the combined polymorphisms for *CYP2E1*, *GSTM1* and *GSTT1* genes (Fustinoni *et al.*, 2002).

The major DNA adducts formed in the liver, lung and kidney of rats and mice exposed to butadiene are at the *N*7 position of guanine. G4 adducts are much more abundant than G1 and G2 adducts, which are derived from epoxybutene (Koc *et al.*, 1999). G4 adducts reach a plateau in rats at 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]. The similarity in the shape of the dose–response curves for the formation of THbVal adducts, G4 adducts and the *Hprt* mutation frequency 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 (Powley *et al.*, 2005). *N*7-Guanine adducts can undergo spontaneous depurination from DNA and create apurinic sites. Epoxide metabolites of butadiene can also react at base-pairing sites to form adducts at *N*3-cytosine, *N*1-adenine, N^6-adenine, *N*1-guanine and N^2-guanine (Selzer & Elfarra, 1996a,b, 1997; Zhao *et al.*, 1998; Zhang & Elfarra, 2004). An increase in *N*1-

trihydroxybutyladenine adducts was detected in lymphocytes of workers occupationally exposed to butadiene (Zhao *et al.*, 2000). Alkylation of *N*1-adenine by epoxybutene followed by hydrolytic deamination to form deoxyinosine has been shown to be highly mutagenic (Rodriguez *et al.*, 2001); this DNA lesion strongly codes for incorporation of cytosine during DNA replication which leads to the generation of A→G mutations. Diepoxybutane is a bifunctional alkylating agent that can form DNA–DNA crosslinks through binding at the *N*7 position of guanine of double-stranded DNA to produce bis-*N*7-guanine-2,3-butanediol (Park & Tretyakova, 2004). Depurination of these interstrand or intrastrand lesions can induce point mutations and large deletion mutations. However, when diepoxybutane alkylates an N^6-adenine in DNA, an exocyclic adenine adduct is formed preferentially to DNA–DNA cross-linked products (Antsypovich *et al.*, 2007).

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 the induction of micronuclei and gene mutations in mammalian cells. Epoxybutanediol, epoxybutene and diepoxybutane induced G→A transition mutations, adenine mutations (A→T and A→G) and deletions (Recio *et al.*, 2001; Lee *et al.*, 2002). The observed base substitution mutations induced by these alkylating agents are consistent with their DNA adduct profiles. A→T and G→C transversions are the most common mutations observed after in-vivo exposure to butadiene or in-vitro exposure to epoxybutene or diepoxybutane.

Markers of individual susceptibility can modulate the genotoxic effects of butadiene. In experimental studies, 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). EH activity varies considerably among individuals. Butadiene-exposed workers with the low EH activity genotype were reported to be more susceptible to butadiene-induced genotoxicity (lymphocyte *HPRT* mutant variant frequency) than individuals with the more common EH genotype (Abdel-Rahman *et al.*, 2001, 2003). In contrast, no significant effects were observed for induction of *HPRT* mutations or sister chromatid exchange in individuals with *GST* (M1 or T1) 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. Several other molecular epidemiological studies report no effect of butadiene on *HPRT* mutations or chromosomal changes at levels of occupational exposures and no significant associations with genotype (Zhang *et al.*, 2004; Albertini *et al.*, 2001, 2007). Discrepancies among these studies may be related to differences in workplace levels of exposure to butadiene, the impact of exposures to butadiene or other genotoxic agents from other sources (e.g. cigarette smoke, automobile exhaust), group size and the level of enzyme activity associated with a particular genotype.

The induction of sister chromatid exchange in human lymphocytes exposed *in vitro* to diepoxybutane was significantly higher in lymphocytes from *GSTT1*-null individuals than *GSTT1*-positive individuals (Wiencke *et al.*, 1995), which indicates that the GST pathway may be important in the detoxification of diepoxybutane released into whole blood.

Epoxybutene can induce sister chromatid exchange and chromosomal aberrations in human peripheral lymphocytes treated *in vitro*; the lack of induction of these effects in G_0 lymphocytes appears to be due to effective DNA excision repair (Kligerman *et al.*, 1999b). Other studies demonstrate the importance of DNA repair in the genotoxicity of butadiene-derived epoxides. For example, mice deficient in nucleotide excision repair activity 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. 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 reactivity and genotoxic effects of butadiene-derived epoxides. A consistent pattern of K-*ras* mutations (G→C transversions at codon 13) was observed in butadiene-induced cardiac haemangiosarcomas, neoplasms of the lung and forestomach 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 had frequent 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 degree of evidence on metabolism, haemoglobin adduct formation and genetic changes in rodents and humans exposed to butadiene is given in Table 33.

Table 33. 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	NI
In-vivo measure of diepoxybutane in blood	Strong	Strong	NI
N-(2,3,4-Trihydroxybutyl)valine haemoglobin adducts	Strong	Strong	Strong
N-(2-Hydroxy-3-butenyl)valine haemoglobin adducts	Strong	Strong	Strong

Table 33 (contd)

Parameter	Rats	Mice	Humans
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]

NI, no information
[a] Possibly due to a lack of adequate studies
[b] Three negative and one positive studies

5. Summary of Data Reported

5.1 Exposure data

1,3-Butadiene is a colourless gas that is produced by three different methods. More than 95% of global production is as a co-product from the industrial synthesis of ethylene. Regardless of the process, the production of butadiene monomer requires the removal of impurities. Butadiene is used primarily (85%) in the production of synthetic rubbers and polymers (polymer production).

The highest exposure to butadiene occurs in occupational settings. No measurements of exposure in butadiene monomer production before the 1970s are available, but levels of exposure have decreased from the late 1970s to the early 2000s from < 20 mg/m^3 to < 2 mg/m^3.

In styrene–butadiene polymer production, the estimated median levels of exposure to butadiene in earlier decades varied from 8 mg/m3 to 20 mg/m^3, while current measurements of exposure in modern facilities in North America and western Europe are generally below 2 mg/m^3. Levels reported in China are somewhat higher (~4 mg/m^3). Regardless of the type of factory, production process or country, some tasks are still characterized by very high exposures (~200 mg/m^3) that are typically short in duration.

Butadiene is a ubiquitous environmental contaminant and levels lower than those found in occupational settings have been reported in ambient air (< 0.02 mg/m^3); these mainly originate from combustion products (e.g. motor vehicle emissions and tobacco smoke).

The American Conference of Governmental Industrial Hygienists has reported occupational exposure limits for butadiene in various countries that range from 1 to 1000 ppm (2–2210 mg/m^3).

5.2 Cancer in humans

The Working Group reviewed studies of three cohorts of workers in the butadiene monomer industry and two cohorts of workers in the styrene–butadiene rubber industry. A study of styrene–butadiene rubber workers by researchers at the University of Alabama at Birmingham was considered by the Working Group to be the most informative. This study examined the mortality rates of approximately 17 000 workers from eight styrene–butadiene rubber facilities in the USA and Canada. Earlier studies of some of the facilities included in this study were carried out by researchers at the National Institute for Occupational Safety and Health and at Johns Hopkins' University.

A limiting factor in the present evaluation was that the diagnosis and classification of lymphatic and haematopoietic malignancies are extremely complex and have undergone several changes over the course of time (see General Remarks).

Although overall mortality from leukaemia was only slightly elevated in the most recent update of the University of Alabama at Birmingham cohort, larger 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 had longer employment (i.e. ≥ 10 years). These excesses were attributable to increased rates of mortality from both chronic lymphocytic and chronic myelogenous leukaemia. Furthermore, a significant exposure–response relationship between cumulative exposure to butadiene and mortality from leukaemia was observed in this study. Exposure–response relationships were apparent for both chronic lymphocytic and chronic myelogenous leukaemia. An exposure–response relationship between cumulative exposure to butadiene and leukaemia was also apparent in an earlier analysis conducted by the University of Alabama at Birmingham that used a different method for the assessment of exposure and in the previous Johns Hopkin's study. While concerns had been raised that these findings might have been due to confounding from exposure to other chemicals in the styrene–butadiene rubber industry, the most recent analyses indicated that the exposure–response relationship for butadiene and leukaemia was independent of exposures to benzene, styrene and dimethyldithiocarbamate.

A slight overall excess of mortality from leukaemia was also observed in two of the studies of the butadiene monomer industry, whereas a small deficit in mortality was observed in the third cohort study. The excess of mortality from leukaemia in one of the monomer industry cohorts was more pronounced among workers who had been exposed during the Second World War when exposures to butadiene had probably been higher. The excess of leukaemia in this cohort did not increase with duration of exposure or cumulative exposure.

The strongest evidence for an association between butadiene and non-Hodgkin lymphoma derives from the studies of workers in the monomer industry. Based on four cases (lymphosarcoma and reticulosarcoma), an approximately sixfold statistically significant excess risk was observed in one of the three studies. An approximately 50% non-significant excess of mortality from non-Hodgkin lymphoma was reported in another study of the monomer industry. Although the excess of mortality from non-Hodgkin lymphoma in this study did not increase 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. The third study of the monomer industry reported only one case of non-Hodgkin lymphoma (0.2 expected). A non-significant twofold excess of mortality from lymphosarcoma and reticulosarcoma was reported in one of the two plants included in the earlier National Institute for Occupational Safety and Health study of styrene–butadiene rubber workers. No overall excess of mortality from non-Hodgkin lymphoma was observed in the University of Alabama at Birmingham study.

Overall, the epidemiological studies provide evidence that exposure to butadiene causes cancer in humans. This excess risk cannot be reasonably explained by confounding, bias or chance. This conclusion is primarily based on the evidence for a significant exposure–response relationship between exposure to butadiene and mortality from leukaemia in the University of Alabama in Birmingham study, which appears to be independent of other potentially confounding exposures. It is also supported by elevated relative risks for non-Hodgkin lymphoma in other studies, particularly in the butadiene monomer production industry. The Working Group was unable to determine the strength of the evidence for particular histological subtypes of lymphatic and haematopoietic neoplasms because of the changes in coding and diagnostic practices for these neoplasms that have occurred during the course of the epidemiological investigations. However, the Working Group considered that there was compelling evidence that exposure to butadiene is associated with an increased risk for leukaemias.

5.3 Cancer in experimental animals

Two bioassays of butadiene by inhalation exposure in mice showed increases in the incidence of lymphoma and neoplasms of the heart, lung, forestomach, liver, Harderian gland, preputial gland and kidney in males and increases in the incidence of lymphomas and neoplasms of the heart, lung, forestomach, liver, Harderian gland, ovary and mammary gland in females. The heart neoplasms were highly malignant and distinctive forms of haemangiosarcoma that were very rare in historical controls. The second bioassay was undertaken at much lower exposure levels than the first, but tumours developed at the same organ sites in both studies. The second study included exposure levels that were comparable with or even lower than historical levels of occupational exposure in humans.

In a single study of inhalation exposure in rats, butadiene caused increases in the incidence of pancreatic exocrine adenomas and carcinomas and interstitial-cell tumours of

the testis in males. In females, increases in the incidence of thyroid follicular-cell tumours, uterine sarcomas, Zymbal gland carcinomas and benign and malignant mammary tumours were observed. This study was conducted at exposure levels that were much higher than those used in the inhalation bioassays in mice.

D,L-Diepoxybutane, a metabolite of butadiene, was tested for carcinogenicity in mice by repeated subcutaneous injection, by repeated intraperitoneal injection and by inhalation exposure. It caused fibrosarcomas at the site of subcutaneous injection and increased the incidence of Harderian gland tumours following inhalation exposure.

D,L-Diepoxybutane was tested for carcinogenicity in rats in one study by inhalation and caused squamous-cell carcinomas of the nasal mucosa. It also induced fibrosarcomas in rats at the site of repeated subcutaneous injection, but did not cause gastric tumours when administered by gavage.

meso-Diepoxybutane was tested for carcinogenicity by skin application in one study in male and one study in female mice in direct comparison with D,L-diepoxybutane. In both studies, *meso*-diepoxybutane caused an increased incidence of squamous-cell papillomas and carcinomas of the skin at the site of application while D,L-diepoxybutane gave negative results.

5.4 Mechanistic and other relevant data

The carcinogenicity of butadiene is most probably mediated by its metabolic intermediates. This assumption is based largely on the fact that butadiene-induced mutagenicity requires metabolic activation and that the DNA-reactive epoxides (stereoisomers of epoxybutene, epoxybutanediol and diepoxybutane) that are formed during the biotransformation of butadiene are direct-acting mutagens. Studies in humans indicate that butadiene is absorbed via inhalation and that the blood:air partition coefficient and alveolar ventilation are important parameters in the determination of uptake. Several studies have quantified the presence of metabolites derived from butadiene in the urine of humans exposed via inhalation in controlled laboratory, environmental or workplace settings. Two urinary metabolites have been identified in humans: 1,2-dihydroxybutyl mercapturic acid and monohydroxy-3-butenyl mercapturic acid, which are derived from the conjugation with glutathione of hydroxymethylvinyl ketone (a metabolite of butenediol) and epoxybutene, respectively.

Several molecular epidemiological studies have assessed the utility of haemoglobin adducts as biomarkers of human exposure to butadiene. The butadiene metabolite, epoxybutene, can react with the *N*-terminal valine of haemoglobin to form *N*-(2-hydroxy-3-butenyl)valine adducts, which have been observed in workers exposed to butadiene. The haemoglobin adduct, *N*-(2,3,4-trihydroxybutyl)valine, which is formed from epoxybutanediol or diepoxybutane, has also been observed in these workers. *N*,*N*-(2,3-Dihydroxy-1,4-butadiyl)valine, which is another adduct formed by the reaction of diepoxybutane with haemoglobin, has not been detected in butadiene-exposed workers. The presence of *N*-(2,3,4-trihydroxybutyl)valine and *N*-(2-hydroxy-3-butenyl)valine in

workers exposed to butadiene demonstrates the systemic availability of the metabolites epoxybutene and epoxybutanediol at occupational exposure levels.

Several studies in humans have demonstrated the DNA-binding properties and clastogenic effects of butadiene. An increase in *N*1-(2,3,4-trihydroxybutyl)adenine adducts — derived from epoxybutanediol or diepoxybutane — was detected in the lymphocytes of workers occupationally exposed to butadiene. One study in workers exposed to butadiene found an increase in the frequency of chromosomal aberrations and sister chromatid exchange. Other studies found no significant increases in chromosomal alterations in workers, although the exposure concentrations were lower than those used in studies with mice.

The enzymes that regulate epoxide formation and elimination are polymorphic in human populations. The extent to which the variabilities of these enzymes modulate the carcinogenicity of butadiene is not known. Butadiene-exposed workers with the low epoxide hydrolase activity genotype were more susceptible to butadiene-induced genotoxicity (frequency of human hypoxanthine–guanine phosphoribosyl transferase gene variants in lymphocytes).

More than 10 urinary metabolites, including the conjugation products monohydroxy-3-butenyl mercapturic acid and 1,2-dihydroxybutyl mercapturic acid, have been identified in butadiene-exposed rats and mice.

At equivalent exposures to butadiene, blood levels of the haemoglobin adducts *N*-(2-hydroxy-3-butenyl)valine and *N,N*-(2,3-dihydroxy-1,4-butadiyl)valine were higher in mice than in rats, while levels of the major adduct, *N*-(2,3,4-trihydroxybutyl)valine, were similar in these species. All of these adducts have been measured in rats and mice exposed to butadiene at concentrations as low as 3 ppm (6 mg/m^3).

Butadiene is clastogenic in mice and induces chromosomal aberrations, micronucleus formation and sister chromatid exchange. It has not been found to be clastogenic in rats.

The most abundant DNA adduct measured in rats and mice exposed to butadiene is *N7*-trihydroxybutylguanine, which is derived from either epoxybutanediol or diepoxybutane. Epoxide metabolites of butadiene can also react at base-pairing sites to form adducts at *N*3-cytosine, *N*1-adenine, N^6-adenine, *N*1-guanine and N^2-guanine. Butadiene and its epoxide metabolites are genotoxic at multiple tissue sites in mice and rats and in a variety of other test systems.

Mutations in *ras* proto-oncogenes and the *p53* tumour-suppressor gene (genes that are involved in the development of a variety of cancers) were identified in several different types of tumour induced by butadiene in mice. A→T and G→C transversions are the most common mutations observed after in-vivo exposure to butadiene or in-vitro exposure to epoxybutene or diepoxybutane.

Although genotoxicity data indicate that diepoxybutane is the most genotoxic epoxide formed from butadiene, the relative contribution of all epoxide metabolites to the mutagenicity and carcinogenicity of butadiene is not known.

6. Evaluation and Rationale

6.1 Carcinogenicity in humans

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

6.2 Carcinogenicity in experimental animals

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

There is *sufficient evidence* in experimental animals for the carcinogenicity of D,L-diepoxybutane.

6.3 Overall evaluation

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

7. References

Abdel-Rahman, S.Z., Ammenheuser, M.M. & Ward, J.B., Jr (2001) Human sensitivity to 1,3-butadiene: Role of microsomal epoxide hydrolase polymorphisms. *Carcinogenesis*, **22**, 415–423

Abdel-Rahman, S.Z., El-Zein, R.A., Ammenheuser, M.M., Yang, Z., Stock, T.H., Morandi, M. & Ward, J.B., Jr (2003) Variability in human sensitivity to 1,3-butadiene: Influence of the allelic variants of the microsomal epoxide hydrolase gene. *Environ. mol. Mutag.*, **41**, 140–146

ACGIH (2001) *1,3-Butadiene. CD ROM*, Cincinnati, OH, American Conference of Government Industrial Hygienists

ACGIH® Worldwide (2005) *Documentation of the TLVs® and BEIs® with Other Worldwide Occupational Exposure Values — 2005 CD-ROM*, Cincinnati, OH, American Conference of Government Industrial Hygienists

Adler, I.-D., Cao, J., Filser, J.G., Gassner, P., Kessler, W., Kliesch, U., Neuhäuser-Klaus, A. & Nüsse, M. (1994) Mutagenicity of 1,3-butadiene inhalation in somatic and germinal cells of mice. *Mutat. Res.*, **309**, 307–314

Adler, I.-D., Filser, J.G., Gassner, P., Kessler, W., Schöneich, J. & Schriever-Schwemmer, G. (1995) Heritable translocations induced by inhalation exposure of male mice to 1,3-butadiene. *Mutat. Res.*, **347**, 121–127

Adler, I.D., Filser, J., Gonda, H., & Schriever-Schwemmer, G. (1998) Dose–response study for 1,3-butadiene-induced dominant lethal mutations and heritable translocations in germs cells of male mice. *Mutat. Res.*, **397**, 85–92

AEA Energy & Environment (2002) [http://www.airquality.co.uk/archive/standards.php; accessed 09.01.2008]

Agency for Toxic Substances and Disease Registry (1992) *Toxicological Profile for 1,3-Butadiene* (Report No. TR-91/07), Atlanta, GA

Albertini, R.J., Sram, R.J., Vacek, P.M., Lynch, J., Wright, M., Nicklas, J.A., Boogaard, P.J., Henderson, R.F., Swenberg, J.A., Tates, A.D. & Ward, J.B., Jr (2001) Biomarkers for assessing occupational exposures to 1,3-butadiene. *Chem.-biol. Interact.*, **135–136**, 429–453

Albertini, R.J., Sram, R.J., Vacek, P.M., Lynch, J., Nicklas, J.A., van Sittert, N.J., Boogaard, P.J., Henderson, R.F., Swenberg, J.A., Tates, A.D., Ward, J.B., Jr, Wright, M., Ammenheuser, M.M., Binkova, B., Blackwell, W., de Zwart, F.A., Krako, D., Krone, J., Megens, H., Musilova, P., Rajska, G., Ranasinghe, A., Rosenblatt, J.I., Rossner, P., Rubes, J., Sullivan, L., Upton, P. & Zwinderman, A.H. (2003) Biomarkers in Czech workers exposed to 1,3-butadiene: A transitional epidemiologic study. *Res. Rep. Health Eff. Inst.*, **116**, 1–141

Albertini, R.J., Sram, R.J., Vacek, P.M., Lynch, J., Rossner, P., Nicklas, J.A., McDonald, J.D., Boysen, G., Georgieva, N. & Swenberg, J.A. (2007) Molecular epidemiological studies in 1,3-butadiene exposed Czech workers: Female–male comparisons. *Chem.-biol. Interact.*, **166**, 63–77

Ammenheuser, M.M., Bechtold, W.E., Abdel-Rahman, S.Z., Rosenblatt, J.I., Hastings-Smith, D.A. & Ward, J.B., Jr (2001) Assessment of 1,3-butadiene exposure in polymer production workers using HPRT mutations in lymphocytes as a biomarker. *Environ. Health Perspect.*, **110**, 1249–1255

Anderson, D., Edwards, A.J. & Brinkworth, M.H. (1993) Male-mediated F_1 effects in mice exposed to 1,3-butadiene. In: Sorsa, M., Peltonen, K., Vainio, H. & Hemminki, K., eds, *Butadiene and Styrene: Assessment of Health Hazards* (IARC Scientific Publications No. 127), Lyon, IARC, pp. 171–181

Anderson, D., Dobrzynka, M.M., Jackson, L.I., Yu, T.W. & Brinkworth, M.H. (1997) Somatic and germ cell effects in rats and mice after treatment with 1,3-butadiene and its metabolites, 1,2-epoxybutene and 1,2,3,4-diepoxybutane. *Mutat. Res.*, **391**, 233–242

Anderson, D., Hughes, J.A., Edwards, A.J. & Brinkworth, M.H. (1998) A comparison of male-mediated effects in rats and mice exposed to 1,3-butadiene. *Mutat. Res.*, **397**, 77–84

Antsypovich, S., Quirk-Dorr, D., Pitts, C. & Tretyakova, N. (2007) Site specific N^6-(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

Anttinen-Klemetti, T., Vaaranrinta, R., Mutanen, P. & Peltonen, K. (2004) Personal exposure to 1,3-butadiene in a petrochemical plant, assessed by use of diffusive samplers. *Int. Arch. occup. environ. Health*, **77**, 288–292

Anttinen-Klemetti, T., Vaaranrinta, R., Mutanen, P. & Peltonen, K. (2006) Inhalation exposure to 1,3-butadiene and styrene in styrene–butadiene copolymer production. *Int. J. Hyg. environ. Health*, **209**, 151–158

Araki, A., Noguchi, T., Kato, F. & Matsushima, T. (1994) Improved method for mutagenicity testing of gaseous compounds by using a gas sampling bag. *Mutat. Res.*, **307**, 335–344

ASTM (2004) *ASTM D2593-93. Standard Test Method for Butadiene Purity and Hydrocarbon Impurities by Gas Chromatography*, West Conshohocken, PA, American Society for Testing and Materials

Austin, C.C., Wang, D., Ecobihon, D.J. & Dussault, G. (2001) Characterization of volatile organic compounds in smoke at municipal structural fires. *J. Toxicol. environ. Health*, **63**, 437–458

Autio, K., Renzi, L., Catalan, J., Albrecht, O.E. & Sorsa, M. (1994) Induction of micronuclei in peripheral blood and bone marrow erythrocytes of rats and mice exposed to 1,3-butadiene by inhalation. *Mutat. Res.*, **309**, 315–320

Belanger, P.L. & Elesh, E. (1980) *Health Hazard Evaluation Determination, Bell Helmets Inc., Norwalk, CA* (Report No. 79-36-646), Cincinnati, OH, National Institute for Occupational Safety and Health

Bevan, C., Keller, D.A., Panepinto, A.S. & Bentley, K.S. (2001) Effect of 4-vinylcyclohexene on micronucleus formation in the bone marrow of rats and mice. *Drug chem. Toxicol.*, **24**, 273–285

Bianchi, A., Boyle, B., Harrison, P., Lawrie, P., Le Lendu, T., Rocchi, P., Taalman, R. & Wieder, W. (1997) *A Review of Analytical Methods and their Significance to Subsequent Occupational Exposure Data Evaluation for 1,3-Butadiene* (Analytical Working Report), Brussels, European Chemical Industry Council

Blair, I.A., Oe, T., Kambouris, S. & Chaudhary, A.K. (2000) 1,3-Butadiene: Cancer, mutations, and adducts. Part IV: Molecular dosimetry of 1,3-butadiene. *Res. Rep. Health Eff. Inst.*, **92**, 151–190

Bolt, H.M. & Jelitto, B. (1996) Biological formation of the 1,3-butadiene DNA adducts 7-*N*-(2-hydroxy-3-buten-1-yl)guanine, 7-*N*-(1-hydroxy-3-buten-2-yl)guanine and 7-*N*-(2,3,4-trihydroxybutyl)guanine. *Toxicology*, **113**, 328–330

Bolt, H.M., Roos, P.H. & 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

Bond, G.G., Bodner, K.M., Olsen, G.W. & Cook, R.R. (1992) Mortality among workers engaged in the development or manufacture of styrene-based products — An update. *Scand. J. Work Environ. Health*, **18**, 145–54

Boogaard, P.J. (2002) Use of haemoglobin adducts in exposure monitoring and risk assessment. *J. Chromatogr. B anal. Technol. biomed. Life Sci.*, **778**, 309–322

Boogaard, P.J., van Sittert, N.J. & Megens, H.J.J.J. (2001a) Urinary metabolites and haemoglobin adducts as biomarkers of exposure to 1,3-butadiene: A basis for 1,3-butadiene cancer risk assessment. *Chem.-biol. Interact.*, **135–136**, 695–701

Boogaard, P.J., van Sittert, N.J., Watson, W.P. & de Kloe, K.P. (2001b) A novel DNA adduct, originating from 1,2-epoxy-3,4-butanediol, is the major DNA adduct after exposure to [2,3-^{14}C]-1,3-butadiene, but not after exposure to [4-^{14}C]-1,2-epoxy-3-butene. *Chem.-biol. Interact.*, **135–136**, 687–693

Boogaard, P.J., de Kloe, K.P., Booth, E.D., & Watson, W.P. (2004) DNA adducts in rats and mice following exposure to [4-^{14}C]-1,2-epoxy-3-butene and to [2,3-^{14}C]-1,3-butadiene. *Chem.-biol. Interact.*, **148**, 69–92

Booth, E.D., Kilgour, J.D. & Watson, W.P. (2004a) Dose responses for the formation of hemoglobin adducts and urinary metabolites in rats and mice exposed by inhalation to low concentrations of 1,3-[2,3-^{14}C]-butadiene. *Chem.-biol. Interact.*, **147**, 213–232

Booth, E.D., Kilgour, J.D., Robinson, S.A. & Watson, W.P. (2004b) Dose responses for DNA adduct formation in tissues of rats and mice exposed by inhalation to low concentrations of 1,3-(2,3-[^{14}C])-butadiene. *Chem.-biol. Interact.*, **147**, 195–211

Boysen, G., Georgieva, N.I., Upton, P.B., Jayaraj, K., Li, Y., Walker, V.E. & Swenberg, J.A. (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

Boysen, G., Georgieva, N.I., Upton, P.B., Walker, V.E. & Swenberg, J.A. (2007) N-Terminal globin adducts as biomarkers for formation of butadiene derived epoxides. *Chem.-biol. Interact.*, **166**, 84–92

Brinkworth, M.H., Anderson, D., Hughes, J.A., Jackson, L.I., Yu, T.W. & Nieschlag, E. (1998) Genetic effects of 1,3-butadiene on the mouse testis. *Mutat. Res.*, **397**, 67–75

Brochot, C., Smith, T.J. & Bois, F.Y. (2007) Development of a physiologically based toxicokinetic model for butadiene and four major metabolites in humans: Global sensitivity analysis for experimental design issues. *Chem.-biol. Interact.*, **167**, 168–183

Bucher, J.R., Melnick, R.L. & Hildebrandt, P.K. (1993) Lack of carcinogenicity in mice exposed once to high concentrations of 1,3-butadiene. *J. natl Cancer Inst.*, **85**, 1866–1867

Burroughs, G.E. (1977) *Health Hazard Evaluation Determination, Firestone Synthetic Rubber Company, Akron, OH* (Report No. 77-1-426), Cincinnati, OH, National Institute for Occupational Safety and Health

Burroughs, G.E. (1979) *Health Hazard Evaluation Determination, Piper Aircraft Corporation, Vero Beach, FL* (Report No. 78-110-585), Cincinnati, OH, National Institute for Occupational Safety and Health

Carmical, J.R., Zhang, M., Nechev, L., Harris, C.M., Harris, T.M. & Lloyd, R.S. (2000a) Mutagenic potential of guanine N^2 adducts of butadiene mono- and diolepoxide. *Chem. Res. Toxicol.*, **13**, 18–25

Carmical, J.R., Nechev, L.V., Harris, C.M., Harris, T.M. & Lloyd, R.S. (2000b) Mutagenic potential of adenine N^6 adducts of monoepoxide and diolepoxide derivatives of butadiene. *Environ. mol. Mutag.*, **35**, 48–56

Carmical, J.R., Kowalczyk, A., Zou, Y., Van Houten, B., Nechev, L.V., Harris, C.M., Harris, T.M. & Lloyd, R.S. (2000c) Butadiene-induced intrastrand DNA cross-links: A possible role in deletion mutagenesis. *J. biol. Chem.*, **275**, 19482–19489

Catallo, W.J., Kennedy, C.H., Henk, W., Barker, S.A., Grace, S.C. & Penn, A. (2001) Combustion products of 1,3-butadiene are cytotoxic and genotoxic to human bronchial epithelial cells. *Environ. Health Perspect.*, **109**, 965–971

Chan, C.C., Shie, R.H., Chang, T.Y. & Tsai, D.H. (2006) Workers' exposures and potential health risks to air toxics in a petrochemical complex assessed by improved methodology. *Int. Arch. occup. environ. Health*, **79**, 135–142

Checkoway, H. & Williams, T.M. (1982) A hematology survey of workers at a styrene–butadiene synthetic rubber manufacturing plant. *Am. ind. Hyg. Assoc. J.*, **43**, 164–169

Cheng, H., Sathiakumar, N., Graff, J., Matthews, R. & Delzell, E. (2007) 1,3-Butadiene and leukemia among synthetic rubber industry workers: Exposure–response relationships. *Chem.-biol. Interact.*, **166**, 15–24

CMAI (Chemical Marketing Associates International) (2004) Product focus. Butadiene. *Chem. Week*, **February 11**, 26

CMAI (Chemical Marketing Associates International) (2006) Product focus. Butadiene. *Chem. Week*, **February 8**, 26

Cochrane, J.E. & Skopek, T.R. (1993) Mutagenicity of 1,3-butadiene and its epoxide metabolite in human TK6 cells and in splenic T cells isolated from exposed B6C3F1 mice. In: Sorsa, M., Peltonen, K., Vainio, H. & Hemminki, K., eds, *Butadiene and Styrene: Assessment of Health Hazards* (IARC Scientific Publications No. 127), Lyon, IARC, pp. 195–204

Cochrane, J.E. & Skopek, T.R. (1994) Mutagenicity of butadiene and its epoxide metabolites: II. Mutational spectra of butadiene, 1,2-epoxybutene and diepoxybutane at the *hprt* locus in splenic T cells from exposed B6C3F1 mice. *Carcinogenesis*, **15**, 719–723

CONCAWE (1987) *A Survey of Exposures to Gasoline Vapour* (Report No. 4/87), The Hague, Conservation of Clean Air and Water in Europe

Cowles, S.R., Tsai, S.P., Snyder, P.J. & Ross, C.E. (1994) Mortality, morbidity, and haematological results from a cohort of long-term workers involved in 1,3-butadiene monomer production. *Occup. environ. Med.*, **51**, 323–329

Csanady, G.A., Guengerich, F.P. & Bond, J.A. (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

Cunningham, M.J., Choy, W.N., Arce, G.T., Rickard, L.B., Vlachos, D.A., Kinney, L.A. & Sarrif, A.M. (1986) In vivo sister chromatid exchange and micronucleus induction studies with 1,3-butadiene in B6C3F1 mice and Sprague-Dawley rats. *Mutagenesis*, **1**, 449–452

Dahl, A.R. & Henderson, R.F. (2000) Comparative metabolism of low concentrations of butadiene and its monoepoxide in human and monkey hepatic microsomes. *Inhal. Toxicol*., **12**, 439–451

Delzell, E., Sathiakumar, N., Hovinga, M., Macaluso, M., Julian, J., Larson, R., Cole, P. & Muir, D.C. (1996) A follow-up study of synthetic rubber workers. *Toxicology*, **113**, 182–189

Delzell, E., Macaluso, M., Sathiakumar, N. & Matthews, R. (2001) Leukemia and exposure to 1,3-butadiene, styrene and dimethyldithiocarbamate among workers in the synthetic rubber industry. *Chem.-biol. Interact.*, **135–136**, 515–534

Delzell, E., Sathiakumar, N., Graff, J., Macaluso, M., Maldonado, G. & Matthew, R. (2006) *An Updated Study of Mortality among North American Synthetic Rubber Industry Workers*, Boston, MA, Health Effects Institute

Divine, B.J. (1990) An update on mortality among workers at a 1,3-butadiene facility — Preliminary results. *Environ. Health Perspect.*, **86**, 119–128

Divine, B.J. & Hartman, C.M. (1996) Mortality update of butadiene production workers. *Toxicology*, **113**, 169–181

Divine, B.J. & Hartman, C.M. (2001) A cohort mortality study among workers at a 1,3-butadiene facility. *Chem.-biol. Interact.*, **135–136**, 535–553

Divine, B.J., Wendt, J.K. & Hartman, C.M. (1993) Cancer mortality among workers at a butadiene production facility. In: Sorsa, M., Peltonen, K., Vainio, H. & Hemminki, K., eds, *Butadiene and Styrene: Assessment of Health Hazards* (IARC Scientific Publications No. 127), Lyon, IARC, pp. 345–362

Dollard, G.J., Dore, C.J. & Jenkin, M.E. (2001) Ambient concentrations of 1,3-butadiene in the UK. *Chem.-biol. Interact.*, **135–136**, 177–206

Downs, T.D., Crane, M.M. & Kim, K.W. (1987) Mortality among workers at a butadiene facility. *Am. J. ind. Med.*, **12**, 311–329

Dubbeld, H. (1998) *Follow-up Study on a Model for Control of Health Hazards Resulting from Exposure to Toxic Substances* (Internal Report 1998-298), Wageningen, Wageningen Agricultural University, Environmental and Occupational Health Group

Duescher, R.J. & Elfarra, A.A. (1994) Human liver microsomes are efficient catalysts of 1,3-butadiene oxidation: Evidence for major roles by cytochromes P450 2A6 and 2E1. *Arch. Biochem. Biophys.*, **311**, 342–349

ECETOC (1997) *1,3-Butadiene OEL Criteria Document* (Special Report No. 12), Brussels, European Centre of Ecotoxicology and Toxicology of Chemicals

Elfarra, A.A., Sharer, J.E. & Duescher, R.J. (1995) Synthesis and characterization of *N*-acetyl-L-cysteine *S*-conjugates of butadiene monoxide and their detection and quantitation in urine of rats and mice given butadiene monoxide. *Chem. Res. Toxicol.*, **8**, 68–76

Elfarra, A.A., Moll, T.S., Krause, R.J., Kemper, R.A. & Selzer, R.R. (2001) Reactive metabolites of 1,3-butadiene: DNA and hemoglobin adduct formation and potential roles in carcinogenicity. *Adv. exp. Med. Biol.*, **500**, 93–103

Eller, P.M., ed. (1994) *NIOSH Manual of Analytical Methods* (DHHS (NIOSH) Publ. No. 94-113), 4th Ed., Cincinnati, OH, National Institute for Occupational Safety and Health [Method 1024]

Environmental Protection Agency Toxic Chemical Release Inventory (2005) [http://www.epa.gov]

Erexson, G.L. & Tindall, K.R. (2000a) Micronuclei and gene mutations in transgenic Big Blue® mouse and rat fibroblasts after exposure to the epoxide metabolites of 1,3-butadiene. *Mutat. Res.*, **472**, 105–117

Erexson, G.L. & Tindall, K.R. (2000b) Reduction of diepoxybutane-induced sister chromatid exchanges by glutathione peroxidase and erythrocytes in transgenic Big Blue® mouse and rat fibroblasts. *Mutat. Res.*, **447**, 267–274

Fajen, J.M. (1985a) *Industrial Hygiene Walk-through Survey Report of Texaco Company, Port Neches, TX* (Report No. 147.14), Cincinnati, OH, National Institute for Occupational Safety and Health

Fajen, J.M. (1985b) *Industrial Hygiene Walk-through Survey Report of Mobil Chemical Company, Beaumont, TX* (Report No. 147.11), Cincinnati, OH, National Institute for Occupational Safety and Health

Fajen, J.M. (1985c) *Industrial Hygiene Walk-through Survey Report of ARCO Chemical Company, Channelview, TX* (Report No. 147.12), Cincinnati, OH, National Institute for Occupational Safety and Health

Fajen, J.M. (1986a) *Industrial Hygiene Walk-through Survey Report of E.I. du Pont deNemours and Company, LaPlace, LA* (Report No. 147.31), Cincinnati, OH, National Institute for Occupational Safety and Health

Fajen, J.M. (1986b) *Industrial Hygiene Walk-through Survey Report of the Goodyear Tire and Rubber Company, Houston, TX* (Report No. 147.34), Cincinnati, OH, National Institute for Occupational Safety and Health

Fajen, J.M. (1988) *Extent of Exposure Study: 1,3-Butadiene Polymer Production Industry*, Cincinnati, OH, National Institute for Occupational Safety and Health

Fajen, J.M., Roberts, D.R., Ungers, L.J. & Krishnan, E.R. (1990) Occupational exposure of workers to 1,3-butadiene. *Environ. Health Perspect.*, **86**, 11–18

Fernandes, P.H., Hackfeld, L.C., Kozekov, I.D., Hodge, R.P. & Lloyd, R.S. (2006) Synthesis and mutagenesis of the butadiene-derived N3 2′-deoxyuridine adducts. *Chem. Res. Toxicol.*, **19**, 968–976

Filser, J.G., Faller, T.H., Bhowmik, S., Schuster, A., Kessler, W., Pütz, C. & Csanady, G.A. (2001) First-pass metabolism of 1,3-butadiene in once-through perfused livers of rats and mice. *Chem.-biol. Interact.*, **135–136**, 249–265

Filser, J.G., Hutzler, C., Meischner, V., Veereshwarayya, V. & Csanady, G.A. (2007) Metabolism of 1,3-butadiene to toxicologically relevant metabolites in single-exposed mice and rats. *Chem.-biol. Interact.*, **166**, 93–193

Food and Drug Administration (1987) 1,3-Butadiene. In: Fazio, T. & Sherma, J., eds, *Food Additives Analytical Manual*, Vol. II, *A Collection of Analytical Methods for Selected Food Additives*, Arlington, VA, Association of Official Analytical Chemists, pp. 58–68

Foureman, P., Mason, J.M., Valencia, R. & Zimmering, S. (1994) Chemical mutagenesis testing in *Drosophila*. IX. Results of 50 coded compounds tested for the National Toxicology Program. *Environ. mol. Mutag.*, **23**, 51–63

Fred, C., Kautiainen, A., Athanassiadis, I. & Tornqvist, M. (2004) Hemoglobin adduct levels in rat and mouse treated with 1,2:3,4-diepoxybutane. *Chem. Res. Toxicol.*, **17**, 785–794

Fred, C., Grawe, J. & Tornqvist, M. (2005) Hemoglobin adducts and micronuclei in rodents after treatment with isoprene monoxide or butadiene monoxide. *Mutat. Res.*, **585**, 21–32

Fustinoni, S., Soleo, L., Warholm, M., Begemann, P., Rannug, A., Neumann, H.G., Swenberg, J.A., Vimercati, L., Foa, V. & Colombi, A. (2002) Influence of metabolic genotypes on biomarkers of exposure to 1,3-butadiene in humans. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 1082–1090

Fustinoni, S., Perbellini, L., Soleo, L., Manno, M. & Foa, V. (2004) Biological monitoring in occupational exposure to low levels of 1,3-butadiene. *Toxicol. Lett.*, **149**, 353–360

Goggin, M., Loeber, R., Park, S., Walker, V., Wickliffe, J. & Tretyakova, N. (2007) HPLC-ESI(+)-MS/MS analysis of N7-guanine–N7-guanine DNA cross-links in tissues of mice exposed to 1,3-butadiene. *Chem. Res. Toxicol.*, **20**, 839–847

Gordon, S.M., Wallace, L.A., Brinkman, M.C., Callahan, P.J. & Kenny, D.V. (2002) Volatile organic compounds as breath biomarkers for active and passive smoking. *Environ. Health Perspect.*, **110**, 689–698

Graff, J.J., Sathiakumar, N., Macaluso, M., Maldonado, G., Matthews, R. & Delzell, E. (2005) Chemical exposures in the synthetic rubber industry and lymphohematopoietic cancer mortality. *J. occup. environ. Med.*, **47**, 916–932

Grasselli, J.G. & Ritchey, W.M., eds (1975) *CRC Atlas of Spectral Data and Physical Constants for Organic Compounds*, Vol. 2, Cleveland, OH, CRC Press, p. 565

Grub, J. & Löser, E. (2005) Butadiene. In: *Ullmann's Encyclopedia of Industrial Chemistry*, 7th Ed., Weinheim, Wiley-VCH Publishers (on line)

Gustafson, P., Barregard, L., Strandberg, B. & Sällsten, G. (2007) The impact of domestic wood burning on personal, indoor and outdoor levels of 1,3-butadiene, benzene, formaldehyde and acetaldehyde. *J. environ. Monit.*, **9**, 23–32

Hayes, R.B., Zhang, L., Swenberg, J.A., Yin, S.N., Xi, L., Wiencke, J., Bechtold, W.E., Yao, M., Rothman, N., Haas, R., O'Neill, J.P., Wiemels, J., Dosemeci, M., Li, G. & Smith, M.T. (2001) Markers for carcinogenicity among butadiene-polymer workers in China. *Chem.-biol. Interact.*, **135–136**, 455–464

Health and Safety Executive (1992) *Methods for the Determination of Hazardous Substances (MDHS) 53—Pumped, Molecular Sieve*, London, Her Majesty's Stationery Office

Health Canada (1999) *Priority Substances List Assessment Report, 1-3 Butadiene*, Ottawa

Heiden Associates (1987) *Additional Industry Profile Data for Evaluating Compliance with Three Butadiene Workplace PEL Scenarios*, Washington DC

Henderson, R.F. (2001) Species differences in the metabolism of olefins: Implications for risk assessment. *Chem.-biol. Interact.*, **135–136**, 53–64

Henderson, R.F., Hahn, F.F., Barr, E.B., Belinsky, S.A., Ménache, M.G. & Benson, J.M. (1999) Carcinogenicity of inhaled butadiene diepoxide in female B6C3F1 mice and Sprague-Dawley rats. *Toxicol. Sci.*, **52**, 33–44

Henderson, R.F., Barr, E.B., Belinsky, S.A., Benson, J.M., Hahn, F.F. & Ménache, M.G. (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

Higashino, H., Mita, K., Yoshikado, H., Iwata, M. & Nakanishi, J. (2006) Exposure and risk assessment of 1,3-butadiene in Japan. *Chem.-biol. Interact.*, **1–3**, 52–62

Himmelstein, M.W., Gladnick, N.L., Donner, E.M., Snyder, R.D. & Valentine, R. (2001) In vitro genotoxicity testing of (1-chloroethenyl)oxirane, a metabolite of beta-chloroprene. *Chem.-biol. Interact.*, **135–136**, 703–713

Hong, H.H.L., Devereux, T.R., Melnick, R.L., Moomaw, C.R., Boorman, G.A. & Sills, R.C. (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

Huff, J.E., Melnick, R.L., Solleveld, H.A., Haseman, J.K., Powers, M. & Miller, R.A. (1985) Multiple organ carcinogenicity of 1,3-butadiene in B6C3F$_1$ mice after 60 weeks of inhalation exposure. *Science*, **227**, 548–549

IARC (1976) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 11, *Cadmium, Nickel, some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics*, Lyon, pp. 115–123

IARC (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 28, *The Rubber Industry*, Lyon

IARC (1986) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 39, *Some Chemicals Used in Plastics and Elastomers*, Lyon, pp. 155–179

IARC (1987a) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 42, *Silica and Some Silicates*, Lyon

IARC (1987b) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, pp. 136–137

IARC (1989) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 45, *Occupational Exposures in Petroleum Refining; Crude Oil and Major Petroleum Fuels*, Lyon, pp. 169–174

IARC (1992) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 54, *Occupational Exposures to Mists and Vapours from Strong Inorganic Acids; and Other Industrial Chemicals*, Lyon, pp. 237–285

IARC (1999) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 71, *Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide*, Lyon, Part 1, pp. 109–225

IARC (2004) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 83, *Tobacco Smoke and Involuntary Smoking*, Lyon

IPCS-CEC (2000) *International Chemical Safety Card 0017*, Geneva, World Health Organization

Irons, R.D. (1990) Studies on the mechanism of 1,3-butadiene-induced leukemogenesis: The potential role of endogenous murine leukemia virus. *Environ. Health Perspect.*, **86**, 49–55

Irons, R.D. & Pyatt, D.W. (1998) Dithiocarbamates as potential confounders in butadiene epidemiology. *Carcinogenesis*, **19**, 539–542

Irons, R.D., Stillman, W.S. & Cloyd, M.W. (1987a) Selective activation of endogenous ecotropic retrovirus in hematopoietic tissues of B6C3F1 mice during the preleukemic phase of 1,3-butadiene exposure. *Virology*, **161**, 457–462

Irons, R.D., Oshimura, M. & Barrett, J.C. (1987b) Chromosome aberrations in mouse bone marrow cells following in vivo exposure to 1,3-butadiene. *Carcinogenesis*, **8**, 1711–1714

Irons, R.D., Cathro, H.P., Stillman, W.S., Steinhagen, W.H. & Shah, R.S. (1989) Susceptibility to 1,3-butadiene-induced leukemogenesis correlates with endogenous ecotropic retroviral background in the mouse. *Toxicol. appl. Pharmacol.*, **101**, 170–176

Irons, R.D., Pyatt, D.W., Stillman, W.S., Som, D.B., Claffey, D.J. & Ruth, J.A. (2000) Comparative toxicity of known and putative metabolites of 1, 3-butadiene in human $CD34^+$ bone marrow cells. *Toxicology*, **150**, 99–106

Irons, R.D., Stillman, W.S., Pyatt, D.W., Yang, Y., Le, A., Gustafson, D.L. & Zeng, J.H. (2001) Comparative toxicity of dithiocarbamates and butadiene metabolites in human lymphoid and bone marrow cells. *Chem.-biol. Interact.*, **135–136**, 615–625

JACA Corp. (1987) *Draft Final Report. Preliminary Economic Analysis of the Proposed Revision to the Standard for 1,3-Butadiene: Phase II*, Fort Washington, PA

Jackson, T.E., Lilly, P.D., Recio, L., Schlosser, P.M. & Medinsky, M.A. (2000) Inhibition of cytochrome P450 2E1 decreases, but does not eliminate, genotoxicity mediated by 1,3-butadiene. *Toxicol. Sci.*, **55**, 266–273

Jauhar, P.P., Henika, P.R., MacGregor, J.T., Wehr, C.M., Shelby, M.D., Murphy, S.A. & Margolin, B.H. (1988) 1,3-Butadiene: Induction of micronucleated erythrocytes in the peripheral blood of B6C3F1 mice exposed by inhalation for 13 weeks. *Mutat. Res.*, **209**, 171–176

Jelitto, B., Vangala, R.R. & Laib, R.J. (1989) Species differences in DNA damage by butadiene: Role of diepoxybutane. *Arch. Toxicol.*, **13** (Suppl.), 246–249

Kanuri, M., Nechev, L.V., Tamura, P.J., Harris, C.M., Harris, T.M. & Lloyd, R.S. (2002) Mutagenic spectrum of butadiene-derived N1-deoxyinosine adducts and N^6,N^6-deoxyadenosine intrastrand cross-links in mammalian cells. *Chem. Res. Toxicol.*, **15**, 1572–1580

Karlsson, A., Söderkvist, P. & Zhuang, S.M. (2002) Point mutations and deletions in the znfn1a1/ikaros gene in chemically induced murine lymphomas. *Cancer Res.*, **62**, 2650–2653

Kauppinen, T., Toikkanen, J., Pedersen, D., Young, R., Ahrens, W., Boffetta, P., Hansen, J., Kromhout, H., Maqueda Blasco, J., Mirabelli, D., de la Orden-Rivera, V., Pannett, B., Plato, N., Savela, A., Vincent, R. & Kogevinas, M. (2000) Occupational exposure to carcinogens in the European Union. *Occup. environ. Med.*, **57**, 10–18 [data partially available on the CAREX web site: http://www.ttl.fi/NR/rdonlyres/407B368B-26EF-475D-8F2B-DA0024B853E0/0/5_exposures_by_agent_and_industry.pdf.]

Kemper, R.A., Krause, R.J. & Elfarra, A.A. (2001) Metabolism of butadiene monoxide by freshly isolated hepatocytes from mice and rats: Different partitioning between oxidative, hydrolytic, and conjugation pathways. *Drug Metab. Dispos.*, **29**, 830–836

Kim, Y.M., Harrad, S. & Harrison, R.M. (2001) Concentrations and sources of VOCs in urban domestic and public microenvironments. *Environ. Sci. Technol.*, **35**, 997–1004

Kim, Y.M., Harrad, S. & Harrison, R.M. (2002) Levels and sources of personal inhalation exposure to volatile organic compounds. *Environ. Sci. Technol.*, **36**, 5405–5410

Kim, Y., Hong, H.H., Lachat, Y., Clayton, N.P., Devereux, T.R., Melnick, R.L., Hegi, M.E., & Sills, R.C. (2005) Genetic alterations in brain tumors following 1,3-butadiene exposure in B6C3F1 mice. *Toxicol. Pathol.*, **33**, 307–312

Kim, M.Y., Tretyakova, N., & Wogan, G.N. (2007) Mutagenesis of the supF gene by stereoisomers of 1,2,3,4-diepoxybutane. *Chem. Res. Toxicol.*, **20**, 790–797

Kligerman, A.D., DeMarini, D.M., Doerr, C.L., Hanley, N.M., Milholland, V.S. & Tennant, A.H. (1999a) Comparison of cytogenetic effects of 3,4-epoxy-1-butene and 1,2:3,4-diepoxybutane in mouse, rat and human lymphocytes following in vitro G_0 exposures. *Mutat. Res.*, **439**, 13–23

Kligerman, A.D., Doerr, C.L. & Tennant, A.H. (1999b) Cell cycle specificity of cytogenetic damage induced by 3,4-epoxy-1-butene. *Mutat. Res.*, **444**, 151–158

Koc, H., Tretyakova, N.Y., Walker, V.E., Henderson, R.F. & Swenberg, J.A. (1999) Molecular dosimetry of N-7 guanine adduct formation in mice and rats exposed to 1,3-butadiene. *Chem. Res. Toxicol.*, **12**, 566–574

Kohn, M.C. & Melnick, R.L. (1993) Species differences in the production and clearance of 1,3-butadiene metabolites: A mechanistic model indicates predominantly physiological, not biochemical, control. *Carcinogenesis*, **14**, 619–628

Kohn, M.C. & Melnick, R.L. (2000) The privileged access model of 1,3-butadiene disposition. *Environ. Health Perspect.*, **108**, 911–917

Kohn, M.C. & Melnick, R.L. (2001) Physiological modeling of butadiene disposition in mice and rats. *Chem.-biol. Interact.*, **135–136**, 285–301

Koivisto, P. & Peltonen, K. (2001) *N*7-Guanine adducts of the epoxy metabolites of 1,3-butadiene in mice lung. *Chem.-biol. Interact.*, **135–136**, 363–372

Koivisto, P., Adler, I.-D., Sorsa, M. & Peltonen, K. (1996) Inhalation exposure of rats and mice to 1,3-butadiene induces N6-adenine adducts of epoxybutene detected by ^{32}P-postlabeling and HPLC. *Environ. Health Perspect.*, **104** (Suppl. 3), 655–657

Koivisto, P., Sorsa, M., Pacchierotti, F. & Peltonen, K. (1997) ^{32}P-Postlabelling/HPLC assay reveals an enantioselective adduct formation in N7 guanine residues *in vivo* after 1,3-butadiene inhalation exposure. *Carcinogenesis*, **18**, 439–443

Koivisto, P., Adler, I.-D., Pacchierotti, F. & Peltonen, K. (1998) DNA adducts in mouse testis and lung after inhalation exposure to 1,3-butadiene. *Mutat. Res.*, **397**, 3–10

Koivisto, P., Kilpeläinen, I., Rasanen, I., Adler, I.-D., Pacchierotti, F. & Peltonen, K. (1999) Butadiene diolepoxide- and diepoxybutane-derived DNA adducts at N7-guanine: A high occurrence of diolepoxide-derived adducts in mouse lung after 1,3-butadiene exposure. *Carcinogenesis*, **20**, 1253–1259

Krause, R.J. & Elfarra, A.A. (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

Kreiling, R., Laib, R.J. & Bolt, H.M. (1986a) Alkylation of nuclear proteins and DNA after exposure of rats and mice to [1,4-^{14}C]1,3-butadiene. *Toxicol. Lett.*, **30**, 131–136

Kreiling, R., Laib, R.J., Filser, J.G. & Bolt, H.M. (1986b) Species differences in butadiene metabolism between mice and rats evaluated by inhalation pharmacokinetics. *Arch. Toxicol.*, **58**, 235–238

Krishnan, E.R., Ungers, L.J., Morelli-Schroth, P.A. & Fajen, J.M. (1987) *Extent-of-exposure Study: 1,3-Butadiene Monomer Production Industry*, Cincinnati, OH, National Institute for Occupational Safety and Health

Kwekkeboom, J. (1996) [A Model for Control of Health Hazards Resulting from Exposure to Toxic Substances (Report V-415)], Wageningen, Wageningen Agricultural University, Department of Air Quality (in Dutch)

Lee, D.H., Kim, T.H., Lee, S.Y., Kim, H.J., Rhee, S.K., Yoon, B., Pfeifer, G.P. & Lee, C.S. (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

Lemen, R.A., Meinhardt, T.J., Crandall, M.S., Fajen, J.M. & Brown, D.P. (1990) Environmental epidemiologic investigations in the styrene–butadiene rubber production industry. *Environ. Health Perspect.*, **86**, 103–106

Lide, D.R., ed. (2005) *CRC Handbook of Chemistry and Physics*, 86th Ed., Boca Raton, FL, CRC Press, pp. 3–72

Lin, Y.S., Smith, T.J., Kelsey, K.T. & Wypij, D. (2001) Human physiologic factors in respiratory uptake of 1,3-butadiene. *Environ. Health Perspect.*, **109**, 921–926

Lin, Y.S., Smith, T.J. & Wang, P.Y. (2002) An automated exposure system for human inhalation study. *Arch. environ. Health*, **57**, 215–223

Loeber, R., Rajesh, M., Fang, Q., Pegg, A.E., & Tretyakova, N. (2006) Cross-linking of the human DNA repair protein O6-alkylguanine DNA alkyltransferase to DNA in the presence of 1,2,3,4-diepoxybutane. *Chem. Res. Toxicol.*, **19**, 645–654

Loughlin, J.E., Rothman, K.J. & Dreyer, N.A. (1999) Lymphatic and haematopoietic cancer mortality in a population attending school adjacent to styrene–butadiene facilities, 1963–1993. *J. Epidemiol. Community Health*, **53**, 283–287

Lovreglio, P., Bukvic, N., Fustinoni, S., Ballini, A., Drago, I., Foa, V., Guanti, G. & Soleo, L. (2006) Lack of genotoxic effect in workers exposed to very low doses of 1,3-butadiene. *Arch. Toxicol.*, **80**, 378–381

Lunsford, R.A., Gagnon, Y.T., Palassis, J., Fajen, J.M., Roberts, D.R. & Eller, P.M. (1990) Determination of 1,3-butadiene down to sub-part-per-million levels in air by collection on charcoal and high resolution gas chromatography. *Appl. occup. environ. Hyg.*, **5**, 310–320

Ma, H., Wood, T.G., Ammenheuser, M.M., Rosenblatt, J.I. & Ward, J.B., Jr (2000) Molecular analysis of hprt mutant lymphocytes from 1, 3-butadiene-exposed workers. *Environ. mol. Mutag.*, **36**, 59–71

Macaluso, M., Larson, R., Delzell, E., Sathiakumar, N., Hovinga, M., Julian, J., Muir, D. & Cole, P. (1996) Leukemia and cumulative exposure to butadiene, styrene and benzene among workers in the synthetic rubber industry. *Toxicology*, **113**, 190–202

Macaluso, M., Larson, R., Lynch, J., Lipton, S. & Delzell, E. (2004) Historical estimation of exposure to 1,3-butadiene, styrene, and dimethyldithiocarbamate among synthetic rubber workers. *J. occup. environ. Hyg.*, **1**, 371–390

Maniglier-Poulet, C., Cheng, X., Ruth, J.A. & Ross, D. (1995) Metabolism of 1,3-butadiene to butadiene monoxide in mouse and human bone marrow cells. *Chem.-biol. Interact.*, **97**, 119–129

Matanoski, G.M. & Schwartz, L. (1987) Mortality of workers in styrene–butadiene polymer production. *J. occup. Med.*, **29**, 675–680

Matanoski, G.M., 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

Matanoski, G., Francis, M., Correa-Villasenor, A., Elliott, E., Santos-Burgoa, C. & Schwartz, L. (1993) Cancer epidemiology among styrene–butadiene rubber workers. In: Sorsa, M., Peltonen, K., Vainio, H. & Hemminki, K., eds, *Butadiene and Styrene: Assessment of Health Hazards* (IARC Scientific Publication No. 127), Lyon, IARC, pp. 363–374

Matanoski, G., Elliott, E., Tao, X., Francis, M., Correa-Villasenor, A. & Santos-Burgoa, C. (1997) Lymphohematopoietic cancers and butadiene and styrene exposure in synthetic rubber manufacture. *Ann. N.Y. Acad. Sci.*, **837**, 157–169

McGregor, D., Brown, A.G., Cattanach, P., Edwards, I., McBride, D., Riach, C., Shepherd, W. & Caspary, W.J. (1991) Responses of the L5178Y mouse lymphoma forward mutation assay: V. Gases and vapors. *Environ. mol. Mutag.*, **17**, 122–129

McMichael, A.J., Spirtas, R. & Kupper, L.L. (1974) An epidemiologic study of mortality within a cohort of rubber workers, 1964–72. *J. occup. Med.*, **16**, 458–464

McMichael, A.J., Spirtas, R., Gamble, J.F. & Tousey, P.M. (1976) Mortality among rubber workers: Relationship to specific jobs. *J. occup. Med.*, **18**, 178–185

McNabola, A., Broderick, B., Johnston, P. & Gill, L. (2006) Effects of the smoking ban on benzene and 1,3-butadiene levels in pubs in Dublin. *J. environ. Sci. Health*, **41**, 799–810

de Meester, C., Poncelet, F., Roberfroid, M. & Mercier, M. (1980) The mutagenicity of butadiene towards *Salmonella typhimurium*. *Toxicol. Lett.*, **6**, 125–130

Meinhardt, T.J., Young, R.J. & Hartle, R.W. (1978) Epidemiologic investigations of styrene–butadiene rubber production and reinforced plastics. *Scand. J. Work Environ. Health*, **4** (Suppl. 2), 240–246

Meinhardt, T.J., Lemen, R.A., Crandall, M.S. & Young, R.J. (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

Melnick, R.L. & Kohn, M.C. (1995) Mechanistic data indicate that 1,3-butadiene is a human carcinogen. *Carcinogenesis*, **16**, 157–163

Melnick, R.L., Huff, J., Chou, B.J. & Miller, R.A. (1990) Carcinogenicity of 1,3-butadiene in C57BL/6 × C3H F_1 mice at low exposure concentrations. *Cancer Res.*, **50**, 6592–6599

Meng, Q., Henderson, R.F., Chen, T., Heflich, R.H., Walker, D.M., Bauer, M.J., Reilly, A.A. & Walker, V.E. (1999a) Mutagenicity of 1,3-butadiene at the *Hprt* locus of T-lymphocytes following inhalation exposures of female mice and rats. *Mutat. Res.*, **429**, 107–125

Meng, Q., Henderson, R.F., Walker, D.M., Bauer, M.J., Reilly, A.A. & Walker, V.E. (1999b) Mutagenicity of the racemic mixtures of butadiene monoepoxide and butadiene diepoxide at the *Hprt* locus of T-lymphocytes following inhalation exposures of female mice and rats. *Mutat. Res.*, **429**, 127–140

Meng, Q., Henderson, R.F., Long, L., Blair, L., Walker, D.M., Upton, P.B., Swenberg, J.A. & Walker, V.E. (2001) Mutagenicity at the Hprt locus in T cells of female mice following inhalation exposures to low levels of 1,3-butadiene. *Chem.-biol. Interact.*, **135–136**, 343–361

Meng, Q., Walker, D.M., Scott, B.R., Seilkop, S.K., Aden, J.K., & Walker, V.E. (2004) Characterization of Hprt mutations in cDNA and genomic DNA of T-cell mutants from control and 1,3-butadiene-exposed male B6C3F1 mice and F344 rats. *Environ. mol. Mutag.*, **43**, 75–92

Meng, Q., Walker, D.M., McDonald, J.D., Henderson, R.F., Carter, M.M., Cook, D.L., Jr, McCash, C.L., Torres, S.M., Bauer, M.J., Seilkop, S.K., Upton, P.B., Georgieva, N.I., Boysen, G., Swenberg, J.A. & Walker, V.E. (2007a) 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

Meng, Q., Redetzke, D.L., Hackfeld, L.C., Hodge, R.P., Walker, D.M. & Walker, V.E. (2007b) Mutagenicity of stereochemical configurations of 1,2-epoxybutene and 1,2:3,4-diepoxybutane in human lymphoblastoid cells. *Chem.-biol. Interact.*, **166**, 207–218

Merritt, W.K., Scholdberg, T.A., Nechev, L.V., Harris, T.M., Harris, C.M., Lloyd, R.S. & Stone, M.P. (2004) Stereospecific structural perturbations arising from adenine N^6 butadiene triol adducts in duplex DNA. *Chem. Res. Toxicol.*, **17**, 1007–1019

Merritt, W.K., Kowalczyk, A., Scholdberg, T.A., Dean, S.M., Harris, T.M., Harris, C.M., Lloyd, R.S. & Stone, M.P. (2005a) Dual roles of glycosyl torsion angle conformation and stereochemical configuration in butadiene oxide-derived N1 beta-hydroxyalkyl deoxyinosine adducts: A structural perspective. *Chem. Res. Toxicol.*, **18**, 1098–1107

Merritt, W.K., Nechev, L.V., Scholdberg, T.A., Dean, S.M., Kiehna, S.E., Chang, J.C., Harris, T.M., Harris, C.M., Lloyd, R.S. & Stone, M.P. (2005b) Structure of the 1,4-bis(2'-deoxyadenosin-N^6-yl)-2R,3R-butanediol cross-link arising from alkylation of the human N-ras codon 61 by butadiene diepoxide. *Biochemistry*, **44**, 10081–10092

Minko, I.G., Washington, M.T., Prakash, L., Prakash, S. & Lloyd, R.S. (2001) Translesion DNA synthesis by yeast DNA polymerase eta on templates containing N^2-guanine adducts of 1,3-butadiene metabolites. *J. biol. Chem.*, **276**, 2517–2522

Moll, T.S., Harms, A.C. & Elfarra, A.A. (2000) A comprehensive structural analysis of hemoglobin adducts formed after in-vitro exposure of erythrocytes to butadiene monoxide. *Chem. Res. Toxicol.*, **13**, 1103–1113

Morrissey, R.E., Schwetz, B.A., Hackett, P.L., Sikov, M.R., Hardin, B.D., McClanahan, B.J., Decker, J.R. & Mast, T.J. (1990) Overview of reproductive and developmental toxicity studies of 1,3-butadiene in rodents. *Environ. Health Perspect.*, **86**, 79–84

Murg, M.N., Schuler, M. & Eastmond, D.A. (1999a) Evaluation of micronuclei and chromosomal breakage in the 1cen-q12 region by the butadiene metabolites epoxybutene and diepoxybutane in cultured human lymphocytes. *Mutagenesis*, **14**, 541–546

Murg, M.N., Schuler, M. & Eastmond, D.A. (1999b) Persistence of chromosomal alterations affecting the 1cen-q12 region in a human lymphoblastoid cell line exposed to diepoxybutane and mitomycin C. *Mutat. Res.*, **446**, 193–203

National Library of Medicine (2008) *Toxic Chemical Release Inventory (TRI87, TRI90, TRI95, TRI05, TRI08) Databases*, Bethesda, MD [available at http://toxnet.nlm.nih.gov]

National Toxicology Program (1984) *Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No. 106-99-0) in B6C3F$_1$ Mice (Inhalation Studies)* (Tech. Rep. Ser. No. 288), Research Triangle Park, NC

National Toxicology Program (1993) *Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No. 106-99-0) in B6C3F$_1$ Mice (Inhalation Studies)* (Tech. Rep. Ser. No. 434), Research Triangle Park, NC

Nazaroff, W.W. & Singer, B.C. (2004) Inhalation of hazardous air pollutants from environmental tobacco smoke in US residents. *J. Expo. Anal. environ. Epidemiol.*, **14**, S71–S77

Nechev, L.V., Zhang, M., Tsarouhtsis, D., Tamura, P.J., Wilkinson, A.S., Harris, C.M. & Harris, T.M. (2001) Synthesis and characterization of nucleosides and oligonucleotides bearing adducts of butadiene epoxides on adenine N^6 and guanine N^2. *Chem. Res. Toxicol.*, **14**, 379–388

NIH/EPA Chemical Information System (1983) *Carbon-13 NMR Spectral Search System, Mass Spectral Search System, and Infrared Spectral Search System*, Arlington, VA, Information Consultants

NOES (1997) *National Occupational Exposure Survey 1981–83*, Unpublished data as of November 1997, Cincinnati, OH, US Department of Health and Human Services, Public Health Service, National Institute for Occupational Safety and Health

Norppa, H. (2004) Cytogenetic biomarkers and genetic polymorphisms. *Toxicol. Lett.*, **149**, 309–334

Occupational Safety and Health Administration (1990a) *OSHA Analytical Methods Manual*, Part 1: *Organic Substances*, Vol. 3, *Methods 55-80*, Salt Lake City, UT [Method 56]

Occupational Safety and Health Administration (1990b) Occupational exposure to 1,3-butadiene. *Fed. Regist.*, **55**, 32736–32826

O'Donoghue, R.T., Gill, L.W., McKevitt, R.J. & Broderick, B. (2007) Exposure to hydrocarbon concentrations while commuting or exercising in Dublin. *Environ. int.*, **33**, 1–8

Oe, T., Kambouris, S.J., Walker, V.E., Meng, Q., Recio, L., Wherli, S., Chaudhary, A.K., & Blair, I.A. (1999) Persistence of N7-(2,3,4-trihydroxybutyl)guanine adducts in the livers of mice and rats exposed to 1,3-butadiene. *Chem. Res. Toxicol.*, **12**, 247–257

Oesch, F. & Daly, J. (1972) Conversion of naphthalene to trans-naphthalene dihydrodiol: Evidence for the presence of a coupled aryl monooxygenase–epoxide hydrase system in hepatic microsomes. *Biochem. biophys. Res. Commun.*, **46**, 1713–1720

O'Neil, M.J., ed. (2006) *Merck Index*, 14th Ed., Whitehouse Station, NJ, Merck, p. 248

Owen, P.E. & Glaister, J.R. (1990) Inhalation toxicity and carcinogenicity of 1,3-butadiene in Sprague-Dawley rats. *Environ. Health Perspect.*, **86**, 19–25

Owen, P.E., Glaister, J.R., Gaunt, I.F. & Pullinger, D.H. (1987) Inhalation toxicity studies with 1,3-butadiene. 3. Two year toxicity/carcinogenicity study in rats. *Am. ind. Hyg. Assoc. J.*, **48**, 407–413

Pacchierotti, F., Adler, I.-D., Anderson, D., Brinkworth, M., Demopoulos, N.A., Lahdetie, J., Osterman-Golkar, S., Peltonen, K., Russo, A., Tates, A. & Waters, R. (1998) Genetic effects of 1,3-butadiene and associated risk for heritable damage. *Mutat. Res.*, **397**, 93–115

Parent, M.E., Hua, Y. & Siemiatycki, J. (2000) Occupational risk factors for renal cell carcinoma in Montreal. *Am. J. ind. Med.*, **38**, 609–618

Park, S. & Tretyakova, N. (2004) Structural characterization of the major DNA–DNA cross-link of 1,2,3,4-diepoxybutane. *Chem. Res. Toxicol.*, **17**, 129–136

Park, S., Hodge, J., Anderson, C. & Tretyakova, N. (2004) Guanine–adenine DNA cross-linking by 1,2,3,4-diepoxybutane: Potential basis for biological activity. *Chem. Res. Toxicol.*, **17**, 1638–1651

Park, S., Anderson, C., Loeber, R., Seetharaman, M., Jones, R. & Tretyakova, N. (2005) Interstrand and intrastrand DNA–DNA cross-linking by 1,2,3,4-diepoxybutane: Role of stereochemistry. *J. Am. chem. Soc.*, **127**, 14355–14365

Perbellini, L., Princivalle, A., Cerpelloni, M., Pasini, F. & Brugnone, F. (2003) Comparison of breath, blood and urine concentrations in the biomonitoring of environmental exposure to 1,3-butadiene, 2,5-dimethylfuran, and benzene. *Int. Arch. occup. environ. Health*, **76**, 461–466

Powley, M.W., Jayaraj, K., Gold, A., Ball, L.M. & Swenberg, J.A. (2003) 1,N^2-Propanodeoxyguanosine adducts of the 1,3-butadiene metabolite, hydroxymethylvinyl ketone. *Chem. Res. Toxicol.*, **16**, 1448–1454

Powley, M.W., Li, Y., Upton, P.B., Walker, V.E. & Swenberg, J.A. (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

Powley, M.W., Walker, V.E., Li, Y., Upton, P.B., & Swenberg, J.A. (2007) The importance of 3,4-epoxy-1,2-butanediol and hydroxymethylvinyl ketone in 3-butene-1,2-diol associated mutagenicity. *Chem.-biol. Interact.*, **166**, 182–190

Ranaldi, R., Bassani, B. & Pacchierotti, F. (2001) Genotoxic effects of butadiene in mouse lung cells detected by an ex vivo micronucleus test. *Mutat. Res.*, **491**, 81–85

Recio, L. & Meyer, K.G. (1995) Increased frequency of mutations at A:T base pairs in the bone marrow of B6C3F1 *lacI* transgenic mice exposed to 1,3-butadiene. *Environ. mol. Mutag.*, **26**, 1–8

Recio, L., Osterman-Golkar, S., Csanady, G.A., Turner, M.J., Myhr, B., Moss, O. & Bond, J.A. (1992) Determination of mutagenicity in tissues of transgenic mice following exposure to 1,3-butadiene and N-ethyl-N-nitrosourea. *Toxicol. appl. Pharmacol.*, **117**, 58–64

Recio, L., Saranko, C.J. & Steen, A.M. (2000) 1,3-Butadiene: Cancer, mutations, and adducts. Part II: Roles of two metabolites of 1,3-butadiene in mediating its in vivo genotoxicity. *Res. Rep. Health Eff. Inst.*, **92**, 49–87

Recio, L., Steen, A.M., Pluta, L.J., Meyer, K.G. & Saranko, C.J. (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

Reilly, M.S. & Grogan, D.W. (2002) Biological effects of DNA damage in the hyperthermophilic archaeon Sulfolobus acidocaldarius. *FEMS Microbiol. Lett.*, **208**, 29–34

Richardson, K.A., Peters, M.M., Megens, R.H., van Elburg, P.A., Golding, B.T., Boogaard, P.J., Watson, W.P. & van Sittert, N.J. (1998) Identification of novel metabolites of butadiene monoepoxide in rats and mice. *Chem. Res. Toxicol.*, **11**, 1543–1555

Richardson, K.A., Peters, M.M., Wong, B.A., Megens, R.H., van Elburg, P.A., Booth, E.D., Boogaard, P.J., Bond, J.A., Medinsky, M.A., Watson, W.P. & van Sittert, N.J. (1999) Quantitative and qualitative differences in the metabolism of ^{14}C-1,3-butadiene in rats and mice: Relevance to cancer susceptibility. *Toxicol. Sci.*, **49**, 186–201

Rinsky, R.A., Ott, G., Ward, E., Greenberg, H., Halperin, W. & Leet, T. (1988) Study of mortality among chemical workers in the Kanawha Valley of West Virginia. *Am. J. ind. Med.*, **13**, 429–438

Ristau, C., Deutschmann, S., Laib, R.J. & Ottenwalder, H. (1990) Detection of diepoxybutane-induced DNA–DNA crosslinks by cesium trifluoracetate (CsTFA) density-gradient centrifugation. *Arch. Toxicol.*, **64**, 343–344

Roberts, D.R. (1986) *Industrial Hygiene Walk-through Survey Report of Copolymer Rubber and Chemical Corporation, Baton Rouge, LA* (Report No. 147.22), Cincinnati, OH, National Institute for Occupational Safety and Health

Rodriguez, D.A., Kowalczyk, A., Ward, J.B., Jr, Harris, C.M., Harris, T.M. & Lloyd, R.S. (2001) Point mutations induced by 1,2-epoxy-3-butene N1 deoxyinosine adducts. *Environ. mol. Mutag.*, **38**, 292–296

Ropert, C.P., Jr (1976) *Health Hazard Evaluation Determination, Goodyear Tire and Rubber Company, Gadsden, AL* (Report No. 74-120-260), Cincinnati, OH, National Institute for Occupational Safety and Health

Rubber Manufacturers' Association (1984) *Requests for Information Regarding 1,3-Butadiene, 49 Fed. Reg. 844 and 845 (Jan. 5 1984)*, Washington DC

Ruhe, R.L. & Jannerfeldt, E.R. (1980) *Health Hazard Evaluation, Metamora Products Corporation, Elkland, PA* (Report No. HE-80-188-797), Cincinnati, OH, National Institute for Occupational Safety and Health

Sadtler Research Laboratories (1995) *The Sadtler Standard Spectra, Cumulative Index*, Philadelphia, PA

Saltzman, B.E. & Harman, J.N. (1989) Direct reading colorimetric indicators. In: Lodge, J.P., Jr, ed., *Methods of Air Sampling and Analysis*, Chelsea, MI, Lewis Publishers, pp. 171–187

Santos-Burgoa, C., Matanoski, G.M., Zeger, S. & Schwartz, L. (1992) Lymphohematopoietic cancer in styrene–butadiene polymerization workers. *Am. J. Epidemiol.*, **136**, 843–854

Sapkota, A., Halden, R.U., Dominici, F., Groopman, J.D. & Buckley, T.J. (2006) Urinary biomarkers of 1,3-butadiene in environmental settings using liquid chromatography isotope dilution tandem mass spectrometry. *Chem.-biol. Interact.*, **160**, 70–79

Saranko, C.J., Pluta, L.J. & Recio, L. (1998) Molecular analysis of lacI mutants from transgenic fibroblasts exposed to 1,2-epoxybutene. *Carcinogenesis*, **19**, 1879–1887

Saranko, C.J., Meyer, K.G., Pluta, L.J., Henderson, R.F. & Recio, L. (2001) Lung-specific mutagenicity and mutational spectrum in B6C3F1 lacI transgenic mice following inhalation exposure to 1,2-epoxybutene. *Mutat. Res.*, **473**, 37–49

Sasiadek, M., Jarventaus, H. & Sorsa, M. (1991a) Sister-chromatid exchanges induced by 1,3-butadiene and its epoxides in CHO cells. *Mutat. Res.*, **263**, 47–50

Sasiadek, M., Norppa, H. & Sorsa, M. (1991b) 1,3-Butadiene and its epoxides induce sister-chromatid exchanges in human lymphocytes *in vitro*. *Mutat. Res.*, **261**, 117–121

Sasiadek, M., Hirvonen, A., Noga, L., Paprocka-Borowicz, M. & Norppa, H. (1999) Glutathione S-transferase M1 genotype influences sister chromatid exchange induction but not adaptive response in human lymphocytes treated with 1,2-epoxy-3-butene. *Mutat. Res.*, **439**, 207–212

Sathiakumar, N. & Delzell, E. (2007) A follow-up study of women in the synthetic rubber industry: Study methods. *Chem.-biol. Interact.*, **166**, 25–28

Sathiakumar, N., Delzell, E., Hovinga, M., Macaluso, M., Julian, J.A., Larson, R., Cole, P. & Muir, D.C. (1998) Mortality from cancer and other causes of death among synthetic rubber workers. *Occup. environ. Med.*, **55**, 230–235

Sathiakumar, N., Graff, J., Macaluso, M., Maldonado, G., Matthews, R. & Delzell, E. (2005) An updated study of mortality among North American synthetic rubber industry workers. *Occup. environ. Med.*, **62**, 822–829

Sathiakumar, N., Delzell, E., Cheng, H., Lynch, J., Sparks, W. & Macaluso, M. (2007) Validation of 1,3-butadiene exposure estimates for workers at a synthetic rubber plant. *Chem.-biol. Interact.*, **166**, 29–43

Schlade-Bartusiak, K., Sasiadek, M. & Kozlowska, J. (2000) The influence of GSTM1 and GSTT1 genotypes on the induction of sister chromatid exchanges and chromosome aberrations by 1,2:3,4-diepoxybutane. *Mutat. Res.*, **465**, 69–75

Schlade-Bartusiak, K., Rozik, K., Laczmanska, I., Ramsey, D. & Sasiadek, M. (2004) Influence of GSTT1, mEH, CYP2E1 and RAD51 polymorphisms on diepoxybutane-induced SCE frequency in cultured human lymphocytes. *Mutat. Res.*, **558**, 121–130

Schmiederer, M., Knutson, E., Muganda, P. & Albrecht, T. (2005) Acute exposure of human lung cells to 1,3-butadiene diepoxide results in G1 and G2 cell cycle arrest. *Environ. mol. Mutag.*, **45**, 354–364

Scholdberg, T.A., Nechev, L.V., Merritt, W.K., Harris, T.M., Harris, C.M., Lloyd, R.S. & Stone, M.P. (2004) Structure of a site specific major groove (2S,3S)-N^6-(2,3,4-trihydroxybutyl)-2′-deoxyadenosyl DNA adduct of butadiene diol epoxide. *Chem. Res. Toxicol.*, **17**, 717–730

Scholdberg, T.A., Nechev, L.V., Merritt, W.K., Harris, T.M., Harris, C.M., Lloyd, R.S. & Stone, M.P. (2005a) Mispairing of a site specific major groove (2S,3S)-N^6-(2,3,4-trihydroxybutyl)-2′-deoxyadenoxyl DNA adduct of butadiene diol epoxide with deoxyguanosine: Formation of a dA(anti).dG(anti) pairing interaction. *Chem. Res. Toxicol.*, **18**, 145–153

Scholdberg, T.A., Merritt, W.K., Dean, S.M., Kowalcyzk, A., Harris, C.M., Harris, T.M., Rizzo, C.J., Lloyd, R.S. & Stone, M.P. (2005b) Structure of an oligodeoxynucleotide containing a butadiene oxide-derived N1 beta-hydroxyalkyl deoxyinosine adduct in the human N-ras codon 61 sequence. *Biochemistry*, **44**, 3327–3337

Selzer, R.R. & Elfarra, A.A. (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

Selzer, R.R. & Elfarra, A.A. (1996b) Synthesis and biochemical characterization of N1-, N2-, and N7-guanosine adducts of butadiene monoxide. *Chem. Res. Toxicol.*, **9**, 126–132

Selzer, R.R. & Elfarra, A.A. (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

Selzer, R.R. & Elfarra, A.A. (1999) In vitro reactions of butadiene monoxide with single- and double-stranded DNA: Characterization and quantitation of several purine and pyrimidine adducts. *Carcinogenesis*, **20**, 285–292

Serrano-Trespalacios, P.I., Ryan, L. & Spengler, J.D. (2004) Ambient, indoor and personal exposure relationships of volatile organic compounds in Mexico City Metropolitan Area. *J. Expo. Anal. environ. Epidemiol.*, **14**, S118–S132

Shimkin, M.B., Weisburger, J.H., Weisburger, E.K., Gubareff, N. & Suntzeff, V. (1966) Bioassay of 29 alkylating chemicals by the pulmonary-tumor response in strain A mice. *J. natl Cancer Inst*., **36**, 915–935

Sills, R.C., Hong, H.L., Boorman, G.A., Devereux, T.R. & Melnick, R.L. (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

Sisk, S.C., Pluta, L.J., Bond, J.A. & Recio, L. (1994) Molecular analysis of lacI mutants from bone marrow of B6C3F1 transgenic mice following inhalation exposure to 1,3-butadiene. *Carcinogenesis*, **15**, 471–477

van Sittert, N.J., Megens, H.J., Watson, W.P. & Boogaard, P.J. (2000) Biomarkers of exposure to 1,3-butadiene as a basis for cancer risk assessment. *Toxicol. Sci.*, **56**, 189–202

Smith, T.J., Lin, Y.S., Mezzetti, M., Bois, F.Y., Kelsey, K. & Ibrahim, J. (2001) Genetic and dietary factors affecting human metabolism of 1,3-butadiene. *Chem.-biol. Interact.*, **135–136**, 407–428

Sprague, C.L. & Elfarra, A.A. (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

Sprague, C.L. & Elfarra, A.A. (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

Šrám, R.J., Rössner, P., Peltonen, K., Podrazilova, K., Mrackova, G., Demopoulos, N.A., Stephanou, G., Vlachodimitropoulos, D., Darroudi, F. & Tates, A.D. (1998) Chromosomal aberrations, sister-chromatid exchanges, cells with high frequency of SCE, micronuclei and comet assay parameters in 1,3-butadiene-exposed workers. *Mutat. Res.*, **419**, 145–154

Šrám, R.J., Beskid, O., Binkova, B., Rossner, P. & Smerhovsky, Z. (2004) Cytogenetic analysis using fluorescence in situ hybridization (FISH) to evaluate occupational exposure to carcinogens. *Toxicol. Lett.*, **149**, 335–344

Startin, J.R. & Gilbert, J. (1984) Single ion monitoring of butadiene in plastics and foods by coupled mass-spectrometry–automatic headspace gas chromatography. *J. Chromatogr*., **294**, 427–430

Stephanou, G., Russo, A., Vlastos, D., Andrianopoulos, C. & Demopoulos, N.A. (1998) Micronucleus induction in somatic cells of mice as evaluated after 1,3-butadiene inhalation. *Mutat. Res.*, **397**, 11–20

Sun, H.N. & Wristers, J.P. (2002) Butadiene. In: *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 4, New York, J. Wiley & Sons, pp. 365–392 (on line)

Sweeney, L.M., Himmelstein, M.W. & Gargas, M.L. (2001) Development of a preliminary physiologically based toxicokinetic (PBTK) model for 1,3-butadiene risk assessment. *Chem.-biol. Interact*., **135–136**, 303–322

Swenberg, J.A., Christova-Gueorguieva, N.I., Upton, P.B., Ranasinghe, A., Scheller, N., Wu, K.Y., Yen, T.Y. & Hayes, R. (2000a) 1,3-Butadiene: Cancer, mutations, and adducts. Part V: Hemoglobin adducts as biomarkers of 1,3-butadiene exposure and metabolism. *Res. Rep. Health Eff. Inst.*, **92**, 191–210

Swenberg, J.A., Ham, A., Koc, H., Morinello, E., Ranasinghe, A., Tretyakova, N., Upton, P.B. & Wu, K.Y. (2000b) DNA adducts: Effects of low exposure to ethylene oxide, vinyl chloride and butadiene. *Mutat. Res.*, **464**, 77–86

Swenberg, J.A., Koc, H., Upton, P.B., Georguieva, N., Ranasinghe, A., Walker, V.E. & Henderson, R. (2001) Using DNA and hemoglobin adducts to improve the risk assessment of butadiene. *Chem.-biol. Interact.*, **135–136**, 387–403

Swenberg, J.A., Boysen, G., Georgieva, N., Bird, M.G. & Lewis, R.J. (2007) Future directions in butadiene risk assessment and the role of cross-species internal dosimetry. *Chem.-biol. Interact.*, **166**, 78–83

Tates, A.D., van Dam, F.J., de Zwart, F.A., van Teylingen, C.M.M. & Natarajan, A.T. (1994) Development of a cloning assay with high cloning efficiency to detect induction of 6-thio-

guanine-resistant lymphocytes in spleen of adult mice following in vivo inhalation exposure to 1,3-butadiene. *Mutat. Res.*, **309**, 299–306

Tates, A.D., van Dam, F.J., van Teylingen, C.M., de Zwart, F.A. & Zwinderman, A.H. (1998) Comparison of induction of *hprt* mutations by 1,3-butadiene and/or its metabolites 1,2-epoxybutene and 1,2,3,4-diepoxybutane in lymphocytes from spleen of adult male mice and rats *in vivo*. *Mutat. Res.*, **397**, 21–36

Thier, R., Bruning, T., Roos, P.H., Rihs, H.P., Golka, K., Ko, Y. & Bolt, H.M. (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

Tice, R.R., Boucher, R., Luke, C.A. & Shelby, M.D. (1987) Comparative cytogenetic analysis of bone marrow damage induced in male B6C3F1 mice by multiple exposures to gaseous 1,3-butadiene. *Environ. Mutag.*, **9**, 235–250

Tommasi, A.M., de Conti, S., Dobrzynska, M.M. & Russo, A. (1998) Evaluation and characterization of micronuclei in early spermatids of mice exposed to 1,3-butadiene. *Mutat. Res.*, **397**, 45–54

Ton, T.V., Hong, H.H., Devereux, T.R., Melnick, R.L., Sills, R.C. & Kim, Y. (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

Tsai, S.P., Wendt, J.K. & Ransdell, J.D. (2001) A mortality, morbidity, and hematology study of petrochemical employees potentially exposed to 1,3-butadiene monomer. *Chem.-biol. Interact.*, **135–136**, 555–567

Urban, M., Gilch, G., Schepers, G., van Miert, E. & Scherer, G. (2003) Determination of the major mercapturic acids of 1,3-butadiene in human and rat urine using liquid chromatography with tandem mass spectrometry. *J. Chromatogr. B*, **796**, 131–140

Valadez, J.G., Liu, L., Loktionova, N.A., Pegg, A.E. & Guengerich, F.P. (2004) Activation of bis-electrophiles to mutagenic conjugates by human O^6-alkylguanine-DNA alkyltransferase. *Chem. Res. Toxicol.*, **17**, 972–982

Van Duuren, B.L., Nelson, N., Orris, L., Palmes, E.D. & Schmitt, F.L. (1963) Carcinogenicity of epoxides, lactones, and peroxy compounds. *J. natl Cancer Inst.*, **3**, 41–55

Van Duuren, B.L., Orris, L. & Nelson, N. (1965) Carcinogenicity of epoxides, lactones, and peroxy compounds. Part II. *J. natl Cancer Inst.*, **35**, 707–717

Van Duuren, B.L., Langseth, L., Orris, L., Teebor, G., Nelson, N. & Kuschner, M. (1966) Carcinogenity 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

Vangala, R.R., Laib, R.J. & Bolt, H.M. (1993) Evaluation of DNA damage by alkaline elution technique after inhalation exposure of rats and mice to 1,3-butadiene. *Arch. Toxicol.*, **67**, 34–38

Verschueren, K. (1996) *Handbook of Environmental Data on Organic Chemicals*, 3rd Ed., New York, Van Nostrand Reinhold, pp. 347–348

Victorin, K., Busk, L., Cederberg, H. & Magnusson, J. (1990) Genotoxic activity of 1,3-butadiene and nitrogen dioxide and their photochemical reaction products in *Drosophila* and in mouse bone marrow micronucleus assay. *Mutat. Res.*, **228**, 203–209

Vodicka, P., Kumar, R., Stetina, R., Musak, L., Soucek, P., Haufroid, V., Sasiadek, M., Vodickova, L., Naccarati, A., Sedikova, J., Sanyal, S., Kuricova, M., Brsiak, V., Norppa, H., Buchancova, J. & Hemminki, K. (2004) Markers of individual susceptibility and DNA repair rate in workers exposed to xenobiotics in a tire plant. *Environ. mol. Mutag.*, **44**, 283–292

Vodicka, P., Stetina, R., Smerak, P., Vodickova, L., Naccarati, A., Barta, I. & Hemminki, K. (2006) Micronuclei, DNA single-strand breaks and DNA-repair activity in mice exposed to 1,3-butadiene by inhalation. *Mutat. Res.*, **608**, 49–57

Walker, V.E. & Meng, Q. (2000) 1,3-Butadiene: Cancer, mutations, and adducts. Part III: In vivo mutation of the endogenous hprt genes of mice and rats by 1,3-butadiene and its metabolites. *Res. Rep. Health Eff. Inst.*, **92**, 89–139

Walles, S.A.S., Victorin, K. & Lundborg, M. (1995) DNA damage in lung cells *in vivo* and *in vitro* by 1,3-butadiene and nitrogen dioxide and their photochemical reaction products. *Mutat. Res.*, **328**, 11–19

Walther, M.W. (2003) *CEH Marketing Research Report – Butadiene*, Zürich, SRI Consulting

Ward, E.M., Fajen, J.M., Ruder, A.M., Rinsky, R.A., Halperin, W.E. & Fessler-Flesch, C.A. (1995) Mortality study of workers in 1,3-butadiene production units identified from a chemical workers cohort. *Environ. Health Perspect.*, **103**, 598–603

Ward, E.M., Fajen, J.M., Ruder, A.M., Rinsky, R.A., Halperin, W.E. & Fessler-Flesch, C.A. (1996) Mortality study of workers employed in 1,3-butadiene production units identified from a large chemical workers cohort. *Toxicology*, **113**, 157–168

Ward, J.B., Ammenheuser, M.M., Whorton, E.B., Jr, Bechtold, W.E., Kelsey, K.T. & Legator, M.S. (1996) Biological monitoring for mutagenic effects of occupational exposure to butadiene. *Toxicology*, **113**, 84–90

Ward, J.B., Jr, Abdel-Rahman, S.Z., Henderson, R.F., Stock, T.H., Morandi, M., Rosenblatt, J.I. & Ammenheuser, M.M. (2001) Assessment of butadiene exposure in synthetic rubber manufacturing workers in Texas using frequencies of hprt mutant lymphocytes as a biomarker. *Chem.-biol. Interact.*, **135–136**, 465–483

White, W.C. (2007) Butadiene production process overview. *Chem.-biol. Interact.*, **166**, 10–14

Wickliffe, J.K., Ammenheuser, M.M., Salazar, J.J., Abdel-Rahman, S.Z., Hastings-Smith, D.A., Postlethwait, E.M., Lloyd, R.S. & Ward, J.B., Jr (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. Mutag.*, **42**, 106–110

Wickliffe, J.K., Galbert, L.A., Ammenheuser, M.M., Herring, S.M., Xie, J., Masters, O.E., III, Friedberg, E.C., Lloyd, R.S. & Ward, J.B., Jr (2006) 3,4-Epoxy-1-butene, a reactive metabolite of 1,3-butadiene, induces somatic mutations in Xpc-null mice. *Environ. mol. Mutag.*, **47**, 67–70

Wickliffe, J.K., Herring, S.M., Hallberg, L.M., Galbert, L.A., Masters, O.E., III, Ammenheuser, M.M., Xie, J., Friedberg, E.C., Lloyd, R.S., Abdel-Rahman, S.Z. & Ward, J.B., Jr (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

Wiencke, J.K., Pemble, S., Ketterer, B. & Kelsey, K.T. (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

Work Environment Fund (1991) *Development and Evaluation of Biological and Chemical Methods for Exposure Assessment of 1,3-Butadiene* (Contract No. 88-0147), Helsinki, Institute of Occupational Health

Xiao, Y. & Tates, A.D. (1995) Clastogenic effects of 1,3-butadiene and its metabolites 1,2-epoxybutene and 1,2,3,4-diepoxybutane in splenocytes and germ cells of rats and mice in vivo. *Environ. Health Perspect.*, **26**, 97–108

Xu, W., Merritt, W.K., Nechev, L.V., Harris, T.M., Harris, C.M., Lloyd, R.S. & Stone, M.P. (2007) Structure of the 1,4-bis(2′-deoxyadenosin-N^6-yl)-2S,3S-butanediol intrastrand DNA cross-link arising from butadiene diepoxide in the human N-ras codon 61 sequence. *Chem. Res. Toxicol.*, **20**, 187–198

Ye, Y., Galbally, I.E. & Weeks, I.A. (1997) Emission of 1,3-butadiene from petrol-driven motor vehicle. *Atmos. Environ.*, **31**, 1157–1165

Zang, H., Harris, T.M. & Guengerich, F.P. (2005) Kinetics of nucleotide incorporation opposite DNA bulky guanine N^2 adducts by processive bacteriophage T7 DNA polymerase (exonuclease-) and HIV-1 reverse transcriptase. *J. biol. Chem.*, **280**, 1165–1178

Zhang, X.Y. & Elfarra, A.A. (2003) Identification and characterization of a series of nucleoside adducts formed by the reaction of 2′-deoxyguanosine and 1,2,3,4-diepoxybutane under physiological conditions. *Chem. Res. Toxicol.*, **16**, 1606–1615

Zhang, X.Y. & Elfarra, A.A. (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

Zhang, X.Y. & Elfarra, A.A. (2005) Reaction of 1,2,3,4-diepoxybutane with 2′-deoxyguanosine: Initial products and their stabilities and decomposition patterns under physiological conditions. *Chem. Res. Toxicol.*, **18**, 1316–1323

Zhang, X.Y. & Elfarra, A.A. (2006) Characterization of 1,2,3,4-diepoxybutane-2′-deoxyguanosine cross-linking products formed at physiological and nonphysiological conditions. *Chem. Res. Toxicol.*, **19**, 547–555

Zhang, L., Hayes, R.B., Guo, W., McHale, C.M., Yin, S., Wiencke, J.K., O'Neill, J.P., Rothman, N., Li, G.L. & Smith, M.T. (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

Zhao, C., Koskinen, M. & Hemminki, K. (1998) ^{32}P-Postlabelling of N^6-adenine adducts of epoxybutanediol in vivo after 1,3-butadiene exposure. *Toxicol. Lett.*, **102–103**, 591–594

Zhao, C., Vodicka, P., Sram, R.J. & Hemminki, K. (2000) Human DNA adducts of 1,3-butadiene, an important environmental carcinogen. *Carcinogenesis*, **21**, 107–111

Zhao, C., Vodicka, P., Sram, R.J. & Hemminki, K. (2001) DNA adducts of 1,3-butadiene in humans: Relationships to exposure, GST genotypes, single-strand breaks, and cytogenetic end points. *Environ. mol. Mutag.*, **37**, 226–230

Zhuang, S.M. & Söderkvist, P. (2000) Genetic analysis of Raf1, Mdm2, c-Myc, Cdc25a and Cdc25b proto-oncogenes in 2′,3′-dideoxycytidine- and 1,3-butadiene-induced lymphomas in B6C3F1 mice. *Mutat. Res.*, **452**, 19–26

Zhuang, S.M., Wiseman, R.W. & 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

Zhuang, S.M., Wiseman, R.W. & 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