1,3-BUTADIENE

This substance (hereinafter referred to as butadiene) was considered by previous Working Groups, in June 1985 (IARC, 1986; see also correction, IARC, 1987a), March 1987 (IARC, 1987b) and October 1991 (IARC, 1992). 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 butadiene, 1,2:3,4-diepoxybutane (hereinafter referred to as diepoxybutane), also was previously evaluated by an IARC Working Group (IARC, 1976), and its reevaluation by the present Working Group is included in this monograph.

1. Exposure Data

1.1 Chemical and physical data

Butadiene

1.1.1 *Nomenclature*

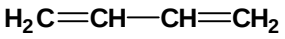
Chem. Abstr. Serv. Reg. No.: 106-99-0

Chem. Abstr. Name: 1,3-Butadiene

IUPAC Systematic Name: 1,3-Butadiene

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*

(*a*) *Description*: Colourless mildly aromatic gas (Budavari, 1996)

(*b*) *Boiling-point*: –4.4°C (Lide, 1995)

(*c*) *Melting-point*: –108.9°C (Lide, 1995)

(*d*) *Density*: d_4^{20} 0.6149 (Lide, 1995)

(*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.

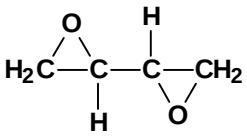
(*f*) *Solubility*: Very slightly soluble in water (735 mg/L at 20°C); soluble in ethanol, diethyl ether, benzene and organic solvents; very soluble in acetone (Lide, 1995; Budavari, 1996; Verschueren, 1996)
(*g*) *Volatility*: Vapour pressure, 120 kPa at 0°C (Lide, 1995); 235 kPa at 20°C (Müller & Löser, 1985); relative vapour density (air = 1), 1.87 (Verschueren, 1996)
(*h*) *Stability*: Flash-point, –76°C; very reactive; may form explosive peroxides upon exposure to air; polymerizes readily, particularly if oxygen is present (Lewis, 1993; Budavari, 1996)
(*i*) *Explosive limits*: Lower, 2.0%; upper, 11.5% (Budavari, 1996)
(*j*) *Conversion factor*: $mg/m^3 = 2.21 \times ppm$[1]

Diepoxybutane

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 1464-53-5
Chem. Abstr. Name: 2,2′-Bioxirane
IUPAC Systematic Name: 1,2:3,4-Diepoxybutane
Synonym: Butadiene dioxide

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 of the pure substance*

(*a*) *Description*: Colourless liquid (Budavari, 1996)
(*b*) *Boiling-point*: 138°C (Budavari, 1996)
(*c*) *Melting-point*: –19°C (Budavari, 1996)
(*d*) *Solubility*: Miscible with water (hydrolyses) (Budavari, 1996)
(*e*) *Vapour pressure*: 918 Pa at 25°C (United States National Library of Medicine, 1997)

[1] Calculated from: mg/m^3 = (relative molecular mass/24.47) × ppm, assuming a temperature of 25°C and a pressure of 101 kPa

1.1.4 *Technical products and impurities*

Butadiene is available commercially as a liquefied gas under pressure. The polymerization grade has a minimum purity of 99%, with acetylene as an impurity in the parts-per-million (ppm) range. Isobutene, 1-butene, butane and *cis*-2- and *trans*-2-butene have been detected in pure-grade butadiene (Miller, 1978). Typical specifications for butadiene are: purity, ≥ 99.5%; inhibitor (*tert*-butylcatechol), 50–150 ppm; impurities (ppm max.): 1,2-butadiene, 20; propadiene, 10; total acetylenes, 20; dimers, 500; isoprene, 10; other C_5 compounds, 500; sulfur, 5; peroxides (as H_2O_2), 5; ammonia, 5; water, 300; carbonyls, 10; nonvolatile residues, 0.05 wt% max.; and oxygen in the gas phase, 0.10 vol% max. (Sun & Wristers, 1992). Butadiene has been stabilized with hydroquinone, catechol and aliphatic mercaptans (IARC, 1986, 1992).

1.1.5 *Analysis*

Selected methods for the analysis of butadiene in various matrices are listed in Table 1. Methods of analysis of butadiene in air have recently been evaluated. There appears to be no single preferred method, but newer methods give higher performance. Thermal desorption methods provide high levels of accuracy and precision (Bianchi *et al*., 1997).

The specificity and detection limits of methods for determining simple, small molecules present in packaging material which migrate into packaged goods have been discussed (Vogt, 1988). Butadiene can be determined in plastic polymers, foods and food simulants by chromatographic methods.

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).

1.2 Production and use

1.2.1 *Production*

Butadiene was first produced in the late nineteenth century by pyrolysis of petroleum hydrocarbons (Kirshenbaum, 1978). Commercial production started in the 1930s.

Butadiene is manufactured primarily as a coproduct of steam cracking of hydrocarbon streams to produce ethylene in the United States, western Europe and Japan. However, in certain parts of the world (e.g., China, India, Poland and Russia) it is still produced from ethanol. The earlier manufacturing processes of dehydrogenation of *n*-butane and oxyhydrogenation of *n*-butenes have significantly declined in importance and output. Efforts have been made to make butadiene from other feedstocks such as other hydrocarbons, coal, shale oil and renewable sources such as animal and vegetable oil, cellulose, hemicellulose and lignin, but in the United States none of these has moved beyond the research and development stage (Müller & Löser, 1985; Sun & Wristers, 1992).

Steam cracking is a complex, highly endothermic pyrolysis reaction. During the reaction, a hydrocarbon feedstock is heated to approximately 800°C and 34 kPa for less

Table 1. Methods for analysis of butadiene

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Adsorb (charcoal); extract (carbon disulfide)	GC/FID	200 µg/m^3	United States 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	United Kingdom Health and Safety Executive (1992)
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	United States Food and Drug Administration (1987)
Solid foods	Cut or mash sample; inject headspace sample	GC/FID	2–20 µg/kg	United States Food and Drug Administration (1987)

[a] Abbreviations GC/FID, gas chromatography/flame ionization detection; GC/MS-SIM, gas chromatography/mass spectrometry with single-ion monitoring

than one second, during which carbon–carbon and carbon–hydrogen bonds are broken. As a result, a mixture of olefins, aromatics, 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, isobutene, 1-butene, 2-butene and some other minor hydrocarbons. The overall process yields of butadiene depend on both the process parameters 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 also have been used for this separation. Another commercial process to separate butadiene from other hydrocarbons uses a solution containing cuprous ammonium acetate, which forms a weak copper(I) complex with butadiene (Müller & Löser, 1985; Sun & Wristers, 1992).

Dehydrogenation of *n*-butane via the Houdry process is carried out under partial vacuum (35–75 kPa) at about 535–650°C with a fixed-bed catalyst. The catalyst contains

aluminium oxide and chromium oxide as the principal components. Normal butenes can also be oxidatively dehydrogenated to butadiene in the presence of a high concentration of steam with fairly high selectivity. The reaction temperature is kept below 600°C to minimize over-oxidation, and the reaction pressure is about 34–103 kPa (Müller & Löser, 1985; Sun & Wristers, 1992).

An estimated 3570 thousand tonnes of butadiene were produced worldwide in 1983 (Anon., 1984). By 1989, that figure had risen to an estimated 6620 thousand tonnes, with the following breakdown by global area (thousand tonnes): North America, 1520; South America, 260; western Europe, 1870; eastern Europe, 1490, Africa and the Middle East, 150; and Asia and the Pacific, 1330 (Sun & Wristers, 1992). Production figures by country for the years 1981–96 are presented in Table 2.

Butadiene remains a major industrial commodity in the United States, ranking 36th among all chemicals produced in 1996 (Anon., 1996a). Seven major producers in the United States, with 10 plant locations, had a total annual capacity of 1900 thousand tonnes in 1996 (Anon., 1996b). Available information indicates that butadiene is produced by seven companies each in Japan and Korea; four companies each in France and Germany; three companies in The Netherlands; two companies each in the Czech Republic and the United Kingdom; one company each in Austria, Canada, Finland, Italy, Mexico, Portugal, Romania, Singapore, Spain and Taiwan; and an undisclosed number of companies in Argentina, Brazil, Bulgaria, China, the Commonwealth of Independent States, India, Poland and Saudi Arabia (Anon., 1996b).

Diepoxybutane is not believed to be produced commercially except in small quantities for research purposes (United States National Library of Medicine, 1997).

Table 2. Butadiene production in selected countries from 1981 through 1996 (thousand tonnes)[a]

Country	1981	1984	1987	1990	1993	1996
Canada	126	127	167	192	174	212
China	NR[b]	141	181	258	NR	NR
China (Taiwan)	NR	NR	NR	NR	90	129
France	266	302	307	281	320	344
Germany	NR	753	700	777	879	673
Italy	163	181	NR	NR	NR	NR
Japan	518	627	707	827	809	1025
Korea (Republic of)	NR	NR	NR	168	486	601
United Kingdom	207	258	231	198	NR	NR
United States	1354	1112	1329	1401	1414	1744

[a] From Anon. (1985, 1988, 1991, 1994, 1997); China National Chemical Information Centre (1993)
[b] NR, not reported

1.2.2 *Use*

Butadiene is used primarily in the production of synthetic rubbers, including styrene–butadiene rubber (SBR), polybutadiene rubber (BR), styrene–butadiene latex (SBL), chloroprene rubber (CR) and nitrile rubber (NR). Important plastics containing butadiene as a monomeric component are shock-resistant polystyrene, a two-phase system consisting of polystyrene and polybutadiene; ABS polymers consisting of acrylonitrile, butadiene and styrene; and a copolymer of methyl methacrylate, butadiene and styrene (MBS), which is used as a modifier for poly(vinyl chloride). It is also used as an intermediate in the production of chloroprene, adiponitrile and other basic petrochemicals. The worldwide use pattern for butadiene in 1981 was as follows (%): SBR + SBL, 56; BR, 22; CR, 6; NR, 4; ABS, 4; hexamethylenediamine, 4; other, 4. The use pattern for butadiene in the United States in 1995 was (%): SBR, 31; BR, 24; SBL, 13; CR, 4; ABS, 5; NR, 2; adiponitrile, 12; and other, 9 (Anon., 1996b).

Diepoxybutane has been proposed for use in curing polymers and cross-linking textile fibres (United States National Library of Medicine, 1997).

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 for 15 countries of the European Union (Kauppinen *et al.*, 1998) and the 1981–83 United States National Occupational Exposure Survey (NOES, 1997), approximately 30 000 workers in Europe and as many as 50 000 workers in the United States were potentially exposed to butadiene (see General Remarks).

Potential exposure to butadiene can occur in the following industrial activities: petroleum refining and related operations (production of C_4 fractions containing butadiene, and production and distribution of gasoline), production of purified butadiene monomer, production of various butadiene-based rubber and plastics polymers and other derivatives, and manufacture of rubber and plastics products (tyres, hoses and a variety of moulded objects).

In the descriptions below, the accuracy of the levels of exposure to butadiene 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 in methods used until the mid-1980s (Lunsford *et al.*, 1990; Bianchi *et al.*, 1997). No measurement data are available on levels of exposure to butadiene before the 1970s, when different processes and working conditions (e.g., during the Second World War) would have resulted in exposure levels different from those now prevalent in developed countries.

(*a*) *Petroleum refining and production of crude butadiene*

Exposure data collected in Europe in 1984–85 suggested that gasoline contains a small percentage of butadiene. Levels of exposure of workers in various job groups in the production and distribution of gasoline are shown in Table 3 (see IARC, 1989). Table 4 shows the exposures since 1984 of workers in different areas of petroleum refineries and petrochemical facilities where crude butadiene is produced (usually a C_4 stream obtained as a by-product of ethylene production). Table 5 shows more recent data from crackers of butadiene production plants for the years 1986–93 (ECETOC, 1997).

Table 3. Personal exposures (mg/m^3) to butadiene associated with gasoline during 1984–85 in 13 European countries (540 measurements)

Activity	Arithmetic mean	Range	Exposure 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

(*b*) *Monomer production*

Detailed industrial hygiene surveys were conducted in the United States 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 originating as ethylene co-product streams (Krishnan *et al.*, 1987). Levels of butadiene to which workers in various job categories were exposed are summarized in Table 6. Jobs that require workers to handle or transport containers, such as voiding sample cylinders or loading and unloading tank trucks or rail cars, present 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 such activities as open-loop sampling and

Table 4. Eight-hour time-weighted average concentrations of butadiene to which workers in different jobs in petroleum refineries and petrochemical facilities were exposed from 1984 to 1987

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	2.9	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

cylinder voiding 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]).

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

A recent study on biological monitoring for 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 *et al*., 1996a). Sorsa *et al*. (1996a) reported that 70% of the samples contained below 0.2 ppm [0.4 mg/m^3] butadiene from two plants in Portugal (personal samples) and Finland (area samples), while 5% and 2% of the samples, respectively, were above 10 ppm [22 mg/m^3].

Monitoring in a Finnish plant generally indicated ambient air levels 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 sample collection. Protective clothing and respirators were used during this operation (Arbetsmiljöfonden, 1991).

Potential exposures in the monomer industry other than to butadiene include extraction solvents and components of the C_4 feedstock. Extraction solvents differ between facilities; some common ones are dimethylformamide, dimethylacetamide, acetonitrile,

Table 5. Personal exposures to butadiene in crackers of butadiene production plants in the European Union

Job category	Year of measurement	Number of people	Number of samples	Personal exposure (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	392	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[a]	0	0
Total	1986–93	1485	1548	1493	28	9	8	4	0	4	2

From ECETOC (1997)

[a] ND, not detected (detection limit not stated)

Table 6. Eight-hour time-weighted average exposure levels in personal breathing zone samples at four butadiene monomer production facilities, United States, 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 voiding	3	126 [277]	7.46 [16.5]	0.42–374 [0.93–826]

From Krishnan *et al.* (1987)

Table 7. Personal exposures to butadiene at 15 monomer extraction sites in the European Union in 1995

Job category	Concentration (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

Table 8. Personal exposures to butadiene in extraction units[a] of butadiene production plants in the European Union

Job category	Year of measurement	Number of people	Number of samples	Personal exposures (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

β-methoxypropionitrile (Fajen, 1985a), furfural and aqueous cuprous ammonium acetate (United States Occupational Safety and Health Administration, 1990b). Stabilizers are commonly used to prevent formation of peroxides in air and polymerization. No information was available on these other exposures, or on exposures 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).

(*c*) *Production of polymers and derivatives*

Detailed industrial hygiene surveys were conducted in 1986 in five of 17 facilities in the United States where butadiene was used to produce SBR, 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 9. 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 to 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.

Table 9. Eight-hour time-weighted average exposure levels in personal breathing-zone samples at five plants producing butadiene-based polymers and derivatives, United States, 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)

More recent data are available from 13 of 27 European sites where synthetic rubber and rubber latex were produced and from on-going exposure surveys in an SBR-producing plant in the Netherlands. Less than 10% of the measured concentrations from the European sites exceeded 5 ppm (Table 10). Data from the Netherlands were available from 1976 onwards, although for the earlier surveys the measurement methods used were unknown and therefore the overview is limited to the period 1983–97. No clear trend can be seen for these years, but average exposures were relatively low (arithmetic mean < 3 ppm [6.6 mg/m^3]) (Table 11).

Other data on levels of exposure to butadiene have been collected during health surveys and epidemiological studies (Table 12). At an SBR manufacturing plant in the United States 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). In samples taken at one of two United States SBR plants in 1976, levels 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). Overall mean 8-h time-weighted average (TWA) exposure levels differed considerably between the two plants, however: 1.24 ppm [2.7 mg/m^3] in one plant and 13.5 ppm [30 mg/m^3] in the other (Meinhardt *et al.*, 1982).

A study by the University of Alabama at Birmingham retrospectively assessed historical exposure to butadiene of SBR workers from eight North American plants using elaborate methods. Estimates of 8-h TWA exposures to butadiene were made for a total of 664 plant-specific work area group–year combinations and ranged from 0 to 64 ppm [0–140 mg/m^3]. The median TWA among groups with any butadiene exposure was below 2 ppm in all plants (Macaluso *et al.*, 1996). The same authors also performed an in-depth study to assess the feasibility of improving the exposure estimation procedures in one of the plants (Macaluso *et al.*, 1997). The revised procedures led to exposure estimates that were higher than the original ones, especially during the 1950s and 1960s. Historical exposure profiles of exposed employees in this plant showed average concentrations of 12–16 ppm [26–35 mg/m^3] in the 1940s, 17–25 ppm [38–55 mg/m^3] in the 1950s and a gradual decline to approximately 2 ppm [4.4 mg/m^3] in the 1980s.

A recent biological monitoring study reported average exposures using personal sampling 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 exposed groups in an SBR plant in Texas (Ward *et al.*, 1996a). A similar study in Europe reported exposure levels of 0.2–2.0 ppm [0.44–4.4 mg/m^3] in about 50% of the samples and 10% of the samples exceeded 10 ppm [22 mg/m^3] in an SBR plant in Poland (Sorsa *et al.*, 1996b).

The manufacture of butadiene-based polymers and butadiene derivatives implies potential occupational exposure to a number of other chemical agents, which vary according to product and process, including 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 were measured in various departments of

Table 10. Eight-hour time-weighted average personal exposures to butadiene in synthetic rubber plants in the European Union (1984–93)

Job category	No. of workers	No. of samples	Personal exposures (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 11. Eight-hour time-weighted average exposure levels of butadiene in personal breathing-zone samples at a plant producing 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) and Dubbeld (1998)

[a] Analytical methods used are described by Bianchi *et al*. (1997). Methods 3M 3500 and 3M 3520 involve absorption onto a 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.

a United States SBR-manufacturing plant in 1979: 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], 8 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 were 0.94 ppm [3.9 mg/m^3] (55 samples) and 1.99 ppm [8.4 mg/m^3] (35 samples) in two SBR-manufacturing plants in 1977; the average benzene level measured in one of the plants was 0.1 ppm [0.3 mg/m^3] (3 samples). Average levels of styrene, toluene, benzene, vinylcyclohexene and cyclooctadiene were reported to be below 1 ppm in another SBR plant in 1977 (Burroughs, 1977).

(*d*) *Manufacture of rubber and plastics products*

Unreacted butadiene was detected as only 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 United States at a rubber tyre plant and an industrial hose plant where SBR, 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 identified as involving potential exposure to butadiene: Banbury operators, mill operators, extruder

Table 12. Eight-hour time-weighted average exposure levels of butadiene measured in two styrene–butadiene rubber manufacturing plants in the United States

Job classification or department	No. of samples	Exposure level		Year of sampling	Reference
		ppm	mg/m^3		
Instrument man	3	58.6	130	1976	Meinhardt *et al.* (1978)
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		
Tank farm	8	20.0	44.3	1979	Checkoway & Williams (1982)
Maintenance	52	0.97	2.14		
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		

operators, curing operators, conveyer operators, calendering operators, wire winders, tube machine operators, tyre builders and tyre repair and buffer workers (Fajen *et al.*, 1990).

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 (Burroughs, 1979; Belanger & Elesh, 1980; Ruhe & Jannerfeldt, 1980). In a polybutadiene rubber warehouse, levels of 0.003 ppm [0.007 mg/m^3] were found in area samples; area and personal samples taken in tyre plants found 0.007–0.05 ppm [0.016–0.11 mg/m^3] (Rubber Manufacturers' Association, 1984). In a tyre and tube manufacturing plant in the United States in 1975, a cutter man/Banbury operator was reported to have been exposed to butadiene at 2.1 ppm [4.6 mg/m^3] (personal 6-h sample) (Ropert, 1976).

Occupational exposures to many other agents in the rubber goods manufacturing industry were reviewed in a previous monograph (IARC, 1982).

1.3.3 *Air*

According to the United States Environmental Protection Agency Toxic Chemical Release Inventory, industrial releases of butadiene to the atmosphere from manufacturing

and processing facilities in the United States were 4415 tonnes in 1987, 2344 tonnes in 1990 and 1321 tonnes in 1995 (United States National Library of Medicine, 1997).

The United States Environmental Protection Agency (1990) estimated that butadiene is emitted in automobile exhaust at 8.9–9.8 mg/mile [5.6–6.1 mg/km] and comprises about 0.35% of total hydrocarbon exhaust emissions.

Sidestream cigarette smoke contains approximately 0.4 mg butadiene per cigarette, and levels of butadiene in smoky indoor environments are typically 10–20 μg/m^3 (IARC, 1992).

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

Reported concentrations of butadiene in urban air generally range from less than 1 to 10 parts per billion [< 2–22 μg/m^3] (IARC, 1992).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for butadiene in several countries are given in Table 13.

2. Studies of Cancer in Humans

Several reviews of the epidemiology of butadiene and cancer have been published, the latest available being by Himmelstein *et al.* (1997). In what follows, ICD codes are given for lymphohaematopoietic cancers in view of the shifting classification with subsequent editions of the International Classification of Diseases.

2.1 Industry-based studies

The most informative industry-based studies of human exposure to butadiene are summarized in Table 14.

In a case–control study nested within a cohort of 6678 male rubber workers in the United States, deaths from cancers at the following sites were compared with a sample of members of the whole cohort (controls): stomach (41 deaths), colorectal (63), respiratory tract (119), prostate (52), urinary bladder (13), lymphatic and haematopoietic (51) and lymphatic leukaemia (14) (McMichael *et al*., 1976). A 6.2-fold increase in risk for lymphatic and haematopoietic cancers (99.9% confidence interval (CI), 4.1–12.5) and a 3.9-fold increase for lymphatic leukaemia (99.9% CI, 2.6–8.0) were found in association with more than five years' work in manufacturing units producing mainly styrene–butadiene rubber during 1940–60. Of the five other cancer sites investigated, only cancer of the stomach was associated with a significant, 2.2-fold increase in risk (99.9% CI, 1.4–4.3). [The Working Group noted that there was no attempt in this study to assess exposure to specific substances; thus, the relevance of the reported findings to the carcinogenicity of butadiene is unknown. A large number of unusually highly significant associations had been reported

Table 13. Occupational exposure limits and guidelines for butadiene[a]

Country	Year	Concentration (mg/m^3)	Interpretation[b]
Australia	1991	22 (C2)	TWA
Belgium	1991	22 (C2)	TWA
Czechoslovakia	1991	20	TWA
		40	Ceiling
Denmark	1993	22 (Ca)	TWA
Finland	1998	2.2	TWA
France	1993	36	TWA
Germany	1998	34 (C1)	TRK
		11	
Hungary	1993	10 (Ca)	STEL
The Netherlands	1996	46	TWA
The Philippines	1993	2200	TWA
Poland	1991	100	TWA
Russia	1991	100	STEL
Sweden	1991	20 (C3)	TWA
		40 (C3)	Ceiling
Switzerland	1991	11 (C)	TWA
Turkey	1993	2200	TWA
United Kingdom	1991	22	TWA
United States			
ACGIH (TLV)[c]	1997	4.4 (A2)	TWA
NIOSH (REL)	1997	(Ca-lfc)	
OSHA (PEL)	1996	2.2	TWA

[a] From International Labour Office (1991); United States Occupational Safety and Health Administration (1996) (OSHA); American Conference of Governmental Industrial Hygienists (1997a,b) (ACGIH); United States National Library of Medicine (1997b); Deutsche Forschungsgemeinschaft (1998); Ministry of Social Affairs and Health (1998)

[b] TWA, time-weighted average; STEL, short-term exposure limit; TRK, technical exposure limit; TLV, threshold limit value; REL, recommended exposure limit; PEL, permissible exposure limit; A2, suspected human carcinogen; C, suspected of being a carcinogen; C1, human carcinogen; C2, probable human carcinogen; C3, suspected of having a carcinogenic potential; Ca, potential occupational carcinogen; lfc, lowest feasible concentration

[c] Countries that follow the ACGIH recommendations for threshold limit values include: Bulgaria, Colombia, Jordan, Korea (Republic of), New Zealand, Singapore and Viet Nam

Table 14. Epidemiological results from the most informative occupational cohorts with exposure to butadiene

Reference	Country	Cohort size/ no. of deaths	Cancer site	Obs. deaths	SMR	95% CI	Comments
Divine & Hartman (1996)	United States	2795/1222	All Lymphohaematopoietic Leukaemia	282 42 13	0.9 1.5 1.1	0.8–1.0 1.1–2.0 0.6–1.9	31 lymphohaematopoietic cancers among those with potentially highest exposure (SMR, 1.7; 95% CI, 1.2–2.4); SMR decreased by duration of employment
Ward *et al*. (1995, 1996b)	United States	364/185	All Lymphosarcoma and reticulosarcoma Stomach cancer Leukaemia	48 4 5 2	1.1 5.8 2.4 1.2	0.8–1.4 1.6–14.8 0.8–5.7 0.2–4.4	All 4 lympho/reticulosarcomas had employment ≥ 2 years (SMR, 8.3; 95% CI, 1.6–14.8), as had the stomach cancers (SMR, 6.6; 95% CI, 2.1–15.3), all occurring in the rubber reserve plant
Delzell *et al*. (1996)	United States and Canada	15 649/3976	All Lymphosarcoma Other lymphopoietic Leukaemia	950 11 42 48	0.93 0.8 1.0 1.3	0.87–0.99 0.4–1.4 0.7–1.3 1.0–1.7	Among so-called 'ever hourly-paid' subjects, there were 45 leukaemia deaths (SMR, 1.4; 95% CI, 1.0–1.9); SMR for hourly subjects having worked for > 10 years and hired ≥ 20 years ago was 2.2 (95% CI, 1.5–3.2) based on 28 leukaemia deaths
Macaluso *et al*. (1996) (overlapping with Delzell *et al*., 1996)	United States and Canada	12 412/3271 exposed to butadiene[a]	Leukaemia deaths by cumulative ppm–years 0 < 1 1–19 20–79 ≥ 80	 8 4 12 16 18	 0.8 0.4 1.3 1.7 2.6	 [0.3–1.5] [0.4–1.1] [0.7–2.3] [1.0–2.7] [1.6–4.1]	Including 7 decedents for whom leukaemia was listed as contributory cause of death, Mantel–Haenszel rate ratios adjusted by race and cumulative exposure to styrene were 1.0, 2.0, 2.1, 2.4 and 4.5 for cumulative ppm–years, respectively

[a] Derived from Table 3 in the publication, 75% of the total cohort of 16 610 being exposed

between employment in different work sectors of this industry and different diseases, both neoplastic and non-neoplastic. The report did not indicate the numbers of subjects with cancers in different work areas and did not provide sufficient information to assess whether the computations of relative risks and confidence intervals were appropriate.]

The mortality in a cohort of workers who manufactured butadiene monomer in Texas, United States (Downs *et al*., 1987) has been continuously updated and the cohort has also been extended (Divine, 1990; Divine *et al*., 1993). The latest available update was published in 1996 (Divine & Hartman, 1996). The cohort then included 2795 male workers regularly employed for at least six months between 1942 and 1994. Exposure assessment was based on job history and industrial hygiene sampling. The number of workers lost to follow-up was 574 (20.5%), all but 28 (1%) of those were known to be alive as of the end of 1993. A total of 1222 deaths were identified through 1994, and death certificates were obtained for all but 20 of the deaths (1.6%). The standardized mortality ratio (SMR) for all causes of death was 0.88 (95% CI, 0.83–0.93) and that for all cancers (282 deaths) was 0.9 (95% CI, 0.8–1.0). There were 42 deaths from lymphohaematopoietic cancers (ICD-8, 200–209; SMR, 1.5; 95% CI, 1.1–2.0), nine observed deaths from lymphosarcoma and reticulosarcoma (ICD-8, 200; SMR, 1.9; 95% CI, 0.9–3.6), 13 observed deaths from leukaemia (ICD-8, 204–207; SMR, 1.1; 95% CI, 0.6–1.9) and 15 observed from cancer of other lymphatic tissues (ICD-8, 202, 203, 208; SMR, 1.5; 95% CI, 0.9–2.5). The SMRs for the lymphohaematopoietic cancers decreased with length of employment. Subcohort analyses were made for groups with background, low and varied exposure, based on industrial hygiene sampling. The background-exposure group included persons in offices, transportation, utilities and warehouse. 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. There were 11 deaths from lymphatic and haematopoietic cancers (ICD-8, 200–209) in the low-exposure group (SMR, 1.0; 95% CI, 0.5–1.8) and 31 in the varied-exposure group (SMR, 1.7; 95% CI, 1.2–2.4); in both groups, the SMR decreased with duration of employment. For lymphosarcoma and reticulosarcoma, there were two deaths (SMR, 1.1; 95% CI, 0.1–4.0) and seven deaths (SMR, 2.5; 95% CI, 1.0–5.1) in the low- and varied-exposure groups, respectively. For leukaemia, there were three cases (SMR, 0.7; 95% CI, 0.1–2.0) in the low-exposure subgroup and 11 cases in the varied-exposure group (SMR, 1.5; 95% CI, 0.8–2.8). Somewhat elevated SMRs were obtained in the low-exposure group also for cancer of the lung (46 cases, SMR, 1.2; 95% CI, 0.9–1.6) and kidney (6 cases; SMR, 2.1; 95% CI, 0.8–4.7). In the varied-exposure group, there were nine kidney cancers (SMR, 1.9; 95% CI, 0.9–3.7) and 18 prostate cancers (SMR, 1.2; 95% CI, 0.7–1.9), both sites with slightly but insignificantly increasing SMRs with duration of employment (> 10 years). The elevated risk for all the lymphohaematopoietic cancers and their subcategories occurred among persons who were first employed before 1950. As an adjunct to the SMR analyses, modelling was done using a qualitative cumulative exposure score as a time-dependent explanatory variable for all lymphohaematopoietic cancers (ICD-8, 200–209), lymphosarcoma (ICD-8, 200) lymphosarcoma and

other lymphoma (ICD-8, 200, 202), multiple myeloma (ICD-8, 203) and leukaemia (ICD-8, 204–207). None of these cancers was significantly associated with the cumulative exposure score and all risk estimates were close to unity.

A relatively small cohort mortality study included 364 men who were assigned to any of three butadiene production units located within several chemical plants in the Kanawha Valley of West Virginia, United States, including 277 men employed in a rubber reserve plant which operated during the Second World War and produced butadiene from ethanol or from olefin cracking (Ward *et al*., 1995, 1996b). The butadiene production units included in this study were selected from an index developed by the Union Carbide Corporation. Departments included in the study were those where butadiene was a primary product and neither benzene nor ethylene oxide was present. The cohort studied was part of a large cohort (with 29 139 individuals) of chemical workers whose mortality experience had been reported earlier, although without regard to particular exposures (Rinsky *et al*., 1988). Three subjects were lost to follow-up (0.8%). A total of 185 deaths were observed; the SMR for all causes of death was 0.9 in comparison with the general population of the United States. There were seven deaths from lymphatic and haematopoietic cancers (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 with the population of the United States as the reference and persisting in an analysis using county referent rates). The four cases all had duration of employment of two or more years (SMR, 8.3; $p < 0.05$). There were two cases of leukaemia (SMR, 1.2; 95% CI, 0.2–4.4). A non-significant excess of stomach cancer was observed in the overall cohort (5 cases; SMR, 2.4; 95% CI, 0.8–5.7). All five stomach cancer cases occurred among workers employed in the rubber reserve plant for two or more years (SMR, 6.6; 95% CI, 2.1–15.3).

Another relatively small retrospective mortality study, along with prospective morbidity and haematological analyses, was performed for male employees at the Shell Deer Park Manufacturing Complex in the United States (Cowles *et al*., 1994). There were 614 male employees who had worked in jobs with potential exposure to butadiene from 1948 to 1989. Eligible for the cohort were those who had worked for five years or more with potential exposure before 1948 and those who later had achieved five years of exposure or half of their employment duration with potential exposure. Follow-up of mortality was almost complete through 31 December 1989. Those lost to follow-up after 1983 were assumed to be alive. Out of the cohort, 438 were employed in 1982 or later and subject to follow-up also regarding morbidity for the period 1982–89. Industrial hygiene data from 1979 to 1992 showed that most butadiene exposures did not exceed 10 ppm [22 mg/m^3] as an 8-h time-weighted average (TWA), and most were below 1 ppm [2.2 mg/m^3], with an arithmetic mean of 3.5 ppm [7.7 mg/m^3]. Twenty-four deaths occurred during the mortality study period, which provided 7232 person–years of follow-up (average 15 years; range < 1 year to 42 years). For all causes of death, the SMR was 0.5 (95% CI, 0.3–0.7) and for all cancers 0.3 ($n = 4$; 95% CI, 0.1–0.9) by comparison with local (county) rates. Two deaths were

due to lung cancer (SMR, 0.4; 95% CI, 0.1–1.5) and none due to lympho-haematopoietic cancer (1.2 expected). Morbidity events of six days or more for the 438 butadiene employees were compared with the unexposed in the rest of the Shell Deer Park Manufacturing Complex. No cause of morbidity was in excess for this group; the all-cause standardized morbidity ratio was 0.85 (95% CI, 0.77–0.93) and that for all neoplasms was 0.5 (95% CI, 0.2–1.0). [The Working Group noted the relatively scanty information on the material and methods and the unusually low SMR for all causes in this study.]

Bond *et al.* (1992) reported a mortality study on workers engaged in the development and manufacture of styrene-based products, including styrene–butadiene latex production. The person-years of follow-up during 1970–86 for workers in this production were 11 754. By comparison with United States mortality rates, the SMR for all causes of death was 0.9, based on 82 deaths. There were 13 cancers in total (SMR, 0.6), with no site having an SMR exceeding unity. There was one death from haemato-lymphatic cancer (ICD-8, 200–209). [The Working Group noted the unusually low SMR for cancer and the limited information relating to butadiene.]

Delzell *et al.* (1996) and more recently also Sathiakumar *et al.* (1998) evaluated the mortality experience of 15 649 men employed for at least one year at any of eight styrene–butadiene rubber plants in the United States and Canada. Seven of these plants had previously been studied by Matanoski and Schwartz (1987), Matanoski *et al.* (1990a, 1993) and Santos-Burgoa *et al.* (1992), and a 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% of the subjects were exposed to butadiene and 83% were exposed to styrene. 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 United States or Ontario (SMR, 0.87; 95% CI, 0.85–0.90). Cancer mortality was slightly lower than expected, with 950 deaths (SMR, 0.93; 95% CI, 0.87–0.99). Eleven lymphosarcomas were observed (SMR, 0.8; 95% CI, 0.4–1.4) and 42 other lymphopoietic cancers (SMR, 1.0; 95% CI, 0.7–1.3). These other lymphopoietic cancers included 17 non-Hodgkin lymphomas, 8 Hodgkin's disease, 14 multiple myelomas, one polycythaemia vera and two myelofibrosis. There were slight increases for lymphosarcoma and these other lymphopoietic cancers in some cohort subgroups, but mortality by number of years worked and process group did not indicate any significant association with occupational exposures. There were 48 observed leukaemia deaths in the overall cohort (SMR, 1.3; 95% CI, 1.0–1.7) and among 'ever hourly-paid' subjects there were 45 deaths (SMR, 1.4; 95% CI, 1.0–1.9). The excess was concentrated among 'ever hourly-paid' subjects with 10 or more years of employment and 20 or more years since hire (28 deaths; SMR, 2.2; 95% CI, 1.5–3.2) and among subjects in polymerization (15 deaths; SMR, 2.5; 95% CI, 1.4–4.1), maintenance labour (13 deaths; SMR, 2.7; 95% CI, 1.4–4.5) and laboratories (10 deaths; SMR, 4.3; 95% CI, 2.1–7.9), which were three areas with potential for relatively high exposure to butadiene or styrene monomers.

Nested case–control studies within the United States and Canadian cohort study have been reported on earlier (Matanoski *et al.*, 1990b; Santos-Burgoa *et al.*, 1992). Macaluso *et al*. (1996) reported an additional analysis of leukaemia mortality 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 in the Delzell *et al*. analysis and another 2350 workers from plants other than styrene–butadiene rubber manufacturing were not included in Delzell *et al*.]. There were 418 846 person–years of follow-up through 1991 and 58 leukaemia deaths, seven of which were reported as contributory ('underlying') cause of death and included only in analyses using internal comparisons. Retrospective quantitative estimates of exposure to butadiene, styrene and benzene were developed and the estimation procedure entailed identifying work areas within each manufacturing process, historical changes in exposure potential and specific tasks involving exposure, and using mathematical models to calculate job- and time period-specific average exposures. The resulting estimates were linked with the subjects' work histories to obtain cumulative exposure estimates, which were employed in stratified and Poisson regression analyses of mortality rates. Mantel–Haenszel rate ratios adjusted by race, age and cumulative styrene exposure increased with cumulative butadiene exposure from 1.0 in the unexposed category through 2.0, 2.1, 2.4 to 4.5 in the exposure categories < 1, 1–19, 20–79 and ≥ 80 ppm–years, respectively (p for trend = 0.01). The trend of increasing risk with butadiene exposure was still present after exclusion of the unexposed category ($p = 0.03$). The risk pattern was less clear and nonsignificant for styrene exposure (rate ratios, 0.9, 5.4, 3.4 and 2.7 in the exposure categories < 5, 5–9, 10–39 and ≥ 40 ppm–years, respectively; p for trend = 0.14) and the association with benzene was nil after controlling for exposure to butadiene and styrene exposure. Irons and Pyatt (1998) suggested that dithiocarbamates, which were used between the early 1950s and 1965 as stopping agents in the cold polymerization reaction for styrene–butadiene rubber production, might interact with butadiene in causing leukaemia in exposed workers. [The Working Group noted that there is no evidence that dithiocarbamates cause leukaemia and that such an interaction, if demonstrated, would not exclude a contribution of butadiene to the carcinogenic process.]

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 to butadiene (minimum purity, > 98.9%) at concentrations of 625 or 1250 ppm [1380 or 2760 mg/m^3] by whole-body inhalation for 6 h per day on five 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. 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, 14/50 low-dose and 30/50 high-dose. As shown in Table 15, butadiene produced haemangiosarcomas originating in the heart with metastases to various organs. The incidence of haemangiosarcomas of the heart in historical controls was 1/2372 in males and 1/2443 in females. Other types of neoplasm for which the incidences were 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 (United States National Toxicology Program, 1984; Huff *et al.*, 1985).

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 five 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 in B6C3F$_1$ mice was 1/60 control, 10/48 exposed for 12 weeks and 34/60 exposed for 52 weeks and, in NIH Swiss mice, 8/57 exposed for 52 weeks. Haemangiosarcomas of the heart were observed in 5/60 B6C3F$_1$ mice and 1/57 NIH Swiss mice (Irons *et al.*, 1989). [The Working Group noted the absence of reporting on NIH Swiss control mice.]

Table 15. Incidences of tumours in B6C3F$_1$ mice exposed to butadiene by inhalation for 61 weeks

	Male			Female		
	0	625 ppm	1250 ppm	0	625 ppm	1250 ppm
Haemangiosarcoma of heart (with metastases)	0/50	16/49 ($p < 0.001$)	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 United States National Toxicology Program (1984); Huff *et al.* (1985)

Groups of 70–90 male and 70–90 female B6C3F$_1$ mice, 6.5 weeks of age, were exposed to butadiene (purity, > 99%) at concentrations of 0, 6.25, 20, 62.5, 200 or 625 ppm [0, 14, 44, 138, 440 or 1380 mg/m^3] for 6 h per day on five days per week for up to two years. Ten 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 at 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 controls, 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 16, exposure to butadiene produced increases in the incidences in both sexes 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 incidences of mammary gland adenocarcinomas and benign and malignant ovarian granulosa-cell tumours were increased in females (Melnick *et al*., 1990).

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 five days per week at 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. After the exposures were terminated, the animals were placed in control chambers for up to 104 weeks. A group of 70 males served as chamber controls (0 ppm). Survival was reduced 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 17, exposure to butadiene produced increases in the incidence of lymphoma, heart haemangiosarcomas, lung alveolar/bronchiolar adenomas and carcinomas, forestomach papillomas and carcinomas, Harderian gland adenomas and adenocarcinomas, preputial gland carcinomas and kidney tubular adenomas (Melnick *et al*., 1990). [The Working Group noted that this study has also been reported by the United States National Toxicology Program (1992) with additional data analyses.]

Groups of 60 male and 60 female B6C3F$_1$ mice, 8–10 weeks old, were exposed to butadiene [purity unspecified] by whole-body inhalation for a single 2-h period at concentrations of 0, 1000, 5000 or 10 000 ppm [0, 2200, 11 000 or 22 000 mg/m^3]. The mice were then held for two years, at which time all survivors were killed and tissues and organs examined histopathologically. Survival, weight gains and tumour incidences of exposed mice were not affected by butadiene exposure (survival: males—28/60 control, 34/60 low-dose, 44/60 mid-dose, 34/60 high-dose; females—45/60, 36/60, 38/60, 45/60) (Bucher *et al*., 1993). [The Working Group noted the single short duration of exposure.]

3.1.2 *Rat*

Groups of 100 male and 100 female Sprague-Dawley rats, five weeks of age, were exposed to butadiene (minimal purity, 99.2%) by whole-body inhalation at concentrations of 0, 1000 or 8000 ppm [0, 2200 or 17 600 mg/m^3] for 6 h per day on five days

Table 16. Tumour incidences (I) and percentage mortality-adjusted tumour rates (R) in mice exposed to butadiene for up to two years

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

From Melnick *et al*. (1990)

[a] Increased compared with chamber controls (0 ppm), $p < 0.05$, based on logistic regression analysis

Table 17. Tumour incidences (I) and percentage mortality-adjusted tumour rates (R) in male mice exposed to butadiene in stop-exposure studies. After exposures were terminated, animals were placed in control chambers until the end of the study at 104 weeks.

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

From Melnick *et al*. (1990)

[a] Increased compared with chamber controls (0 ppm), $p < 0.05$, based on logistic regression analysis

per week for 111 weeks (males) or 105 weeks (females). Survival was reduced in low- and high-dose females and in high-dose males; 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 significantly increased incidence in males were pancreatic exocrine adenomas and carcinomas (3 control, 1 low-dose, 10 ($p < 0.05$) high-dose) and interstitial-cell tumours of the testis (0 control, 3 low-dose, 8 ($p < 0.01$) high-dose). Those that occurred at significantly increased incidence (Fisher's exact test) in females were follicular-cell adenomas and carcinomas of the thyroid gland (0 control, 4 low-dose, 11 ($p < 0.001$) high-dose) with a significant, dose-related trend ($p < 0.001$). Tumours that occurred with positive trends (Cochran–Armitage trend test) only in females were sarcomas of the uterus ($p < 0.05$; 1 control, 4 low-dose, 5 high-dose), carcinomas of the Zymbal gland ($p < 0.01$; 0 control, 0 low-dose, 4 high-dose), and benign and malignant mammary tumours ($p \leq 0.001$; 50 control, 79 low-dose and 81 high-dose). Mammary adenocarcinomas were found in 18 control, 15 low-dose and 26 high-dose rats (Owen *et al*., 1987). [The Working Group noted that differences in tumour incidence between groups were not analysed using statistical methods that took into account differences in mortality between control and treated groups.]

3.2 Carcinogenicity of metabolites

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

A group of 30 male Swiss mice was treated with undiluted epoxybutene, the initial monoepoxide metabolite of butadiene, by skin application at a dose of 100 mg three times per week for life. The median survival time was 237 days and four skin tumours were observed (Van Duuren *et al*., 1963). [The Working Group noted that this incidence was similar to that in control groups that were either administered solvents or left untreated.]

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

D,L-Diepoxybutane and *meso*-diepoxybutane induced skin papillomas and squamous-cell carcinomas when applied to the skin of female Swiss mice at a dose of approximately 3 or 10 mg in 100 mg acetone three times per week for life (Van Duuren *et al*., 1963, 1965). Subcutaneous injection of 0.1 mg D,L-diepoxybutane in 0.05 mL tricaprylin once per week for more than one year induced local fibrosarcomas in female Swiss mice; no tumour was observed in three solvent-treated control groups. Similar findings were seen in female Sprague-Dawley rats (Van Duuren *et al*., 1966).

L-Diepoxybutane was administered by intraperitoneal injection (12 injections thrice weekly) to male and female strain A mice at total doses ranging from 1.7 to 192 mg/kg bw in water or tricaprylin. It increased the incidence and multiplicity of lung tumours (Shimkin *et al*., 1966).

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

The toxicokinetics and toxicology of 1,3-butadiene have been reviewed recently (ECETOC, 1997; Himmelstein *et al.*, 1997).

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No measured data are available on butadiene in exposed humans.

Metabolites

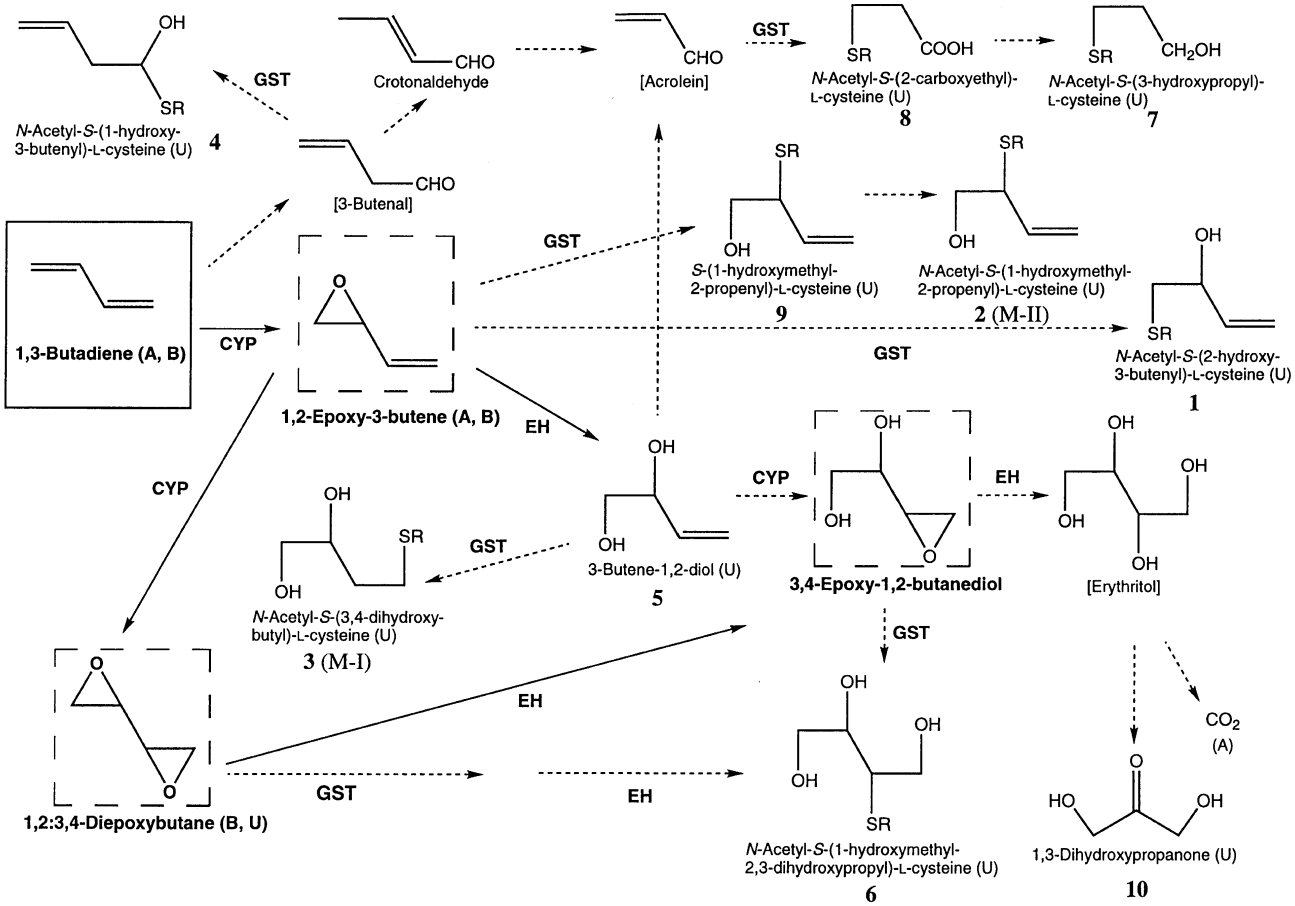
The currently known metabolic pathways of butadiene in man, cynomolgus monkeys, rats and mice are presented in Figure 1.

In seven employees working in production areas with atmospheric concentrations of 3–4 ppm [6.6–8.8 mg/m^3] butadiene over the previous six months, Bechtold *et al.* (1994) detected urinary excretion of the metabolite *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine (M-I, no. 3 in Figure 1) (3.2 ± 1.6 μg/mL) but not of *N*-acetyl-*S*-(1-hydroxymethyl-2-propenyl)-L-cysteine (M-II, no. 2 in Figure 1). In 10 unexposed employees and nine outside controls, urinary M-I concentrations were 0.63 ± 0.19 and 0.32 ± 0.07 μg/mL. M-I was assumed to result from the conjugation of glutathione (GSH) with 3-butene-1,2-diol (butenediol) and M-II from conjugation of GSH with 1,2-epoxy-3-butene (epoxybutene). From the absence of M-II in human urine, it was concluded that epoxybutene is metabolically eliminated in humans predominantly by epoxide hydrolase and not by direct GSH conjugation. Hallberg *et al.* (1997) found the concentration of M-I in urine samples of 24 workers exposed to 2.4 ± 1.8 ppm [5.3 ± 4.0 mg/m^3] butadiene (time-weighted average) to be 2.4 ± 1.9 μg/mL. In 19 controls (butadiene exposure below detection limit of 0.3 ppm [0.66 mg/m^3]), urinary M-I concentrations of 0.69 ± 0.37 μg/mL were measured. In both groups there was no significant difference between smokers and non-cigarette smokers.

Haemoglobin adducts

N-(2-Hydroxy-3-butenyl)valine (HOBVal) as a reaction product of epoxybutene with N-terminal valine in haemoglobin has been found in workers exposed to butadiene. Osterman-Golkar *et al*. (1993) recorded adduct levels of 1.1–2.6 pmol HOBVal/g globin in four nonsmoking workers exposed to about 1 ppm [2.2 mg/m^3] butadiene as estimated from exposure measurements made three to nine months earlier. A haemoglobin binding index of 0.004 pmol HOBVal/(g globin per ppm.h) was estimated from these preliminary results. In nonsmoking workers exposed outside the production area to an environmental butadiene concentration of about 0.03 ppm [0.07 mg/m^3], the adduct levels were below the detection limit of 0.5 pmol HOBVal/g globin. Based upon data from a more recent

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



A, B, U: metabolites in exhaled air, blood, urine, respectively; CYP, cytochrome P450; GST, glutathione-*S*-transferase; EH, epoxide hydrolase; dashed frame: metabolites forming DNA or haemoglobin adducts; [ ]: proposed metabolites not yet detected**;** dashed lines, assumed pathways; number assignment according to Nauhaus *et al.* (1996)

study (Osterman-Golkar *et al.*, 1996; Sorsa *et al.*, 1996), an even lower binding index of 0.0005 pmol/(g globin per ppm.h) was calculated (Osterman-Golkar & Bond, 1996).

After improving the method to reduce the detection limit to ~0.03–0.05 pmol HOBVal/g globin, Osterman-Golkar *et al*. (1996) measured adduct levels in controls, either five nonsmokers (≤ 0.05 pmol HOBVal/g globin) or four smokers (0.04–0.13 pmol HOBVal/g globin). Similar values were found in laboratory and maintenance workers (≤ 0.06 and ≤ 0.07 pmol HOBVal/g globin in four nonsmokers and three smokers, respectively) exposed to 0.6 ± 0.9 mg/m^3 butadiene. In plant workers exposed to 11.2 ± 18.6 mg/m^3 butadiene, adduct levels were higher (0.2–0.32 and 0.02–0.24 pmol HOBVal/g in three nonsmokers and seven smokers, respectively) [these values were read from a graph]. The mean adduct level given for all 10 workers was 0.16 ± 0.099 pmol HOBVal/g. The authors calculated the amount of butadiene inhaled from the mainstream smoke of 30 cigarettes per day to be equal to that inhaled during an 8-h exposure to 0.1 ppm [0.22 mg/m^3] butadiene. In another plant, measurements of HOBVal were made at two time points (Sorsa *et al*., 1996). In the first investigation, butadiene concentrations at the workplace were > 3 ppm [6.6 mg/m^3], and in the second < 3 ppm. The mean adduct levels were 2 ± 3.6 (n = 12) and 0.54 ± 0.33 pmol HOBVal/g globin (n = 4), respectively. In controls, the mean levels were 0.13 ± 0.35 (n =14) and 0.12 ± 0.05 pmol HOBVal/g globin (n = 8), respectively.

van Sittert and van Vliet (1994) were unable to detect haemoglobin adducts in workers exposed in butadiene manufacture or in smokers.

Pérez *et al*. (1997) found two stereoisomers of *N*-(2,3,4-trihydroxybutyl)valine (THBVal) in haemoglobin resulting from the reaction of 3,4-epoxy-1,2-butanediol (epoxybutanediol) with the N-terminal valine. Theoretically, epoxybutanediol can be formed by oxidation of dihydroxybutene and/or hydrolysis of diepoxybutane. THBVal could also form by direct binding of diepoxybutane to haemoglobin with subsequent hydrolysis of the second epoxide ring. In two workers exposed to a median concentration of 1 ppm butadiene (see Osterman-Golkar *et al*., 1996), the levels of THBVal for one of these isomers were 10 and 14 pmol/g globin, whereas in two control workers the corresponding adduct levels were 1.8 and 3.3 pmol/g globin. These THBVal values were 70-fold higher than corresponding values of HOBVal in the same subjects.

4.1.2 *Experimental systems*

Butadiene

Male Sprague-Dawley rats (Bolt *et al*., 1984) and B6C3F$_1$ mice (Kreiling *et al*., 1986a) were exposed in closed chambers to initial butadiene concentrations in the atmosphere ranging from about 100 to 12 000 ppm [220–26 500 mg/m^3] (rats) or to 5000 ppm [11 000 mg/m^3] (mice) or were treated by intraperitoneal injection of about 1 μL butadiene gas/g bw (rats). The resulting concentration–time courses in the chamber atmosphere revealed linear kinetics below 1000 ppm [2200 mg/m^3] and saturation of metabolism above 2000 ppm [4400 mg/m^3], with maximum rates (μmol/h/kg bw) of 220 in rats and 400 in mice. In the linear range, rates of metabolism per kg body weight were 1.6-fold higher in

mice than in rats. The whole body : air concentration ratio of butadiene at steady state was 0.5 in rats and 1 in mice. Due to metabolic elimination, these values were below the thermodynamic whole body:air partition coefficient, which was determined to be 2.7 in mice and 2.3 in rats. Following induction of metabolizing enzymes by pretreatment of rats with Aroclor 1254, no saturation was observed within the exposure range studied. From these data it was concluded that the rate of butadiene metabolism in the linear range was limited by the uptake from the gas phase into the organism. Metabolism in both species was inhibited effectively by pretreatment with diethyldithiocarbamate. Medinsky *et al*. (1994) also exposed male B6C3F$_1$ mice and Sprague-Dawley rats to butadiene in closed chambers at initial concentrations of up to 5000 ppm. In animals pretreated with pyrazole (32 mg/kg bw) and exposed to initial concentrations of 1200 ppm [2650 mg/m^3] butadiene, metabolism was inhibited completely in rats and the V_{max} was reduced by 87% in mice. Using a dynamic chamber, Leavens *et al*. (1996a) determined the rate of butadiene metabolism in male B6C3F$_1$ mice from the uptake during steady-state exposures (8 h) to 100 or 1000 ppm [220 or 2200 mg/m^3] butadiene. Their value of 246 ± 19 μmol/h/kg bw during exposure to 1000 ppm was close to that of about 270 μmol/h/kg bw given by Kreiling *et al*. (1986) for the same exposure concentration. Bond *et al*. (1986) determined the retention of [1-^{14}C]-butadiene in male B6C3F$_1$ mice and Sprague-Dawley rats exposed via the nose only for 6 h to various concentrations of butadiene. The percentage of ^{14}C retained decreased from 16–20% at 0.14–13 mg/m^3 to 4% at 1800 mg/m^3 in mice and from 17% at 0.14 mg/m^3 to 2.5% at 1800 mg/m^3 in rats, indicating saturation of metabolic elimination. Within the exposure range up to 1800 mg/m^3, the inhaled doses were on average 1.8-fold higher in mice than in rats, when normalized to body surface area. Nose-only exposures (2 h) of male cynomolgus monkeys to [1-^{14}C]butadiene gave much lower retention of butadiene (2.9% at 10.1 ppm [18 mg/m^3], 1.5% at 310 ppm [560 mg/m^3] and 1.7% at 7760 ppm [14 000 mg/m^3]) than in mice and rats. As determined by vacuum-line cryogenic distillation of radioactive compounds, blood concentrations of butadiene in monkeys reached 0.009 μmol/L at 10.1 ppm, 0.6 μmol/L at 310 ppm and 32 μmol/L at 7760 ppm. The resulting blood/air concentration ratios were 0.03, 0.06 and 0.12, respectively, the increase reflecting saturation of butadiene metabolism (Dahl *et al*., 1991). Using headspace gas chromatography, Himmelstein *et al*. (1994) measured the blood concentration of butadiene in male B6C3F$_1$ mice and Sprague-Dawley rats during nose-only exposure (6 h) to butadiene. A steady state was reached in blood after 2 h, giving butadiene concentrations (μmol/L) of 2.4, 37, 58 in mice and 1.3, 18, 37 in rats at 62.5, 625 and 1250 ppm [138, 1380 and 2760 mg/m^3], respectively. These values indicate nearly linear relationships between butadiene concentrations in blood and air, with blood concentrations in mice being about twice those in rats. Blood concentrations declined within minutes after exposure ceased.

Since GSH conjugation is an important pathway in butadiene metabolism, several laboratories have investigated the GSH-depleting effect of butadiene.

Deutschmann and Laib (1989) determined the non-protein sulfhydryl (NPSH) content in lung, liver and heart of male B6C3F$_1$ mice and Sprague-Dawley rats exposed for 7 h to constant butadiene concentrations between 10 and 2000 ppm [22–4400 mg/m^3]. In rats,

hepatic NPSH (% of control) was depleted to 70–80% at 250–1000 ppm and to 40% at 2000 ppm. An NPSH reduction in rat lung to about 80% and 70% was observed only at 1000 and 2000 ppm, respectively, whereas NPSH in rat heart did not change. In mice, hepatic NPSH content began to decrease significantly (70%) at 250 ppm butadiene, and fell to 40% at 1000 ppm and 20% at 2000 ppm. In mouse lung, marked depletion of NPSH (about 50%) occurred at 500 ppm and reached about 10% at 2000 ppm. NPSH content in the heart was reduced to about 75% at 1000 ppm and 30% at 2000 ppm. At conditions of maximum rate of butadiene metabolism (exposure concentration, > 2000 ppm), Kreiling *et al*. (1988) and Laib *et al*. (1990) observed depletion of control levels of hepatic NPSH content in male B6C3F_1 mice to 20% and 4% after 7 h and 15 h, respectively, of butadiene exposure. In contrast, hepatic NPSH content in male Wistar and Sprague-Dawley rats decreased to about 65% and 80%, respectively, after 7 h of exposure; no major change occurred after 15 h.

Following 6 h of exposure to 1250 ppm [2760 mg/m^3] butadiene, Himmelstein *et al*. (1995) found hepatic NPSH to decrease to 57 ± 18% and 62 ± 3% in male B6C3F_1 mice and Sprague-Dawley rats, respectively. In rats exposed to 8000 ppm [17 700 mg/m^3], no further depletion occurred. In lungs of mice, 65% depletion of NPSH was already observed at 62.5 ppm [138 mg/m^3] butadiene, the maximum reduction to 26 ± 13% being reached at 1250 ppm. In rat lung, NPSH was significantly depleted (74 ± 5%) only at 1250 ppm, with a similar value at 8000 ppm.

In male Wistar rats exposed for two weeks (6 h per day, five days per week) to butadiene, urinary mercapturic acids resulting from the conjugation of epoxybutene with GSH were qualitatively analysed by gas chromatography/mass spectrometry after deacetylation as heptafluorobutanoic anhydride derivatives of the cysteine conjugates. The major product formed was assumed to be *S*-(2-hydroxy-3-butenyl)-L-cysteine. Quantitation of the cysteine conjugates as phthaldialdehyde derivatives by high-performance liquid chromatography revealed a nearly linear relationsphip between the amount of cysteine conjugates in afternoon samples [sampling period not given] and the exposure concentration, with a maximum value of about 16 μmol at the highest exposure concentration of 1000 ppm butadiene [2200 mg/m^3] (Osterman-Golkar *et al*., 1991).

Nose-only exposures of B6C3F_1 mice, Sprague-Dawley and Fischer 344/N rats, Syrian hamsters [sexes not specified] to 7600 ppm [14 150 mg/m^3] and male cynomolgus monkeys over 2 h to 8000 ppm [17 700 mg/m^3] [1-^{14}C]butadiene (Sabourin *et al*., 1992) and of male B6C3F_1 mice and Fischer 344/NtacfBR rats over 4 h to 11.7 ppm [26 mg/m^3] butadiene (Bechtold *et al*., 1994) resulted in urinary excretion of two major metabolites identified as *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine (M-I) and *N*-acetyl-*S*-(1-hydroxymethyl-2-propenyl)-L-cysteine (M-II). The ratio of M-I to M-I + M-II was 0.2 in mice, 0.3–0.5 in rats, about 0.4 in hamsters and about 0.9 in monkeys, compared with a value of nearly 1 in humans (see Section 4.1.1). This ratio was positively correlated with the epoxide hydrolase activity in the livers of the different species, suggesting that in these species, as in human metabolism (see Section 4.1.1), hydrolysis of epoxybutene to butenediol precedes the formation of M-I and that M-II is the mercapturate formed from the conjugate of GSH with epoxybutene.

Nauhaus *et al*. (1996) analysed butadiene metabolites in urine of male B6C3F$_1$ mice and Sprague-Dawley rats exposed via the nose only for up to 5 h to 800 ppm [1770 mg/m^3] [1,2,3,4-^{13}C]butadiene. The metabolites identified and their relative quantities are listed in Table 18. Metabolites 1, 2 and 9, derived from epoxybutene via the glutathione pathway, amounted to 70% in mice and 61% in rats, whereas the hydrolytic product butenediol (5) reached only 2.9% in mice and 5% in rats. Metabolite 3, formed by conjugation of butenediol with glutathione, was found in three- to four-fold higher amounts in rats than in mice. Metabolite 6, assumed to be derived from diepoxybutane, was found in small amounts only in mice, as was metabolite 4, which was attributed to the hemithioacetal product of 3-butenal. [Metabolite 6 might also be formed via the oxidation of butenediol to epoxybutanediol.] Metabolites 7 and 8, present only in mouse urine, could not be attributed to a single pathway (via metabolite 3), but the involvement of conversion of butadiene to acrolein has been speculated, too. Rats but not mice excreted 1,3-dihydroxypropanone (10) in urine, probably generated from the postulated erythritol via the pentose phosphate pathway. [The authors assumed erythritol to be derived from diepoxybutane. It might, however, also be formed via the oxidation of butenediol to epoxybutanediol.]

Interaction between butadiene, styrene and benzene

Like butadiene, styrene is metabolized in a first step by cytochrome P450-dependent monooxygenases (Nakajima *et al*., 1994). Co-exposure could therefore lead to mutual influences on the rates of metabolism. Laib *et al*. (1992) co-exposed male Sprague-Dawley rats to butadiene (20, 100, 500, 1000, 3000, 6000 ppm [44, 220, 1100, 2200, 6600, 13 300 mg/m^3] and styrene (0, 20, 100, 250, 500 ppm [0, 85, 430, 1070, 2130 mg/m^3]). Analysing the measured data by means of a toxicokinetic two-compartment model (Filser, 1992), biotransformation rates of both compounds were determined as functions of the exposure concentrations. Whereas butadiene did not affect the metabolic rate of styrene, competitive inhibition of butadiene metabolism by styrene occurred up to a styrene concentration of 90 ppm [380 mg/m^3]. Higher styrene concentrations resulted in only a small additional inhibition. These findings led to the hypothesis that butadiene is metabolized by at least two different cytochrome 450-dependent monooxygenases, only one of which is inhibited by styrene. The presence of several butadiene-metabolizing monooxygenases was later verified by studies *in vitro* (Csanády *et al*., 1992; Duescher & Elfarra, 1994). The lack of inhibition of styrene metabolism by butadiene was attributed to the higher enrichment of inhaled styrene in the body compared to that of inhaled butadiene. Using the data of Laib *et al*. (1992), a physiological toxicokinetic model was developed in order to predict interactions between butadiene and styrene in humans (Filser *et al*., 1993). For low exposure by inhalation to both compounds, biotransformation appeared to be limited by transport to the metabolizing enzymes. Inhibition of butadiene metabolism by styrene in co-exposed people was predicted to occur.

Bond *et al*. (1994) simulated interactions of butadiene with styrene or with benzene in rats using their own physiological toxicokinetic model for butadiene and published

Table 18. Butadiene metabolites in urine of mice and rats exposed to 800 ppm [1770 mg/m^3] [1,2,3,4-^{13}C]butadiene

Metabolite	Percentage of total metabolites	
	Mouse	Rat
1. *N*-Acetyl-*S*-(2-hydroxy-3-butenyl)-L-cysteine	43.9	8.0
2. *N*-Acetyl-*S*-(1-hydroxymethyl-2-propenyl)-L-cysteine	21.6	52.8
3. *N*-Acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine	7.1	26.4
4. *N*-Acetyl-*S*-(1-hydroxy-3-butenyl)-L-cysteine	3.7	Not detected
5. 3-Butene-1,2-diol	2.9	5.0
6. *N*-Acetyl-*S*-(1-hydroxymethyl-2,3-dihydroxypropyl)-L-cysteine	4.6	Not detected
7. *N*-Acetyl-*S*-(3-hydroxypropyl)-L-cysteine	5.4	Not detected
8. *N*-Acetyl-*S*-(2-carboxyethyl)-L-cysteine	4.8	Not detected
9. *S*-(1-Hydroxymethyl-2-propenyl)-L-cysteine	4.7	Not detected
10. 1,3-Dihydroxypropanone	Not detected	5.3

Metabolite numbers correspond to those in Figure 1.

models for styrene (Ramsey & Andersen, 1984) and benzene (Medinsky *et al*., 1989), assuming competitive mutual inhibition of the metabolism of butadiene and styrene and of butadiene and benzene. Whereas the metabolism of butadiene was predicted to be reduced by co-exposure to styrene or benzene, no effect of butadiene on the metabolism of styrene and of benzene was predicted. This was explained by the low solubility of butadiene compared with styrene and benzene.

Leavens *et al.* (1996a, 1997) and Leavens and Bond (1996) further explored the metabolic interactions between butadiene and styrene in male B6C3F$_1$ mice exposed to mixtures of butadiene and styrene by inhalation. At steady state, significant inhibition of butadiene metabolism by styrene was observed with mixtures of 1000 ppm butadiene and 250 ppm styrene, but not with 100 ppm butadiene and 250 ppm styrene. Inhibition by butadiene of styrene metabolism was evidenced by the significant increase in styrene blood concentrations (42% above that in mice exposed to styrene only) in the exposure to 1000 ppm butadiene and 250 ppm styrene. These authors concluded that while exposure to mixtures of styrene and butadiene results in inhibition of metabolism of both styrene and butadiene, interactive effects are seen only at high concentrations that are of little relevance to human exposure.

In order to analyse these observations, Leavens and Bond (1996) developed a physiological toxicokinetic model based on the model of Medinsky *et al*. (1994) for butadiene and the model of Csanády *et al*. (1994) for styrene. As previously found by Laib *et al*. (1992), a reasonable model prediction of the reduced butadiene uptake was obtained only by including two oxidation pathways for both butadiene and styrene, one

catalysed by the same CYP isoenzyme with competitive interaction and another by separate CYP isoenzymes without interaction between the two compounds.

Metabolites in vitro

As in humans, two important metabolites of butadiene are epoxybutene and diepoxybutane (Figure 1).

The half-life of the spontaneous hydration of epoxybutene in water (pH 7) has been calculated using rate constants given in Ross *et al*. (1982) to be 13.7 h and that of diepoxybutane, using rate constants given in Ehrenberg and Hussain (1981), to be 100 h (Gervasi *et al*., 1985).

Epoxybutene is the main first product of the NADPH-dependent metabolism of butadiene in postmitochondrial liver and lung fractions of mouse, rat, monkey and man (Schmidt & Loeser, 1985) and more specifically in the microsomal fraction of mouse liver (Wistuba *et al*., 1989; Elfarra *et al*., 1991; Csanády *et al*., 1992; Duescher & Elfarra, 1992; Recio *et al*., 1992; Sharer *et al*., 1992; Maniglier-Poulet *et al*., 1995), of mouse lung (Csanády *et al*., 1992; Sharer *et al*., 1992), of mouse kidney and testis (Sharer *et al*., 1992), of rat liver (Malvoisin *et al*., 1979; Bolt *et al*., 1983; Wistuba *et al*., 1989; Csanády *et al*., 1992; Cheng & Ruth, 1993; Maniglier-Poulet *et al*., 1995), of rat lung (Csanády *et al*., 1992; Sharer *et al*., 1992), of rat kidney and testis (Sharer *et al*., 1992), of human liver (Csanády *et al*., 1992; Duescher & Elfarra, 1994) and of human lung (Csanády *et al*., 1992). From the correlations with the activity of human liver microsomes to the specific substrates chlorzoxazone (Csanády *et al*., 1992; Duescher & Elfarra, 1994) and coumarin (Duescher & Elfarra, 1994), CYP2E1 and CYP2A6 were concluded to be the major isoenzymes catalysing the oxidation of butadiene, CYP2E1 at low and CYP2A6 at high butadiene concentrations. This was confirmed using microsomal preparations from six human B-lymphoblastoid cell lines, each expressing a particular human cDNA encoding specific CYP isoenzymes (Duescher & Elfarra, 1994).

The rate of butadiene metabolism in diverse cell fractions has been investigated in various species and is characterized by the Michaelis–Menten parameters V_{max} and apparent K_m (K_{mapp}). Three methods have been used to determine these parameters:

(i) loss of butadiene in the headspace of a closed vial due to metabolism in the incubate; analysis using a two-compartment model (Filser *et al.*, 1992);

(ii) formation of epoxybutene, measured in the headspace of a closed vial; analysis using a two-compartment model taking into account the further metabolism of epoxybutene (Csanády *et al*., 1992; Recio *et al*., 1992).

(iii) formation of epoxybutene, measured in the incubate without consideration of hydrolysis or vaporization (Malvoisin *et al*., 1979; Elfarra *et al*., 1991; Sharer *et al*., 1992; Cheng & Ruth, 1993; Duescher & Elfarra, 1994; Maniglier-Poulet *et al*., 1995).

The differences in methodology complicate the direct comparison of the results. For physiological toxicokinetic modelling, the data obtained by the first two methods were used.

Tables 19, 20 and 21 present the V_{max}/K_{mapp} values which were used in physiological toxicokinetic models developed for the formation and degradation of epoxybutene and diepoxybutane, all of which were obtained from in-vitro measurements. Although these parameters were obtained in different laboratories, the similarity of the data is striking. Most interestingly, in liver microsomes of NMRI mice, CYP-dependent monooxygenase-mediated oxidation of butadiene was about 10 times lower than in liver microsomes from $B6C3F_1$ mice. However, oxidative metabolism of inhaled butadiene was accurately predicted for conditions *in vivo* using both values. This can be explained by the fact that, over a broad concentration range, the first step in the metabolism of inhaled butadiene is not limited by enzymic capacity but by uptake into the blood and transport through the metabolizing organs (Filser *et al*., 1993). Furthermore, only part of the metabolized inhaled butadiene is systemically available as epoxybutene (Filser & Bolt, 1984; Johanson & Filser, 1993; Csanády *et al*., 1996; Sweeney *et al*., 1997).

Molecular modelling of butadiene oxidation by CYP2E1 has indicated that species differences in the kinetic parameters might be explained by a non-conservative change from Thr-437 to His-437 between rodents and humans and by a conservative change from Ile-438 to Val-438 (Lewis *et al*., 1997).

Table 19. V_{max}/K_{mapp} values of the NADPH-dependent oxidation of butadiene and epoxybutene, as determined in cell fractions and used for physiological toxicokinetic modelling

	V_{max}/K_{mapp} (nmol.L/mg protein/min/mmol)	Reference
Oxidation of butadiene to epoxybutene		
NMRI mouse, liver microsomes	134	Filser *et al*. (1992)
Sprague-Dawley rat, liver microsomes	62	
Human (n = 1), liver microsomes	111	
$B6C3F_1$ mouse		Csanády *et al*. (1992)
Liver microsomes	1295	
Lung microsomes	461	
Sprague-Dawley rat		
Liver microsomes	157	
Lung microsomes	21	
Human		
Liver microsomes (n = 12)	230	
Lung microsomes (n = 5)	75	
Oxidation of epoxybutene to diepoxybutane		
$B6C3F_1$ mouse, liver microsomes	12.8	Csanády *et al*. (1992)
$B6C3F_1$ mouse, liver microsomes	9.2	Seaton *et al*. (1995)
Sprague-Dawley rat, liver microsomes	2.8	
Human (n = 4), liver microsomes	0.15–3.8	

Table 20. V_{max}/K_{mapp} values of the epoxide hydrolase and glutathione *S*-transferase catalysed epoxybutene metabolism, as determined in cell fractions

	V_{max}/K_{mapp} (nmol.L/mg protein/min/mmol)	Reference
Epoxide hydrolase		
NMRI mouse, liver microsomes	13	Kreuzer *et al*. (1991)
Sprague-Dawley rat, liver microsomes	24	
Human (n = 1), liver microsomes	28	
B6C3F$_1$ mouse, liver microsomes	3.6	Csanády *et al*. (1992)
Sprague-Dawley rat, liver microsomes	9.5	
Human (n = 3), liver microsomes	32–38	
Glutathione *S*-transferase		
NMRI mouse, liver cytosol	15	Kreuzer *et al*. (1991)
Sprague-Dawley rat, liver cytosol	11	
Human (n = 1), liver cytosol	8	
B6C3F$_1$ mouse		Csanády *et al*. (1992)
Liver cytosol	14	
Lung cytosol	7.5	
Sprague-Dawley rat		
Liver cytosol	17	
Lung cytosol	2.5	
Human (n = 2), Liver cytosol	4.3	

Liver microsomes from male Sprague-Dawley rats convert butadiene into the *R*- and *S*-enantiomers of epoxybutene (Bolt *et al*., 1983). The ratios of *R*- to *S*-epoxybutene in butadiene-exposed liver microsomes [concentration not specified] were 1 and 1.6 (phenobarbital treatment) in mice [strain not specified], 0.33 and 0.43 (phenobarbital treatment) in rats [strain not specified] and 1.08–1.27 (n = 4) in humans (Wistuba *et al.*, 1989). Exposure of liver microsomes from male Sprague-Dawley rats to 25 000 ppm [55 300 mg/m^3] butadiene gave ratios of *R*- to *S*-epoxybutene that varied with incubation time from about 0.3 at 5 min to about 1 at 30 min (Nieusma *et al.*, 1997). A nearly constant value of 0.75 was determined in liver microsomes from male B6C3F$_1$ mice.

Crotonaldehyde was formed NADPH-dependently as a minor metabolite of butadiene (partial pressure of 48–52 cm Hg = 660 000 ppm) in microsomes obtained from liver, lung or kidney of male B6C3F$_1$ mice (Sharer *et al*., 1992) or human liver (Duescher & Elfarra, 1994), the formation rate being 20–50 times lower than that of epoxybutene. 3-Butenal was suggested as an intermediate metabolite. No crotonaldehyde formation was observed with microsomes from mouse testis or with microsomes of testis, liver, lung or kidney of male Sprague-Dawley rats (Sharer *et al*., 1992).

Table 21. Kinetic constants of epoxide hydrolase- and glutathione *S*-transferase-catalysed metabolism of diepoxybutane in cell fractions

	V_{max} (nmol/mg protein/min)	K_{mapp} (mmol/L)	V_{max}/K_{mapp} (nmol.L/mg protein/min/mmol)
Epoxide hydrolase (Boogaard & Bond, 1996)			
$B6C3F_1$ mouse			
Liver microsomes	32.0 ± 6.0	8.1 ± 1.8	3.93
Lung microsomes	49.8 ± 9.7	7.5 ± 1.7	6.65
Sprague-Dawley rat			
Liver microsomes	52.9 ± 3.5	2.76 ± 0.22	19.2
Lung microsomes	19.3 ± 7.8	7.1 ± 3.4	2.71
Human (n = 6)			
Liver microsomes	155.8 ± 9.8	4.8 ± 0.41	32.5
Lung microsomes	21.7 ± 1.9	2.83 ± 0.37	7.66
Glutathione *S*-transferase (Boogard *et al.*, 1996)			
$B6C3F_1$ mouse			
Liver cytosol	162 ± 16	6.4 ± 1.6	25.3
Lung cytosol	38.5 ± 2.5	1.70 ± 0.37	21.0
Sprague-Dawley rat			
Liver cytosol	186 ± 37	24 ± 6	7.62
Lung cytosol	17.1 ± 3.0	4.2 ± 1.7	4.10
Human (n = 6)			
Liver cytosol	6.4 ± 1.9	2.1 ± 1.4	3.04

Segments from different airway regions or whole airways obtained from male $B6C3F_1$ mice and Sprague-Dawley rats were incubated in headspace vials with 10 000 ppm butadiene gas (34 μmol/L buffer). Epoxybutene formation in tissues from mice was two-fold higher than in rats. The quantity of epoxybutene measured was doubled in the presence of the epoxide hydrolase inhibitor trichloropropene oxide, but remained unchanged following addition of the GSH depletor diethyl maleate, indicating that epoxide hydrolase contributes more than glutathione conjugation to epoxybutene detoxification (Seaton *et al*., 1996).

Bone-marrow cells of $B6C3F_1$ mice do not contain CYP2E1 (Genter & Recio, 1994). Nevertheless, bone-marrow cells of $B6C3F_1$ mice and humans can oxidize butadiene to epoxybutene, the activity being increased two-fold by 1 mmol hydrogen peroxide/L. The metabolic rate in hydrogen peroxide-fortified lysates of mouse cells (0.0053 nmol/min/mg protein) was two orders of magnitude lower than in mouse and rat liver microsomes (Maniglier-Poulet *et al*., 1995).

Incubation of butadiene with human myeloperoxidase (from polymorphonuclear leukocytes) in the presence of hydrogen peroxide (1 mmol/L) yielded epoxybutene and small amounts of crotonaldehyde by direct oxygen transfer (Duescher & Elfarra, 1992; Maniglier-Poulet *et al*., 1995). Addition of chloride in the hundred millimolar range led to the formation of 1-chloro-2-hydroxy-3-butene as the major metabolite (Duescher & Elfarra, 1992).

The kinetics of epoxybutene oxidation to diepoxybutane were investigated by Csanády *et al*. (1992) in liver microsomes of male B6C3F$_1$ mice and by Seaton *et al*. (1995) and Krause and Elfarra (1997) in those of male B6C3F$_1$ mice, male Sprague-Dawley rats and human subjects. Similar ratios of V_{max}/K_{mapp} relevant at low epoxybutene concentrations were measured by Csanády *et al*. (1992) and Seaton *et al*. (1995), whereas Krause and Elfarra (1997) found ratios that were one order of magnitude lower than those of Seaton *et al*. (1995) in all three species. [One possible explanation of this difference could be that Krause and Elfarra (1997) determined the kinetic parameters at epoxybutene concentrations that were two to four orders of magnitude higher than those found in the blood of rodents exposed to butadiene under conditions of metabolic saturation. Thus Krause and Elfarra (1997) may have characterized a low-affinity enzyme that is not relevant for in-vivo conditions.] The parameters published by Csanády *et al*. (1992) and Seaton *et al*. (1995) were used for physiological toxicokinetic modelling (see Table 19). [The data of Seaton *et al*. (1995) had to be corrected for hydrolytic loss of diepoxybutane (Sweeney *et al*., 1997).]

Krause and Elfarra (1997) detected NADPH-dependent formation of *meso*- and (±)-diepoxybutane from racemic epoxybutene in mice, rats and humans.

Whereas liver microsomes from male Sprague-Dawley rats formed nonsignificantly higher amounts of diepoxybutane from *R*- than from *S*-epoxybutene, in those of male B6C3F$_1$ mice, the yield was significantly higher from the *S*-isomer than from the *R* (Nieusma *et al*., 1997).

Seaton *et al*. (1995) and Krause and Elfarra (1997) used human B-lymphoblastoid cell lines from the same source each expressing a cDNA of one of eight different human CYP isoenzymes. Epoxybutene at 80 µmol/L was oxidized only by CYP2E1, whereas at 5 mmol/L CYP3A4 was similarly active (Seaton *et al*., 1995). Krause and Elfarra (1997) found CYP2E1 to oxidize epoxybutene at 5 mmol/L nearly four- and six-fold faster than CYP2C9 and 2A6, respectively, whereas in contrast to the Seaton *et al.* (1995) study, CYP3A4 was inactive. Diepoxybutane was hydrolysed in human liver microsomes, the *meso* form being preferred over the two other stereoisomers (Krause & Elfarra, 1997).

In summary, in-vitro results suggest that the rate of cytochrome P450-mediated epoxidation of butadiene to epoxybutene and to diepoxybutane is highest in mice compared with rats and humans and that the rate in humans varies widely (Seaton *et al*., 1995; see Table 19).

Investigations *in vitro* have demonstrated that epoxybutene is eliminated by microsomal epoxide hydrolase and by cytosolic glutathione *S*-transferase (GST). Epoxide hydro-

lase activity was determined in liver of mouse, rat and man (Kreuzer *et al*., 1991, Csanády *et al*., 1992; Krause *et al*., 1997), in lung of mouse, rat and man (Csanády *et al*., 1992) and in liver of mouse (Recio *et al*., 1992). GST activity was determined in liver of mouse, rat and man (Kreuzer *et al*., 1991; Csanády *et al*., 1992), in lung of mouse, rat and man (Csanády *et al*., 1992), and in liver, lung, testis and kidney of mouse and rat (Sharer *et al*., 1992). Sharer *et al*. (1991) purified π-class GST from human placenta for kinetic studies. The Michaelis–Menten parameters obtained by Kreuzer *et al*. (1991) and Csanády *et al*. (1992) have been used for physiological toxicokinetic modelling (see Table 20).

Hydrolysis of *R*- and *S*-epoxybutene to the respective enantiomer of 3-butene-1,2-diol (butenediol) is nearly completely stereospecific in liver microsomes from male Sprague-Dawley rats, whereas in liver microsomes from male $B6C3F_1$ mice, an inversion of the configuration of 16% (*S*-epoxybutene) and 24% (*R*-epoxybutene) was observed (Nieusma *et al*., 1997).

Epoxybutene is also metabolized by human θ-class GST purified from placenta. Products formed were *S*-(1-hydroxy-3-buten-2-yl)glutathione [*S*-(1-hydroxymethyl-2-propenylglutathione, using the nomenclature of Figure 1] and *S*-(2-hydroxy-3-buten-1-yl)glutathione. The latter product is in 1:1 equilibrium with the relatively stable sulfurane tautomer formed by intramolecular displacement of the hydroxyl group by the sulfur atom (Sharer *et al*., 1991).

Diepoxybutane, like epoxybutene, is eliminated by microsomal epoxide hydrolase in liver and lung of mouse, rat and man (Boogard & Bond, 1996) and by cytosolic GST in liver and lung of mouse and rat and in liver of man (Boogard *et al*., 1996).

In summary, the elimination of epoxybutene and diepoxybutane by GSH conjugation appears to be faster in rodents than in humans. Epoxybutene and diepoxybutane hydrolysis appears to be fastest in humans (see Tables 20 and 21).

Rydberg *et al*. (1996) investigated the reaction of diepoxybutane with valinamide *in vitro* (40°C, pH > 9, 100 h) as a model for the N-terminal valine in haemoglobin. The main products at the lowest diepoxybutane concentration (1 mmol/L) were *N*-(2,3,4-trihydroxybutyl)valinamide and erythritol, formed with similar yields. The amount of a ring-closed pyrrolidine derivative (2,2-*N*,*N*-(2,3-dihydroxybuta-1,4-diyl)valinamide) was three-fold lower. A cross-linked 2,2′-*N*,*N*-(2,3-dihydroxybuta-1,4-diyl)bis-valinamide was detectable at 100 mmol diepoxybutane/L.

Rat θ class GST 5-5 (Thier *et al*., 1995) and human θ class GSTT1-1 (Thier *et al*., 1996), both expressed in *Salmonella typhimurium* TA1535, enhanced the mutagenicity of diepoxybutane but not of epoxybutene. The formation of a reactive glutathione conjugate of the bifunctional diepoxybutane was assumed, possibly a five-membered thialonium ion or a thiiranium (episulfonium) ion. On the other hand, a close correlation was found between the diepoxybutane-dependent induction of sister chromatid exchanges (SCE) (Kelsey *et al*., 1995; Norppa *et al*., 1995; Wiencke *et al*., 1995; Landi *et al*., 1996; Pelin *et al*., 1996) and of micronuclei (Vlachodimitropoulos *et al*., 1997) in human peripheral blood lymphocytes and the homozygous deletion of GSTT1, suggesting detoxification by GSTT1.

Butenediol can be oxidized to 3,4-epoxybutanediol (epoxybutanediol), as has been shown in rat liver microsomes. Incubation for 30 min with butadiene gave concentrations of butenediol and epoxybutanediol which were nearly three-fold and 10-fold, respectively, higher than the corresponding concentration of epoxybutene (Cheng & Ruth, 1993). Epoxybutanediol can, however, also be a product of diepoxybutane hydrolysis.

Kemper and Elfarra (1996) demonstrated the oxidation of butenediol by hepatic alcohol dehydrogenase (ADH), yielding 1-hydroxy-2-butanone as a single stable metabolite; various intermediates have been proposed. For the ADH-dependent oxidation of racemic butenediol in liver cytosol of male B6C3F_1 mice, male Sprague-Dawley rats and three humans, saturation kinetics were found. The ratio V_{max}/K_{mapp} was similar in these species. ADH purified from horse liver oxidized butenediol in a stereoselective manner, since V_{max} was about seven times higher for the *S*- than for the *R*-enantiomer.

The fate of epoxybutanediol has not been studied *in vitro*.

Metabolites in vivo

Bolt *et al*. (1983) exposed male Sprague-Dawley rats in a closed system to initial butadiene concentrations of 6000–7000 ppm [13 300–15 500 mg/m^3] and found exhaled epoxybutene to accumulate in the atmosphere up to 2–4 ppm within 15 h. In further studies, animals were exposed for up to 17 h to butadiene concentrations above 2000 ppm [4400 mg/m^3] under conditions of maximum metabolism of butadiene (Filser & Bolt, 1984). Exhaled epoxybutene accumulated in the air of the closed system, reaching a plateau of about 3.7 ppm. Toxicokinetic analysis with a two-compartment model revealed that only 29% of biotransformed butadiene was systemically available as epoxybutene. From these results, the authors deduced the existence of an intrahepatic first-pass effect for epoxybutene formed from butadiene. Using the same experimental design, Kreiling *et al*. (1987) exposed male B6C3F_1 mice to butadiene at > 2000 ppm; exhaled epoxybutene accumulated in the atmosphere up to about 10 ppm. From the steady-state concentration of epoxybutene in the atmosphere of the closed chamber containing rats or mice exposed to butadiene under conditions of metabolic saturation and using thermodynamic body/air partition coefficients of 37 for rats (Filser & Bolt, 1984) and 42.5 for mice (Kreiling *et al*., 1987), the average concentration of epoxybutene in the body was calculated to be 5.5 μmol/L in rats and 17 μmol/L in mice.

In the experiments of Bond *et al*. (1986) described on p. 140, at the end of 6-h exposure to butadiene, blood concentrations of epoxybutene reached values of 0.4 and 4 μmol/L in rats at 130 and 1800 mg/m^3 and 0.7, 0.9 and 15 μmol/L in mice at 13, 130 and 1800 mg/m^3, respectively. In the cynomolgus monkey, Dahl *et al*. (1991) found blood concentrations of only 1.6, 500 and 1100 nmol epoxybutene/L following 2-h exposures to 10, 310 and 7760 ppm [18, 560 and 14 000 mg/m^3] butadiene, respectively, using the same method as Bond *et al*. (1986). [The Working Group noted that due to the unspecific determination of radioactivity in cryogenic traps, contamination of epoxybutene with other metabolic products of butadiene cannot be excluded.]

More recently, concentrations of butadiene epoxides were determined by gas chromatography–mass spectrometry in blood and tissues of $B6C3F_1$ mice and Sprague-Dawley rats exposed via the nose only to butadiene. Losses of the volatile epoxybutene that may occur in the time between sacrifice and organ dissection have been modelled (Sweeney *et al.*, 1996). These simulations predicted that epoxybutene concentrations in the liver can decrease by orders of magnitude within minutes. Such losses might differ between large and small organs and between those of mouse and rat. However, Himmelstein *et al.* (1994) removed blood from the animal while it was still breathing the exposure atmosphere, so it is unlikely that epoxybutene was lost during sampling. At exposure concentrations of 62.5, 625 and 1250 ppm [138, 1380 and 2760 mg/m^3] butadiene, they found steady-state concentrations (6 h exposure) in blood of 0.56, 3.7 and 8.6 μmol epoxybutene/L in mice and of only 0.07, 0.94 and 1.3 μmol epoxybutene/L in rats. Diepoxybutane reached concentrations of 0.65, 1.9 and 2.5 μmol/L in mice, but was not detected in rats. Bechtold *et al*. (1995) measured epoxybutene concentrations in blood of 0.38 and 0.1 μmol/L in mice and rats, respectively, exposed for 4 h to 100 ppm [220 mg/m^3] butadiene. Diepoxybutane reached 0.33 μmol/L in mice but was not found in rats. Following 6-h exposures to 625 and 1250 ppm butadiene, Himmelstein *et al*. (1995) found epoxybutene concentrations of 0.58 and 0.93 nmol/g (mice) and 0.06 and 0.16 nmol/g (rats) in liver and 2.6 and 3.7 nmol/g (mice) and 0.16 and 0.31 nmol/g (rats) in lung, respectively. Diepoxybutane was detected in mouse lung at concentrations of 0.71 and 1.5 nmol/g tissue at 625 and 1250 ppm butadiene, respectively. Even at 8000 ppm [17 700 mg/m^3] butadiene, no diepoxybutane was detected in rat lung; the detection limit was 0.04 nmol/g.

Thornton-Manning *et al*. (1995a) exposed male mice and rats for up to 4 h to 62.5 ppm [138 mg/m^3] butadiene. Using a highly sensitive method, the authors detected epoxybutene and diepoxybutane in tissues of both species (Table 22). The tissue concentrations of epoxybutene varied considerably between tissues but in general were 3–10 times higher in mice than in rats. With the exception of liver, as the main metabolizing organ, and bone marrow, diepoxybutane reached similar concentrations in all mouse tissues. Corresponding concentrations in rat lung were up to two orders of magnitude lower. The homogeneous distribution of diepoxybutane in the body is also reflected by the similar tissue:hexane partition coefficients determined experimentally (Table 23; Sweeney *et al*., 1997). Thornton-Manning *et al*. (1995b) found tissue concentrations of epoxybutene to be similar in female and male rats exposed for 6 h to 62.5 ppm (Table 22). However, corresponding concentrations of diepoxybutane were three to five times higher in females than in males.

In a further study, Thornton-Manning *et al*. (1997) investigated the disposition of butadiene epoxides in female $B6C3F_1$ mice and Sprague-Dawley rats following single and repeated (10 days) nose-only exposures (6 h) to 62.5 ppm [138 mg/m^3] butadiene (Table 24). With the exception of lung, tissue and blood concentrations of epoxybutene in rats and mice were higher after repeated exposures. Whereas repeated exposures of rats did not lead to changes in diepoxybutane concentrations, a reduction of up to 30% was observed in mice.

Table 22. Tissue concentrations of epoxybutene and diepoxybutane in rats and mice after inhalation of butadiene

Tissue	Epoxybutene (pmol/g)			
	4 h exposure (Thornton-Manning *et al.*, 1995a)		6 h exposure (Thornton-Manning *et al.*, 1995b)	
	Male mice	Male rats	Male rats	Female rats
Blood	295 ± 27	36 ± 7	25.9 ± 2.9	29.4 ± 2
Liver	8 ± 4	Not detected	n.d.	n.d.
Lung	33 ± 9	Not detected	12.7 ± 5	2.7 ± 4.3
Fat	1302 ± 213	267 ± 14	175 ± 21	203 ± 13
Heart	120 ± 15	40 ± 16	n.d.	n.d.
Spleen	40 ± 19	7 ± 6	n.d.	n.d.
Thymus	104 ± 55	12.5 ± 3.2	n.d.	n.d.
Bone marrow	2.3 ± 1.5[a]	0.2 ± 0.1	9.3; 9.7 (femur)	10.4 ± 1 (femur)
Mammary	n.d.	n.d.	n.d.	57.4 ± 4
	Diepoxybutane (pmol/g)			
Blood	204 ± 15	5 ± 1	2.4 ± 0.4	11.4 ± 1.7
Liver	20 ± 4	Not detected	n.d.	–
Lung	114 ± 37	0.7 ± 0.2	1.4 ± 0.8	4.8 ± 0.7
Fat	98 ± 15	2.6 ± 0.4	1.1 ± 0.1	7.7 ± 1.3
Heart	144 ± 16	3 ± 0.4	n.d.	–
Spleen	95 ± 12	1.7 ± 0.5	n.d.	–
Thymus	109 ± 19	2.7 ± 0.7	n.d.	–
Bone marrow	1.4 ± 0.3[a]	Not detected	1.1; 1.8	7.1 ± 1.3
Mammary	n.d.	n.d.	n.d.	10.5 ± 2.4

$B6C3F_1$ mice and Sprague-Dawley rats inhaled butadiene via the nose only. Three animals were used for each experiment.
n.d., not determined
[a] pmol/mg protein

Inhalation kinetics of epoxybutene were investigated in Sprague-Dawley rats (Filser & Bolt, 1984; Kreiling *et al.*, 1987) and in male $B6C3F_1$ mice (Kreiling *et al.*, 1987) using closed chambers. Animals were exposed to initial concentrations of epoxybutene ranging from 10 to 5000 ppm [22–11 000 mg/m^3] (rats) and 100 to 2000 ppm [220–4400 mg/m^3] (mice). The exhalation of intraperitoneally administered epoxybutene (45.6 µL/kg bw) by rats was also determined (Filser & Bolt, 1984). In rats, first-order kinetics were observed over the whole exposure range. In mice, initial enrichment phases were seen. The further shape of the concentration–time curves was interpreted as showing saturation kinetics. In a

Table 23. Measured partition coefficients of butadiene, epoxybutene and diepoxybutane

	Mouse	Rat	Rat	Man
Butadiene tissue:air	(Medinsky *et al*., 1994)	(Medinsky *et al*., 1994)	(Johanson & Filser, 1993)	(Filser *et al*., 1993)
Blood	1.34	1.49	3.03	1.00
Fat	19.2	22.2	21.9	22.5
Muscle	4.01	1.47	0.73	0.88
Liver	1.35	1.19	0.94	0.68
Lung	1.47	0.92	n.d.	0.48
Kidney	n.d.	n.d.	0.92	0.86
Brain	n.d.	n.d.	0.43	1.05
Spleen	n.d.	n.d.	0.87	n.d.
Epoxybutene tissue:air	(Medinsky *et al*., 1994)	(Medinsky *et al*., 1994)	(Johanson & Filser, 1993)	(Csanády *et al*., 1996)
Blood	36.6	50.4	83.4	93.3
Fat	91.2	138	155	168
Muscle	23.6	19.8	59.9	45.8
Liver	42.1	72.0	53.7	55.3
Lung	56.3	54.7	n.d.	n.d.
Kidney	n.d.	n.d.	70.2	n.d.
Brain	n.d.	n.d.	51.6	n.d.
Diepoxybutane tissue:hexane	(Sweeney *et al*., 1997)			
Blood	0.437			
Fat	0.959			
Muscle	0.795			
Liver	0.615			
Kidney	0.672			

n.d., not determined

later publication, however, it was explained by depletion of GSH at high exposure concentrations, resulting in a loss of GST-mediated detoxification (Johanson & Filser, 1993), on the basis of GSH measurements in tissues of rats and mice exposed to epoxybutene (Deutschmann & Laib, 1989).

Valentine *et al*. (1997) studied the kinetics of epoxybutene and diepoxybutane in blood following intravenous administration to male Sprague-Dawley rats. The following toxicokinetic parameters were obtained for epoxybutene at 71, 143, 286 μmol/kg bw,

Table 24. Tissue concentrations of epoxybutene and diepoxybutane in female mice and rats exposed to butadiene[a]

Tissue	Epoxybutene (pmol/g)			
	Mouse		Rat	
	Single exposure	Multiple exposure	Single exposure	Multiple exposure
Blood	239 ± 24	317 ± 19	44 ± 7	64 ± 8
Lung	~ 25[a]	~ 150[a]	~ 5[a]	Not detected
Mammary	~ 700[a]	~ 1200[a,b]	~ 80[a]	~ 300[a,b]
Fat	~ 1150[a]	~ 1650[a,b]	~ 200[a]	~ 430[a,b]
Femur	~ 56 [a]	Not reported	~ 10[a]	~ 15[a,b]
	Diepoxybutane (pmol/g)			
	Mouse		Rat	
	Single exposure	Multiple exposure	Single exposure	Multiple exposure
Blood	345 ± 33	247 ± 32	14 ± 2	17 ± 2
Lung	219 ± 33	144 ± 13 [b]	5 ± 1	4 ± 0.3
Mammary	265 ± 11	191 ± 17 [b]	11 ± 2	15 ± 1
Fat	203 ± 2	173 ± 11 [b]	8 ± 1	13 ± 0.4 [b]
Ovary	169 ± 13	152 ± 16	6 ± 2	10 ± 7
Femur	214 ± 27	184 ± 19	7 ± 1	9 ± 1

Female B6C3F$_1$ mice and Sprague-Dawley rats were exposed to 62.5 ppm butadiene for 6 h via the nose only, either on one day only or on 10 successive days. Three or four animals were used for each experiment (Thornton-Manning *et al.*, 1997).

[a] Read from diagram

[b] Significantly different from single exposure value, $p \leq 0.05$

respectively: distribution half-lives of 1.4, 1.8, 1.4 min, terminal half-lives of 5.7, 7.0, 8.5 min, systemic clearance of 104, 114, 67 mL/min/kg bw and volume of distribution at steady state of 0.59, 0.58, 0.53 L/kg bw. The corresponding values for diepoxybutane at a dose of 523 μmol/kg bw were: distribution half-life of 2.7 min, terminal half-life of 14 min, systemic clearance of 76 mL/min/kg bw and volume of distribution at steady state of 0.73 L/kg bw. These values were interpreted as demonstrating the similarity of disposition of the two epoxides in rats.

When treated intraperitoneally with epoxybutene (71.3 to 285 μmol/kg bw), male B6C3F$_1$ mice and Sprague-Dawley rats excreted butenediol in urine, the amount within 24 h being less than 1% of the administered dose (Krause *et al.*, 1997).

Conjugation of epoxybutene with GSH in the liver *in vivo* was demonstrated by Sharer and Elfarra (1992) in male Sprague-Dawley rats which excreted *S*-(2-hydroxy-3-

buten-1-yl)glutathione and *S*-(1-hydroxymethyl-2-propenyl)glutathione in a 3:1 ratio in the bile within 60 min following intraperitoneal injection of epoxybutene (14.3–286 μmol/kg bw). The total amount of conjugates excreted was linearly related to dose, indicating no saturation, but accounted for only 7.6 ± 4.2% of the dose.

Following single intraperitoneal administrations of epoxybutene (71.5, 143 or 285 μmol/kg) to male B6C3F_1 mice or Sprague-Dawley rats, diastereomeric pairs of *N*-acetyl-*S*-(2-hydroxy-3-buten-1-yl)-L-cysteine (1 in Figure 1) and *N*-acetyl-*S*-(1-hydroxymethyl-2-propenyl)-L-cysteine (2 in Figure 1) were excreted in the urine within 8 h. In rats, linear dose–response relationships were observed with respect to the excretion of metabolites 1 and 2 (mean, 17% of epoxybutene dose), the amount of metabolite 1 being two to three times higher than that of metabolite 2. In mice, an overproportional increase in the excretion of metabolites 1 and 2 occurred at the highest dose (mean 26%, compared with 7 and 9% at the lower doses, respectively), the amount of metabolite 1 being about one half to one third of that of metabolite 2. The amount per body weight of metabolites 1 and 2 in rats was approximately twice as high as in mice at the lower doses and similar in both species at the high dose (Elfarra *et al*., 1995).

Haemoglobin adducts

Using haemoglobin and epoxybutene, Osterman-Golkar *et al*. (1991) observed the formation of two diastereomeric pairs of adducts to the N-terminal valine of haemoglobin namely *N*-(2-hydroxy-3-buten-1-yl)valine and *N*-(1-hydroxy-3-buten-2-yl)valine. These findings were corroborated by Richardson *et al*. (1996), who incubated erythrocyte suspensions obtained from mice, rats and humans with epoxybutene. The second-order rate constant of adduct formation for the sum of both adducts (HOBVal) was determined *in vitro* at 37°C to be 0.29×10^{-4} L/g globin/h with erythrocytes isolated from mice (Recio *et al*., 1992; value corrected by the same authors to the one quoted here, Osterman-Golkar *et al*., 1993).

In male Wistar rats exposed for two weeks (6 h per day, five days per week) to butadiene, covalent binding of epoxybutene (mainly at C-1) to the N-terminal valine of haemoglobin was observed. Total adduct levels (nmol/g haemoglobin) and the daily average increment (nmol/g haemoglobin) at day 12 were 0.5 and 0.06 at 250 ppm [550 mg/m^3], 1.5 and 0.17 at 500 ppm [1100 mg/m^3] and 3.0 and 0.33 at 1000 ppm [2200 mg/m^3] butadiene. Seventeen days after the end of exposure, the levels had decreased to nearly two thirds of the original values (Osterman-Golkar *et al*., 1991).

Osterman-Golkar *et al*. (1993) observed a linear increase in the HOBVal level up to about 4 nmol/g globin following exposure of male B6C3F_1 mice over four weeks (6 h per day, five days per week) to butadiene (0, 2, 10 and 100 ppm [0, 4, 22 and 220 mg/m^3]). In Sprague-Dawley rats, the increase of HOBVal was linear up to 10 ppm butadiene, amounting to about 0.2 nmol/g globin and reached a value of about 1 nmol/g globin at 100 ppm. The authors also summarized haemoglobin binding indices resulting from butadiene exposure (pmol HOBVal/g globin per ppm.h) in different species as ~0.5 in B6C3F_1 mice, ~0.3 in CD2F_1 mice (from Recio *et al*., 1992), ~0.09 in Wistar rats

(from Osterman-Golkar *et al*., 1991), and ~0.3 and ~0.1 in Sprague-Dawley rats at 0–10 ppm and 10–100 ppm butadiene, respectively. For humans, a value of ~0.004 and an even lower value of 0.0005 have been estimated (see Section 4.1.1).

Albrecht *et al*. (1993) determined HOBVal in female $CB6F_1$ mice, male and female C3H × 101/EL mice and female Wistar rats exposed (6 h per day for five days) to butadiene concentrations of 0, 50, 200 and 500 ppm [0, 110, 440 and 1100 mg/m^3]. Additionally, animals were exposed to 1300 ppm [2870 mg/m^3] butadiene, with the exception of male C3H × 101/EL mice. In mice, background levels of HOBVal were between 1 and 8 nmol/g globin. Up to 200 ppm butadiene, a steep increase of the HOBVal levels was observed, reaching values between 10 and 16 nmol/g globin. At higher butadiene concentrations, the slope of the curve flattened and at 1300 ppm, the HOBVal value reached about 25 nmol/g globin. No significant strain or sex difference was observed. In rats, background levels were between 1.3 and 2.2 nmol/g globin. Following exposure, the adduct levels were distinctly lower than in mice. The slope of the dose–response curve between 0 and 200 ppm, reaching a level of about 3 nmol/g globin, was somewhat steeper than between 200 and 1300 ppm, at which the level reached about 5 nmol/g globin.

Pérez *et al.* (1997) exposed male Wistar rats for five consecutive days (6 h per day) to constant butadiene concentrations of 0, 50, 200 and 500 ppm [0, 110, 440 and 1100 mg/m^3]. On day 6, animals were killed; the levels of HOBVal were 0.6, 21, 88 and 180 pmol/g.

Osterman-Golkar *et al.* (1998) investigated the dose–response relationships for adduct formation and persistence in rats and mice during long-term low-level exposure to butadiene by inhalation. Values reported by Osterman-Golkar *et al*. (1993) were also recalculated. HOBVal levels were measured in male $B6C3F_1$ mice and Sprague-Dawley rats following exposure to 0, 2, 10 or 100 ppm [0, 4, 22 or 220 mg/m^3] butadiene for 6 h per day on five days per week for one, two, three or four weeks. The increase and decrease, respectively, of the adduct levels during and three weeks after the end of the four-week exposure indicated that adducts are chemically stable *in vivo* and that elimination follows the turnover of red blood cells. Adduct levels increased linearly with butadiene concentration in mice, whereas a deviation from linearity between 10 and 100 ppm butadiene (decrease in slope) was observed in rats. Blood concentrations of epoxybutene estimated from haemoglobin adduct levels were in general agreement with those reported in mice and rats exposed to 62.5 ppm butadiene, indicating that HOBVal adduct levels can be used to predict blood concentrations of epoxybutene in rats and mice.

After intraperitoneal administration of epoxybutene (10, 20, 40 and 60 mg/kg bw) to male $B6C3F_1$ mice and Sprague-Dawley rats, HOBVal levels increased with dose approximately linearly in rats and sublinearly in mice. At the highest dose, the binding efficiency in mice was twice that in rats, HOBVal levels reaching about 950 and 460 pmol/g globin in mice and rats, respectively (Richardson *et al*., 1996).

Tretyakova *et al*. (1996) exposed female and male $B6C3F_1$/CrlBR mice and Crl:CDBR rats to 1000 ppm [2200 mg/m^3] butadiene (6 h per day, 5 days per week, for

13 weeks). Two isomers of HOBVal were found [not further specified], the level of isomer I being 1.3–1.5-fold that of isomer II. HOBVal levels (means of isomer I up to 11 190 and of isomer II up to 8660 pmol/g globin in female mice) were three to four times higher in mice than in rats, the mean levels in females being about twice those in males.

N-(2,3,4-Trihydroxybutyl)valine (THBVal) in haemoglobin is regarded as a reaction product of epoxybutanediol with N-terminal valine. This adduct could, however, form by direct binding of diepoxybutane to haemoglobin with subsequent hydrolysis of the second epoxide (see Section 4.1.1). Two isomers of this adduct were found in male Sprague-Dawley rats 24 or 48 h following intraperitoneal treatment with epoxybutene (78.3 mg/kg bw), epoxybutanediol (30 and 60 mg/kg bw) or diepoxybutane (16.7 and 33.4 mg/kg bw) or after exposure to butadiene. Adduct levels were reported only for 'adduct II'. Compared with a control level of about 2 pmol/g globin, THBVal reached a maximum level of 2800 pmol/g globin after 33.4 mg/kg bw diepoxybutane. As calculated from THBVal levels, diepoxybutane had higher haemoglobin binding indices (pmol THBVal/g globin per μmol/kg bw) of 9.3 and 7.2 (at 16.7 and 33.4 mg/kg doses, respectively) than epoxybutanediol (3.4 and 4.0 at 30 and 60 mg/kg doses, respectively) and epoxybutene (0.07). In Wistar rats killed one day after exposure (6 h per day, for five days) to 0, 50, 200 or 500 ppm [0, 110, 440 or 1100 mg/m^3] butadiene, the highest THBVal levels of 1190 pmol adduct/g globin were found at 200 ppm (controls: 9 pmol THBVal/g globin). The binding index (pmol THBVal/g globin per ppm × h) decreased from 0.5 at 50 ppm to 0.04 at 500 ppm. Parallel determination of the levels of HOBVal determined in the same rats were three-fold (500 ppm) to about 32-fold (50 ppm) lower than the THBVal levels (Pérez *et al*., 1997).

Physiological toxicokinetic models

Physiological toxicokinetic (or pharmacokinetic) models represent descriptions of biological systems and can be used to describe the behaviour of chemicals in the intact animal. Such models have been used to predict the disposition of butadiene and metabolites in rats, mice, and humans. For the case of rats and mice, these predictions can be compared with experimental data. In some cases (see below), the models successfully describe (and accurately predict) the disposition of butadiene and metabolites. Human physiological toxicokinetic model predictions normally cannot be verified due to lack of experimental data.

Several models have been developed to simulate the absorption, distribution, metabolism and excretion of butadiene, some of its metabolites and its adducts to haemoglobin in mouse, rat and man. Critical aspects are discussed in Csanády *et al.* (1996) and in Himmelstein *et al*. (1997). Basically, the models consist of a number of compartments representing diverse tissues and organs, several of which are grouped together. These compartments are linked by blood flow. The main differences between models are the number of metabolizing and nonmetabolizing compartments, the mechanisms of metabolism, the metabolites taken into consideration, and the values of the biochemical,

physiochemical and physiological parameters. The first group of parameters is represented by apparent Michaelis constants, maximum rates of metabolism, tissue concentrations of GSH and turnover rates. The second group consists of the blood:air, tissue:air and tissue:blood partition coefficients of butadiene and selected metabolites. The structure of the tissue compartments, blood flow rates and alveolar ventilation belong to the third group.

Physiological toxicokinetic models have been presented describing the behaviour of inhaled butadiene in the human body. Partition coefficients for tissue:air and tissue:blood, respectively, had been measured directly using human tissue samples or were calculated based on theoretical considerations. Parameters of butadiene metabolism were obtained from in-vitro studies in human liver and lung cell constituents and by extrapolation of parameters from experiments with rats and mice *in vivo* (see above). In these models, metabolism of butadiene is assumed to follow Michaelis–Menten kinetics.

By means of an apparent Michaelis constant (K_{mapp}) together with a maximum rate (V_{max}) of butadiene metabolism both obtained with human liver microsomes (Filser *et al.*, 1992), Filser *et al.* (1993) constructed a human model which was later extended by Csanády *et al*. (1996) for the butadiene metabolites epoxybutene and diepoxybutane. For butadiene and epoxybutane, the required human tissue:air partition coefficients were measured using autopsy material (Table 23). Filser *et al.* (1993) investigated the influence of styrene co-exposure on butadiene metabolism by assuming competitive interaction. Simulations for a 70-kg man exposed over 8 h to 5 or 15 ppm [11 or 33 mg/m^3] butadiene indicated total amounts of butadiene metabolized of 0.095 and 0.285 mmol, respectively, reduced by about 19% and 37% as a result of co-exposure to 20 and 50 ppm styrene, respectively. No influence of butadiene on styrene metabolism was noted.

Kohn and Melnick (1993) and Medinsky *et al.* (1994) used in their models values of K_{mapp} and V_{max} which had been determined by Csanády *et al.* (1992) with microsomes from human liver and lung. The tissue:blood partition coefficients used by Kohn and Melnick (1993) were theoretically derived and were 5–10 times higher than those derived from the tissue:air partition coefficients measured by Filser *et al.* (1992) in human tissues and by Johanson and Filser (1993) and by Medinsky *et al.* (1994) in rodent tissues. Simulations of human exposure to butadiene under workplace conditions (8 h per day, five days per week) indicated that high accumulation in fat would occur, with levels increasing about three-fold during the week. For their model, Medinsky *et al.* (1994) used either their own partition coefficients determined experimentally in mouse tissues or for comparison those which had been published by Kohn and Melnick (1993). Simulations of concentration–time courses in fat tissue resulting from human exposure to 54 ppm [120 mg/m^3] butadiene for 6 h yielded about three-fold lower peak concentrations and an area under the concentration–time curve (AUC) several times lower when the mouse values were used. With the latter values, which are close to those obtained by Filser *et al.* (1992) in human tissues, no suggestion of accumulation during the working week was found.

Internal burdens of epoxybutene in humans resulting from exposure to butadiene were predicted from models by Kohn and Melnick (1993), Johanson and Filser (1996) and Csanády *et al.* (1996) and were compared with simulations for rats and mice. In the model of Kohn and Melnick (1993), metabolic parameters were incorporated which had been obtained by Csanády *et al.* (1992) by measuring butadiene and epoxybutene oxidation and epoxybutene hydrolysis in human liver and lung microsomes *in vitro*, and conjugation of epoxybutene with glutathione in human liver and lung cytosol. Tissue:blood partition coefficients were theoretically derived. The body burden of epoxybutene following exposure to 100 ppm butadiene for 6 h was predicted to be 0.056 μmol/kg in humans.

Johanson and Filser (1996) used metabolic parameters which had been obtained for enzymic butadiene oxidation (Filser *et al.*, 1992) and epoxybutene hydrolysis (Kreuzer *et al*., 1991) in human liver microsomes and for enzymic conjugation of epoxybutene with glutathione in human liver cytosol (Kreuzer *et al.*, 1991). Tissue:air partition coefficients had been determined experimentally for butadiene in human tissues (Filser *et al.* 1993a) and for epoxybutene in rat tissues (Johanson & Filser, 1993). For an eight-hour exposure to 10 ppm butadiene, the model predicted a blood concentration of epoxybutene of about 0.08 μmol/L in a man (Johanson & Filser, 1996). Csanády *et al*. (1996) simulated an exposure to 10 ppm (22 mg/m^3) butadiene over 8 h and predicted an AUC of epoxybutene in blood of 0.27 μmol.h/L. Most of the model parameters used by these authors were identical to those of Johanson and Filser (1996). Tissue:air partition coefficients for epoxybutene in humans used by Csanády *et al*. (1996) were measured with human tissue samples (Table 23). The values suggest an almost homogeneous distribution of epoxybutene in the body, with about twofold enrichment in fat tissue. The models of Johanson and Filser (1996) and Csanády *et al*. (1996) predict AUCs of epoxybutene in humans about one order of magnitude higher than those from the model of Kohn and Melnick (1993). The main reason for this difference might lie in the very high theoretically derived fat:air partition coefficient for butadiene which was used by the latter authors, leading to prediction of storage of inhaled butadiene in fat tissue, resulting in reduced availability for biotransformation to epoxybutene during the time span of a single exposure over 6 h.

Physiological toxicokinetic models for experimental systems

Models presented for mice and rats (Evelo *et al*., 1993; Filser *et al*., 1993; Johanson & Filser, 1993; Kohn & Melnick, 1993; Bond *et al*., 1994; Medinsky *et al*., 1994; Csanády *et al*., 1996; Sweeney *et al.*, 1997) predicted, species specifically, similar toxicokinetic behaviour of butadiene. The only exception was the first model of Kohn and Melnick (1993), which contained much higher theoretically derived partition coefficients than the experimentally determined ones, leading to prediction of butadiene storage in fat tissue. In a second, extended version, the authors used average values of the partition coefficients determined experimentally by Johanson and Filser (1993) and Medinsky *et al*. (1994).

The influence of metabolism in the lung with respect to the toxicokinetics of butadiene was simulated in the models of Evelo *et al*. (1993), Kohn and Melnick (1993), Medinsky *et al*. (1994) and Sweeney *et al.* (1997). The model of Evelo *et al*. (1993) yielded the surprising result that the total metabolic activity in lung of mice exposed to 1 ppm [2.2 mg/m^3] butadiene in air would be nearly equal to that in liver. Experimental data confirming this model prediction have not been published. Under similar conditions, the ratios of lung to liver metabolic activity in rats and humans were around 0.2 and 0.08, respectively. This ratio decreased in all species by 30–50% at 1000 ppm [2200 mg/m^3] exposure. The simulations indicated a strong first-pass effect of butadiene in the lung at low concentrations. The model of Kohn and Melnick (1993) predicted that most butadiene (85–95%) would be metabolized in the liver of the three species, whereas metabolism in the lung accounted for only 4% in mice and 1% in rats and humans. From model simulations of their own closed-chamber uptake data, Medinsky *et al*. (1994) suggested that lung metabolism of butadiene might be important for the total body clearance in mice but not in rats. At lower butadiene concentrations, lung metabolism was predicted to become more important relative to metabolism in the liver, which was attributed to the limitation of hepatic metabolism by the blood flow through the liver.

Physiological toxicokinetic models of butadiene metabolite disposition

Epoxybutene was included as the first metabolite of butadiene in several models. The models of Medinsky *et al*. (1994) and Kohn and Melnick (1996) overpredicted the burden of epoxybutene in rodents two- to three-fold, since it was assumed that biotransformed butadiene would become fully systemically available as epoxybutene. Under the same assumption, the model of Kohn and Melnick (1993) yielded reasonable simulations of experimental data, but the predicted rates of butadiene metabolism in this model were much lower than those which had been determined experimentally (Bolt *et al*., 1984; Kreiling *et al*., 1986; Leavens *et al*., 1996a). In the model of Sweeney *et al*. (1997), epoxybutene formation was reasonably simulated by adjusting the systemic availability of epoxybutene to measured data. It was assumed that only a fraction of the butadiene metabolized was transformed to epoxybutene. Further intermediates formed within the first step of butadiene catabolism not leading to epoxybutene were postulated. The fraction of butadiene oxidized to epoxybutene was estimated to be 0.19 in mice and 0.24 in rats. This fraction is consistent with the 'extraction factor' of 0.29 reported by Filser and Bolt (1984). These authors interpreted their findings as indicative of an intrahepatic first-pass effect for the epoxybutene formed, since only 29% of this metabolite entered systemic circulation. This effect was considered in the models of Johanson and Filser (1993, 1996) and Csanády *et al*. (1996), in which the liver was modelled as consisting of cytosol containing GST and endoplasmic reticulum containing a cytochrome P450–epoxide hydrolase complex. Such a complex was proposed to explain the biotransformation of a series of olefinic hydrocarbons including naphthalene *in vitro* and *in vivo* (Oesch, 1973). Evidence supporting the existence of such a complex comes from the demonstration that the rat microsomal epoxide hydrolase (mEH) and a CYP2B1–mEH

fusion protein, in which the CYP2B1 membrane anchor signal sequence replaced the N-terminal 20 amino acid residues of mEH, could be co-translationally inserted into dog pancreas microsomes, whereas truncated mEH, in which the N-terminal 20 amino acids were deleted, was not co-translationally inserted (Friedberg *et al.*, 1994). The biochemical parameters of butadiene and epoxybutene metabolism incorporated in the model of Johanson and Filser (1993, 1996) were derived from in-vitro data for butadiene (Filser *et al*., 1992) and epoxybutene (Kreuzer *et al*., 1991). The model overpredicted epoxybutene formation by a factor of about two.

In the model of Csanády *et al*. (1996), the biochemical parameters for butadiene in rats and mice were obtained by fitting model simulations to in-vivo data of Bolt *et al*. (1984) and Kreiling *et al*. (1986). The biochemical parameters for epoxybutene were identical to those of Johanson and Filser (1993, 1996). This model accurately predicted experimental data on epoxybutene. The most advanced models are those of Csanády *et al*. (1996) and Sweeney *et al*. (1997), since they can simulate both epoxybutene and diepoxybutane as metabolites of butadiene. The tissue:blood partition coefficients for diepoxybutane were estimated by Csanády *et al*. (1996) to have a value of 1 for all tissues. Sweeney *et al.* (1997) obtained tissue:blood partition coefficients from in-vitro measurements (Table 23). Both models yielded good predictions for mice and rats for both metabolites. For humans, no measured data have been reported against which the predictions could be validated. In addition, the model of Csanády *et al*. (1996) predicted accurately the measured haemoglobin adduct levels (Osterman-Golkar *et al*., 1993; Albrecht *et al*., 1993) of epoxybutene in rodents following exposure to butadiene. None of the models published has included the formation and elimination of epoxybutanediol.

4.1.3 *Comparison of rodent and human data*

By comparing butadiene metabolites in urine of butadiene-exposed mice and humans, Bechtold *et al*. (1994) concluded that in humans epoxybutene was metabolically eliminated predominantly by epoxide hydrolase. In rats, GSH conjugation and hydration pathways were about equal and in mice direct GSH conjugation was more important.

No measured data have been published on the burden of butadiene and its epoxy metabolites in exposed humans that can be used for comparison with the rodent data. However, the models of Kohn and Melnick (1993), Johanson and Filser (1996) and Csanády *et al*. (1996) predict significantly lower body burdens of epoxybutene, based on data derived from human tissues (Tables 19, 20 and 21).

The partition coefficients measured in rodent and human tissue samples (Table 23) suggest that there would be no substantial difference between rodents and humans with respect to distribution of butadiene and its metabolite epoxybutene.

Model predictions have been made of the disposition of butadiene and epoxybutene in rodents and humans. Kohn and Melnick (1993) predicted that the cumulative body burden of epoxybutene after a 6-h exposure to 100 ppm butadiene '(area under the epoxybutene versus time curve from 0 to 6 h)' in humans would be 7- and 35-fold lower than in rat and mouse, respectively. For a 12-h exposure to 10 ppm butadiene, the model

of Johanson and Filser (1996) predicted the internal dose of epoxybutene in humans to be only 3.3- and 5.3-fold lower than in rat and mouse, respectively. Similar results were obtained with the model of Csanády *et al*. (1996) for exposure to 10 ppm butadiene over 8 h: the AUCs of epoxybutene in blood were 3.7- and 4.8-fold lower in humans than in rat and mouse, whereas the AUCs of butadiene in blood were about three-fold lower in humans than in both rodent species.

Osterman-Golkar *et al.* (1993) summarized data on the formation of HOBVal, the adduct of epoxybutene at the N-terminal valine of haemoglobin, in rodents exposed experimentally and in subjects exposed at the workplace to butadiene. The binding indices (pmol HOBVal/g globin per ppm.h) were ~0.5 in $B6C3F_1$ mice, ~0.3 in $CD2F_1$ mice (Recio, 1992), ~0.09 in Wistar rats (Osterman-Golkar *et al*., 1991), ~0.3 and ~0.1 in Sprague-Dawley rats at 0–10 ppm and 10–100 ppm [0–22 and 22–220 mg/m^3] butadiene, respectively, and, as a preliminary value, ~0.004 in humans. [The latter value was estimated assuming an average exposure concentration of 1 ppm [2.2 mg/m^3], but the exposure concentrations were mostly below this value (Osterman-Golkar, 1993; Sorsa *et al*., 1996).] In a later publication, Osterman-Golkar *et al*. (1996) reported a median HOBVal level of 0.16 pmol/g globin in 10 workers exposed to a median butadiene concentration of 2.1 mg/m^3 [0.93 ppm]. Considering a ratio of 3:1 of the C-1:C-2 isomers of the epoxybutene-valine adducts at the N-terminal valine of haemoglobin (Richardson *et al.*, 1996) and assuming a workplace exposure of 8 h per day for five days, a binding index of $(4/3) \times 0.16/(0.93 \times 8 \times 63) \sim 0.0004$ pmol HOBVal/(g globin per ppm.h) can be calculated. An identical low binding index was calculated from data given in a review by Osterman-Golkar and Bond (1996).

Taking all these data together, it can be concluded that exposure of humans to butadiene leads to lower body burdens of the reactive metabolite epoxybutene than in similarly exposed rats and mice. No comparative data are available concerning the intermediate diepoxybutane. Only limited data have been published on the adducts of epoxybutanediol at the N-terminal valine of haemoglobin resulting in *N*-(2′,3′,4′-trihydroxybutyl)valine (Pérez *et al*., 1997).

4.2 Toxic effects

4.2.1 *Humans*

Butadiene

The toxic effects of combined exposures to butadiene and other agents (e.g., styrene, chloroprene, hydrogen sulfide, acrylonitrile) have been reviewed (Parsons & Wilkins, 1976). Concentrations of several thousand parts per million of butadiene irritate the skin, eyes, nose and throat (Carpenter *et al*., 1944; Wilson *et al*., 1948; Parsons & Wilkins, 1976).

Several studies have been reported on the effects of occupational exposure to butadiene, mainly from the USSR and Bulgaria. Few are substantiated by details on the atmospheric concentration or duration of exposure, and control data are generally not provided. The effects reported include haematological disorders (Batkina, 1966; Volkova & Bagdinov, 1969), kidney malfunction, laryngotracheitis, irritation of the upper respiratory tract,

conjunctivitis, gastritis, various skin disorders, a variety of neurasthenic symptoms (Parsons & Wilkins, 1976) and hypertension and neurological disorders (Spasovski *et al*., 1986).

Checkoway and Williams (1982) reported minimal changes in haematological indices among eight workers exposed to about 20 ppm [44 mg/m^3] butadiene, 14 ppm [60 mg/m^3] styrene and 0.03 ppm [0.1 mg/m^3] benzene, compared with those among 145 workers exposed to less than 2 ppm [4.4 mg/m^3] butadiene, 2 ppm [8.5 mg/m^3] styrene and 0.1 ppm [0.3 mg/m^3] benzene. Changes included a slight decrease in haemoglobin level and a slight increase in red-cell mean corpuscular volume. [The Working Group considered that these changes cannot be interpreted as an effect of butadiene on the bone marrow, particularly as alcohol intake was not evaluated.]

Diepoxybutane

Diepoxybutane is highly irritant to the eyes and respiratory tract of accidentally exposed workers (IARC, 1976).

4.2.2 *Experimental systems*

Butadiene

LC_{50} values for butadiene were reported to be 270 000 mg/m^3 in mice exposed for 2 h and 285 000 mg/m^3 in rats exposed for 4 h; after 1 h of exposure, rats were in a state of deep narcosis (Shugaev, 1969). Oral LD_{50} values of 5.5 g/kg bw for rats and 3.2 g/kg bw for mice have been reported (United States National Toxicology Program, 1984).

In female rats exposed to 1–30 mg/m^3 butadiene for 81 days, morphological changes were observed in liver, kidney, spleen, nasopharynx and heart (G.K. Ripp, reported in Crouch *et al*., 1979). In groups of 24 rats exposed to 600–6700 ppm [1300–14 800 mg/m^3] butadiene for 7.5 h per day on six days per week for eight months, no adverse effect was noted, except for a slight retardation in growth at the highest concentration (Carpenter *et al*., 1944). Rats exposed to 2200–17 600 mg/m^3 butadiene for 6 h per day on five days per week for three months showed no treatment-related effect other than increased salivation in females (Crouch *et al*., 1979).

Groups of 110 male and 110 female CD Sprague-Dawley rats were exposed to atmospheres containing 0, 1000 or 8000 ppm [0, 2200 or 17 600 mg/m^3] butadiene for 6 h per day on five days per week. The study was terminated when it was predicted that survival would drop to 20–25% (105 weeks for females, 111 weeks for males). Ten animals of each sex from each group were killed at 52 weeks. Treatment was associated with changes in clinical condition and lowering of body weight gain during the first 12 weeks, then nonsignificant changes, reduced survival and increases in certain organ weights and in the incidence of uncommon tumour types (for details, see Section 3.1.2). Increased mortality in the high-dose males was accompanied by an increase in the severity of nephropathy (Owen *et al*., 1987; Owen & Glaister, 1990).

B6C3F$_1$ mice exposed to 0, 625 or 1250 ppm [0, 1380 or 2760 mg/m^3] butadiene for 6 h per day on five days per week for 60–61 weeks had increased prevalence of atrophy of the ovary and testis, atrophy and metaplasia of the nasal epithelium, hyperplasia of the

respiratory and forestomach epithelium and liver necrosis (see also Section 3.1.1) (United States National Toxicology Program, 1984).

Haematological changes in male B6C3F$_1$ mice exposed to 62.5, 200 or 625 ppm [138, 440 or 1380 mg/m^3] butadiene for 6 h per day on five days per week for 40 weeks included decreased red blood cell count, haemoglobin concentration and packed red cell volume and increased mean corpuscular volume. Similar changes occurred in female mice exposed to 625 ppm butadiene (for details, see Section 3.1.1) (Melnick *et al*., 1990).

The role of murine retroviruses in induction of leukaemias and lymphomas following inhalation of butadiene was evaluated in a series of studies reviewed by Irons (1990). Exposure of groups of male B6C3F$_1$ mice, which have the intact ecotropic murine leukaemia virus, to 1250 ppm [2750 mg/m^3] butadiene for 6 h per day on six days per week for 6–24 weeks resulted in decreases in the number of circulating erythrocytes, in total haemoglobin and in haematocrit and an increase in mean corpuscular volume. Leukopenia, due primarily to a decrease in the number of segmented neutrophils, and an increase in the number of circulating micronuclei were observed (Irons *et al*., 1986a). Persistent immunological defects were not detected after this treatment (Thurmond *et al*., 1986). Exposure of male NIH Swiss mice, which do not possess intact endogenous ecotropic murine leukaemia virus, produced similar results (Irons *et al*., 1986b).

A further study was conducted to examine the expression and behaviour of endogenous retroviruses in these strains during the preleukaemic phase of butadiene exposure. Chronic exposure of B6C3F$_1$ mice to butadiene (1250 ppm [2760 mg/m^3]) for 6 h per day on five days per week for 3–21 weeks increased markedly the quantity of ecotropic retrovirus recoverable from the bone marrow, thymus and spleen. Expression of other endogenous retroviruses (xenotropic, MCF-ERV) was not enhanced. No virus of any type was found in similarly treated NIH Swiss mice (Irons *et al*., 1987a).

Enhanced susceptibility to butadiene-induced leukaemogenesis as a result of an ability to express the retrovirus was suggested by the finding that exposure to 1250 ppm butadiene for one year resulted in a 57% incidence of thymic lymphoma in B6C3F$_1$ mice (with expression of the virus) and a 14% incidence in NIH Swiss (without viral expression) (Irons *et al*., 1989).

Groups of 70 male and 69 female B6C3F$_1$ mice were exposed to 0, 6.25, 20, 62.5, 200 or 625 ppm [0, 14, 44, 138, 440 or 1380 mg/m^3] butadiene for 6 h per day on five days per week for up to 103 weeks. Groups of 10 males and 10 females were killed at 40 and 65 weeks. Ovarian atrophy was noted in female mice at 65 weeks of exposure (after completion of the reproductive life of this species) at 20 ppm and higher. Testicular atrophy occurred after 65 weeks in the male mice at 625 ppm (Melnick & Huff, 1992).

The effect of butadiene exposure on arteriosclerotic plaque development was assessed in white leghorn cockerels exposed for 6 h per day on five days per week for 16 weeks to 20 ppm (44 mg/m^3) butadiene. Plaque frequency and size in the abdominal aorta wall were determined for butadiene-exposed animals and controls. Plaques were larger for butadiene-exposed animals than for corresponding air controls and the authors concluded that butadiene exposure markedly accelerated arteriosclerotic plaque development. Since

one epidemiological study has suggested a link between death from arteriosclerotic heart disease and chronic occupational exposure to butadiene, the authors suggested that their animal model could be used to further investigate this disease (Penn & Snyder, 1996).

Diepoxybutane

Diepoxybutane is highly toxic to rats (oral LC_{50} 78 mg/kg bw), mice and rabbits. Among surviving animals, there was eye, skin and respiratory tract damage. Intramuscular injection of rabbits with 25 mg/kg bw produced leukopenia and lymphopenia. However, once weekly gavage dosing of rats for one year with 5 mg D,L-diepoxybutane was not toxic (IARC, 1976).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Butadiene

The reproductive and developmental toxicity of butadiene has been reviewed (Melnick & Huff, 1992; Christian, 1996).

Fertility was reported to be unimpaired in mating studies in rats, guinea-pigs and rabbits exposed to 600, 2300 or 6700 ppm [1300, 5000 or 14 800 mg/m^3] butadiene by inhalation for 7.5 h per day on six days per week for eight months (Carpenter *et al*., 1944). [The Working Group noted the incomplete reporting of this study].

Pregnant Sprague-Dawley rats (24–28 per group) and Swiss (CD-1) mice (18–22 per group) were exposed to atmospheric concentrations of 0, 40, 200 or 1000 ppm [0, 88, 440 or 2200 mg/m^3] butadiene for 6 h per day on days 6–15 of gestation and killed on gestation day 18 (mice) or 20 (rats). Subsequently, the uterine contents were evaluated; individual fetal body weights were recorded, and external, visceral and skeletal examinations were performed. In rats, maternal toxicity was observed in the 1000-ppm group in the form of reduced extragestational weight gain and, during the first week of treatment, decreased body weight gain. Under these conditions, there was no evidence of developmental toxicity. Maternal toxicity was observed in mice given 200 and 1000 ppm butadiene, while 40 ppm and higher concentrations of butadiene caused significant exposure-related reduction in the mean body weights of male fetuses. Mean body weights of female fetuses were reduced at the 200 and 1000 ppm exposure levels. No increased incidence of malformations was observed in either species. The frequency of fetal variations (supernumerary ribs, reduced sternebral ossification) was significantly increased in mice exposed to 200 and 1000 ppm. In a study of sperm-head morphology, groups of 20 male B6C3F$_1$ mice were exposed to atmospheric concentrations of 0, 200, 1000 or 5000 ppm [0, 440, 2200 or 11 000 mg/m^3] butadiene for 6 h per day for five consecutive days. Small, concentration-related increases in the frequency of abnormal sperm morphology were seen five weeks after exposure (the only time of examination) (Hackett *et al*., 1987; Morrissey *et al*., 1990).

[The Working Group noted that sequential examinations were not conducted after exposure to determine the effect of butadiene on all stages of gamete development].

Female Sprague-Dawley rats were exposed to 0, 200, 1000 or 8000 ppm [0, 440, 2200 or 17 700 mg/m^3] butadiene for 6 h per day for 10 days on days 6–15 of gestation. Maternal body weight gain was significantly reduced at all exposure concentrations, with weight loss at 8000 ppm. Uterine implantation was unaffected. At 8000 ppm, there was a significant reduction in fetal body weights, delay in ossification of the ribs (wavy ribs) and the thoracic centra and incomplete ossification of the sternum. There were no teratogenic effects that were significant or outside the historical control range. The no-observed-effect level (NOEL) was reported as 200 ppm for maternal toxicity and 1000 ppm for developmental effects (Christian, 1996).

Epoxybutene

Groups of 10 female B6C3F$_1$ mice and 10 Sprague-Dawley rats were administered 0.005, 0.02, 0.09, 0.36 or 1.43 mmol/kg bw [0.35, 1.4, 6.3, 25 or 100 mg/kg bw] epoxybutene in sesame oil by intraperitoneal injection daily for 30 days. There was a 10% body weight decrement among the highest-dose mice at the end of the experiment, but there was no body weight effect in rats. Ovarian and uterine weights also were reduced in mice at the highest dose, with an accompanying reduction in the number of developing follicles and absence of primordial follicles, but there was no effect in rats (Doerr *et al.*, 1996).

Diepoxybutane

There are two reports of reproductive toxicity of diepoxybutane in experimental systems. In the first study, groups of 10 female B6C3F$_1$ mice and 10 Sprague-Dawley rats were administered 0.002, 0.009, 0.036, 0.14 or 0.29 mmol/kg bw [0.17, 0.78, 3.1, 12, 25 mg/kg bw] diepoxybutane in sesame oil by intraperitoneal injection daily for 30 days. Diepoxybutane exposure depressed growth at the two highest doses in both rats and mice. Since rats were extremely sensitive to the high-dose diepoxybutane treatment (0.29 mmol/kg), the diepoxybutane was administered to rats at this dose for only 25 days. At day 25, body weights of these rats were 50% of controls and only four of ten rats were alive at day 30. Animals in this group exhibited signs of severe gastrointestinal toxicity as evidenced by diarrhoea. Ovarian toxicity was determined by assessing reproductive organ weights and ovarian follicle number. Although diepoxybutane was ovotoxic in both species, it was more potent in mice than rats. At a dose of 0.14 mmol/kg bw, the ovary was depleted of 83% of the small follicles and 52% of the growing follicles in mice. Only 31% and 40% of these follicle populations were depleted in rats at that dose. A decrease in ovarian and uterine weights with increasing dose was observed in mice at the 0.14 and 0.29 mmol/kg bw doses. Similar observations were also seen in rats (Doerr *et al.*, 1996).

The effects of diepoxybutane on male reproductive cells were investigated by flow cytometric and histological description of testicular cell populations and alterations of chromatin packaging. Male B6C3F$_1$ mice were treated with a single intraperitoneal

injection of diepoxybutane in saline at doses of 8.5, 17, 26, 34, 43 and 52 mg/kg bw. Groups were killed at intervals of 7, 14, 21, 28 and 35 days after treatment. One group was injected with 78 mg/kg bw for observation at three weeks. The treated animals did not show any clinical signs of toxicity on daily observation. Cytotoxic damage to all post-stem cell stages was assessed by alterations in relative ratios of haploid, diploid, and tetraploid testicular cells and by the reduction of relative percentages of cell populations. Dose-dependent reductions of tetraploid cells, round spermatids and elongated spermatids were detected at 7, 21 and 28 days, respectively, reflecting cytotoxic damage on the differentiating spermatogonia compartment. The dose necessary to reduce the number of differentiating spermatogonia to half the control value was 55 mg/kg bw. Stem cells were not affected by this treatment. Depletion of spermatids and reduction of the secondary spermatocyte layers were noted in the seminiferous tubules (Spano *et al.*, 1996).

4.4 Genetic and related effects

The genetic toxicology of butadiene and of its major metabolites, epoxybutene and diepoxybutane, has been reviewed (Adler *et al*., 1995; Jacobson-Kram & Rosenthal, 1995). Additional information is available in a more recent review of the toxicology and epidemiology of butadiene (Himmelstein *et al*., 1997) and a compilation of publications (Adler & Pacchierotti, 1998).

Butadiene

4.4.1 *Humans*

In a small pilot study, the *hprt* locus mutation frequencies in lymphocytes of eight male workers from a high-exposure area in a butadiene production plant in Texas, USA, were compared with those of five (four male and one female) low-exposure workers and six male control area personnel (Ward *et al.*, 1994, 1996a). All subjects were non-smokers. Butadiene concentrations were measured in both area and personal samples, which gave values of 3.5 ± 7.25 ppm [7.7 ± 16.6 mg/m^3] and 0.03 ± 0.03 ppm [0.07 ± 0.07 mg/m^3] for the high-exposure (production) and the control areas, respectively. The levels in the majority of the production area samples were below 1 ppm. Urinary concentrations (mean ± S.D. in ng/mg creatinine) of the butadiene metabolite, 1,2-dihydroxy-4-(*N*-acetylcysteinyl)butane (*N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine, using the nomenclature of Figure 1 (Metabolite 3)), were 1690 ± 201.3, 355 ± 250 and 580 ± 191 for the high- and low-exposure area and control area personnel, respectively. The *hprt* locus mutation frequencies ($\times 10^{-6} \pm$ standard deviation (SD)), determined by an autoradiographic technique, were 3.99 ± 2.81, 1.20 ± 0.51 and 1.03 ± 0.12, respectively, in the three groups. The value for the high-exposure group was significantly higher ($p < 0.05$) than those for the other groups. A second study was conducted at the same plant eight months later in which exposures were determined from 8-h personal breathing zone air samplers (Ward *et al.*, 1996a). Three exposure groups were compared (high, intermediate and low, there being no control group), for which the average butadiene concentrations were 0.30 ± 0.59, 0.21 ± 0.21 and 0.12 ± 0.27 ppm, respectively

[0.66 ± 1.30, 0.46 ± 0.46 and 0.27 ± 0.60 mg/m^3]. The corresponding urinary concentrations of 1,2-dihydroxy-4-(*N*-acetylcysteinyl)butane were 761 ± 245, 596 ± 155 and 684 ± 176 ng/mg creatinine. The frequencies of *hprt* locus mutations were 5.33 ± 3.76, 2.27 ± 0.99 and 2.14 ± 0.97, respectively, in the three groups. The value for the high-exposure group was significantly higher ($p < 0.05$) than those for the other groups.

Preliminary data from an on-going population study of rubber plant workers exposed to butadiene and styrene (16 high-exposure (including five smokers) versus nine low-exposure (including three smokers)) are also available (Ward *et al.*, 1996a). Passive badge dosimeters were used to measure butadiene and styrene concentrations in the air. The butadiene detection limit was 0.25 ppm [0.55 mg/m^3] over an 8-h period. Half of the 40 samples collected in the high-exposure areas exceeded the detection limit and 11 were greater than 1 ppm [2.2 mg/m^3]. None of the samples collected in low-exposure areas exceeded the detection limit. The styrene concentration averaged 25% of that of butadiene and only one sample from the high-exposure area had > 1 ppm styrene. The frequencies of *hprt* locus mutations for the non-smokers were 7.47 ± 5.69 and 1.68 ± 0.85 for the high- and low-exposure groups, respectively, and, for the smokers, 6.24 ± 4.37 and 3.42 ± 1.57, respectively. The values for the high-exposure groups were significantly higher ($p < 0.01$) than those for the low-exposure groups.

The *hprt* mutation frequency was also evaluated in two studies using the T-lymphocyte clonal assay. The mutation frequency for 41 workers (15 male, 26 female) exposed to butadiene (1–3.5 ppm [2.2–7.7 mg/m^3]) at a polybutadiene rubber production facility in China was not significantly different from that of the 38 (14 male, 24 female) controls. Mutation frequency decreased with cloning efficiency, increased with age and was moderately higher in women than in men. After adjustment for age, sex and cloning efficiency by multiple regression analysis, the mean mutation frequency was 32% higher in exposed workers than in controls, but this difference was not significant ($p = 0.13$) and was due largely to the greater values among exposed women (Hayes *et al.*, 1996). The *hprt* locus mutation frequencies were measured in blood samples collected twice (in 1993 and 1994) from 19 workers exposed to butadiene and 19 matched controls from a butadiene production plant in the Czech Republic (Tates *et al.*, 1996). Three exposed and three control subjects were the same in 1993 and 1994. Personal passive dosimetry was performed in 1993 and twice in 1994 on the day preceding blood sampling. About half of the 1993 samples were lost, so that five exposed and 13 control lymphocyte samples remained for analysis. The mean exposure level in 1994 was 1.76 ± 4.20 ppm (SD) [3.9 ± 9.3 mg/m^3] and tabulated individual exposure levels ranged from < 0.024 ppm to 10.2 ppm [0.053 and 22.6 mg/m^3]. Using the clonal assay (Tates *et al.*, 1994), the geometric mean of *hprt* mutation frequencies ($\times 10^{-6}$ ± SD) adjusted for cloning efficiency, age and smoking were, respectively, 7.85 ± 7.09 and 10.14 ± 9.16 in pooled (1993 plus 1994) exposed and control subjects. The difference was not significant. A similar result was obtained for the 1994 subjects alone. There was no difference between adjusted geometric mean mutation frequencies of exposed and unexposed non-smokers or between exposed and unexposed smokers.

Cytogenetic analysis of peripheral blood lymphocytes of butadiene production workers showed that occupational exposure to butadiene (median concentration, 1–3.5 ppm [2.2–7.7 mg/m^3]) did not induce chromosomal aberrations, micronuclei, sister chromatid exchanges, DNA strand breaks or alkali-labile sites (Comet assay). These results were obtained from workers in three butadiene production facilities in the United States (Legator *et al*., 1993; Kelsey *et al*., 1995; Hallberg *et al*., 1997), one in Portugal and one in the Czech Republic (Sorsa *et al*., 1994). Lymphocyte cultures from control and exposed subjects from two of these study groups were also irradiated with γ-rays in a challenge assay and chromosomal damage was assessed. The results indicated that butadiene exposure reduced DNA repair competence of the cells (Au *et al*., 1995; Hallberg *et al*., 1997).

As part of the same study in the Czech Republic factory described above (Tates *et al*., 1996), analysis of chromosomal aberrations in lymphocytes from 1994 subjects indicated that the percentage of aberrant cells was slightly, but significantly, enhanced in exposed subjects compared with the controls (3.11 ± 1.33 and 2.03 ± 1.53, respectively, $p < 0.01$), these data being very similar to those from the earlier study conducted in the same factory (Sorsa *et al*., 1994), which did not provide evidence for a clastogenic effect (2.9 ± 1.5 and 2.1 ± 1.4, respectively). Frequencies of micronuclei in cytochalasin-B blocked binucleate lymphocytes in 1994 exposed and unexposed workers were not significantly different and there was no evidence for differences in the levels of DNA damage, as provided by the single-cell gel electrophoresis assay.

4.4.2 *Experimental systems* (see Table 25 for references)

In all of the following tests, exposure was to gaseous butadiene unless otherwise indicated. Butadiene induced gene mutations in *Salmonella typhimurium* strains TA100 and TA1535 in the presence of phenobarbital- or 5,6-benzoflavone-induced rat liver S9. It was also weakly mutagenic to TA1535 in the presence of Aroclor 1254-induced rat liver S9, uninduced rat S9 or uninduced mouse S9, but was not mutagenic with uninduced human S9. Mutations were induced in strain TA1530 in the presence of phenobarbital- or Aroclor 1254-induced rat liver S9 but not uninduced S9. Butadiene was not mutagenic to other *Salmonella* strains or to *Escherichia coli*.

Butadiene did not induce somatic cell mutation and recombination or sex-linked recessive lethal mutation in *Drosophila melanogaster*.

Butadiene did not cause DNA single-strand breaks in mouse alveolar macrophage cultures, and was not active in the L5178Y mouse lymphoma ($tk^{+/-}$) assay. A weak positive response was reported for induction of sister chromatid exchanges in Chinese hamster ovary CHO cells exposed to butadiene dissolved in ethanol in the presence of Aroclor 1254-induced rat liver S9. In the same laboratory, sister chromatid exchanges were induced weakly in human whole blood lymphocytes after butadiene dissolved in ethanol was added to the culture medium in the presence or in the absence of Aroclor-1254-induced rat liver S9. In a second study, in which S9 from a variety of sources including mouse and human was used, no sister chromatid exchange was induced in human lymphocyte cultures after exposure to gaseous butadiene.

Table 25. Genetic and related effects of butadiene

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1300 ppm	Arce *et al*. (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	1080 ppm	Araki *et al*. (1994)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	–	+	86 ppm	de Meester *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	(+)	650 ppm	Arce *et al*. (1990)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	216 ppm	Araki *et al*. (1994)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1080 ppm	Araki *et al*. (1994)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1300 ppm	Arce *et al*. (1990)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1080 ppm	Araki *et al*. (1994)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	1300 ppm	Arce *et al*. (1990)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	1080 ppm	Araki *et al*. (1994)
DMM, *Drosophila melanogaster*, somatic mutation or recombination	–		10000 ppm inh	Victorin *et al*. (1990)
DMX, *Drosophila melanogaster*, sex-linked recessive leathal mutations	–		500 ppm inh	Foureman *et al*. (1994)
DIA, Single-strand breaks, NMRI mouse alveolar macrophages *in vitro*	–	NT	40 ppm	Walles *et al*. (1995)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	650 ppm	McGregor *et al*. (1991)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	(+)	1.35	Sasiadek *et al*. (1991a)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	–	2160 ppm	Arce *et al*. (1990)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	+	108	Sasiadek *et al*. (1991b)
DVA, DNA cross-links, B6C3F$_1$ mouse liver *in vivo*	+		450 ppm inh 7 h	Jelitto *et al*. (1989)
DVA, DNA cross-links, B6C3F$_1$ mouse liver *in vivo*	–		2070 ppm inh 8 h/d, 7 d	Ristau *et al*. (1990)
DVA, DNA cross-links, B6C3F$_1$ mouse lung, liver *in vivo*	+		250 ppm inh 7 h	Vangala *et al*. (1993)
DVA, DNA single-strand breaks, B6C3F$_1$ mouse liver *in vivo*	+		2000 ppm inh 7 h/d, 7 d	Vangala *et al*. (1993)
DVA, DNA single-strand breaks, NMRI mouse lung and liver *in vivo*	+		200 ppm inh 16 h	Walles *et al*. (1995)
DVA, DNA cross-links, Sprague-Dawley rat liver *in vivo*	–		550 ppm inh 7 h	Jelitto *et al*. (1989)
DVA, DNA cross-links, Sprague-Dawley rat liver *in vivo*	–		1240 ppm inh 8 h/d, 7 d	Ristau *et al*. (1990)
DVA, DNA cross-links, Sprague-Dawley rat liver, lung *in vivo*	–		2000 ppm inh 7 h	Vangala *et al*. (1993)
DVA, DNA single-strand breaks, Sprague-Dawley rat liver *in vivo*	+		2000 ppm inh 7 h/d, 7 d	Vangala *et al*. (1993)

Table 25 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
DVA, 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)
DVA, DNA damage, CD-1 mouse testicular cells *in vivo*	+[c]		125 ppm inh 6 h	Brinkworth *et al.* (1998)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		4000[c] inh	Arce *et al*. (1990)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		4000[d] inh	Arce *et al*. (1990)
UVM, Unscheduled DNA synthesis, B6C3F$_1$ mouse hepatocytes *in vivo*	–		11600[c] inh	Arce *et al*. (1990)
UVM, Unscheduled DNA synthesis, B6C3F$_1$ mouse hepatocytes *in vivo*	–		11600[d] inh	Arce *et al*. (1990)
GVA, Gene mutation, *lac*Z mouse bone marrow *in vivo*	+		625 ppm inh 6 h/d, 5 d/wk, 1 wk	Recio *et al*. (1992)
GVA, 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)
GVA, 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)
GVA, Gene mutation, *lac*I mice *in vivo*	+		62.5 ppm inh 6 h/d, 5 d/wk, 4 wk	Sisk *et al*. (1994)
GVA, 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)
GVA, Gene mutation, *lac*I mice *in vivo*	+		1250 ppm inh 6 h/d, 5 d/wk, 4 wk	Recio & Meyer (1995)
GVA, Gene mutation, (102/E1 × C3H/E1)F$_1$ mouse splenocytes, *hprt* locus *in vivo*	+[c]		500 ppm inh 6 h/d, 5 d	Tates *et al.* (1998)
GVA, 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)
MST, Mouse spot test, female T-stock mice	+		500 ppm inh 6 h/d, 5 d/wk, 1 wk	Adler *et al*. (1994)
SVA, Sister chromatid exchange, B6C3F$_1$ mouse bone marrow *in vivo*	+		116 ppm inh 6 h	Cunningham *et al*. (1986)

Table 25 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SVA, Sister chromatid exchange, Sprague-Dawley rat bone marrow *in vivo*	–		4000 ppm inh 6 h	Cunningham *et al*. (1986)
SVA, 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)
MVM, Micronucleus test, B6C3F_1 mouse bone marrow *in vivo*	+		116 ppm inh 6 h	Cunningham *et al*. (1986)
MVM, Micronucleus test, B6C3F_1 mouse peripheral blood *in vivo*	+		70 ppm inh 6 h/d, 5 d/wk, 2 wk	Tice *et al*. (1987)
MVM, Micronucleus test, B6C3F_1 mouse peripheral blood *in vivo*	+		7 ppm inh 6 h/d, 5 d/wk, 13 wk	Jauhar *et al*. (1988)
MVM, Micronucleus test, NMRI mouse bone marrow *in vivo*	+		35 ppm inh 23 h	Victorin *et al*. (1990)
MVM, Micronucleus test, CB6F_1 mice *in vivo*	+		50 ppm inh 6 h/d, 5 d/wk	Autio *et al*. (1994)
MVM, Micronucleus test, (102/E1 × C3H/E1)F_1 mice *in vivo*	+		50 ppm inh 6 h/d, 5 d/wk	Adler *et al*. (1994)
MVM, Micronucleus test, (102 × C3H) mice *in vivo*	+		200 ppm inh 6 h/d, 5 d/wk	Xiao & Tates (1995)
MVM, Micronucleus test, (102/E1 × C3H/E1)F_1 mouse splenocytes *in vivo*	+		130 ppm inh 6 h/d, 5 d	Stephanou *et al.* (1998)
MVM, Micronucleus test (102/E1 × C3H/E1)F_1 mouse spermatids *in vivo*	+		250 ppm inh 6 h/d, 5 d	Tommasi *et al.* (1998)
MVR, Micronucleus test, Sprague-Dawley rat bone marrow *in vivo*	–		4000 ppm inh 6 h/d, 2 d	Cunningham *et al*. (1986)
MVR, Micronucleus test, Sprague-Dawley rats *in vivo*	–		500 ppm 6 h/d, 5 d/wk	Autio *et al*. (1994)
CBA, Chromosomal aberrations, B6C3F_1 mouse bone marrow *in vivo*	+		1500 ppm inh 6 h	Irons *et al*. (1987b)
CBA, Chromosomal aberrations, NIH mouse bone marrow *in vivo*	+		1500 ppm inh 6 h	Irons *et al*. (1987b)
CBA, Chromosomal aberrations, B6C3F_1 mouse bone marrow *in vivo*	+		700 ppm inh 6 h/d, 5 d/wk, 2 wk	Tice *et al*. (1987)
AVA, Aneuploidy, B6C3F_1 mouse bone marrow *in vivo*	–		1500 ppm inh 6 h	Irons *et al.* (1987b)
AVA, Aneuploidy, NIH mouse bone marrow *in vivo*	–		1500 ppm inh 6 h	Irons *et al.* (1987b)

Table 25 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
COE, Chromosomal aberrations, (102/E1 × C3H/E1)F_1 mouse embryo *in vivo*	+		130 ppm inh 6 h/d, 5 d	Pachierotti *et al.* (1998)
DLM, Dominant lethal test, male CD-1 mice	+		233 ppm inh 6 h/d, 5 d/wk, 1 wk	Morrissey *et al*. (1990)
DLM, Dominant lethal test, CD-1 mice	+		1250 ppm inh 6 h/d, 5 d/wk, 10 wk	Anderson *et al*. (1993)
DLM, Dominant lethal test, CD-1 mice	–		6250 ppm inh 6 h	Anderson *et al*. (1993)
DLM, 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)
DLM, Dominant lethal test (102/E1 × C3H/E1)F_1 mice	+		500 ppm inh 6 h/d, 5 d	Adler *et al.* (1998)
DLM, Dominant lethal test, CD-1 mice	+		65 ppm inh 6 h/d, 5 d/wk, 4 wk	Anderson *et al*. (1998)
DLM, Dominant lethal test, CD-1 mice	+		125 ppm inh 6 h/d, 5 d/wk, 10 wk	Brinkworth *et al*. (1998)
DLR, Dominant lethal test, Sprague-Dawley rats	–		1250 ppm inh 6 h/d, 5 d/wk, 10 wk	Anderson *et al.* (1998)
MHT, Mouse (C3H/E1) heritable translocation test	+		1300 ppm inh 6 h/d, 5 d/wk, 1 wk	Adler *et al*. (1995)
MHT, Mouse (102/E1 × C3H/E1)F_1 heritable translocation test	+		500 ppm inh 6 h/d, 5 d	Adler *et al.* (1998)
BVD, Binding to DNA, male B6C3F_1 mouse or male Wistar rat liver *in vivo*	+		13 ppm inh 4–6.6 h	Kreiling *et al.* (1986b)
BVD, Binding to DNA at N7 of guanine, male B6C3F_1 mouse liver *in vivo*	+		450 ppm inh 7 h	Jelitto *et al*. (1989)
BVD, Binding to DNA at N7 of guanine, male B6C3F_1 mouse liver *in vivo*	+		NG	Bolt & Jelitto (1996)
BVD, 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)
BVD, Binding to DNA at N7 of guanine, male Wistar rat liver *in vivo*	–		550 ppm inh 7 h	Jelitto *et al*. (1989)

Table 25 (contd)

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

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

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw /day, inh, inhalation exposure; NG, not given

[c] Exposed 6 h on day 1, 3 h on day 2, livers sampled 2 h later

[d] Exposed 6 h on day 1 and 2, livers sampled 18 h later

DNA–DNA and DNA–protein cross-links were formed in the livers and lungs of mice exposed to butadiene at 250, 500 or 1000 ppm [550, 1100 or 2200 mg/m^3] for 7 h. Exposures of up to 2000 ppm [4400 mg/m^3] for 8 h per day for seven days did not induce cross-links in the liver or lung DNA of rats. Single-strand breaks were induced in mouse and rat liver DNA following exposure to 2000 ppm for 7 h per day for seven days and in mouse lung and liver following a 16-h exposure to 200 ppm [440 mg/m^3] of butadiene.

Unscheduled DNA synthesis was not evident in the livers of Sprague-Dawley rats or B6C3F$_1$ mice after exposure to 10 000 ppm [22 000 mg/m^3] butadiene for 6 h per day for two days.

Mutations were induced at the *hprt* locus in mice exposed to butadiene for 6 h per day on five days per week at 625 ppm [1380 mg/m^3] for two weeks or at 1300 ppm [2760 mg/m^3] for one week. Butadiene was mutagenic in the mouse spot test (500 ppm [1100 mg/m^3] 6 h per day for five days) and in two transgenic mouse models. Exposure to 62.5 or 1250 ppm [138 or 2760 mg/m^3] butadiene for 6 h per day on five days per week for four weeks increased the frequency of mutations induced at A:T base pairs in bone marrow of *lac*I mice, while exposure to 625 ppm for 6 h per day for five days increased the *lacZ* mutation frequency in lung but not liver or bone marrow of the MutaMouse®.

Butadiene increased the frequency of sister chromatid exchanges and micronuclei in mouse but not rat bone marrow. Micronucleus frequency also increased in peripheral erythrocytes and splenocytes. Butadiene also induced chromosomal aberrations in mouse bone marrow, and dominant lethal mutations, heritable translocations and sperm-head abnormalities in mice. It did not induce aneuploidy in bone marrow cells *in vivo*.

In a study by Sisk *et al.* (1994), male B6C3F$_1$ *lacI* transgenic mice were exposed by inhalation to 0, 62.5, 625 or 1250 ppm [0, 138, 1380 or 2760 mg/m^3] butadiene for four weeks (6 h per day, five days per week). Animals were killed 14 days after the last exposure and *lacI* mutants were recovered from the DNA according to established protocols. A 2.5- and 3-fold increase in the *lacI* mutant frequency was observed in the bone marrow of mice exposed to 625 or 1250 ppm butadiene, respectively, compared with air-exposed control mice. DNA sequence analysis of *lacI* mutants recovered from the bone marrow of mice exposed to 625 ppm butadiene demonstrated that there was a shift in the spectrum of base substitution mutations at A:T base pairs in butadiene-exposed mice (6/26, 23%), compared to air control mice (2/45, 4%). Recio and Meyer (1995) examined the *lacI* mutational spectrum in the bone marrow of mice exposed to 1250 ppm butadiene in the above study. DNA sequence analysis of *lacI* mutants revealed an increase in mutations at A:T base pairs (9/49, 20%) similar to that observed by Sisk *et al.* (1994).

Recio *et al.* (1998) also examined the *lacI* mutagenicity and mutational spectrum in the spleen of mice exposed to butadiene in the above study. The authors reported three- and four-fold increases in the *lacI* mutant frequency in mice exposed to 625 or 1250 ppm butadiene, respectively, compared with air control mice. DNA sequence analysis of *lacI* mutants recovered from the spleen of mice exposed to 1250 ppm butadiene once again revealed an increase in mutations at A:T base pairs (10/57, 18%) in butadiene-exposed mice compared with air control mice (3/41, 7%). In addition, an increased frequency of

G:C→A:T transitions occurred at non-5′CpG-3′ sites in butadiene-exposed mice. The increased frequency of specific mutations at G:C base pairs was not observed in bone marrow from the same animals; there seem therefore to be tissue-specific differences in the butadiene mutational spectrum.

To examine the effect of exposure time on the *lacI* mutant frequency in butadiene-exposed mice, Recio *et al.* (1996) exposed male B6C3F$_1$ *lacI* transgenic mice by inhalation to 625 or 1250 ppm butadiene for 6 h per day for five days. Mice were killed 14 days following the last exposure and mutant frequency in the bone marrow was determined. The authors reported a five-fold increase in the *lacI* mutant frequency in mice exposed to 625 ppm butadiene compared with air control mice. These results demonstrated that there was little difference in the bone marrow *lacI* mutant frequency between a short-term exposure and the long-term exposure used in the previous study.

Butadiene metabolites

Epoxybutene

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 26 for references)

Epoxybutene was mutagenic to bacteria in the absence of an exogenous metabolic activation system. It did not induce DNA strand-breaks in mouse splenocytes nor unscheduled DNA synthesis in mouse or rat hepatocytes. In one study, it did increase the frequency of sister chromatid exchanges in Chinese hamster ovary CHO cells *in vitro*. However, it had no effect on sister chromatid exchanges or chromosomal aberrations in rat or mouse splenocytes, nor did it induce micronuclei in rat spermatids. Gene mutations at the *tk* and *hprt* loci were observed in human TK6 cells treated with epoxybutene and sister chromatid exchanges were induced in human lymphocyte cultures. In single studies, treatment with epoxybutene *in vivo* induced *hprt* mutations in mouse splenic T cells, and sister chromatid exchanges and chromosomal aberrations in mouse bone marrow. Micronucleus frequencies were also elevated in splenocytes and spermatids of mice and rats following in-vivo exposure to epoxybutene.

Epoxybutanediol

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental data* (see Table 27 for references)

In a single study, epoxybutanediol induced gene mutations in *Salmonella typhimurium* strain TA100 in the presence or absence of an exogenous metabolic activation system. It did not induce micronuclei in Sprague-Dawley rat spermatids *in vitro*.

A marginal response was reported for induction of micronuclei in the bone marrow of rats exposed for 48 h to epoxybutanediol. A positive response was observed in the

Table 26. Genetic and related effects of epoxybutene

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	350	de Meester *et al*. (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	26	Gervasi *et al*. (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	(+)	175	Adler *et al*. (1997)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	+	NT	175	de Meester *et al*. (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	1750	de Meester *et al*. (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	140	Thier *et al*. (1995)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	NT	8750	de Meester *et al*. (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	8750	de Meester *et al*. (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	8750	de Meester *et al*. (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	105	Gervasi *et al*. (1985)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	+	NT	NG	Hemminki *et al*. (1980)
KPF, *Klebsiella pneumonia*, forward mutation	+	NT	70	Voogd *et al*. (1981)
DIA, DNA single-strand breaks, CD-1 mouse splenocytes *in vitro*	–	NT	65	Kligerman *et al*. (1996)
DIA, DNA single-strand breaks, CD rat splenocytes *in vitro*	–	NT	65	Kligerman *et al*. (1996)
URP, Unscheduled DNA synthesis, Sprague-Dawley rat hepatocytes *in vitro*	–	NT	1000	Arce *et al*. (1990)

Table 26 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
UIA, Unscheduled DNA synthesis, B6C3F$_1$ mouse hepatocytes *in vitro*	–	NT	1000	Arce *et al*. (1990)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	0.07	Sasiadek *et al*. (1991a)
SIM, Sister chromatid exchange, CD-1 mouse splenocytes *in vitro*	–	NT	65	Kligerman *et al*. (1996)
SIR, Sister chromatid exchange, CD rat splenocytes *in vitro*	–	NT	65	Kligerman *et al*. (1996)
MIA, Micronucleus test, Sprague-Dawley rat spermatids *in vitro*	–	NT	70	Sjoblom & Lahdetie (1996)
CIM, Chromosomal aberrations, CD-1 mouse splenocytes *in vitro*	–	NT	65	Kligerman *et al*. (1996)
CIR, Chromosomal aberrations, CD rat splenocytes *in vitro*	–	NT	65	Kligerman *et al*. (1996)
GIH, Gene mutation, human TK6 cells, *tk* locus *in vitro*	+	NT	17.5	Cochrane & Skopek (1993)
GIH, Gene mutation, human TK6 cells, *hprt* locus *in vitro*	+	NT	10.5	Cochrane & Skopek (1993)
GIH, Gene mutation, human TK6 cells, *hprt* locus *in vitro*	+	NT	28	Steen *et al*. (1997b)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	1.75	Sasiadek *et al*. (1991b)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	35	Wiencke & Kelsey (1993)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	3.5	Uuskula *et al*. (1995)
DVA, DNA strand breaks, CD-1 mouse testis *in vivo*	(+)		120 ip × 1	Anderson *et al*. (1997)

Table 26 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
DVA, DNA strand breaks, Sprague-Dawley rat bone marrow *in vivo*	+		80 ip × 1	Anderson *et al.* (1997)
GVA, Gene mutation, B6C3F$_1$ mouse splenic T cells, *hprt* locus *in vivo*	+		100 ip × 3	Cochrane & Skopek (1993)
GVA, Gene mutation, B6C3F$_1$ mouse splenic T cells, *hprt* locus *in vivo*	+		60 ip × 3	Cochrane & Skopek (1994)
GVA, Gene mutation, (102/El × C3H/El)F$_1$ mouse splenocytes, *hprt* locus *in vivo*	+[c]		100 ip × 3	Tates *et al.* (1998)
GVA, Gene mutation, (102/El × C3H/El)F$_1$ mouse splenocytes, *hprt* locus *in vivo*	–		100 ip × 1	Tates *et al.* (1998)
GVA, Gene mutation, Lewis rat splenocytes, *hprt* locus *in vivo*	–		100 ip × 1	Tates *et al.* (1998)
SVA, Sister chromatid exchange, C57BL/6 mouse bone marrow *in vivo*	+		25 ip × 1	Sharief *et al.* (1986)
MVM, Micronucleus test, (102 × C3H)F$_1$ mouse splenocytes *in vivo*	+		40 ip × 1	Xiao & Tates (1995)
MVM, Micronucleus test, (102 × C3H)F$_1$ mouse spermatids *in vivo*	+		40 ip × 1	Xiao & Tates (1995)
MVM, Micronucleus test, (102/El × C3H/El)F$_1$ mouse bone marrow *in vivo*	+		20 ip × 1	Adler *et al.* (1997)
MVM, Micronucleus test, CD-1 mouse bone-marrow *in vivo*	+		40 ip × 1	Anderson *et al.* (1997)
MVM, Micronucleus test, BALB/c mouse lymphocytes *in vivo*	+		25 ip × 1	Russo *et al.* (1997)
MVM, Micronucleus test, BALB/c mouse spermatids *in vivo*	(+)		73 ip × 1	Russo *et al.* (1997)
MVR, Micronucleus test, Lewis rat spermatids *in vivo*	+		40 ip × 1	Xiao & Tates (1995)
MVR, Micronucleus test, Lewis rat splenocytes *in vivo*	+		80 ip × 1	Xiao & Tates (1995)
MVR, Micronucleus test, Sprague-Dawley rat bone-marrow *in vivo*	(+)		120 ip × 1	Anderson *et al.* (1997)

Table 26 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
MVR, Micronucleus test, Sprague-Dawley rat spermatids *in vivo*	+		13 ip × 1	Lahdetie *et al*. (1997)
MVR, Micronucleus test, Sprague-Dawley rat bone marrow *in vivo*	–		78 ip × 1	Lahdetie & Grawe (1997)
CBA, Chromosomal aberrations, C57BL/6 mouse bone marrow *in vivo*	+		25 ip × 1	Sharief *et al*. (1986)
DLM, Dominant lethal test, (102/El × C3H/El)F_1 mice *in vivo*	–		120 ip × 1	Adler *et al*., 1997
BID, Binding (covalent) to DNA, salmon testis *in vitro*	+	NT	NG	Citti *et al*. (1984)
BID, Binding (covalent) to DNA, calf thymus *in vitro*	+	NT	21 700	Tretyakova *et al*. (1997)

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

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw /day; ; ip, intraperitoneal; NG, not given

Table 27. Genetic and related effects of epoxybutanediol

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	100	Adler *et al*. (1997)
MIA, Micronucleus test, Sprague-Dawley rat spermatocytes treated, spermatids scored *in vitro*	–	NT	10	Sjoblom & Lahdetie (1996)
MVM, Micronucleus test, (102/El × C3H/El)F_1 mouse bone marrow *in vivo*	+		120 ip × 1	Adler *et al*. (1997)
MVR, Micronucleus test, Sprague-Dawley rat bone marrow *in vivo*	(+)		30 ip × 1	Lahdetie & Grawe (1997)
MVR, Micronucleus test, Sprague-Dawley rat spermatogonia treated *in vivo*, spermatids scored	–		30 ip × 1	Lahdetie *et al*. (1997)
MVR, Micronucleus test, Sprague-Dawley rat spermatocytes treated *in vivo*, spermatids scored	+		30 ip × 1	Lahdetie *et al*. (1997)
DLM, Dominant lethal test, (102/El × C3H/El)F_1 mice *in vivo*	–		240 ip × 1	Adler *et al*. (1997)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw /day; ip, intraperitoneal dose

spermatids after treatment of rat spermatocytes, but not of spermatogonia. Epoxybutanediol induced micronuclei in (102/E1 × C3H/E1)F_1 mouse bone marrow samples 24 h after intraperitoneal injection, but no dominant lethal effects were induced in mice.

Diepoxybutane

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 28 for references)

Diepoxybutane was genotoxic *in vitro* without the addition of exogenous metabolic activation. In bacteria, it induced prophage, DNA repair and gene mutations (positive in *Salmonella* strains TA100 and TA1535 but not TA1537, TA1538 or TA98). The insertion of a rat GST 5-5+ or human GSTT1 plasmid vector in TA1535 increased the activity of diepoxybutane as much as 10-fold. It also induced mutation, gene conversion and mitotic recombination and crossing-over in yeast and reverse mutation in fungi. Diepoxybutane caused both somatic and sex-linked recessive lethal mutations as well as small chromosomal deletions and heritable translocations in *Drosophila melanogaster*.

Costa *et al*. (1997) reported that DNA–protein cross-links were produced by diepoxybutane in cultured human lymphoma cells. DNA cross-links were induced in mouse hepatocytes, but DNA strand breaks and/or alkali-labile sites were not detected in mouse or rat splenocytes *in vitro*. Unscheduled DNA synthesis was induced in Syrian hamster but not rat primary hepatocytes. Diepoxybutane enhanced gene mutations in Chinese hamster ovary CHO and lung V79 cells (*hprt* locus) and in mouse lymphoma L5187Y cells at the *tk* locus. It induced dose-related increases in the frequency of sister chromatid exchanges in CHO cells and in mouse and rat splenocyte cultures and, in a single study, it induced micronuclei in rat spermatids *in vitro*. It also induced chromosomal aberrations in rat and mouse splenocytes and in rat liver epithelial cell cultures. Gene mutations at the *tk* and *hprt* loci were induced in human TK6 cell cultures and dose-related increases were induced by diepoxybutane in sister chromatid exchanges in cultures of human lymphocytes from healthy donors and from patients with a variety of solid tumours, but not from Fanconi's anaemia homozygotes or heterozygotes. A bimodal distribution of sensitivity to induction of sister chromatid exchanges by diepoxybutane was observed in lymphocytes from healthy donors: lymphocyte populations from donors with GSTT1 null genotype showed greater sensitivity to diepoxybutane than those from donors with the GSTT1 gene. No correlation was seen between GSTM1 genotype and sister chromatid exchange induction by diepoxybutane. Chromosomal aberrations were induced in cultures of skin fibroblasts from Fanconi's anaemia heterozygotes, in primary lymphocytes from Fanconi's anaemia homo- and heterozygotes, and in lymphoblastoid cell lines from normal donors, Fanconi's anaemia homo- and heterozygotes and patients with xeroderma pigmentosum and ataxia telangiectasia. Positive results were also reported in one of two studies using lymphocytes from healthy donors. Diepoxybutane caused a weak increase in the frequencies of chromosomal

Table 28. Genetic and related effects of diepoxybutane

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
PRB, Prophage, induction, *Bacillus megaterium*	+	NT	NG	Lwoff (1953)
PRB, Prophage, induction, *Pseudomonas pyocyanea*	+	NT	NG	Lwoff (1953)
PRB, Prophage induction, *Escherichia coli* K-12	+	NT	7.5	Heinemann & Howard (1964)
ECB, *Escherichia coli* H540, DNA repair induction	+	NT	2500	Thielmann & Gersbach (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	(+)	50	Dunkel *et al*. (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	20	Gervasi *et al*. (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	–	38	Zeiger & Pagano (1989)
SA0, *Salmonella typhimurium*, TA100, reverse mutation	+	+	26	Adler *et al*. (1997)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	25	McCann *et al*. (1975)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	5	Rosenkranz & Poirier (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	5	Dunkel *et al*. (1984)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	2.5	Zeiger & Pagano (1989)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	4.3	Thier *et al*. (1995)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	8.6	Thier *et al*. (1996)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	167	Dunkel *et al*. (1984)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	50	Rosenkranz & Poirier (1979)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	167	Dunkel *et al*. (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	167	Dunkel *et al*. (1984)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	60	Gervasi *et al*. (1985)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	(+)	(+)	167	Dunkel *et al*. (1984)
ECR, *Escherichia coli* B, reverse mutation	+	NT	1720	Glover (1956)
ECR, *Escherichia coli* B/r, reverse mutation	+	NT	860	Glover (1956)
KPF, *Klebsiella pneumonia*, fluctuation test	+	NT	4	Voogd *et al*. (1981)
SCH, *Saccharomyces cerevisiae* D4, mitotic gene conversion	+	NT	430	Zimmermann (1971)
SCH, *Saccharomyces cerevisiae* D81, mitotic crossing-over	+	NT	2000	Zimmermann & Vig (1975)
SCH, *Saccharomyces cerevisiae* D3, mitotic recombination	+	+	400	Simmon (1979)
SCH, *Saccharomyces cerevisiae* D7, gene conversion	+	+	130	Sandhu *et al.* (1984)
SCH, *Saccharomyces cerevisiae* D7, mitotic crossing-over	+	+	130	Sandhu *et al.* (1984)
SCF, *Saccharomyces cerevisiae*, mitochondrial mutation	+	NT	4000	Polakowska & Putrament (1979)
SCF, *Saccharomyces cerevisiae*, cytoplasmic petite mutation	–	NT	4000	Polakowska & Putrament (1979)
SCR, *Saccharomyces cerevisiae*, reverse mutation	+	NT	4000	Polakowska & Putrament (1979)
SCR, *Saccharomyces cerevisiae* D7, reverse mutation	+	+	130	Sandhu *et al.* (1984)
NCR, *Neurospora crassa*, reverse mutation	+	NT	1720	Pope *et al*. (1984)
NCR, *Neurospora crassa*, reverse mutation	+	NT	4300	Kolmark & Westergaard (1953)
DMM, *Drosophila melanogaster*, somatic mutation	+		430 feed	Olsen & Green (1982)
DMM, *Drosophila melanogaster*, somatic mutation	+		1000 feed	Graf *et al*. (1983)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		100 inj	Bird & Fahmy (1953)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		1000 inj	Fahmy & Fahmy (1970)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		175 feed	Sankaranarayanan *et al*. (1983)
DMC, *Drosophila melanogaster*, chromosome aberrations	+		1000 inj	Fahmy & Fahmy (1970)
DMH, *Drosophila melanogaster*, heritable translocations	+		1000 inj	Denell *et al*. (1978)
DIA, DNA–DNA cross-links, B6C3F_1 mouse liver DNA *in vitro*	+	NT	4	Ristau *et al*. (1990)
DIA, DNA single-strand breaks, male CD rat and male CD-1 mouse splenocytes *in vitro*	–	NT	13.7	Kligerman *et al*. (1996)
URP, Unscheduled DNA synthesis, male Sprague-Dawley rat primary hepatocytes *in vitro*	–	NT	8.6	Kornbrust & Barfknecht (1984)
UIA, Unscheduled DNA synthesis, Syrian hamster hepatocytes *in vitro*	+	NT	0.86	Kornbrust & Barfknecht (1984)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	+	NT	2.15	Zhu & Zeiger (1993)
G9H, Gene mutation, Chinese hamster V79 cells, *hprt* locus *in vitro*	(+)	NT	2	Nishi *et al*. (1984)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	0.3	McGregor *et al*. (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	0.025	Perry & Evans (1975)
SIC, Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+	NT	0.1	Nishi *et al*. (1984)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	0.01	Sasiadek *et al*. (1991a)
SIM, Sister chromatid exchange, CD-1 mouse splenocytes *in vitro*	+	NT	0.43	Kligerman *et al*. (1996)
SIR, Sister chromatid exchange, CD rat splenocytes *in vitro*	+	NT	0.86	Kligerman *et al*. (1996)
MIA, Micronucleus test, rat spermatids (spermatocytes treated) *in vitro*	+	NT	0.43	Sjoblom & Lahdetie (1996)
CIM, Chromosomal aberrations, CD-1 mouse splenocytes *in vitro*	+	NT	3.44	Kligerman *et al*. (1996)
CIR, Chromosomal aberrations, Carworth Farm E rat liver epithelial (RL_1) cells *in vitro*	+	NT	0.1	Dean & Hodson-Walker (1979)
CIR, Chromosomal aberrations, CD rat splenocytes *in vitro*	+	NT	6.88	Kligerman *et al*. (1996)
TCM, Cell transformation, C3H 10T1/2 mouse cells *in vitro*	+	NT	0.0001	Nelson & Garry (1983)
TCL, Cell transformation, Syrian hamster lung epithelial M3E3/C3 cells *in vitro*	+	NT	0.009	Lichtenberg *et al*. (1995)
GIH, Gene mutation, human TK6 cells, *tk* locus *in vitro*	+	NT	0.2	Cochrane & Skopek (1993)
GIH, Gene mutation, human TK6 cells, *hprt* locus *in vitro*	+	NT	0.3	Cochrane & Skopek (1993)
GIH, Gene mutation, human TK6 cells, *hprt* locus *in vitro*	+	NT	0.34	Steen *et al*. (1997a)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.125	Wiencke *et al*. (1982)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.01	Porfirio *et al*. (1983)
SHL, Sister chromatid exchange, human lymphocytes[c] *in vitro*	–	NT	0.01	Porfirio *et al*. (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.04	Sasiadek *et al*. (1991b)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+[d]	NT	0.13	Wiencke *et al*. (1991)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+[e]	NT	0.17	Landi *et al*. (1995)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+[e]	NT	0.17	Norppa *et al*. (1995)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+[e]	NT	0.5	Wiencke *et al*. (1995)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.17	Landi *et al*. (1996a,b)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.172	Pelin *et al*. (1996)
MIH, Micronucleus test, human blood lymphotyctes *in vitro*	+	NT	172	Vlachadimitropoulos *et al*. (1997)
CHF, Chromosome aberrations, human skin fibroblasts[f] *in vitro*	+	NT	0.01	Auerbach & Wolman (1976)
CHF, Chromosome aberrations, human skin fibroblasts *in vitro*	–	NT	0.01	Auerbach & Wolman (1976)
CHL, Chromosome aberrations, human lymphocytes[g] *in vitro*	+	NT	0.01	Cohen *et al*. (1982)
CHL, Chromosome aberrations, human lymphocytes *in vitro*	(+)	NT	0.1	Marx *et al*. (1983)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
CHL, Chromosome aberrations, human lymphocytes[c] *in vitro*	+	NT	0.1	Marx *et al*. (1983)
CHL, Chromosome aberrations, human lymphocytes *in vitro*	–	NT	0.01	Porfirio *et al*. (1983)
CHL, Chromosome aberrations, human lymphocytes[c] *in vitro*	+	NT	0.01	Porfirio *et al*. (1983)
CHL, Chromosome aberrations, human lymphocytes *in vitro*	+	NT	0.5	Wiencke *et al*. (1991)
CIH, Chromosome aberrations, human bone-marrow cells *in vitro*	(+)	NT	0.1	Marx *et al*. (1983)
CIH, Chromosome aberrations, human bone-marrow cells[c] *in vitro*	(+)	NT	0.1	Marx *et al*. (1983)
HMM, Host-mediated assay, reverse mutation in *Salmonella typhimurium* TA1530 in Swiss-Webster mice	+		444 im	Simmon *et al*. (1979)
HMM, Host-mediated assay, mitotic recombination in *Saccharomyces cerevisiae* D3 in Swiss-Webster mice	–		56 po	Simmon *et al*. (1979)
DVA, DNA single-strand breaks, male CD-1 mouse bone marrow and estis *in vivo*	+		15 ip × 1	Anderson *et al*. (1997)
DVA, DNA single-strand breaks, male Sprague-Dawley rat bone marrow *in vivo*	(+)		50 ip × 1	Anderson *et al*. (1997)
GVA, Gene mutation, B6C3F$_1$ mice, splenic T cells, *hprt* locus *in vivo*	+		21 ip × 3	Cochrane & Skopek (1993)
GVA, Gene mutation, B6C3F$_1$ mice, splenic T cells, *hprt* locus *in vivo*	+		7 ip × 3	Cochrane & Skopek (1994)
GVA, Gene mutation, male Lewis rats *in vivo* (*hprt* locus)	–		40 ip × 1	Tates *et al*. (1998)
GVA, Gene mutation, male (102/El × C3H/El)F$_1$ mice *in vivo* (*hprt* locus)	–		40 ip × 1	Tates *et al*. (1998)
GVA, Gene mutation, C57BL mice *in vivo* (*hprt* locus)	–		14 ip × 3	Tates *et al*. (1998)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SVA, Sister chromatid exchange, Swiss-Webster mouse bone marrow *in vivo*	+		1 ip × 1	Conner *et al*. (1983)
SVA, Sister chromatid exchange, Swiss-Webster mouse alveolar macrophages *in vivo*	+		1 ip × 1	Conner *et al*. (1983)
SVA, Sister chromatid exchange, Swiss-Webster mouse regenerating liver cells *in vivo*	+		1 ip × 1	Conner *et al*. (1983)
SVA, Sister chromatid exchange, NMRI mouse bone-marrow cells *in vivo*	+		22 inh 2 h	Walk *et al*. (1987)
SVA, Sister chromatid exchange, NMRI mouse bone-marrow cells *in vivo*	+		29 ip × 1	Walk *et al*. (1987)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells *in vivo*	+		34 inh 2 h	Walk *et al*. (1987)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells *in vivo*	+		32 ip × 1	Walk *et al*. (1987)
MVM, Micronucleus test, $(102 \times C3H)F_1$ mouse splenocytes *in vivo*	+		15 ip × 1	Xiao & Tates (1995)
MVM, Micronucleus test, $(102 \times C3H)F_1$ mouse spermatids *in vivo*	(+)		30 ip × 1	Xiao & Tates (1995)
MVM, Micronucleus test, $(102/El \times C3H/El)F_1$ mouse bone marrow *in vivo*	+		9 ip × 1	Adler *et al*. (1995b)
MVM, Micronucleus test, male CD-1 mouse bone marrow *in vivo*	+		30 ip × 1	Anderson *et al*. (1997)
MVM, Micronucleus test, mouse spermatids and peripheral blood lymphocytes *in vivo*	+		15 ip × 1	Russo *et al*. (1997)
MVM, Micronucleus test, male $(102/El \times C3H/El)F_1$ mice *in vivo*	+		30 ip × 1	Tates *et al*. (1998)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
MVR, Micronucleus test, Lewis rat spermatids *in vivo*	+		20 ip × 1	Xiao & Tates (1995)
MVR, Micronucleus test, Lewis rat splenocytes *in vivo*	+		40 ip × 1	Xiao & Tates (1995)
MVR, Micronucleus test, male Sprague-Dawley rat bone marrow *in vivo*	+		25 ip × 1	Anderson *et al.* (1997)
MVR, Micronucleus test, Sprague-Dawley rat bone-marrow *in vivo*	+		17 ip × 1	Lahdetie & Grawe (1997)
MVR, Micronucleus test, Sprague-Dawley rat spermatids *in vivo*	+		16.7 ip × 1	Lahdetie *et al*. (1997)
CBA, Chromosomal aberrations, NMRI mouse bone marrow *in vivo*	+		22 inh 2 h	Walk *et al*. (1987)
CBA, Chromosomal aberrations, NMRI mouse bone marrow *in vivo*	+		29 ip × 1	Walk *et al*. (1987)
CBA, Chromosomal aberrations, Chinese hamster bone marrow *in vivo*	+		34 inh 2 h	Walk *et al*. (1987)
CBA, Chromosomal aberrations, Chinese hamster bone marrow *in vivo*	+		32 ip × 1	Walk *et al*. (1987)
COE, Chromosomal aberrations, zygotes of (102/El × C3H/El)F_1 mice *in vivo*	+		17 ip × 1	Adler *et al*. (1995)
DLM, Dominant lethal test, (102/El × C3H/El)F_1 mice *in vivo*	+		18 ip × 1	Adler *et al*. (1995b)
BID, Binding (covalent) to DNA, Chinese hamster ovary AA8 cells *in vitro*	+	NT	43	Leuratti *et al*. (1993)
BID, Binding (covalent) to DNA, CHO AA8 cells *in vitro* (adenine adduct N6)	+	NT	43	Leuratti *et al*. (1994)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	NT	3400	Mabon *et al*. (1996)
BVD, Binding (covalent) to DNA, female ICR mouse skin *in vivo*	+		6.5 skin paint	Mabon *et al*. (1996)
BVD, Binding (covalent) to DNA, female ICR mouse skin *in vivo*	+		60 skin paint	Mabon & Randerath (1996)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw /day; inj, injection; im, intramuscular; po, oral; ip, intraperitoneal; inh, inhalation
[c] Fanconi's anaemia (homozygotes and heterozygotes)
[d] Bimodal response, 24% positive, 76% negative; no correlation to GSTM1 deficiency
[e] Positive response correlates with GSTT1 deficiency
[f] Fanconi's anaemia (heterozygotes)
[g] Fanconi's anaemia (homozygotes and heterozygotes), ataxia telangiectasia, xeroderma pigmentosum, normal

aberrations in bone-marrow cultures from Fanconi's anaemia patients and normal individuals.

In the mouse host-mediated assay, diepoxybutane induced mutation in *S. typhimurium* TA1530 but did not induce mitotic recombination in *Saccharomyces cerevisiae* D3.

In a single study, gene mutations were induced at the *hprt* locus in splenic T cells of mice following intraperitoneal injection with diepoxybutane. Micronucleus frequencies were increased in splenocytes as well as spermatids of both mice and rats treated with a single intraperitoneal injection of diepoxybutane. Dose-related increases in the frequency of sister chromatid exchanges were observed in bone marrow and in alveolar macrophages from both intact and partially hepatectomized mice and in the regenerating livers of hepatectomized mice. Diepoxybutane also induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster and NMRI mouse bone-marrow following exposure by inhalation or intraperitoneal injection. In one study, chromosomal aberrations were observed in zygotes from matings of untreated female mice with male mice exposed to diepoxybutane seven days earlier. Negative results were reported for the 14–28-day mating periods, indicating that only early spermatozoa were affected. Dominant lethal mutations were also induced in this study. The dominant lethal effect was restricted to spermatozoa for the two lower doses and only late spermatids could be evaluated at the highest dose because the number of pregnancies during the first eight mating days following treatment was greatly reduced.

Mechanism of mutation induction

The mechanisms by which epoxybutene and diepoxybutane induce mutagenicity have been examined using human and *lacI* transgenic cells in culture. By determining the spectra of mutations induced, the contribution of each metabolite to the genotoxic properties of the parent butadiene can be assessed.

Steen *et al.* (1997a,b) assessed the mutagenicity of epoxybutene and diepoxybutane at *hprt* in human TK6 lymphoblastoid cells exposed to 400 μM (epoxybutene) or 4 μM (diepoxybutane) for 24 h. These concentrations of epoxybutene and diepoxybutane resulted in approximately 10% survival relative to media controls and induced a five-fold increase in *hprt* mutant frequency. Molecular analysis of epoxybutene-induced *hprt* mutants revealed an increase (39/50, 78%) in single-base substitution mutations compared with media controls (22/43, 51%), and a shift in the spectrum of base substitution mutations at A:T base pairs (21/50, 42%) compared with media controls (8/43, 18%). The most significant change was a five-fold increase in A:T→T:A transversions among the epoxybutene-induced mutants. The DNA sequence context of the mutations at A:T base pairs among the epoxybutene-induced mutants showed a strand bias; in 19/21 (90%) of these mutants, the A was located in the non-transcribed DNA strand. All of the epoxybutene-induced A:T→T:A transversions displayed this strand bias. Molecular analysis of diepoxybutane-induced *hprt* mutants revealed an increase in 5′ partial deletion of the *hprt* gene (7/51, 14%) compared with media controls (1/43, 2%). Diepoxybutane-induced mutants also had an increased frequency of A:T→T:A transversions (9/51, 18%) compared with media controls

(2/43, 5%), but with the opposite strand bias compared with epoxybutene; in 8/11 (78%) of these mutants the T was located in the non-transcribed strand.

Saranko and Recio (1998) examined the mutagenicity of diepoxybutane at the *lacI* gene in Rat2 *lacI* transgenic fibroblasts exposed to 0, 2, 5 or 10 μM for 24 h. These concentrations of diepoxybutane resulted in approximately 100, 30 and 10% survival respectively, compared with media controls. There was no significant increase in *lacI* mutant frequency following exposure to 2, 5 or 10 μM diepoxybutane. However, all three of these exposure concentrations resulted in significant increases in the formation of micronuclei (2-, 2.5-, and 3.5-fold, respectively) in Rat2 cells. These experiments demonstrated the insensitivity of the lambda shuttle vector-based *lacI* transgenic system to the clastogenic effects of diepoxybutane. The inability of diepoxybutane to induce a mutational response in the Rat 2 *lacI* transgenic fibroblasts is probably due to the poor recovery of deletions by the *lacI* transgenic assay.

The results of these in-vitro studies demonstrate that epoxybutene and diepoxybutane differ in their mutagenic potency and mechanism of action. Epoxybutene is effective at producing base substitutions as well as large deletions and micronuclei.

DNA adducts

Butadiene

After exposure of $B6C3F_1$ mice and Wistar rats for up to 6.6 h to [1,4-^{14}C]butadiene (uptake of 0.24 mmol/kg), Laib *et al*. (1990) found alkylation of DNA to be similar in both species and a level of alkylation of nuclear proteins which was about twice as high in mice as in rats.

Acid hydrolysis of hepatic DNA isolated from mice exposed to [^{14}C]butadiene yielded two alkylation products: 7-*N*-(hydroxy-3-buten-2-yl)guanine and 7-*N*-(2,3,4-trihydroxybutyl)guanine. These were not found in similarly exposed rats (Jelitto *et al.*, 1989; Bolt & Jelitto, 1996). DNA adducts at the N7 position of guanine were also detected using ^{32}P-postlabelling in $B6C3F_1$ mouse lung following inhalation exposure to 200 ppm [440 mg/m^3] butadiene for 6 h per day for five days and in lung and liver of Sprague-Dawley rats treated under the same exposure conditions (Koivisto *et al*., 1996, 1997).

CB6 F_1 mice [sex not indicated] were exposed (6 h per day for five days) to 0, 50, 200, 500 and 1300 ppm [0, 110, 440, 1100 and 2870 mg/m^3] butadiene. In addition, Wistar rats [sex not indicated] were exposed up to 500 ppm butadiene. Using a post-labelling assay, dose-dependent formation of epoxybutene adducts at N^6 of adenine was found in lung DNA of both species at the higher concentrations. The mean adduct levels (fmol adducts/100 nmol 3′-dAMP) were similar in mouse lung (up to about 2.6 at 500 ppm) and rat lung (up to about 2.3 at 500 ppm), mean background levels being 0.5 in mice and 0.7 in rats (Koivisto *et al*., 1996; Sorsa *et al*., 1996). Corresponding mean adduct levels in the liver DNA of the rats exposed to 500 ppm were about 30, whereas background levels were about 2 (Sorsa *et al*., 1996).

Enantio- and regioisomeric formation of the epoxybutene adduct at guanine N7 of liver DNA (7.2 fmol/10 μg DNA = 2.4 adducts/10^7 nucleotides) was determined in male

Sprague-Dawley rats exposed for five days (6 h per day) to 200 ppm [440 mg/m^3] butadiene. The relative formation of the different isomers were 47, 22, 18 and 14%, corresponding to the adducts derived from *R*-epoxybutene (C-2″, C-1″) and from *S*-epoxybutene (C-2″, C-1″), respectively (Koivisto *et al.*, 1997).

After single exposures (7 h) of male B6C3F$_1$ mice to butadiene (100–2000 ppm [220–4400 mg/m^3]), dose-dependent DNA–DNA and DNA–protein cross-link formation was suggested from alkaline-elution profiles, the effect being stronger in lung DNA than in liver DNA. No cross-linking activity was found in Sprague-Dawley rats similarly exposed to butadiene (Vangala *et al.*, 1993).

Metabolites

Epoxybutene reacts with free DNA bases, nucleosides and DNA to form covalent adducts. Citti *et al*. (1984) characterized adducts formed *in vitro* between epoxybutene and deoxyguanosine or DNA (pH 7.2). They found *N*7-(2′-hydroxy-3′-buten-1′-yl)-guanine and *N*7-(1′-hydroxy-3′-buten-2′-yl)guanine in ratios of 59:4 using the nucleosides and of 54:46 using DNA. These results together with those of later investigations are summarized in Table 29. Selzer and Elfarra (1996a,b, 1997a,b) determined the pseudo-first-order rate constants from the in-vitro reactions between epoxybutene and guanosine, adenosine, deoxycytidine and thymidine at pH 7.4 and 37°C; they ranged from 2.67×10^{-4} to 2.63×10^{-2} per hour. Comparison of these rate constants indicates that the order of adduct formation at the various sites on the bases is likely to be as follows: α and β *N*7-guanosine > β *N*^{6}-adenosine, β *N*3-deoxyuridine, β *N*3-deoxycytidine > α *N*^{1}- and α *N*^{2}-guanosine, α *N*^{6}-adenosine, α *N*^{1}-inosine > β *N*3-thymidine, β *O*^{2}-deoxycytidine, α *N*3-deoxyuridine > α *N*3-thymidine. Thus, the pseudo-first-order constants suggest that the *N*3-thymidine adducts are among the least abundant under these in-vitro conditions. This order of formation may or may not be replicated in reactions of epoxybutene with DNA, where the molecular structure and hydrogen bonding at various sites may modify reactivity. The finding that thymidine adducts are likely to be less abundant than other adducts does not necessarily exclude them as mutagenic precursors, since analysis of *lacI* mutants from the bone marrow of B6C3F$_1$ mice exposed to butadiene showed an increase in mutations at A:T base pairs, with A:T→T:A transversions apparently occurring only in exposed mice (Sisk *et al.*, 1994).

Diepoxybutane also reacts with nucleosides, nucleotides and DNA. Adducts at N^6 of adenine were identified in incubations (pH 7) containing deoxyadenosine, deoxyadenosine monophosphate or poly(dA-dT)(dA-dT), as determined by mass spectrometry, or calf thymus DNA as determined by a high-performance liquid chromatography/^{32}P-postlabelling method (Leuratti *et al*., 1994). By the latter method, the authors demonstrated adduct formation to N^6 of adenine in DNA from Chinese hamster ovary cells incubated with diepoxybutane at 37°C.

In calf thymus DNA incubated with diepoxybutane, *N*7-(2′-hydroxy-3′,4′-epoxybut-1′-yl)guanine (Tretyakova *et al*., 1997b) and *N*7-(2′,3′,4′-trihydroxybut-1′-yl)guanine (Tretyakova *et al*., 1996, 1997b) [enantiomers not resolved] were formed, as characterized

Table 29. Reactivity of epoxybutene with DNA bases *in vitro*

Targets	Adducts formed	Kinetics	Comments (References)
Deoxyguanosine; Salmon testis DNA type III	*N*7-(2-Hydroxy-3-buten-1-yl)guanosine (I) *N*7-(1-Hydroxy-3-buten-2-yl)guanosine (II)	Half-lives of spontaneous depurination of I and II in DNA 50 h (pH 7.2; 37 °C).	NMR, MS HPLC, UV (Citti *et al.*, 1984)
Guanosine; Deoxyguanosine; Calf thymus DNA	Diastereomeric pairs of *N*7-(2-Hydroxy-3-buten-1-yl)guanosine (I) *N*7-(1-Hydroxy-3-buten-2-yl)guanosine (II)	Half-lives of spontaneous depurination of I and II in DNA 48 h (pH 7.4).	HPLC, UV, ECD, NMR, MS (Neagu *et al.*, 1995)
Deoxyadenosine; Deoxyadenosine monophosphate; Calf thymus DNA	Diastereomeric pairs of N^6-(1-Hydroxy-3-buten-2-yl)adenosine (III) N^6-(2-Hydroxy-3-buten-1-yl)adenosine (IV)		HPLC/^{32}P-post-labelling, MS/MS, CD, NMR (Koivisto *et al.*, 1995, 1996)
Guanosine	Diastereomeric pairs of *N*7-(2-Hydroxy-3-buten-1-yl)guanosine (I) *N*7-(1-Hydroxy-3-buten-2-yl)guanosine (II) N^2-(1-Hydroxy-3-buten-2-yl)guanosine (III) N^1-(1-Hydroxy-3-buten-2-yl)guanosine (IV)	Pseudo-first-order formation rate constant (pH 7.4; 37°C) at N7 about 10-fold higher than at N2 or N1. Half-lives of decomposition (pH 7.4; 37°C) of I 50 h, of II 90 h. III and IV stable up to 192 h (pH 7.4; 37°C).	HPLC, UV, NMR, FAB-MS (Selzer & Elfarra, 1996a)
Deoxyguanosine monophosphate; Salmon testis DNA	Diastereomeric pairs of *N*7-(2-Hydroxy-3-buten-1-yl)dGMP (I) *N*7-(1-Hydroxy-3-buten-2-yl)dGMP (II)	Half-lives of decomposition (pH 9.6; 37°C) of I 4.5 h, of II 5 h.	HPLC/^{32}P-postlabelling (Kumar *et al.*, 1996)
Adenine; Adenosine; Calf thymus DNA	N^1-(2-Hydroxy-3-buten-1-yl)adenine (I) N^1-(1-Hydroxy-3-buten-2-yl)adenine (II) *N*3-(2-Hydroxy-3-buten-1-yl)adenine (III) *N*3-(1-Hydroxy-3-buten-2-yl)adenine (IV)	Formation in DNA (pH 7.2; 37°C); V and VI 8-fold > IV; IV 2-fold > III; III 3-fold > I and II.	HPLC, UV, NMR, ESI^+-MS (Tretyakova *et al.*, 1997a)
Guanosine; Calf thymus DNA	*N*7-(2-Hydroxy-3-buten-1-yl)guanine (V) *N*7-(1-Hydroxy-3-buten-2-yl)guanine (VI)		

Table 29 (contd)

Targets	Adducts formed	Kinetics	Comments (References)
Adenosine	Diastereomeric pairs of N^1-(1-Hydroxy-3-buten-2-yl)adenosine (I) N^1-(2-Hydroxy-3-buten-1-yl)adenosine (II) N^6-(1-Hydroxy-3-buten-2-yl)adenosine (III) N^6-(2-Hydroxy-3-buten-1-yl)adenosine (IV) N^1-(1-Hydroxy-3-buten-2-yl)inosine (V)	Pseudo-first-order formation rate constants (pH 7.4; 37°C) of the sum of III and IV about 3-fold higher than of V. Half-lives of decomposition (pH 7.4; 37°C) of I 7 h, of II 9.5 h. III, IV, V stable up to 7 days (pH 7.4; 37°C). Dimroth rearrangement of I and II to III and IV (pH 7.4; 37°C). Deamination of I and II to V (pH 7.4; 80°C).	HPLC, UV, NMR, FAB-MS (Selzer & Elfarra, 1996b)
Thymidine	Diastereomeric pairs of *N*3-(2-Hydroxy-3-buten-1-yl)thymidine (I) *N*3-(1-Hydroxy-3-buten-2-yl)thymidine (II)	Pseudo-first-order formation rate constant (pH 7.4; 37°C) of I about 5- to 6-fold higher than of II. I and II stable up to 7 days (pH 7.4; 37°C).	HPLC, UV, NMR, FAB-MS (Selzer & Elfarra, 1997a)
Deoxycytidine	Diastereomeric pairs of *N*3-(2-Hydroxy-3-buten-1-yl)deoxycytidine (I) *N*3-(2-Hydroxy-3-buten-1-yl)deoxyuridine (II) *N*3-(1-Hydroxy-3-buten-2-yl)deoxyuridine (III) O^2-(2-Hydroxy-3-buten-1-yl)deoxycytidine (IV)	Pseudo-first-order formation rate constant (pH 7.4; 37°C) of I about 5- to 6-fold higher than of III and IV. Deamination of I to II (pH 7.4; 37°C). Half-lives of decomposition (pH 7.4; 37°C) of I c. 2.4 h, of IV 11 h; II and III stable up to 168 h (pH 7.4; 37°C).	HPLC, UV, ^{1}H-NMR, FAB-MS (Selzer & Elfarra, 1997b)

dGMP, deoxyguanosine monophosphate; NMR, nuclear magnetic resonance; MS, mass spectrometry; HPLC, high-performance liquid chromatography; ECD, electrochemical detection; FAB, fast atom bombardment; ESI^+, electron spray ionization; CD, circular dichroism

by UV spectrophotometry, electron spray ionization mass spectrometry and nuclear magnetic resonance. Incubation of diepoxybutane (methanol/Tris-HCl buffer 1:1; pH 7.2) with adenine yielded *N*3-, *N*7- and *N*9-(2′-hydroxy-3′,4′-epoxybut-1′-yl)adenine, which hydrolysed to the corresponding trihydroxybutyl adducts [enantiomers not resolved]. 2′-Deoxyadenosine reacted in aqueous solution with diepoxybutane, probably forming an N1 adduct, which after acid hydrolysis and heating yielded trihydroxybutyl adducts at N^6 through Dimroth rearrangement. Trihydroxybutyl adducts were also found at N3- and N^6 of adenine in calf thymus DNA following acidic hydrolysis (Tretyakova *et al*., 1997c). The molar ratios of adduct formation at N7 of guanine to N3 of adenine in calf thymus DNA were similar for epoxybutene (Tretyakova *et al*., 1997a) and diepoxybutane (Tretyakova *et al*., 1997b,c).

Skin application of diepoxybutane for three days to female ICR mice with a daily dose of 1.9–153 μmol per mouse led to the formation of three adenine adducts in skin DNA, as determined by ^{32}P-postlabelling. The relative adduct labelling values correlated linearly with dose, reaching a mean maximum value of 185.6 total adducts per 10^8 DNA nucleotides after application of 153 μmol [13–17 mg] diepoxybutane per mouse per day (Mabon *et al*., 1996; Mabon & Randerath, 1996).

Alterations of oncogenes and suppressor genes in tumours

Mouse tumours from the study of Melnick *et al*. (1990) were evaluated for the presence of oncogenes. Activated K-*ras* oncogenes were detected in 6/9 lung adenocarcinomas, 3/12 hepatocellular carcinomas and 2/11 lymphomas obtained from $B6C3F_1$ mice exposed to butadiene. A specific codon 13 mutation (guanine to cytosine transversion) was found in most of the activated K-*ras* genes (Goodrow *et al*., 1994). Activated K-*ras* genes have not been found in spontaneously occurring liver tumours or lymphomas from $B6C3F_1$ mice (Reynolds *et al*., 1987; Goodrow *et al*., 1994) and were observed in only 1/10 spontaneous lung tumours in this strain of mice (Goodrow *et al*., 1994).

Mutations of the *p53* and *ras* genes were also detected in lymphomas from butadiene-treated mice by Zhuang *et al*. (1997). Most of the lymphomas with *ras* mutations at codon 13 (CGC) were from the low-dose group (< 200 ppm [440 mg/m^3]) or from the high-dose group with shortened treatment time (26 weeks), while those with *p53* mutations were from the high-dose (625 ppm [1380 mg/m^3]) continuous-exposure group. These results suggest that the *ras* genes may be involved in the early stages of butadiene-induced lymphomagenesis, while the *p53* gene appears to be more involved with the late-stage progression of these tumours.

4.5 Mechanistic considerations

Mechanistic studies conducted in whole animals and in rodent and human tissues using biochemical and molecular biological approaches have provided important insights into the likely critical steps in the initiation of butadiene carcinogenicity and the identity of the most likely chemical species responsible for the development of tumours.

The initial step is metabolic activation of butadiene to its reactive epoxide metabolites by multiple cytochrome P450 enzymes, including cytochrome P450 2E1 (CYP2E1). Butadiene is bioactivated to at least two genotoxic metabolites, epoxybutene and diepoxybutane. These two metabolites have been studied in detail by numerous laboratories. A third genotoxic epoxide metabolite of butadiene, epoxybutanediol, has not been quantified in animals but adducts to haemoglobin that are presumed to be derived from this epoxide have been detected in rats and humans exposed to butadiene.

Following inhalation exposure to butadiene, blood concentrations of epoxybutene were up to eight-fold higher in mice than in rats and blood concentrations of diepoxybutane were 40-fold higher in mice than in rats. Further, tissue concentrations of epoxybutene were 3–10 times higher in mice than in rats and tissue concentrations of diepoxybutane were up to 100 times higher in mice than in rats. Mice are much more susceptible to the carcinogenic effects of butadiene than are rats, with female $B6C3F_1$ mice developing tumours at butadiene concentrations as low as 6.25 ppm [13.8 mg/m^3]. Rats, in contrast, developed tumours after exposure to butadiene at concentrations of 1000 and 8000 ppm [2200 and 17 700 mg/m^3]. Considering the higher mutagenic potency of diepoxybutane as compared with epoxybutene and epoxybutanediol, the correlation between the measured circulating blood and tissue levels of the epoxides, especially diepoxybutane, and the observed development of tumours is suggestive of the role of diepoxybutane in the initiation of cancers in rodents exposed to butadiene.

Data on the metabolism of butadiene *in vitro*, including activation and detoxication, indicate significant species differences, and suggest that levels of epoxides *in vivo* should be higher in mice than in rats. The data on metabolism and tissue concentrations of epoxybutene and diepoxybutane in mice and rats *in vivo* following inhalation exposures to butadiene are consistent with results *in vitro*. The substantial variation in enzymatic activity between tissues from humans for the conversion of epoxybutene to diepoxybutane suggests the potential for large interindividual variation among humans in susceptibility to the potential genotoxic effects of butadiene. Bioactivation of butadiene at low concentrations to epoxybutene and diepoxybutane is mediated primarily by CYP2E1, so that this isoenzyme may play a key role in mediating differences between species in response to butadiene.

Studies on the induction of mutations by epoxybutene and diepoxybutane and the resulting mutational spectra have demonstrated clear mechanistic differences between epoxybutene- and diepoxybutane-induced mutational events. The concentrations of diepoxybutane that are genotoxic *in vitro* are within the range of concentrations measured in the blood and tissues of mice exposed to butadiene by inhalation, while the concentrations of epoxybutene that are genotoxic *in vitro* are 10- to 100-fold greater than concentrations observed in blood of mice exposed to butadiene. The characterization of molecular events induced by epoxybutene and diepoxybutane indicates that epoxybutene-induced genotoxicity is primarily due to point mutations and small deletion events. Diepoxybutane induces not only point mutations and small deletions, but also large-scale deletions involving hundreds or thousands of base pairs at an equal frequency.

The molecular biology data suggest involvement of at least diepoxybutane in the development of cancer in rodents following butadiene exposure. However, the additive or possible synergistic involvement of one or both of the other butadiene epoxides cannot be discounted.

Haemoglobin binding indices of epoxides which are formed as metabolic intermediates in the butadiene pathway can be regarded as dose surrogates of the internal body burden of these compounds. The haemoglobin binding index of *N*-(2-hydroxy-3-butenyl)-valine, the adduct with epoxybutene, was about 1.5–5 times higher in butadiene-exposed mice than in rats. In exposed humans, the corresponding binding index was between 25 and 250 times lower than in rats. There are only two preliminary reports on the formation in butadiene-exposed rats and humans of haemoglobin adduct of epoxybutanediol which can arise from the oxidation of dihydroxybutene and/or the hydrolysis of diepoxybutane. Based on these data, binding indices can be estimated to be more than one order of magnitude lower in exposed humans than in exposed rats. Together with model predictions which are based on in-vitro data obtained with tissues of mouse, rat and human, the available in-vivo data indicate a considerably lower body burden of butadiene-derived epoxides in butadiene-exposed humans than in rats and mice.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

1,3-Butadiene is a monomer used in high volume in the manufacture of a wide range of polymers, including styrene–butadiene rubber, polybutadiene, nitrile rubber, acrylonitrile–butadiene–styrene resins and styrene–butadiene latexes. It is also an intermediate in the production of various other chemicals.

Occupational exposure to 1,3-butadiene occurs in the production of monomeric 1,3-butadiene and of 1,3-butadiene-based polymers and 1,3-butadiene-derived products. The mean full-shift, time-weighted average exposure levels measured for workers in these industries have usually been below 10 ppm [22 mg/m^3], although that level may be exceeded during some short-term activities. Recent data from monomer extraction and styrene–butadiene rubber plants showed lower average concentrations (< 5 ppm [< 11 mg/m^3]). 1,3-Butadiene is not usually found at detectable levels in workplace air during manufacture of finished rubber and plastic products.

The general population may be exposed to very low levels of 1,3-butadiene due to its occurrence in engine exhausts and cigarette smoke.

5.2 Human carcinogenicity data

One cohort study of workers in the United States who manufactured 1,3-butadiene monomer showed a moderate and significant excess of lymphohaematopoietic cancers based on 42 deaths. Persons employed before 1950 were especially at increased risk, but there was no convincing association with a cumulative exposure score. A total of

13 leukaemia cases only slightly and insignificantly contributed to the excess of the lymphohaematopoietic cancers.

A small cohort study of 1,3-butadiene production workers showed a significant excess of lymphosarcoma and reticulosarcoma, based on four cases. There was also an excess of stomach cancer, although represented by only five cases. Two leukaemia cases were found: this was slightly more than expected.

Several reports have been published on follow-up of styrene–butadiene rubber workers at eight plants in the United States and Canada. The most recent follow-up showed a consistent excess of leukaemia and a significant dose–response relationship with cumulative exposure to 1,3-butadiene, which remained after adjustment for exposure to styrene.

Evaluation of the human carcinogenicity of 1,3-butadiene hinges on evidence regarding leukaemia risks from one large and well conducted study and two smaller studies. The smaller studies neither support nor contradict the evidence from the larger study. The larger, United States–Canada study shows that workers in the styrene–butadiene rubber industry experienced an excess of leukaemia and that those with apparently high 1,3-butadiene exposure had higher risk than those with lower exposure. The evidence from this study strongly suggests a hazard, but the body of evidence does not provide an opportunity to assess the consistency of results among two or more studies of adequate statistical power. Further, while 1,3-butadiene was a major exposure in this cohort, there were others, and it remains possible that even if there is an increased risk of cancer in the styrene–butadiene rubber industry, it may be due to occupational exposures other than 1,3-butadiene.

5.3 Animal carcinogenicity data

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

In the studies in mice, tumours were induced in multiple organs at all exposure concentrations studied, ranging from 6.25 to 1250 ppm [13.8–2760 mg/m^3]. The tumours induced included malignant lymphomas and heart haemangiosarcomas. Neoplasms at multiple organ sites were induced in mice after as little as 13 weeks of exposure at exposure levels of 625 ppm.

In one inhalation study in rats, 1,3-butadiene increased the incidence of tumours at several sites. The tumour increases were mainly in organs in which tumours develop spontaneously. The response was seen mainly at 8000 ppm [17 700 mg/m^3].

The initial metabolite of 1,3-butadiene, 1,2-epoxy-3-butene, yielded equivocal results in carcinogenicity tests, whereas the subsequent metabolite, 1,2:3,4-diepoxybutane, was carcinogenic to mice and rats when administered by skin application or by subcutaneous injection.

5.4 Other relevant data

1,3-Butadiene is metabolized in experimental animals and human liver microsomes to epoxide metabolites, initially 1,2-epoxy-3-butene and subsequently 1,2:3,4-

diepoxybutane, by cytochrome P450. The epoxides can be inactivated by epoxide hydrolase and glutathione *S*-transferases. Adducts formed by reaction of 1,2-epoxy-3-butene and 3,4-epoxy-1,2-butanediol with haemoglobin and urinary mercapturic acids derived from 1,2-epoxy-3-butene have been detected in 1,3-butadiene-exposed workers. There are significant species differences in the metabolism of 1,3-butadiene both *in vitro* and *in vivo*. The in-vitro data are consistent with modelled and measured concentrations of 1,2-epoxy-3-butene and 1,2:3,4-diepoxybutane in 1,3-butadiene-exposed mice and rats. In these animals, blood and tissue levels of 1,2-epoxy-3-butene are several times higher in mice than in rats and those of 1,2:3,4-diepoxybutane up to 100 times higher in mice than in rats. There is considerable interindividual variability in the ability of human liver microsomes to metabolize 1,3-butadiene and 1,2-epoxy-3-butene *in vitro.* Mechanistic data suggest that the much higher carcinogenic potency of 1,3-butadiene in mice than in rats results predominantly from the high burden of 1,2:3,4-diepoxybutane.

The haemoglobin-binding index of 1,2-epoxy-3-butene can be considered as a dose surrogate for this metabolite; corresponding haemoglobin-binding indices have been published for mouse and rat. Haemoglobin-binding indices in occupationally exposed humans have also been estimated. In agreement with model predictions, these data demonstrate binding indices for 1,3-butadiene-exposed humans more than one order of magnitude lower than those in exposed rats.

There are conflicting results on whether 1,3-butadiene increases *hprt* mutations in lymphocytes from 1,3-butadiene-exposed humans compared with non-exposed controls. Sister chromatid exchanges, micronuclei, chromosomal aberrations and DNA strand breaks were not significantly elevated above control levels in peripheral blood lymphocytes of occupationally exposed workers. 1,3-Butadiene induced DNA adducts and damage in both mice and rats *in vivo*, although the damage was significantly greater in mice than in rats. 1,3-Butadiene is mutagenic in virtually all test systems both *in vitro* and *in vivo*. Where a direct comparison between rats and mice could be made for the same end-point, positive effects were observed primarily in mice.

Activated K-*ras* oncogenes have been detected in lymphomas and in liver and lung tumours induced in mice by 1,3-butadiene. Mutations in the *p53* tumour-suppressor gene have been detected in mouse lymphomas.

1,2-Epoxy-3-butene was directly mutagenic in bacteria and induced gene mutations, chromosomal aberrations and sister chromatid exchanges *in vivo* in rodents. Micronuclei were induced in both somatic and germ cells of mice and rats *in vivo*. It induced gene mutations and sister chromatid exchanges in cultured human lymphocytes but did not induce unscheduled DNA synthesis, micronuclei or chromosomal aberrations in mouse or rat cells *in vitro.*

1,2:3,4-Diepoxybutane is a potent bifunctional alkylating agent which reacts with DNA *in vitro* and *in vivo*. As a result, it is mutagenic in virtually all test systems including effects in somatic and germ cells of mammals exposed *in vivo*. *In vivo*, it induced DNA adducts, dominant lethal mutations and gene mutations in mice; chromosomal aberrations

and sister chromatid exchanges in Chinese hamsters and mice; and micronuclei in splenocytes and spermatids of rats and mice. It induced gene mutations, chromosomal aberrations and sister chromatid exchanges in human and mammalian cell cultures. In one study, 1,2:3,4-diepoxybutane induced DNA–DNA cross-links in murine hepatocytes *in vitro*. It induced somatic and sex-linked recessive lethal mutations, chromosomal deletions and heritable translocations in *Drosophila.* Gene mutations were induced in bacteria in the mouse host-mediated assay and *in vitro*. 1,2:3,4-Diepoxybutane also induced bacterial prophage and DNA repair.

5.5 Evaluation

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

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 1,2:3,4-diepoxybutane.

Overall evaluation

1,3-Butadiene is *probably carcinogenic to humans (Group 2A)*.

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