Special Report No. 12

1,3-Butadiene OEL Criteria Document (Second Edition)

CAS No. 106-99-0

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ECETOC Special Report No. 12

PREFACE

This report has been prepared by ECETOC for use by the Commission of the EU (DG V) and its Scientific Committee for Occupational Exposure Limits to Chemical Agents. It has been compiled in accordance with guidance provided (CEC, 1992) and contains a review and assessment of toxicological data (Chapter 7-9) and quantitative risk assessments (Chapter 10) to provide a scientific basis for an occupational exposure limit for 1,3-butadiene. Information on occurrence, production and use, exposure and uptake, and measurement techniques (Chapter 3-6) has been drawn largely from existing reviews.

A summary evaluation of the health significance of 1,3-butadiene and a recommendation for an occupational exposure limit are presented in Section 12.3 and 12.4.

This review has been based on an earlier criteria document on 1,3-butadiene (ECETOC, 1993), supplemented by data that have subsequently become available.

1,3-Butadiene OEL Criteria Document (Second Edition)

CAS No. 106-99-0

CONTENTS

SPECIAL ABBREVIATIONS

1,3-BD	1,3-butadiene
BSO	L-buthionine-[*S,R*]-solfoximine
CAG	Carcinogen Risk Assessment Group (US EPA)
CFU	colony-forming assay of stem cells (CFU-S) or granulocytes/ macrophages (CFU-GM)
Crude C_4 fraction	A mixture of C_4 hydrocarbons, consisting predominantly of 1,3-BD and isomers of butene
DEB	1,2:3,4-diepoxybutane
EB	1,2-epoxybutene-3
FID	flame ionisation detector
GC	gas chromatography
GSH	glutathione
GST	GSH *S*-transferase
HPLC	high-pressure/performance liquid chromotography
LHC	lymphohaematopoietic cancer
OEL	occupational exposure limit (value)
MS	mass spectrometry
NHL	non-Hodgkin's lymphoma
NOEL	no-observed effect level
NPSH	non-protein sulphydryl
NTP I	first mouse study by US National Toxicology Programme (NTP, 1984; Huff *et al*, 1985)
NTP II	second mouse study by NTP (Melnick *et al*, 1990a,b; Melnick and Huff, 1992), not quoted: NTP, 1992
PB-PK	physiologically-based pharmacokinetic (model)
PRA	Poisson regression analysis
RR	rate ratio (from Poisson regression analysis)
SBR	styrene-butadiene rubber
SMR	standard mortality ratio (of observed over expected deaths)
SCE	sister-chromatid exchange
SLRL	sex-linked recessive lethal
SMART	somatic mutation and recombination test
STEL	short-term exposure limit (15 min, unless specified)
TWA	time-weighted average (concentration)
UDS	unscheduled DNA synthesis
VOC	volatile organic carbon

1. SUBSTANCE IDENTIFICATION

1.1 IDENTITY

Common name:	1,3-butadiene
CAS name:	1,3-butadiene
CAS registry No:	106-99-0
EEC No:	601-013-00-X, nota D
EEC classification:	F+ ; R 12 / Carc. Cat. 2; R 45
EEC labelling:	R: 45-12 / S: 53-9-16-33
RTECS No:	EI 9275000
IUPAC name:	1,3-butadiene
EINECS name:	buta-1,3-diene
EINECS No:	203-450-8
Synonyms and trade names:	
DA:	1,3-butadien
DE:	1,3-Butadien
EL:	1,3-βουταδιένιο
EN:	biethylene bivinyl butadiene butadiene, inhibited butadiene-1,3 a,g-butadiene *trans*-butadiene diethylene divinyl erythrene NCI-C50602 pyrrolylene vinylethylene
ES:	1,3-butadieno
FR:	1,3-butadiène
IT:	1,3-butadiene
N:	1,3-butadien
NL:	1,3-butadieen
PT:	1,3-butadieno
S:	1,3-butadien
SF:	1,3-butadieeni
Chemical group:	unsaturated hydrocarbons
Formula:	C_4H_6
Structure:	CH_2=CH–CH=CH_2

Molecular mass:	54.09
Purity of technical product:	99.8% (min. 99.5%) (ICI, 1992)
Impurities of technical product:	1,2-butadiene, max. 20 ppm
	peroxides (measured as H_2O_2), max. 5 ppm
	acetylene, max. 25 ppm
	sulphur, max. 2 ppm
	C5's, max. 0.1% w/w
	butadiene dimer, max. 0.05% w/w
	non-volatile residues (such as trimer), max. 500 ppm
	Carbonyl (as acetaldehyde), max. 25 ppm
	propadiene, max. 10 ppm
	water, some (ICI, 1992)
Inhibitor:	75-150 ppm of *p-tert*-butyl catechol (ICI, 1992)

2. CHEMICAL AND PHYSICAL PROPERTIES

1,3-Butadiene (1,3-BD) is a colourless, non-corrosive gas with a mild aromatic, gasoline-like odour. It is a highly reactive material which can dimerise to 4-vinylcyclohexene. 1,3-BD polymerises readily, especially in the presence of oxygen. In air, it can form acrolein and explosive peroxides (Sax, 1991). Other chemical and physical properties are given in Table I.

TABLE I: Chemical and Physical Properties

Parameter, units	Value	Reference
Boiling temperature, °C at 1,013 hPa	–4.4	Weast *et al*, 1988
Melting temperature, °C at 1,013 hPa	–108.9	Weast *et al*, 1988
Relative density of liquid D_4^{20} (water at 4 °C = 1,000 kg/m^3)	621.1	Weast *et al*, 1988
Vapour pressure, hPa at 20 °C	2,477	Weast *et al*, 1988
Saturation concentration in air, g/ml at 20 °C	5.4	Calculated
Vapour density at 20 °C (air = 1)	1.87	Verschueren, 1983
Threshold odour concentration, ppm (odour: mildly aromatic)	0.5-1.6	Amoore and Hautala, 1983; DECOS, 1990
Solubility in water, g/kg at 20 °C	0.74	Verschueren, 1983
Solubility in alcohol, ether, acetone and benzene	Yes	Weast *et al*, 1988
Partition coefficient, at 20 °C log P_{ow} (octanol/water)	1.99	Hansch and Leo, 1979[a]
Flash point, closed cup, °C	–85	Hüls, 1994
Explosion limits in air[b], %	2.0-11.5	BASF, 1992
Auto-flammability, ignition temp., °C	429	DECOS, 1990
	420	Sax, 1991

a As quoted in Hazardous Substances Database (HSDB, 1992)
b Temperature range not specified

The technical product is transported as a liquefied gas under pressure with an inhibitor such as aliphatic mercaptans, *o*-dihydroxybenzene (Windholz *et al*, 1976) or *p-tert*-butyl catechol (ICI, 1992) to prevent polymerisation and/or peroxide formation. Other inhibitors are mentioned in IARC (1986, 1992).

2.1 CONVERSION FACTORS

Conversion factors for 1,3-BD concentrations in air, calculated at 20 °C and 1,013 hPa are:

1 mg/m^3 = 0.445 ppm
1 ppm = 2.249 mg/m^3

3. OCCURRENCE

3.1 EMISSIONS

1,3-BD does not occur as a natural product (Howard, 1990).

1,3-BD may be formed during combustion processes and has been reported following forest fires (Howard, 1990). It has been identified in automobile exhaust (Hoekman, 1992; Stump *et al*, 1989) and cigarette smoke (0.4 mg/cigarette) (Löfroth *et al*, 1989 as quoted in IARC, 1992).

1,3-BD is a contaminant in some gasolines (Stump *et al*, 1989). Depending on the manufacturing process, liquefied petroleum gas (LPG) may contain a small percentage 1,3-BD (Concawe, 1992).

Small amounts of 1,3-BD can be produced by the thermal degradation of polyurethane-coated wire during electrical overload (Rigby, 1981 as quoted in ATSDR, 1991) and when 1,3-BD based-plastics or rubbers are burnt (Miller *et al*, 1978 as quoted in ATSDR, 1991). 1,3-BD has also been detected in smoke generated during house fires (up to 15 ppm) (Berg *et al*, 1978 as quoted in IARC, 1992).

Industrial emissions arise during (i) production of crude 1,3-BD and petroleum refining, (ii) 1,3-BD monomer extraction, (iii) production of 1,3-BD containing polymers and derivatives, and (v) plastic products manufacturing.

According to a 1984 survey by the US EPA, atmospheric emissions of 1,3-BD from facilities which produced or processed 1,3-BD were approximately 5kt/year; 70% of these were attributed to equipment leaks and 30% to process venting (Mullins, 1990). In the EU, emissions to air and waste water are reported by DoE (1996).

3.2 OCCURRENCE AT THE WORKPLACE

In man, exposure to 1,3-BD occurs during the production of 1,3-BD and at user sites (see Section 5.1 for details).

3.3 BACKGROUND ENVIRONMENT

Due to its high volatility and low water solubility, environmentally-released 1,3-BD partitions almost entirely into the atmosphere (Section 5.3.1). Destruction of 1,3-BD in the atmosphere occurs by rapid reaction

with photochemically produced hydroxyl radicals, nitrate radicals, ozone and molecular oxygen (Guicherit and Schulting, 1985; ATSDR, 1992). Acrolein and formaldehyde are the major photo-oxidation reaction products of 1,3-BD (Maldotti *et al*, 1980).

The non-occupational daily intake has been calculated to be 2.62 µg/person, assuming a mean urban air concentration of 0.29 ppb (Section 5.3.1) and human air intake of 20 m^3/day (ATSDR, 1992). Very low levels may be ingested from contaminated food and drinking water, and inhalation of gasoline vapours, automobile exhaust or cigarette smoke (Section 5.3.2); quantification is not possible for lack of data (ATSDR, 1992).

4. PRODUCTION AND USE DATA

4.1 PRODUCTION

1,3-BD is a major commodity chemical of the petrochemical industry. It is produced largely as a co-product in the catalytic steam cracking of petroleum fractions (light oil and naphta) during the manufacture of ethylene. During steam cracking a crude C_4 fraction consisting predominantly of 1,3-BD and isomers of butene is produced. This C_4 stream is removed and transported by pipeline or container to a monomer purification unit where 1,3-BD is isolated by solvent extraction. Other production methods exist, such as catalytic dehydrogenation of butene, butane-butene mixtures of alcohol, but steam cracking is predominant in Europe (Slooff *et al*, 1994).

The annual quantities of 1,3-BD produced in Western Europe are detailed in Table II.

TABLE II: Quantities (kt/y) of 1,3-BD Produced in Western Europe
(APPE, 1995a,b)

Country or area	1988	1989	1990	1991	1992	1993	1994
Benelux	421	384	439	387	397	339	369
Germany	539	533	547	534	528	528	549
France	334	329	284	286	332	321	351
United Kingdom	186	178	146	164	224	167	201
Austria, Finland	a	a	a	b	b	b	b
Italy, Spain, Portugal	405	457	489	442	370	388	423
Total western Europe	1,885	1,881	1,905	1,813	1,851	1,743	1,893

a Included in figures for Italy, Spain, Portugal
b Included in figures for UK

4.2 USE

The major use of 1,3-BD is in the manufacture of synthetic rubbers such as styrene-butadiene rubber (SBR) and polybutadiene rubber used in tyres and tyre products. It is also used in manufacturing of thermoplastic resins (such as acrylonitrile-butadiene-styrene (ABS) for automotive parts and business machines) and styrene-butadiene latex suspensions (for paints and carpet backing). It is used as a chemical intermediate in the production of neoprene (for industrial and automotive rubber goods) and adiponitrile (a nylon precursor) (Slooff *et al*, 1994).

The use pattern in the USA in 1990 is shown in Table III. European data are not available.

TABLE III: Use Pattern of 1,3-BD in the USA in 1990
(Anonymous, 1991)

Production of	%
Styrene-butadiene rubber	30
Polybutadiene rubber	20
Adiponitrile/hexamethylene diamine	15
Styrene-butadiene latex	10
Neoprene rubber	5
Acrylonitrile-butadiene styrene resins	5
Nitrile rubber	3
Speciality polymers and non-polymers	12

5. QUANTITATIVE INFORMATION ON EXPOSURE AND UPTAKE

5.1 EXPOSURE LEVELS AT THE WORKPLACE

The predominant route of occupational exposure to 1,3-BD is by inhalation. Dermal contact with the liquefied gas may occur during loading and unloading of tanks, or by the accidental rupture of tanks (ATSDR, 1992).

5.1.1 Production of Crude 1,3-BD and Petroleum Refining

During the production of crude 1,3-BD (C_4 fraction) in German petroleum refineries (crackers) exposure levels were mostly below 5 ppm with levels up to 30 ppm at a few places (mean values from 93 shifts consisting of personal samples and background concentration measurements) (Deutscher Ausschuß für Gefahrstoffe, 1992). Data from other European countries are not available.

1,3-BD production facilities in the EU were surveyed over the years 1986-1993. At the cracker plants with crude 1,3-BD containing streams, 1,584 people were exposed, almost all (1,493) to concentrations < 1 ppm (CEFIC, 1993). Detailed data are given in Table IV.

TABLE IV: Personal 1,3-BD Exposure Levels in Crackers[a] of 1,3-BD Production Plants
(CEFIC, 1993)

Job category	Year of measurement	Number of people	Number of samples	< 1 ppm	1-2 ppm	2-3 ppm	3-4 ppm	4-5 ppm	5-10 ppm	10-25 ppm	≥ 25 ppm
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
			Area[b]: 6	6							
Maintenance	1986-92	282	371	364	5	0	1	0	0	1	0
Other	1990-92	467	509	487	18	2	1	1	ND[c]	0	0
			Area: 21	20	1	0	0	0	0	0	0
Total	1986-93	1,485	1,548	1,493	28	9	8	4	0	4	2
			Area: 27	26	1	0	0	0	0	0	0

a 1,3-BD containing product streams
b Area = background monitoring at the work place
c ND = not detectable (detection limit not stated)

In the USA in 1984, the following exposure levels were identified at petroleum refineries and petrochemical facilities producing crude 1,3-BD (Table V).

TABLE V: Exposure of Workers Involved in Petroleum Refining and Production of Crude C_4 (USA, 1984)
(Heiden Associates, 1987 as quoted in IARC, 1992)

Job category	N° of facilities	Concentration (ppm)	
		Mean[a]	Range
Production	7	0.24	0.008-2.0
Maintenance	6	0.11	0.02-0.37
Distribution	1	2.9	-
Laboratory	4	0.18	0.07-0.4

a Mean time-weighted average (TWA) concentration for an 8-hour working period, weighted by number of exposed workers (at each facility)

Levels of 1,3-BD to which workers have been exposed in the production and distribution of gasoline have been reported (Table VI).

TABLE VI: Personal Exposures Associated with Gasoline Production and Handling
(after CONCAWE, 1987 as quoted in IARC, 1989)

Location	Concentration				Duration
	Mean	Range	Mean	Range	
	(mg/m^3)[a]		(ppm)[b]		
Production on-site (refining)	0.3	ND[c]-11.4	0.13	ND[c]-5.07	8 h
Production off-site (refining)	0.1	ND-1.6	0.04	ND-0.71	8 h
Loading ships (closed system)	6.4	ND-21.0	2.85	ND-9.35	8 h
Loading ships (open system)	1.1	ND-4.2	0.49	ND-1.87	8 h
Loading barges	2.6	ND-15.2	1.16	ND-6.76	8 h
Jettyman	2.6	ND-15.9	1.16	ND-7.08	8 h
Bulk loading road tankers					
Top loading < 1 h	1.4	ND-32.3	0.62	ND-14.37	< 1 h
Top loading > 1 h	0.4	ND-4.7	0.18	ND-2.09	8 h
Bottom loading < 1 h	0.2	ND-3.0	0.09	ND-1.34	< 1 h
Bottom loading > 1 h	0.4	ND-14.1	0.18	ND-6.27	8 h
Road tanker delivery (bulk plant to service station)	ND		ND		
Railcar top loading	0.6	ND-6.2	0.27	ND-2.76	8 h
Drumming	ND		ND		
Service station attendant (dispensing fuel)	0.3	ND-1.1	0.13	ND-0.49	8 h
Self-service station (filling tank)	1.6	ND-10.6	0.71	ND-4.72	2 min

a As quoted
b Calculated using conversion factor from Section 2.1
c Not detected

5.1.2 1,3-BD Monomer Extraction

CEFIC (1996) data on occupational exposure during 1,3-BD monomer production in the EU in 1995 are summarised in Table VII. The data come from 15 of 16 producers in Europe, covering 16 extraction units and 2 derivative units (18 sites). Although there were differences in the methods of sampling and analysis, and the reporting of results, the available data indicate that in general, exposure levels were below 5 ppm. During certain operations, short-term exposure levels may be much higher.

TABLE VII: Occupational Exposure Levels at 15 Monomer Extraction Sites
(CEFIC, 1996)

Job category	Year of measurement	Sample type[a]	Concentration (ppm)		
			TWA	Range	Short-term[b]
Production					
Extraction	1995	Personal	< 0.01-2	(0-14)	0-100
Derivation[c]	1995	Personal	1.4-3.4	(0.07-60)	0-177
Storage and filling	1995	Personal	< 0.02-5	(0-18.1)	NS
Transport	1995	Personal	< 0.1-0.7	(0.02-1.2)	0-114
Laboratory	1995	Personal	0.03-1	(0-13.1)	0.1-24.6

a Personal = monitoring in the worker's breathing zone
b Short-term level = exposure concentration during short (unspecified) periods
NS = not stated
c Integrated monomer extraction and SBR production on same site

An earlier survey of measurements taken at EU 1,3-BD production facilities over the years 1986-1993 indicated that in extraction plants 986 people in different jobs were exposed to 1,3-BD, most (843) to concentrations < 1 ppm (CEFIC, 1993). Detailed data are given in Table VIII.

TABLE VIII: Personal 1,3-BD Exposure Levels in Extraction Units[a] of 1,3-BD Production Plants
(CEFIC, 1993)

Job category	Year of measurement	Number of people	Number of samples	< 1 ppm	1-2 ppm	2-3 ppm	3-4 ppm	4-5 ppm	5-10 ppm	10-25 ppm	≥ 25 ppm
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
			Area: 45	13	4	2	4	4	5	4	9
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
			Area: 24	15	4	0	0	0	4	1	0
Total	1984-93	986	1,035	843	49	32	17	16	25	23	30
			69	28	8	2	4	4	9	5	9

a Isolation of 1,3-BD from C_4 stream
b Area = background monitoring at the work place
c ND = not detectable (detection limit not stated)

In a Finnish plant producing purified 1,3-BD, levels were generally < 10 ppm at different sites of the plant (33 samples; mean sampling time 5.3 h). In personal samples of 16 process workers, the concentration ranged from < 0.1 to 447 ppm (mean 11.5 ppm, median < 0.1 ppm; 46 samples, mean sampling time 2.5 h), with highest concentrations during sample collection. Protective clothing and respirators were used during this operation (Arbetsmiljöfonden, 1991 as quoted in IARC, 1992). In another study at the same plant, ambient air concentrations were generally below 10 ppm (both stationary and personal samples) with peak concentrations up to 300 ppm (personal samples) in a few cases. Workers used protective clothing and respirators during the operations (Ahlberg *et al*, 1991).

During the production of pure 1,3-BD in Germany, the mean exposure level was about 5 ppm, with peak levels of up to 30 ppm (personal samples; mean values from 88 shifts) (Deutscher Ausschuß für Gefahrstoffe, 1992).

In the USA, between 1984 and 1987, NIOSH conducted in-depth industrial hygiene surveys at four 1,3-BD monomer extraction plants. Airborne exposure concentrations of 1,3-BD were determined for various job categories by personal sampling. The samples were analysed by NIOSH method 1024 that is sensitive to 0.2 mg/sample (i.e. the detection limit was 0.005 ppm in 25 litres of air). The 1,3-BD concentrations in 88 personal full-shift air samples are presented in Table IX. High short-term personal exposures (23 samples) occurred during open-loop cylinder sampling (up to 147 ppm), cylinder voiding (up to 108 ppm) and maintenance (up to 16.8 ppm); short-term levels during processing were lower (up to 0.36 ppm). Area samples (97 full-shift and 3 short-term) indicated high 1,3-BD concentrations in rail-car terminals (mean 10.5 ppm, range 0.09-64.3 ppm) and tank storage farms (mean 7.80 ppm; range 0.15-23.8 ppm) (Fajen *et al*, 1993).

TABLE IX: Personal 1,3-BD Exposure Levels at 4 US Momomer Extraction Plants
(Fajen *et al*, 1993)

Job	No. of samples	Concentration (ppm)				
		Range	Arithmetic mean	SD	Geometric mean	SD
Laboratory technician	29	0.03-6.31	1.06	1.61	0.40	4.36
bomb voiding	3	0.42-374	126	215	7.43	33.60
Process technician						
control	10	< 0.02-1.87	0.45	0.72	0.09	7.93
loading	12	0.08-124	11.60	35.30	1.00	7.82
production	29	< 0.06-34.9	2.16	6.38	0.60	4.46
storage	5	< 0.05-1.53	0.44	0.62	0.21	4.26
Total	88	< 0.02-374	7.00	41.8	0.47	6.49

Detailed industrial hygiene surveys were conducted by NIOSH in 1985 in four of 10 1,3-BD monomer extraction plants in the USA. Levels of 1,3-BD to which workers in various job categories were exposed are summarised in Table X. 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 for exposure. Average exposure levels for other job categories were below 1 ppm. Activities such as open-loop sampling and cylinder voiding were associated with peak exposures of 100 ppm. Ambient concentrations of 1,3-BD were greatest in the railcar terminals (geometric mean, 1.77 ppm) and in the tank storage farm (2.12 ppm) (Krishnan *et al*, 1987 as quoted in IARC, 1992).

TABLE X: 1,3-BD Exposure Levels[a] at Four US Monomer Extraction Plants
(Krishnan *et al*, 1987 as quoted in IARC, 1992)

Job category	N° of samples	Concentration (ppm, 8-h TWA))		
		Arithmetic mean	Geometric mean	Range
Process technician/ control room	10	0.45	0.09	< 0.02-1.87
Process technician process area	28	2.23	0.64	< 0.08-34.9
Process technician/ loading area				
- railcar	9	14.64	1.00	0.12-123.57
- tank truck	3	2.65	1.02	0.08-5.46
- tank farm	5	0.44	0.20	< 0.04-1.53
Laboratory technician	29	1.06	0.40	0.03-6.31
Laboratory technician/ cylinder voiding	3	125.52	7.46	0.42-373.54

a Personal breathing zone samples

In 1984, the US Chemical Manufacturers' Association obtained data on personal exposure to 1,3-BD before 1984 from 13 companies extracting 1,3-BD monomer from crude C_4 streams, categorised broadly by job type (Table XI). These data were collected by an older method than the data reported in Table VI and provide a historical perspective. The highest exposures were in maintenance and distribution. Out of a total of 1,287 samples, 91% were less than or equal to 10 ppm and 68% were less than 5 ppm. Factors that limit generalization of these data are unspecified sampling and analytical techniques, lack of detailed job descriptions and different or unspecified average times of sampling (JACA Corp., 1987 as quoted in IARC, 1992).

TABLE XI: Personal 1,3-BD Exposure Levels at 13 Momoner Extraction Plants in the USA
(JACA Corp., 1987 as quoted in IARC, 1992)

Category	N° of samples	1,3-BD TWA concentration (ppm)					
		0.00-5.00	5.01-10.00	10.01-25.00	25.01-50.00	50.01-100.00	> 100.00
Production	562	446	111	5			
(%)		(79.4)	(19.7)	(0.9)			
Maintenance	329	247		47	35		
(%)		(75.1)		(14.3)	(10.6)		
Supervisory	64	60	4				
(%)		(93.8)	(6.2)				
Distribution	206	60	121	16	5	2	2
(%)		(29.1)	(58.7)	(7.8)	(2.4)	(1.0)	(1.0)
Laboratory	126	58	68				
(%)		(46.0)	(54.0)				
Total	1,287	871	304	68	40	2	2
(%)		(67.8)	(23.6)	(5.3)	(3.1)	(0.1)	(0.1)

5.1.3 Transfer of 1,3-BD

Large quantities of 1,3-BD are transferred through pipelines, either as a crude C_4 fraction or as purified 1,3-BD. High exposure may occur during the connection or filling of the pipes. Data from such operations in Germany (173 mean values from personal shift-samples) showed exposure levels between 5 and 50 ppm with concentrations as high as 500 ppm in some cases (Deutscher Ausschuß für Gefahrstoffe,1992).

5.1.4 Production of 1,3-BD Polymers, Derivatives, Rubber and Plastic Products

The International Institute of Synthetic Rubber Producers (IISRP) surveyed 1,062 workers in the synthetic rubber and rubber latex producing industry from 13 of 27 sites operated by 26 European producers over the years 1984-1993. The data were collected by a variety of sampling and analytical techniques, and are summarised in Table XII; data collected by inappropriate methods were excluded. The table shows that 71.1% of exposures did not exceed a level of 1 ppm 1,3-BD; 93.3% did not exceed 5 ppm (8-h TWA). High exposures may occur during loading/unloading and storage, recovery, polymerisation, in the laboratory and during maintenance (IISRP, 1994).

TABLE XII: 1,3-BD Exposure in Synthetic Rubber Plants (1984-1993)
(IISRP, 1994)

Job category	Number of workers	Number of samples	Concentration (8-h TWA)								
			< 0.5 ppm	0.51-1 ppm	1.01-2 ppm	2.01-3 ppm	3.01-4 ppm	4.01-5 ppm	5.01-10 ppm	10.01-25 ppm	> 25 ppm
Unloading, loading and storage	132	77	47	1	8	6	3	0	5	5	2
Polymerisation	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	1,062	661	407	63	59	49	26	13	22	19	3
Percentage			61.6	9.5	8.9	7.4	3.9	2	3.3	2.9	0.5
Percentile			61.6	71.1	80	87.4	91.3	93.3	96.6	99.5	100

In-depth industrial hygiene surveys were conducted by the US National Institute of Occupational Safety and Health (NIOSH) at four monomer and five polymer manufacturing plants. Occupational exposure to 1,3-BD in most process areas was less than 10 ppm. However, in operations involving decontamination and maintenance of process equipment, sampling and analysing of quality control samples, and loading or unloading of tank trucks or rail cars for example, maximum 8-hour TWA exposure was frequently between 10 and 125 ppm (in one case as high as 374 ppm) (Fajen *et al*, 1990).

NIOSH surveyed five 1,3-BD polymer plants in the USA between 1984 and 1987. Airborne exposure concentrations of 1,3-BD were determined for various job categories by personal sampling (limit of detection 0.005 ppm in 25 litres of air). The 1,3-BD concentrations in 438 personal full-shift air samples are presented in Table XIII. The highest short-term personal exposure (14 samples) was 280 ppm (mean 48.7 ppm) for a process technician sampling 1,3-BD for quality contnrol. Maintenance workers experienced short-term exposure up to 14.4 ppm (mean 4.50 ppm). Area samples (132) showed full-shift exposures of up to 9.01 ppm (mean 3.02) in the laboratory, caused by a faulty threaded connection of the cylinder to the gas chromatograph (GC). In the 51 samples taken at locations near the plant perimeter, the mean 1,3-BD concentration was 0.03 ppm (range < 0.006-0.170 ppm) (Fajen *et al*, 1993).

TABLE XIII: 1,3-BD Concentrations in Personal Full-Shift Air Samples in the Polymer Industry (Fajen *et al*, 1993)

Job	No. of samples	Concentration (ppm)				
		Range	Arithmetic mean	SD	Geometric mean	SD
Laboratory technician	49	< 0.006-37.9	3.09	6.91	0.332	12.1
Tank farm operator	23	0.009-24.0	1.97	5.01	0.260	9.59
Front end (reaction)	108	< 0.006-24.7	1.80	4.02	0.151	12.1
Maintenance technician	42	0.012-42.9	1.84	6.85	0.144	7.44
Back end (finishing)	79	< 0.005-7.12	0.351	1.07	0.037	7.13
Other	137	< 0.005-0.167	0.035	0.032	0.022	3.03
Total	438	< 0.005-42.9	1.14	4.02	0.072	9.27

Concentrations at German facilities producing 1,3-BD polymer varied greatly, depending on the type and conditions of the process of polymerisation and/or preparation. Results from 465 mean shift-values (personal samples) and 691 background measurements by GC showed that most exposure levels were between 10 and 20 ppm; at a few places concentrations were as high as 50 ppm (Deutscher Ausschuß für Gefahrstoffe, 1992).

Detailed industrial hygiene surveys were conducted in 1986 in five of 17 facilities in the USA where 1,3-BD was used to produce SBR, nitrile-butadiene rubber, poly-butadiene rubber, neoprene and adiponitrile. Levels of 1,3-BD to which workers in various job categories have been exposed are summarized in Table XIV. Process technicians in unloading, the tank farm, purification, polymerization and reaction, laboratory technicians and maintenance technicians were exposed to the highest levels. Short-term sampling showed that activities such as sampling a barge or laboratory work were associated with peak exposures of more than 100 ppm. Full-shift area sampling indicated that geometric mean ambient concentrations of 1,3-BD were less than 0.5 ppm and usually less than 0.1 ppm in all locations at the five plants (Fajen, 1988 as quoted in IARC, 1992).

TABLE XIV: Full-shift 1,3-BD TWA Exposure Levels[a] at Five US Plants Producing 1,3-BD-based Polymers and Derivatives (1986)
(Fajen, 1988 as quoted in IARC, 1992)

Job category	N° of samples	Exposure level (ppm)		
		Arithmetic mean	Geometric mean	Range
Process technician				
- unloading area	2	14.6	4.69	0.770-28.5
- tank farm	31	2.08	0.270	< 0.006-23.7
- purification	18	7.80	6.10	1.33-24.1
- polymerisation or reaction	81	0.414	0.062	< 0.006-11.3
- solutions and coagulation	33	0.048	0.029	< 0.005-0.169
- crumbing and drying	35	0.033	0.023	< 0.005-0.116
- packaging	79	0.036	0.022	< 0.005-0.154
- warehouse	20	0.020	0.010	< 0.005-0.068
- control room	6	0.030	0.019	< 0.012-0.070
Laboratory technician	54	2.27	0.213	< 0.006-37.4
Maintenance technician	72	1.37	0.122	< 0.006-43.2
Utilities operator	6	0.118	0.054	< 0.006-0.304

a Personal breathing zone samples

Eight-hour TWA exposures to 1,3-BD in the polymer industry were obtained by personal sampling in 11 North American synthetic rubber plants in 1978-84 by the IISRP in 1984 (Table XV). The highest exposures were found for tank car loaders (15% of exposures, > 10 ppm), reactor operators (18% > 10 ppm) and laboratory technicians (6% > 10 ppm). Sampling and analytical techniques and job descriptions were not available (JACA Corp., 1987 as quoted in IARC, 1992).

TABLE XV: Occupational Exposure to 1,3-BD in the Polymer Industry[a]
(JACA Corp., 1987 as quoted in IARC, 1992)

Occupational group	N° of samples	Concentration (ppm, 8-h TWA)							
		0.00-5.00	5.01-10.00	10.01-25.00	25.01-50.00	50.01-100.00	100.01-200.00	200.01-500.00	500.01-1000.00
Tank-car loader (%)	102	78 (76.5)	9 (8.8)	9 (8.8)	4 (3.9)	2 (2.0)			
Vessel cleaner (%)	214	199 (93)	9 (4.2)	4 (1.9)	2 (0.9)				
Charge solution make-up (%)	89	83 (93.2)	3 (3.4)		2 (2.3)	1 (1.1)			
Reactor operator (%)	190	133 (70)	22 (11.6)	14 (7.4)	7 (3.7)	7 (3.7)	5 (2.6)	1 (0.5)	1 (0.5)
Recovery operator (%)	108	100 (92.6)	5 (4.6)	2 (1.9)	1 (0.9)				
Coagulation operator (%)	185	173 (93.5)	9 (4.9)	2 (1.1)	1 (0.5)				
Dryer operator (%)	85	84 (98.8)	1 (1.2)						
Baler and packager (%)	167	164 (98.2)	2 (1.2)	1 (0.6)					
Warehouse-man (%)	22	22 (100)							
Laboratory technician (%)	116	103 (88.8)	6 (5.2)	6 (5.2)	1 (0.9)				
Maintenance technician (%)	262	241 (92.0)	12 (4.6)	4 (1.5)	2 (0.8)	3 (1.1)			
Supervisor (%)	123	111 (90.2)	6 (4.9)	6 (4.9)					
Waste-treatment operator (%)	9	9 (100)							
Total (%)	1,672	1,500 (89.7)	84 (5.0)	48 (2.9)	20 (1.2)	13 (0.78)	5 (0.30)	1 (0.06)	1 (0.06)

a Based on 1978-84 data obtained from 11 North American synthetic rubber producers.

Data from more than 800 exposures obtained from two SBR plants in the USA in 1987 showed that approximately 70% of all synthetic rubber workers were exposed to 1,3-BD at levels below 5 ppm, and 85% to less than 10 ppm (Table XVI).

TABLE XVI: 1,3-BD Exposure Data from Two SBR Plants[a] (1981-1987)
(Tozzi, 1988)

Job Category[b]	Concentration (ppm)								
	< 0.50	0.51-1	1.01-2	2.01-3	3.01-4	4.01- 5	5.01-10	10.01-25	> 25
Unloading/ loading/ storage	2.3%	-	6.8%	9.2%	6.8%	6.8%	22.7%	22.7%	22.7%
Polymerisation operations	21.7%	17.1%	15.8%	8.6%	5.9%	5.9%	12.5%	7.9%	4.6%
Recovery operations	10.7%	8.3%	12.4%	9.5%	11.2%	15.7%	9.5%	13.2%	9.5%
Finishing operations	78.4%	5.2%	5.2%	5.2%	3.4%	-	1.7%	-	.9%
Laboratory and sampling	13.3%	10.4%	8.8%	13.3%	8.2%	10.4%	15.6%	16.3%	3.7%
Maintenance	30.7%	15.4%	15.4%	9.9%	2.2%	5.5%	13.2%	4.4%	3.3%
Total exposures	25.3%	10.2%	11.4%	9.4%	7.2%	6.9%	13.1%	10.2%	6.3%

a Percentage within job category

b < 5% excluded because of job classification difficulties.

Other historical data on 1,3-BD exposure levels have been collected during health surveys or epidemiological studies. In a US SBR manufacturing plant in 1979, 1,3-BD levels were greater than 10 ppm at the tank farm (53.4 ppm) and during maintenance (20.7 ppm) (Checkoway and Williams, 1982 as quoted in IARC,1992). In a US SBR plant in 1976, levels above 100 ppm were encountered by technical services personnel (114.6 ppm) and an instrument man (174.1 ppm) (Meinhardt *et al*, 1978 as quoted in IARC,1992). Mean 8-h TWA exposure levels differed considerably between the two plants: 1.24 ppm and 13.5 ppm (Meinhardt *et al*, 1982 as quoted in IARC,1992).

No data are available on levels of exposure to 1,3-BD before the 1970s, but different processes and working conditions would have resulted in exposure levels higher than now prevalent in developed countries (IARC, 1992).

Unreacted 1,3-BD was detected as only a trace (0.04-0.2 ng/mg) in 15 of 37 bulk samples of polymers and other chemicals synthesized from 1,3-BD and analyzed in 1985-86. Only two samples contained measurable amounts of 1,3-BD: tetrahydro-phthalic anhydride (53 ng/mg) and vinyl pyridine latex (16.5 ng/mg) (JACA Corp., 1987 as quoted in IARC, 1992).

Between 1984 and 1987, NIOSH surveyed a rubber tyre plant (90 personal full-shift samples) and an industrial hose plant (34 personal full-shift samples) using SBR, polybutadiene and acrylonitrile-butadiene rubber. No 1,3-BD was detected (detection limit 0.005 ppm in 25 litres of air) in any of the

following job categories: Banbury operators, mill operators, extruder operators, curing operators, conveyer operators, calendering operators, wire winders, tube machine operators, tyre builders and tyre repair and buffer workers (Fajen *et al*, 1990 as quoted in IARC, 1992; Fajen *et al*, 1993).

Measurements taken in 1978 and 1979 in personal 8-hour samples in companies where acrylonitrile-butadiene-styrene moulding operations were conducted showed levels of < 0.05-1.9 mg/m^3 (< 0.02-0.85 ppm) (Belanger and Elesh, 1980; Ruhe and Jannerfeldt, 1980; both as quoted in IARC, 1992). In a polybutadiene rubber warehouse, levels of 0.003 ppm were found in area samples; area and personal samples taken in tyre plants contained 0.007-0.05 ppm (Rubber Manufacturers' Association, 1984 as quoted in IARC, 1992). In a US tyre and tube manufacturing plant in 1975, a cutter man/Banbury operator was reported to have been exposed to 2.1 ppm (personal 6-hour sample) (Ropert, 1976 as quoted in IARC, 1992).

5.1.5 Summary of Exposures

There are data on exposure to 1,3-BD during both its production and use.

The most recent data from the European 1,3-BD production industry indicates that the vast majority of exposures are below 1 ppm, although there are a number of job categories that appear to be associated with higher exposures. These are most often those jobs which involve the breaking of 1,3-BD containment, specifically maintenance, sampling, and transfer operations. This pattern is also reflected by the available short-term exposure data from European industry, and extensive short- and long-term data from the US production industry. The absolute values given for the US industry are higher, with a number of job categories where exposures were between 1 and 5 ppm (8-hour TWA). However, it is unclear whether this represents a significant difference, as the two sets of data are not directly comparable. Not only do they span a period during which new controls were introduced, but the use of different sampling techniques and analytical procedures will also have introduced variation in the figures.

Exposure levels during the use of 1,3-BD in the production of various polymers have been measured in both Europe and the USA. In Europe, the available data indicate that 71.1% of all measurements did not exceed 1 ppm and 93.3% did not exceed 5 ppm (8-h TWA). However, higher exposures may occur during some tasks. An extensive study in the USA showed that the majority of exposures was in the range 0-5 ppm (8-h TWA), the lowest exposure band. Again, the job categories with highest short- and long-term exposures were those involving maintenance and sampling.

5.2 BIOMONITORING

No methods for biological monitoring have been established, and data on levels in human tissues, secreta or excreta are not available. So far, the methods studied have not given a consistent measure of exposure. Further validation of these methods will be required before they can be used for monitoring occupational exposure to 1,3-BD.

Biological monitoring methods are being developed and validated in the EU under the Environment Programme, and in the USA at the Inhalational Toxicology Research Institute (ITRI) and the Chemical Industry Institute of Toxicology (CIIT).

5.2.1 Biochemical Effect Monitoring

Although haemoglobin (Hb) adducts have been reported in populations occupationally exposed to 1,3-BD, a consistent correlation with exposure has not been demonstrated. Two of three studies have reported an increase in Hb adducts in exposed individuals, whilst the third did not demonstrate adducts in workers exposed to up to 14 ppm 1,3-BD for 8 hours (Section 7.1.5 and 7.2.4.2 *Humans*.)

Mercapturic acids of 1,3-BD metabolites have been studied in urine samples from humans occupationally exposed to 1,3-BD, as well as from individuals with no known exposure. Employees who worked in production areas with historical atmospheric concentrations of 3 to 4 ppm 1,3-BD were compared to intermediate exposed workers (spending variable amounts of time in low and high exposure areas) and low exposed workers (working in areas with historical TWA concentrations of less than 0.1 ppm 1,3-BD). Urine from control persons who had no known occupational exposure to 1,3-BD was also analysed. One conjugated metabolite (M1) could be quantified in all urine samples, but another (M2) could not. The mean concentration of M1 in the high exposure group was 3,200 ng/ml. The mean concentrations in the other exposure groups were estimated at 1,200 ng/ml, 800 ng/ml and 600 ng/ml for the intermediate, low and unexposed groups respectively (Bechtold *et al*, 1994; Section 7.1.4.).

5.2.2 Biological Effect Monitoring

Sister-chromatid exchanges (SCEs) and micronuclei have not been demonstrated in exposed populations, and although chromosomal aberrations have been reported in one study, a consistent exposure-effect relationship has not been established. One preliminary study has demonstrated a possible link between HPRT and exposure, but in contrast two other studies which assessed larger

populations exposed to higher 1,3-BD concentrations did not detect any increase in HPRT levels (Section 7.2.4.2 *Humans*).

5.3 ENVIRONMENTAL LEVELS

5.3.1 Ambient Air

In the UK, continuous routine monitoring for 1,3-BD is carried out at several locations, including industrial and urban locations. Typically, average urban levels are up to 1 $\mu g/m^3$ (0.5 ppb), with average rural levels being lower at up to around 0.09 $\mu g/m^3$ (0.04 ppb). Certain weather conditions and traffic flows have been found to influence the measured levels. For instance, in London in December 1991 heavy traffic and very cold weather produced a pollution episode where the hourly average concentration of 1,3-BD was around 22 $\mu g/m^3$ (9.7 ppb) The large difference in concentrations between rural and urban locations indicates that road traffic emissions are a significant source of 1,3-BD in urban areas. The low levels in rural areas indicates that 1,3-BD in the atmosphere degrades rapidly and is not transported over significant distances from release (DoE, 1994 as quoted in BRE, 1995).

The concentration of 1,3-BD in the air outside a tavern was 0.45 ppb (Löfroth *et al*, 1989 as quoted in DoE, 1996).

Several monitoring studies in the Netherlands have been summarised by Slooff (1994). Based on the data, the average Dutch 1,3-BD concentration was 0.2 $\mu g/m^3$ (0.09 ppb). Road transport was the main contributor to airborne concentrations.

Road traffic is expected to contribute less to ambient air 1,3-BD concentrations since increasing numbers of cars are fitted with conversion catalysts (DoE, 1996). It is also likely that enhanced fuel economy and improved catalysts will lower any volatile organic carbon (VOC), including 1,3-BD, emissions from cars (CONCAWE, 1995) as well as legislative reductions in tailpipe emissions standards over time (McArragher, 1991)

A comparison of UK and Dutch monitoring reviews with mainly US results presented in earlier publications (e.g. ATSDR, 1992 and IARC, 1992) indicates that current European levels are similar or slightly lower than those measured in the USA in the late 1980s.

In the vicinity of a petrochemical complex in Allendale (Texas), the average 1,3-BD concentration was 100 ppb (maximal average 143 ppb/day and 905 ppb/h) in 1986. Within one mile of another

petrochemical plant, the maximal average concentrations were 240 ppb/12 h and 642 ppb/h (Texas Control Board, 1990 as quoted in ATSDR, 1992).

5.3.2 Indoor Air

1,3-BD concentrations of 4.98 ppb and 8.60 ppb were measured in the indoor air of a tavern; cigarette smoke was the most likely source of the 1,3-BD. The concentration of 1,3-BD in the outdoor air was 0.45 ppb (Löfroth *et al*, 1989 as quoted in DoE, 1996). The concentration of 1,3-BD in a public building in California in 1965 was 9.0 ppb; source unspecified (Stephens and Burleson, 1967 as quoted in DoE, 1996).

5.3.3 Soil

No data are available.

Given the physical nature of 1,3-BD, it is not expected to be present in the soil compartment (DoE, 1996).

5.3.4 Water

In a 1975-76 survey of 14 heavily industrialised US river basins, 2 µg 1,3-BD/l was found at one out of 204 sites. Waste water from synthetic rubber plants contained no 1,3-BD (Ewing *et al*, 1977 as quoted in DoE, 1996 and HSDB, 1992).

To refine the EU Existing Substance Risk assessment on 1,3-BD, a European inter-industry initiative has been initiated to gather information on potential concentrations in receiving water around the facilities where 1,3-BD is produced or used. Monitoring results on the streams that enter the environment are not yet available (ECETOC, 1996a).

However, several companies measured the 1,3-BD content of the waste water streams from plants in which 1,3-BD is made or processed. The preliminary results indicate that 1,3-BD concentrations vary from 0 to 7 ng/l (CEFIC, 1996). Thus most 1,3-BD is lost from wastewater by volatilisation.

5.3.5 Levels in Food and Drinking Water

Olive oil stored in 1,3-BD rubber-modified acrylonitrile bottles contained 8-9 µg 1,3-BD/kg, (6 samples; 3 of 3 brands), the bottles themselves containing residues of up to 6,600 µg/kg. No 1,3-BD could be detected in vegetable oil packaged in 1,3-BD rubber-modified PVC (2 samples) or in yoghurt packaged in polystyrene with 1,3-BD rubber-modified polystyrene lids (2 samples); the detection limit was 1 µg/kg. Residues of the monomer were not detectable in chewing gum based on 1,3-BD rubber (McNeal and Breder, 1987 as quoted in ATSDR, 1992 and HSDB, 1992).

Plastic tubs containing margarine (5 major UK brands) contained < 5 to 310 µg 1,3-BD/kg; no monomer was found in the margarine (detection limit 0.2 µg/kg). Similarly, plastic tubs for potato salad, cottage cheese and yoghurt had residual levels of 21-1,700 µg/kg, but no 1,3-BD was detected in the foodstuffs packaged in them (detection limit 1 µg/kg) (Startin and Gilbert, 1984 as quoted in IARC, 1992 and ATSDR, 1992).

1,3-BD has been detected qualitatively in drinking water in the USA (EPA, 1978; Kraybill, 1980; both as quoted in IARC, 1992 and ATSDR, 1992).

5.3.6 Hobbies and Lifestyle

Very low levels of 1,3-BD may be inhaled from gasoline vapours, automobile exhaust or cigarette smoke (Section 3.3 and 5.3.2).

6. MEASUREMENT TECHNIQUES AND ANALYTICAL METHODS

6.1 MONITORING AT THE WORKPLACE

Selected methods for the analysis of 1,3-BD levels in air at the workplace are listed in Table XVII.

Methods in use by the 1,3-BD monomer production facilities and SBR plants in the EU are currently being investigated by CEFIC (European Chemical Industry Council) and ESRA (European Synthetic Rubber Association) in a joint Task Force. This study involves a comparison of sampling and analytical techniques.

TABLE XVII: Methods for the Analysis of 1,3-BD in Air
(adapted from IARC, 1992)

Sample preparation	Assay[a]	Limit of detection	Reference
Collect on solid sorbent tube; desorb with dichloromethane; chill in ice	GC-FID	0.044 mg/m^3	Eller, 1987[b]
Collect on solid sorbent tube of charcoal coated with *tert*-butylcatechol; desorb with carbon disulphide	GC-FID	0.35 mg/m^3	Hendricks and Schulz, 1986 (OSHA method)[b]
Collect ambient air; inject sample in GC using a temperature-programmed, fused- silica porous layer, open tubular (PLOT) Al_2O_3/KCl column	GC-FID	0.01 ppm (0.01 µl/l)	Locke *et al*, 1987[b]
Collect air sample; assay directly	FT-IR	5 ppm (10 mg/m^3)	Harman, 1987[b]
Collect 3-10 l air on solid sorbent tube; desorb thermically onto cold trap; inject sample in GC using methylsilicone capillary column at –35 °C	GC-FID	0.01-100 ppm	Bianchi and Cook, 1988 (CONCAWE method)
Collect 5 l air on solid sorbent tube; desorb thermically onto cold trap; inject sample in GC using ballistically heated PLOT fused silica capillary column	GC-FID	0.2-100 mg/m^3 (0.1-50 ppm)	HSE, 1986, 1989 (UK method MDHS 53, 63)

a FT-IR, Fourier transform-infrared absorption spectroscopy; GC-FID, GC equipped with flame ionisation detector
b As quoted in IARC, 1992

Techniques routinely used for the determination of hydrocarbons in environmental air may be applied equally for detecting low concentrations of 1,3-BD at the workplace. These methods involve the

collection of a large volume of air and concentration of the volatile components, which are then separated, identified and quantified by a GC equipped with a suitable detector or combination of GC and mass spectrometry (GC-MS) (ATSDR, 1992).

The determination of 1,3-BD in personal air can be obtained using the procedure outlined in NIOSH Method 1024 (NIOSH, 1987). The air sample is obtained by passing a known volume of air (3-25 l) through a set of tandem coconut charcoal tubes, which adsorb 1,3-BD and remove it from the air stream. The collected 1,3-BD is then removed from the adsorption tube by extraction with methylene chloride. Injection of the methylene chloride solution into a GC equipped with a FID combination separates 1,3-BD from any interfering compounds that may be present. The choice of chromatography column is not crucial for this determination, as long as 1,3-BD is clearly separated from other compounds. The estimated detection limit of this method is 0.02 µg/ml, with an applicable range of 1-480 µg per sample (approximately 0.04-19.2 ppm). The precision of this method appears to change as a function of the concentration being measured, due to desorption efficiencies changing as a function of sample concentration. With increasing concentration, the preparation of a standard becomes more difficult. In NIOSH Method 1024, quantitation of 1,3-BD is accomplished by comparing the area under the sample's signal to that of a known amount of 1,3-BD. The preparation and injection of a gaseous 1,3-BD standard is a difficult procedure; it must be performed carefully or erroneous results will occur. Sample storage appears to dramatically affect the results of the measurement. Samples stored at –4 °C displayed an average recovery of between 93% and 98% over a 21-day period, while samples stored at room temperature ranged from 61% to 95%. Literature methods for the determination of 1,3-BD in personal air samples overcome some of these problems (Hendricks and Schultz, 1986; Lunsford, 1987; Lunsford and Gagnon, 1987; all as quoted in ATSDR, 1992).

The Swedish National Occupational Board of Occupational Safety and Health use the following methods. A GC method has been evaluated for air concentrations of 1,3-BD in the range 1,065 to 4,590 mg/m^3 (474 to 2,043 ppm). The 1,3-BD is adsorbed on carbon in a tube through which the air is pumped, desorbed with carbon disulfide, and the desorbate analyzed with GC (NIOSH, 1977; Swedish National Board of Occupational Safety and Health, 1979; both as quoted in Lundberg, 1986). The method is reported to be applicable in the range 200 to 6,600 mg/m^3 (89 to 2,937 ppm), but is considered likely to work at much lower concentrations. One prerequisite is that the desorption losses be determined. When measurements were made with this method and also with a diffusion sampler, results have been in agreement in the range 0.1 to 100 mg/m^3 (0.0445 to 44.5 ppm) (Checkoway and Williams, 1982 as quoted in Lundberg, 1986).

Infrared spectrophotometry can be used for continuous monitoring of 1,3-BD in air. Disturbance from other sources can usually be minimized by suitable choice of wavelength (Lundberg, 1986).

A method for monitoring 1,3-BD in air has been developed at the Finnish Institute of Occupational Health. This involves sampling by means of carbon tubes and analysis by GC with flame ionisation detection (FID). The detection limit is 0.8 $\mu g/m^3$ and the recovery 90% (600 cm^3 of air; 2 h sampling time). The active metabolite, butenemonoxide, can also be detected, albeit at extremely low concentrations (0.1%). The detection limit for the epoxide is 0.01 $\mu g/m^3$ with approximately 90% recovery, using GC with ECD (electron capture device) (Ahlberg *et al*, 1991).

CONCAWE recommends a method (modified 8/86) that entails drawing air through a double packed stainless steel sorbent tube (i.e. Perkin-Elmer ATD 50). The tube is packed with 300 mg of activated coconut charcoal and 200 mg of chromosorb-106. The sampling rate is approximately 20-50 ml/min using a low-flow sampling pump. The sampling tube is thermally desorbed onto a cold trap held at –30 °C. The sample is then ballistically heated and reinjected on to a 50 m BP-1 methylsilicone (chemically bonded) capillary column held at –35 °C. Separation of 1,3-BD from other C_4 components takes place at –35 °C whilst providing good chromotography. The method is suitable for the measurement of airborne 1,3-BD in the range 0.01-100 ppm for samples of 3-10 l of air. The GC parameter variant has been published by Bianchi and Cook (1988).

The UK HSE recommended method involves sampling air using a low flow personal sampling pump (10-50 ml/min) on to a Perkin Elmer ATD 50 sorbent tube packed with 900 mg of Molecular Sieve 13X. The tube is thermally desorbed on to a cold trap held at –30 °C. The trapped components are then re-injected by ballistic heating on to a 50 m Porous-Layer-Open-Tubular (PLOT) fused silica capillary column held isothermally at 130 °C. 1,3-BD is completely eluted and separated from all other C4 isomers. The method is suitable for the measurement of airborne 1,3-BD in the range 0.2 to 100 mg/m^3 (0.1-50 ppm) for samples of 5 l of air (HSE, 1986, 1989).

6.2 ENVIRONMENTAL (AREA) MONITORING

The Dutch Expert Committee for Occupational Standards (DECOS) has reviewed methods for environmental (area) monitoring as follows. By means of a sampling pump 25 l air is pumped over a solid sorbent (coconut charcoal). The upper limit of the sampler is 220 mg/m^3; the measurement range covers 0.044 to 19 mg/m^3. Desorption is performed with methylene chloride; below 0.9 mg/m^3, the desorption efficiency falls below 75%. 1,3-BD is analysed by GC-FID. Interferences are: pentane, methyl acetylene and vinylidene chloride at high levels. High humidity (> 80% RH) or other hydrocarbons present at permissible levels may significantly decrease the sampler's capacity for 1,3-BD (NIOSH, 1987 as quoted in DECOS, 1990). Checkoway and Williams (1982) were able to detect with this method concentrations as low as 0.03 ppm (0.066 mg/m^3) of 1,3-BD.

Stephens and Burleson (1967 as quoted in DECOS, 1990) developed a procedure for the analysis of trace quantities of light hydrocarbons in air. A freeze-trap filled with chromatographic packing was installed in place of the sample loop of a FID chromatograph. An air sample of 0.1-0.5 l was passed through the trap which was chilled with liquid oxygen. After the contents had been swept into the column the minimum detectable concentration was below 1 ppb for 1,3-BD (2.2 $\mu g/m^3$). A long-term (8 h) indicator tube is commercially available. It is specific for 1,3-BD and the detection range is 0.067-5.836 mg (Gentry and Walsh, 1987 as quoted in DECOS, 1990).

There are several gas detector tubes that use common colorimetric reactions to detect 1,3-BD. These reactions include the reduction of chromate or dichromate to chromous ion and the reduction of ammonium molybdate plus palladium sulphate to molybdenum blue (Saltzman and Harman, 1989 as quoted in IARC, 1992).

Alternative methods, based upon current occupational exposure methodologies, are currently being validated under the European Diffusive Sampling Initiative (EDSI).

6.3 BIOLOGICAL TISSUES AND FLUIDS

No standardised method for assaying 1,3-BD and its metabolites in biological tissues, secreta or excreta is available. Methods for biological monitoring have yet to be validated. Biological monitoring methods are being developed and validated in the EU under the Environment Programme, and in the USA at the Inhalational Toxicology Research Institute (ITRI) and the Chemical Industry Institute of Toxicology (CIIT).

Some groups have reported preliminary measurements of 1,3-BD exposure by analysis of Hb adducts or urinary excretion of 1,3-BD metabolites (Section 5.2).

Methods for determination of Hb adducts involve isolation of globin which is then derivatised with pentafluorophenylisothiocyanate prior to GC-MS. The derivative of the 1,3-BD-valine adduct is measured from characteristic peaks at *m/z* 318 and 374 (and also 323 in one method). The methods all use an internal standard of *N*-terminal valine-labelled globin, but the nature of this label varies: one method uses 2-hydroxyethylvaline, whilst the others use 2-hydroxypropylvaline (Section 7.1.5).

Urinary metabolites (Section 7.1.4) are determined by GC or GC-MS.

7. TOXICOLOGY

7.1 TOXICOKINETICS

The relevant route for exposure to 1,3-BD is inhalation. Accordingly, studies on toxicokinetics with oral or dermal exposure of experimental animals have not been conducted. Human toxicokinetic *in vivo* data are not available, however *in vitro* data on biotransformation were obtained using fractions of liver and lung and preliminary Hb adduct data were obtained from workers exposed to 1,3-BD.

7.1.1 Uptake

The distribution (partition) coefficient for 1,3-BD between rabbit blood and air was 0.645 measured *in vivo* at an exposure concentration of 250,000 ppm. The distribution coefficient between rabbit blood and air measured *in vitro* was 0.603. The good agreement between these values suggests a simple passive diffusion of the gas from the alveoli to the blood (Carpenter *et al*, 1944). The blood/air coefficients for Sprague-Dawley rats and $B6C3F_1$ mice were 1.49 and 1.34, respectively (Csanády *et al*, 1992a).

The uptake of 1,3-BD by Sprague-Dawley rats and $B6C3F_1$ mice from the gas phase of closed inhalation chambers was measured and the uptake data obtained were analysed using a two-compartment model developed by Filser and Bolt (1981) (see also Bolt *et al*, 1984; Kreiling *et al*, 1986). The elimination of 1,3-BD by rats or mice can be described by a first-order process. Saturation of 1,3-BD metabolism is observed in both species at about 2,000 ppm. The standardised clearance rate from the gas phase was 10,280 ml/h for the mouse and 5,750 ml/h for the rat, showing that 1,3-BD is metabolised approximately twice as fast by mice as by rats.

Rats and mice were exposed in a nose-only device to ^{14}C-labelled 1,3-BD for 6 hours. Concentrations were 0.08 ppm to 1,000 ppm for mice and 0.08 ppm to 7,100 ppm for rats. The amount of ^{14}C retained at the end of the exposure period ranged from 1.5% of radioactivity (7,100 ppm) to 17% (0.8 ppm) in rats and 4% (1,000 ppm) to 20% (7 ppm or less) in mice. There was a significant decrease in the percentage of retained 1,3-BD with increasing exposure concentration for both rats and mice. When the total amount of ^{14}C retained at 6 hours of exposure was normalised to body weight, mice retained about 4 to 7 times more 1,3-BD and its metabolites than rats (Bond *et al*, 1986).

Three male monkeys (*Macaca fascicularis*) were exposed by inhalation (nose-only) to concentrations ranging from 10 to 7,760 ppm ^{14}C-labelled 1,3-BD for 2 hours (Sun *et al*, 1989a; Dahl *et al*, 1991).

The uptake of 1,3-BD in monkeys was calculated from the total 1,3-BD metabolites formed and excreted within a 96-hours observation period after the exposure. Residual ^{14}C retained in the monkeys at the end of the 96-hour observation period was not determined. Total metabolites formed, expressed as a percentage of the total 1,3-BD inhaled, were converted to the absolute amount of metabolites formed. This value was normalised to the duration and concentration of exposure, and to body weight, in order to facilitate comparisons with earlier rodent studies. The calculated uptake rates for monkeys did not include metabolites remaining in the animals' bodies at the end of the 96-hours post-exposure collection period. The uptake rates calculated by this procedure were 0.13, 0.07 and 0.05 nmol/[min x kgbw x ppm] at exposure concentrations of 10 ppm, 300 ppm and 8,000 ppm respectively. The authors compared these values with data for mice and rats derived from Laib *et al* (1988). The corresponding values for mice were 5.2, 5.2, and 0.8, and for the rats 2.8, 2.8, and 0.4 nmol/[min x kgbw x ppm] at the exposure concentrations given above. For the monkeys, uptake expressed as percentage of inhaled 1,3-BD was 2.9, 1.5, and 1.7 at exposure concentrations of 10, 300, and 8,000 ppm respectively. The corresponding percentages for mice were 12, 12, and 1.8, and for rats 15, 15, and 2.3. These data show that the metabolic uptake of the primates studied was several fold lower than that of rats or mice.

7.1.2 Distribution

Bond *et al* (1987) exposed male Sprague-Dawley rats and B6C3F$_1$ mice nose-only for 3.4 hours to ^{14}C-labelled 1,3-BD. The exposure concentrations, 670 ppm for rats and 65 ppm for mice, were selected on the basis of previous studies which demonstrated that they result in a similar retention of 1,3-BD and its metabolites (expressed as mg/kgbw; Bond *et al*, 1986). Radioactivity was distributed widely in tissues immediately following exposure of both rats and mice. In both species, lung, trachea, nasal turbinates, small and large intestine, liver, kidneys, urinary bladder, and pancreas contained high concentrations of radioactivity within one hour after the end of exposure. At this time, rats had concentrations ranging from 10 (bone marrow) to 960 (bladder) nmol ^{14}C-1,3-BD equivalents/g tissue. In mice, the values ranged from 0.6 (bone marrow) to 1,300 (bladder) nmol ^{14}C-1,3-BD equivalents/g tissue. The data were normalised and the results were expressed as ^{14}C-1,3-BD [equivalents/g tissue x µmol 1,3-BD inhaled]. Mouse tissues contained from 15 to 100 times more ^{14}C than rat tissues. In summary, the data indicated that there was no apparent difference between the amount of ^{14}C derived from 1,3-BD deposited in the tissues of rats (exposed to 670 ppm) and mice (exposed to 65 ppm), but that tissues of mice attained significantly greater concentrations of ^{14}C than those of rats when expressed in terms of inhaled 1,3-BD.

7.1.3 Biotransformation

7.1.3.1 *In Vitro*

Malvoisin *et al* (1979) incubated liver microsomes from Wistar rats with 1,3-BD in the presence of an NADPH-generating system. 1,2-Epoxybutene-3 (EB) was identified as one of the major metabolites. Pretreatment of the rats with phenobarbital increased the microsomal metabolism of 1,3-BD about 2-fold, whereas 3-methylcholanthrene pretreatment had no effect. When the mixed function oxidase inhibitor SKF-525A was added to the incubation mixture, the 1,3-BD epoxidase activity was inhibited by 50%. These results suggest the involvement of cytochrome P_{450}-dependent monooxygenases in the metabolism of 1,3-BD.

These results were corroborated by Bolt *et al* (1983) who also found that the presence of an epoxide hydrolase inhibitor, 1,1,1-trichloropropene oxide, increased the concentration of EB in the incubation mixture. In contrast, the presence of glutathione decreased the epoxide concentration in the incubation mixture. The authors detected both enantiomers of the epoxide.

Further studies with liver microsomes from male Wistar rats led to the tentative identification of 3-butene-1,2-diol, the 2 stereoisomers of DL-diepoxybutane (1,2:3,4-diepoxybutane, DEB), and 2 stereoisomers of 3,4-epoxy-1,2-butanediol as metabolites of EB (Malvoisin *et al*, 1982; Malvoisin and Roberfroid, 1982).

Species differences in the formation of EB from 1,3-BD were investigated by Schmidt and Loeser (1985) using liver preparations from Sprague-Dawley rats, NMRI and $B6C3F_1$ mice, Rhesus monkeys and humans (one sample). The sequence of epoxide formation was mice > rat > human > monkey with a ratio between mouse and monkey of 7:1. With the exception of the monkey the amount of epoxide detected was proportional to the monooxygenase activity. The authors also investigated homogenates from lung tissue. Only tissues from mice and rats produced measurable epoxide concentrations.

Pretreatment (by inhalation, nose only, 6 h/d) of male Sprague-Dawley rats or male $B6C3F_1$ mice with 7,600 and 740 ppm 1,3-BD for 5 days had no effect on the ability of the isolated liver microsomes to metabolise 1,3-BD. However, there was a significant depression of 1,3-BD metabolism *in vitro* in microsomes from lungs obtained from both pretreated rats and mice, compared to non-exposed controls (Bond *et al*, 1988).

Wistuba *et al* (1989) investigated the enantio-selectivity of the *in vitro* conversion of aliphatic alkenes into oxiranes by liver microsomes of untreated or phenobarbital induced rats, of untreated or phenobarbital or benzo(a)pyrene induced mice, and of humans. In rat microsomes, 29% *R*- and 71% *S*-enantiomer was formed, a ratio that was not affected by phenobarbital pretreatment. In mouse microsomes, 46% *R*- and 54% *S*-enantiomer was formed. Benzo(a)pyrene pretreatment did not affect this ratio, but phenobarbital pretreatment changed the ratio to 61% *R*- and 39% *S*-enantiomer. The liver microsomes from 4 individual humans produced between 52 and 56% *R*- and between 44 and 48% *S*-enantiomer. Consequently, there appear to be no substantial differences in the ratio of optical isomers formed between species.

Using male $B6C3F_1$ mouse liver microsomes, Elfarra *et al* (1991) confirmed the cytochrome P_{450}-mediated formation of EB as the primary metabolic pathway of 1,3-BD. Crotonaldehyde was identified as an additional metabolite. The ratio between EB and crotonaldehyde was about 50:1 and was constant over the incubation time, suggesting a common intermediate for both metabolites. In a subsequent study, 3-butenal was identified as intermediate between EB and crotonaldehyde (Duescher and Elfarra, 1993).

The conjugation of EB with glutathione (GSH) was investigated by Sharer *et al* (1991). From the 3 possible regio-isomers that may be formed only *S*-(2-hydroxy-3-buten-1-yl)glutathione and *S*-(1-hydroxy-3-buten-2-yl)glutathione were formed *in vitro* through the action of human placental glutathione-*S*-transferase (GST).

The metabolism of EB was also investigated by Kreuzer *et al* (1991) in liver fractions from male NMRI mice, male Sprague-Dawley rats, and humans (one sample). Only EB hydrolysis was observed in microsomes, NADPH-dependent metabolism of the epoxide, with subsequent formation of DEB, was not detected. In the concentration range examined, metabolism by mouse liver microsomes was strikingly lower than by those derived from rats and humans. The apparent K_m values for the epoxide hydrolase activity were 1.5, 0.7 and 0.5 mmol EB/l incubate for mice, rats and humans respectively; the corresponding V_{max} values were 19, 17 and 14 nmol EB/[mg protein x min] for mice, rats and humans. The conjugation of EB with GSH catalysed by GST in cytosolic fractions followed first order kinetics in the measured range. The derived ratios V_{max}/K_m were 15, 11 and 8 for mice, rats, and humans respectively. On the basis of these *in vitro* results, the investigators attempted to estimate the relative importance of epoxide hydrolysis and glutathione conjugation in the *in vivo* metabolism of EB for the three species. Standardised to 1 kgbw body weight and a level of EB in the liver of 0.5 mmol/l tissue, the authors estimated that the rate of EB metabolism in humans would be between 2 and 7 times slower than in rats, whereas in mice it would be 1.3 times faster than in rats. The ratios between the

epoxide hydrolase and the GST pathways were estimated to be 0.21, 0.38, and 0.6 for mouse, rat and humans, respectively.

Filser *et al* (1992) measured enzyme specific kinetics of 1,3-BD in liver microsomes from mice, rats and one human sample. From the *in vitro* data the authors extrapolated maximum rates for the 1,3-BD metabolism to EB *in vivo*, resulting in 243, 157, and 99 µmol/h/kgbw for mice, rats and humans, respectively. The calculated values were similar to data determined for mice and rats *in vivo* (Kreiling *et al*, 1986, see below).

Sharer *et al* (1992) investigated the metabolism of 1,3-BD to EB in liver, lung, testis and kidney microsomes from male $B6C3F_1$ mice and male Sprague-Dawley rats. Testis microsomes were inactive. Overall, oxidation rates by mouse microsomes were higher than those of rat tissues. Furthermore, crotonaldehyde was detected with mouse tissues only. With mouse microsomes, a 4-fold higher rate was measured with kidney compared to liver or lung, which exhibited similar rates. With rat microsomes, the rate obtained with liver was 2- and 6-fold higher than those of lung and kidney. EB glutathione conjugation rates with liver cytosol of both species were similar, whereas conjugation rates with mouse lung and kidney cytosol were 4- and 2-fold higher than those of rat lung and kidney, respectively.

An alternative pathway for 1,3-BD oxidation to EB was described by Duescher and Elfarra (1992). The authors demonstrated that human myeloperoxidase is capable of oxidising 1,3-BD to EB, crotonaldehyde and 1-chloro-2-hydroxy-3-butene in the presence of H_2O_2. Metabolite formation was dependent on incubation time, pH, KCl, 1,3-BD, and H_2O_2 concentrations. The significance of the results for the *in vivo* metabolism of 1,3-BD is not clear.

The species differences between mice, rats, and humans were also investigated by Csanády *et al* (1992a, 1993) using liver and lung fractions obtained from male Sprague-Dawley rats, male $B6C3F_1$ mice, and humans (12 liver samples, 5 lung samples). Maximum rates for 1,3-BD oxidation to EB (V_{max}) were highest for mouse liver microsomes (2.6 nmol/mg protein/min) compared to humans (1.2) and rats (0.6). The V_{max} for 1,3-BD oxidation by mouse lung microsomes was similar to that of mouse liver but approximately 10-fold higher than the reaction in human or rat lung microsomes. The K_m values were 5.14, 2.0, and 3.75 µmol/l for the reaction in human, mouse, and rat microsomes. The V_{max}/K_m ratios for 1,3-BD oxidation in liver microsomes were 1,295, 230, and 157 for mice, humans, and rats, respectively and 461, 75, and 21 for lung microsomes. Correlation analysis revealed that cytochrome P_{450} 2E1 is the major isoenzyme responsible for 1,3-BD oxidation in human liver samples. This finding is in agreement with the general hypothesis that low molecular weight compounds are often substrates of this isoenzyme (Guengerich *et al*, 1991).

The *in vitro* metabolism of the monoepoxide was also investigated by Csanády *et al* (1992a, 1993). Only mouse liver microsomes displayed rates of EB oxidation to the diepoxide, DEB, which allowed determination of kinetic constants, but the diepoxide was also detected in human and rat microsomal incubations. The identity of the diepoxide was verified by MS. The V_{max} for this reaction was 0.2 nmol/mg protein/min with a V_{max}/K_m ratio of 13. Human liver microsomes displayed the highest rate of EB hydrolysis. The V_{max} for this reaction ranged from 9 to 58 nmol/mg protein/min and was at least 2-fold higher than the V_{max} observed in mouse and rat liver microsomes. The median K_m value was 0.58 mmol/l in human microsomes, and 1.59 and 0.26 mmol/l for mouse and rat microsomes, respectively, resulting in V_{max}/K_m ratios of about 32, 3.6, and 9.5 for the reaction in human, mouse and rat microsomes. The kinetic constants for the conjugation of EB with glutathione in hepatic cytosolic fractions were: V_{max} 45 (human), 500 (mouse), 241 (rat) [nmol/(mg protein x min)]; K_m 10.4 (human), 35.3 (mouse), 13.8 (rat) mmol/l. These results demonstrate that in general the K_ms for the detoxification reactions were about 1,000-fold higher than the K_ms for the oxidation reaction. *In vivo* clearance constants were calculated from the *in vitro* data for 1,3-BD oxidation, and EB oxidation, hydrolysis, and GSH conjugation. Comparison of the overall activation/detoxification ratio revealed that mice have a significantly higher ratio of activation/deactivation (12.5) than rats (1.3) and humans (4.4). Despite these differences, the steady state concentration of EB in the blood of mice upon exposure to 70 ppm 1,3-BD *in vivo* was estimated only to be twice the rat concentration (5.1 µmol/l and 10 µmol/l, respectively). These values calculated from *in vitro* data, although higher than the *in vivo* values measured at the same exposure concentration, reflect the same species difference (see 7.1.3.2, Bond *et al*, 1986).

Rat liver microsomal incubations were analysed for EB, crotonaldehyde, 3-butene-1,2-diol, DEB, and 3,4-epoxy-1,2-butanediol after incubation with 1,3-BD (Cheng and Ruth, 1993). 1,3-BD was added to the incubation mixture that was kept in head space vials via a gas syringe (5 ml, 0.2 mmol/l). According to the authors this amount was sufficient to saturate the incubation mixture with 1,3-BD. After 30 min the amounts of EB, crotonaldehyde, 3-butene-1,2-diol, DEB and 3,4-epoxy-1,2-butanediol formed were 11.0, 0.72, 28.2, 0, and 113.5 nmol/mg protein, respectively. Only one diastereomer of the two possible epoxydiols was observed.

Duescher and Elfarra (1994) investigated the oxidation of 1,3-BD to EB by human, mouse and rat microsomes. In addition, cDNA-expressed human P_{450} isoenzymes were used to identify the isoenzymes responsible for 1,3-BD oxidation in humans. P_{450} 2E1 and P_{450} 2A6 were the most active isoenzymes for 1,3-BD oxidation. Additional experiments with inhibitors indicated that both isoenzymes may be active over a wide range of 1,3-BD concentrations, but that P_{450} 2E1 predominates at low, and P_{450} 2A6 at high 1,3-BD concentrations. At a given 1,3-BD concentration, a 3-fold interindividual variation in the EB rate formed was found. Apparent kinetic constants were

determined in microsomes from two human liver donors. The V_{max} values were 22.8 and 10.4 nmol/mg protein/min and the K_m values were 0.39 and 0.20 mmol/l, respectively. The corresponding values for mouse liver and rat liver microsomes were 9.2 and 2.0 nmol/mg/min and 0.16 and 0.12 mmol/l, respectively. The resulting V_{max}/K_m ratios were about 55 for human, 17 for rat and 58 for mouse microsomes. The results are in contrast to the results reported by other authors with regard to the human activity. It is not clear what the reason for this discrepancy is. All other data available so far indicate that in humans 1,3-BD metabolism to EB is not as rapid as in mice.

Bone marrow of $B6C3F_1$ mice was analysed for the presence of the cytochrome P_{450} dependent monooxygenase isoenzyme 2E1, believed to be the major P_{450} isoenzyme responsible for the conversion of 1,3-BD into EB (Genter and Recio, 1994). The basis for this investigation was the observation that the concentrations of EB are greater in bone marrow than in blood following inhalation exposure (Maples *et al*, 1993). Analysis by Western blot and immunohistochemistry did not detect the isoenzyme in bone marrow. The authors concluded that EB is formed outside the bone marrow and is subsequently concentrated in bone marrow, or that the conversion to EB occurs by an alternate enzymatic pathway. However, as the observations of Maples *et al* (1993) were not confirmed in a subsequent study (Thornton-Manning *et al*, 1996), there is some question as to whether EB concentrates in the bone marrow.

Maniglier-Poulet *et al* (1995) investigated the formation of EB from 1,3-BD in mouse and human bone marrow cells. EB was formed in vitro in both mouse and human bone marrow cells via a NADPH-independent reaction indicating the involvement of peroxidases. Cytochrome P_{450} -dependent enzymes were not involved. Metabolism of 1,3-BD to EB in human bone marrow was more than two orders of magnitude lower than in hepatic mouse or rat microsomes and was demonstrated at very high 1,3-BD concentrations. It is not clear whether the oxidation of 1,3-BD by human myeloperoxidase is a relevant pathway *in vivo*.

The acid-catalysed hydrolysis of EB and DEB and the reactivity towards deoxyguanosine, guanosine and calf thymus DNA were investigated by Bolt *et al* (1995). The data show a much higher chemical stability of DEB compared to EB and more than 10-fold higher second-order rate constants for reaction of EB with nucleophiles compared to DEB.

The conjugation of DEB with GSH by cytosolic GSTs was characterised using cytosol from human, mouse and rat liver and lung (Boogaard *et al*, 1996). DEB conjugated rapidly with GSH via a spontaneous reaction resulting in the formation of the two possible isomers, which were hydrolysed subsequently to the corresponding triol compounds. The enzyme-catalysed reaction led only to one isomer (*S*-(1-hydroxy-3,4-epoxy-2-butyl)glutathione). It was calculated that the spontaneous reaction is

negligible in the intact organism. The enzyme mediated conjugation was well described by Michaelis-Menten kinetics. The conjugation rates (V_{max}) in rat and mouse liver were similar and over 10-fold greater than in human liver. The K_m-values of the hepatic GST for DEB increased from 2.1 mmol/l (human) to 6.4 mmol/l (mouse) to 24 mmol/l (rat). The initial rates of conjugation in the liver as expressed by V_{max}/K_m increased from humans to rats to mice and the initial rate of conjugation in the lung was much higher in mice than in rats. The results indicate that the higher DEB blood concentrations observed in other experiments *in vivo* are not due to differences in hepatic or pulmonary GSH conjugation of DEB.

Seaton *et al* (1995) investigated the epoxidation of EB to DEB using microsomes prepared from human β-lymphoblastoid cells expressing cDNAs encoding individual human cytochrome P_{450} isoenzymes to identify the isoenzymes responsible for this reaction. In addition, microsomal preparations from 10 human donors, from male Sprague-Dawley rats and from male $B6C3F_1$ rats were used to determine the kinetic constants for the reaction. The results identified human cytochrome P_{450} isoenzymes 2E1 and 3A4 as the hepatic isoforms involved in the oxidation of EB to DEB. At low concentrations, as would be encountered in a relevant human exposure scenario, 2E1 appeared to predominate. At 80 mmol/l EB only 2E1 was active whereas at 5.0 mmol/l 2E1 and 3A4 were active. At 80 mmol/l EB, the experiments with microsomes from human donors revealed a 60-fold variation in activity (0.005 - 0.324 nmol/mg protein/min). In pooled mouse and rat microsomal preparations the rates were 0.473 and 0.166 nmol/mg/min, respectively. Kinetic constants were determined for 4 human samples and the pooled mouse and rat microsomes with detailed investigations at varying EB concentrations. The apparent K_m for human samples ranged from 0.304 to 0.880 mmol/l, and V_{max} values ranged from 0.38 to 1.2 nmol/mg/min. In mouse microsomes the K_m was 0.141 mmol/l and the V_{max} was 1.303 nmol/mg/min. In rat microsomes the K_m was 0.145 mmol/l and the V_{max} was 0.408 nmol/mg/min. The resulting V_{max}/K_m ratio for mice was 2.4 to 61-fold greater than the highest and lowest human ratio, respectively, and 3.3-fold greater than the rat ratio. Coupled with the V_{max}/K_m ratios calculated by Csanády *et al* (1992a, 1993) for 1,3-BD activation to EB and EB deactivation this suggests that at concentrations below the K_m, mice have a significantly higher 1,3-BD activation to EB compared to rats and humans and that the resulting higher concentrations of EB are more efficiently converted to DEB in mice compared with rats and humans.

7.1.3.2 *In Vivo*

The first evidence that EB is also formed *in vivo* upon 1,3-BD exposure was provided by Bolt *et al* (1983). Male Sprague-Dawley rats were exposed to initial 1,3-BD concentrations between 6,000 and 7,000 ppm and the exhaled epoxide was measured. At the same time an increase of acetone was noted in the chamber atmosphere.

To compare the metabolic elimination rates of 1,3-BD in male Sprague-Dawley rats and male B6C3F_1 mice, the animals were placed in separate closed chambers with fixed concentrations of 1,3-BD in the air (Bolt *et al*, 1984; Kreiling *et al*, 1986). The decline in the 1,3-BD concentration was measured over time and the resulting concentration/time curve was analysed using a two compartment kinetic model. The calculated metabolic elimination rates of 1,3-BD for rats and mice were dependent on the atmospheric concentration of the compound. Up to ambient concentrations of about 1,000 ppm metabolic elimination was proportional to the exposure concentration in mice and rats. Above 1,000 ppm saturation kinetics of 1,3-BD metabolism became apparent in both species. The metabolic elimination rate of 1,3-BD in mice was about twice that in rats, under conditions of both low and high exposure concentrations.

With the same methods the inhalation toxicokinetics of EB were analysed in male Sprague-Dawley rats and male B6C3F_1 mice (Kreiling *et al*, 1987). At lower exposure concentrations, mice showed a higher metabolic clearance for EB than rats (24,000 ml/h/kg versus 13,400 ml/h/kgbw). EB metabolism in rats is linearly dependent on the atmospheric concentration of the compound up to exposure concentrations of about 5,000 ppm. In mice, saturation of EB metabolism was observed at about 500 ppm. The maximal metabolic rate V_{max} was 350 µmol/h/kgbw in mice and > 2,600 µmol/h/kgbw in rats. Thus, with increasing concentrations the metabolic capacity for EB becomes rate-limiting in mice but not in rats. When mice were continuously exposed to high 1,3-BD concentrations above 2,000 ppm (Filser and Bolt, 1984; Kreiling *et al*, 1987), the exhalation of EB could be measured. Exhalation of the epoxide by mice led to an increase of epoxide concentration in the exposure system up to a peak concentration of 10 ppm after 10 h. In rats, the exhaled EB reaches a plateau concentration of about 4 ppm after 2 hours of exposure to 1,3-BD. From 12 hours onward mice showed signs of acute toxicity and hepatic non-protein sulphydryl (NPSH) content of the animals was virtually depleted. In rats using the same protocol the hepatic NPSH content showed no major depletion and no toxicity was observed (Kreiling *et al*, 1988). From these results the authors concluded that the EB metabolism in mice is predominantly via the GST pathway as compared to the epoxide hydrolase pathway.

The distribution of ^{14}C in blood of male Sprague-Dawley rats and male B6C3F_1 mice was investigated after exposure to 7, 70, and 1,000 ppm ^{14}C-labelled 1,3-BD for 6 hours (Bond *et al*, 1986). Samples of blood were analysed by vacuum line-cryogenic distillation at 2, 4, and 6 hours after start of exposure. The temperatures of the traps were chosen to trap CO_2 (–195 °C), 1,3-BD (–130 °C), EB (–95 °C) and DEB and 1,2-butene-3,4-diol (–45 °C). The largest percentage of ^{14}C in the blood was associated with non-volatile material, which typically accounted for approximately 60 to 80% of the total ^{14}C in the blood. Quantities of metabolites in mice and rats increased with an increase in exposure concentration, although the increases were not proportional to the exposure concentration. At

concentrations of 70 and 1,000 ppm, mice had 2 to 5 times higher concentrations of EB than rats. Similar concentrations of 1,3-BD and DEB were measured in the blood of mice and rats. However, identification of these metabolites was solely based on co-distillation with authentic standards, rather than chemical characterisation. In addition, the authors noted that significantly higher concentrations of $^{14}CO_2$ were found in the blood of the rats than in that of the mice.

The effects of different exposure concentrations of 1,3-BD on the cellular content of liver, lung, and heart were investigated in male Sprague-Dawley rats and male B6C3F$_1$ mice exposed in an open exposure system to 10, 50, 100, 500, 1,000, and 2,000 ppm for 7 hours (Deutschmann and Laib, 1989). A concentration dependent NPSH depletion was observed in mice for all tissues examined. In rats, depletion of NPSH content showed a major reduction above 1,000 ppm only. In mice, depletion of NPSH content of liver, lung, and heart tissue is only observed at exposure concentrations of 250 ppm and greater. A reduction in the NPSH content of about 80% is observed for lung tissue at 1,000 ppm and for liver and heart tissue at exposure concentrations of 2,000 ppm.

Three male cynomolgus monkeys (*Macaca fascicularis*) were exposed to ^{14}C-1,3-BD at concentrations of 10.1, 310, or 7,760 ppm for 2 hours (Sun *et al*, 1989a; Dahl *et al*, 1991). Exhaled air and excreta were collected up to 96 hours after the end of the exposure. The exhaled air was led through –45, – 5, –160, and –195 °C traps. These traps were calibrated with CO_2 (trapped at –195 °C), 1,3-BD (– 60 °C), EB (–95 °C), and DEB and its diol (–45 °C). Other potential metabolites that might have been trapped at these temperatures were not used for calibration. The majority of the volatile material found in the blood immediately after the exposure was CO_2 or 1,3-BD, while the majority of the radioactivity associated with metabolites in the blood at the 2 lower concentrations was non-volatile. In contrast, at the highest exposure concentration, 1,3-BD was the major blood component, probably indicating saturation of the 1,3-BD metabolism at that concentration. The authors compared the distribution of volatile and non-volatile radioactive metabolites in the blood of monkeys with that of rats and mice after 2 hours exposure derived with the same technique (Bond *et al*, 1986). Although the comparison was hampered by differences in both method and exposure between the monkey study and the rodent studies, it was concluded that for equivalent inhalation exposures the concentrations of total 1,3-BD derived metabolites in the blood were 5 to 50 times lower in the monkey than in the mouse, and 4 to 14 times lower than in the rat. However, from the above cited data it is questionable whether steady state conditions were met.

Male Sprague-Dawley rats, B6C3F$_1$ mice, Syrian hamsters, and cynomolgus monkeys were exposed for 2 hours to 8,000 ppm ^{14}C-labelled 1,3-BD and 24 hours urine samples were analysed for metabolites (Sabourin *et al*, 1992, Section 7.1.4).

The bioavailability of EB was assessed using the formation of the Hb adduct of EB with the *N*-terminal valine as dosimeter (Albrecht *et al*, 1993). Female $CB6F_1$ mice and female Wistar rats were exposed (6 h/d) to 0, 50, 200, 500 or 1,300 ppm 1,3-BD for 5 consecutive days. Globin was isolated 18 hours after the last exposure. Adduct levels were about 5 times higher in mice than in rats (17 and 3.5 nmol/g globin, respectively, at 500 ppm). Adduct levels increased linearly with dose in rats, whereas in mice the slope of the curve became flatter at 500 ppm and became steeper again at 1,300 ppm. This behaviour may be explained by saturation of 1,3-BD metabolism at 500 ppm followed by saturation of GSH detoxification of epoxides at higher concentration. Similar results were obtained in male and female C3H x 101/EL mice.

The bioavailability of EB was also assessed in humans using the formation of EB-Hb adduct (Osterman-Golkar *et al*, 1993; see Section 7.1.5)

Male $B6C3F_1$ mice and male Sprague-Dawley rats were exposed to 1,000 ppm 1,3-BD or EB for 4 - 6 hours in closed chambers (Leavens *et al*, 1993). The loss of 1,3-BD or EB from the chamber atmosphere was measured in animals pretreated with the cytochrome P_{450} 2E1 inhibitors pyrazole or diethyldithiocarbamate (DTC). Oxidation of 1,3-BD to EB was inhibited in both species. Clearance of 1,3-BD was reduced in rats from 3.23 to 0.163 l/h/kgbw after pretreatment with either inhibitor. In mice, clearance was reduced from 32.5 to 1.83 or 0.5 l/kgbw/h by pyrazole and DTC, respectively. Inhibition of EB metabolism was not observed in rats, while EB metabolism was partially inhibited in DTC-treated mice (1.12 versus 1.38 l/kgbw/h in controls).

Indirect evidence for the *in vivo* formation of DEB can be derived by measuring DNA adduct levels detected after exposure of male Wistar rats and male $B6C3F_1$ mice to initial concentrations of 500 ppm ^{14}C-labelled 1,3-BD (Jelitto *et al*, 1989; Bolt and Jelitto, 1996). After isolation, purification, and hydrolysis, liver DNA hydrolysates of the exposed animals were separated by column chromatography. The radioactivity of the eluted fractions was measured, and the observed peaks were identified by co-elution with non-labelled authentic marker compounds. 7-*N*-(2,3,4-trihydroxybutyl)guanine, an expected reaction product of DEB with guanine bases, and 7-*N*-(1-hydroxy-3-butene-2-yl)guanine, one of the expected reaction products of EB, were detected in mouse liver DNA hydrolysates but not in rat liver DNA hydrolysates. The DEB-derived adduct was the most common DNA adduct in mice *in vivo*. In contrast, *in vitro* experiments with rat liver microsomes and DNA conducted by the same group indicated that the EB-derived adducts are more prominent.

The same authors investigated DNA-DNA and DNA-protein crosslinks after exposure of male Sprague-Dawley rats and male $B6C3F_1$ mice to 250, 500, and 1,000 ppm of 1,3-BD for 7 h. Immediately after exposure, nuclear DNA from liver and lung cells were isolated and subjected to

alkaline elution. The curves obtained from mouse tissues show the occurrence of protein-DNA and DNA-DNA crosslinks from about 250 ppm onwards. No crosslinking activity of 1,3-BD was observed for rats. The crosslinking activity of 1,3-BD in the mouse was attributed to its bifunctional-alkylating intermediate DEB.

In contrast, Ristau *et al* (1990) could not detect DNA-DNA crosslinks in liver DNA from male $B6C3F_1$ mice or male Sprague-Dawley rats exposed (8 h/d) to 2,000 ppm 1,3-BD for 7 days. The authors who used caesium trifluoroacetate density-gradient centrifugation for the detection and quantification, were able to demonstrate that DNA-DNA crosslinks were formed after *in vitro* incubation of DNA with DEB.

Maples *et al* (1993) found that in $B6C3F_1$ mice exposed to 100 ppm 1,3-BD for 4 hours the concentrations of EB were substantially higher in bone marrow than in blood. Details are not available. However, the observation of increased EB levels in bone marrow was not confirmed in a subsequent study (Thornton-Manning *et al*, 1996).

A highly sensitive and selective GC-MS method was developed for analysis of 1,3-BD, EB and DEB in blood (Bechtold *et al*, 1995). It was applied to blood obtained from 6 male $B6C3F_1$ mice or 6 male Sprague-Dawley rats exposed to 100 ppm 1,3-BD for 4 hours via nose-only inhalation. Mean blood levels of 1,3-BD and EB in rats were 4.1 and 0.1 mmol/l, respectively. Levels of DEB were below the limit of detection in rats (0.01 mmol/l). In mice, mean blood levels for 1,3-BD, EB and DEB were 2.9, 0.38 and 0.33 mmol/l, respectively.

Rats were exposed (6 h/d) by inhalation to 500 ppm 1,3-BD for 5 days, and simultaneously to acetone and/or L-buthionine-[*S*,*R*]-sulphoximine (BSO) in drinking water (Elovaara *et al*, 1994). The effect of the treatments on NPSH content, the formation of EB-Hb adducts and on cytochrome P_{450}-dependent monooxygenase activities in liver and lung were investigated. BSO, which inhibits GSH synthesis, lowered the levels of NPSH in liver, lung and blood and increased the level of EB-Hb adducts in animals treated with 1,3-BD and acetone. Cytochrome P_{450} 2E1 and epoxide hydrolase were induced by 1,3-BD exposure in the liver. In the lungs, 2E1 was not detected and 2B1/2 the main pulmonary P_{450} form was not induced. The rate of styrene-7,8-oxide formation was enhanced 1.5-fold in liver microsomes, but less in lung microsomes, from rats exposed to 1,3-BD only.

Himmelstein *et al* (1994) measured 1,3-BD, EB and DEB concentrations in blood from Sprague-Dawley rats and $B6C3F_1$ mice during and following 6 hours nose-only exposure to 1,3-BD at 62.5, 625, or 1250 ppm. The concentration of 1,3-BD in blood was not directly proportional to the inhaled concentration of 1,3-BD suggesting that the uptake of 1,3-BD was saturated at the highest concentration. In both rats and mice, 1,3-BD and EB blood concentrations were at steady state at 2,

3, 4, and 6 hours of exposure and declined rapidly after termination of exposure. Steady state blood concentrations of 1,3-BD were 2.4, 37, and 58 mmol/l in mice and 1,3, 18, and 37 mmol/l in rats exposed to 62.5, 625 and 1250 ppm 1,3-BD, respectively. The respective steady-state blood concentrations of EB were 0.6, 3.7, and 8.6 mmol/l in mice and 0.07, 0.94, and 1.3 mmol/l in rats. Mice, but not rats, had quantifiable levels of DEB in the blood. The peak concentrations of DEB in mouse blood were 0.65, 1.9, and 2.5 mmol/l.

Himmelstein *et al* (1995) also measured the concentrations of EB and DEB in lung and liver of male Sprague-Dawley rats and B6C3F_1 mice exposed to 0, 62.5, 625, 1250, or 8,000 (rats only) ppm 1,3-BD, 6 hours via nose-only inhalation. Samples of lung and liver were collected at 3 and 6 hours during and at 6 and 12 min after the exposure. In the 62.5 ppm groups, no epoxides were detected due to analytical problems. Exposure to 625 ppm and higher levels resulted in higher EB concentrations in lungs and livers of mice as compared to rats. The mean peak concentrations for EB at 625 ppm and 1250 ppm in mouse lungs were 2.6 and 3.7 nmol/g tissue, in mouse liver 0.58 and 0.93 nmol/g tissue were measured. In rats, the corresponding values were 0.16 and 0.31 (lungs) and 0.06 and 0.16 nmol/g tissue (livers). In rats exposed to 8000 ppm 1,3-BD, the maximum concentrations were 1.3 (lungs) and 1.2 nmol/g tissue (livers). DEB was quantified in lungs (0.71 and 1.5 nmol/g tissue) but not livers of mice exposed to 625 or 1250 ppm. DEB was not detected in lungs or livers of rats exposed to any of the 1,3-BD concentrations tested. GSH depletion was also determined in the individual tissues and was found to be dependent on both the concentration and duration of 1,3-BD exposure. The lungs of mice sustained the greatest depletion (26% of control at 1250 ppm).

Thornton-Manning *et al* (1995a) exposed male Sprague-Dawley rats and male B6C3F_1 mice to 62.5 ppm 1,3-BD for 2 or 4 hours, after which tissues and blood were either taken immediately or after the animals had been held without exposure for 0.5 or 1 h. Blood, heart, lung, liver, adipose tissue, spleen, thymus and bone marrow were analysed for the presence of EB and DEB. Background levels of EB (0.05 - 64 pmol/g tissue) were found in all tissues. Thus it is possible that EB is present in tissues from unexposed animals. Exposure to 1,3-BD increased EB levels above background levels in all tissues in mice, but only in blood, adipose tissue, spleen, and thymus in rats, with no increase in lungs. DEB was increased in the blood of rats and mice at 2 and 4 hours. In mice DEB was detected in all tissues examined, whereas in rats it was detected in blood, heart, lung, adipose tissue, spleen and thymus, although at levels 40- to 160-fold lower than those seen in mice. Immediately after exposure the mean blood level in mice was 204 pmol/g, but only 5 pmol/g in rats. In the sensitive mouse target organs, heart and lungs, levels of DEB exceeded EB levels immediately after exposure. The highest EB concentrations were detected in adipose tissue, indicating fat storage of this metabolite. The results of Maples *et al* (1993), who described much higher EB levels in bone marrow compared to blood, were not confirmed in this study, which is the first to examine thoroughly the

distribution of EB and DEB in rats and mice. 1,3-BD is metabolised by similar metabolic pathways in both species, but substantial quantitative differences were observed. The high concentrations of DEB in rat and mouse tissues which are targets for 1,3-BD-induced carcinogenicity suggest that this metabolite is particularly important in the aetiology of these lesions.

Gender differences in 1,3-BD metabolism in Sprague-Dawley rats were also studied by Thornton-Manning *et al* (1995b). Male and female rats were exposed to 62.5 ppm 1,3-BD for 6 hours. After exposure blood, bone marrow, lung, and adipose tissue samples were removed from all animals and mammary tissue was removed from females. The tissues were analysed for EB and DEB concentrations. The concentration of EB in blood and all other tissues investigated was similar in males and females, but DEB levels in the blood of females were 5-fold greater than in males. DEB levels in the other tissues examined were consistently higher in females. For instance, the mean fat DEB concentration in females was 7.7 pmol/g tissue, whereas in males it was 1.1 pmol/g tissue. Mammary tissue levels contained 10.5 pmol/g, a concentration slightly lower than that observed in blood. The ratios of the two epoxides differed markedly between males and females in all tissues, but differences were most pronounced in lung and adipose tissue, where EB/DEB ratios were 9 and 0.6 (lung) and 159 and 26 (fat) for males and females respectively. The authors suggested that the greater production of DEB in females was associated with the increased incidence of mammary tumours after chronic exposure to 1,3-BD.

The toxicokinetic interaction between 1,3-BD and styrene has been investigated (Laib *et al*, 1992, Leavens *et al*, 1995; see 7.1.3.4).

7.1.3.3 *Physiologically-Based Pharmacokinetic Models*

On the basis of the kinetic data accumulated by Filser and his co-workers over the last decade, Johanson and Filser (1992, 1993, 1995) developed a 2-compartment physiologically-based pharmacokinetic (PB-PK) model to describe the interaction between 1,3-BD, EB, and glutathione in mice and rats, which they extrapolated to man. The model describes the uptake, distribution and elimination of 1,3-BD together with the formation, distribution and elimination of EB via the three metabolic pathways. In order to describe GSH conjugation, a compartment for production and non-epoxybutene dependent elimination of GSH was included in the model. The 1,3-BD and EB compartments were linked via a common intrahepatic compartment which also described the epoxide hydrolase pathway for EB (intrahepatic first-pass effect). Most parameters included in this model were experimentally determined (e.g., partition coefficients) or derived from experimental data (e.g. from the kinetic constants obtained by analysis of gas-uptake studies via a 2 compartment model). The model was validated by comparison of simulated concentration-time courses with experimentally

determined 1,3-BD uptake curves, EB exhalation curves and liver GSH depletion data. The authors claimed that the predicted concentration-time curves agreed well with the experimental data. The model predicts a 1.5-fold difference between the body burden of EB in mice and rats at exposure concentrations below 1,000 ppm 1,3-BD, increasing to 3-fold at higher concentrations. At 10 ppm 1,3-BD, the model predicts EB steady state concentrations of about 0.005, 0.27 and 0.4 mmol/l for man, rat and mouse.

A refinement of the model was described by Csanády *et al* (1995). In addition to the features described above, the model covered disposition and metabolism of DEB in mice and included simulation of Hb adducts of EB in rats and mice. The model successfully described Hb adduct levels at 1,3-BD concentrations ranging from 2 ppm to 100 ppm in male Sprague-Dawly rats and male $B6C3F_1$ mice, and in female Wistar rats ranging from 50 ppm to 1312 ppm. In addition, EB exhalation as well as 1,3-BD and EB blood concentrations were simulated and compared to experimental results. In mice, DEB blood concentrations were also simulated. The areas under the concentration versus time curves for EB in humans were estimated to be only one third to one fifth of those for EB in rodents. The authors stated that the results of their simulation could not explain the vast differences in species susceptibility.

This model has also been used to investigate possible interactions between 1,3-BD and styrene in humans (Filser *et al*, 1993a; see 7.1.3.4).

Another PB-PK model developed by Evelo *et al* (1993) also predicted that in mice, especially at low concentrations, metabolic elimination of 1,3-BD by the lung is significant. However, the authors did not validate their predictions with experimental data.

Kohn and Melnick (1993, 1995) have developed and refined another PB-PK model. Initially, they used theoretically derived partition coefficients instead of measured values and came to the conclusion that formation and retention of EB are controlled to a much greater extent by physiological rather than biochemical parameters, and that storage in fat represents a significant fraction of the retained 1,3-BD. Medinski *et al* (1994) demonstrated that these concusions were at least partially a consequence of the use of theoretically derived partition coefficients. It is not clear whether the partition coefficients have been changed in the updated model, but a number of modifications were introduced, including additional compartments for arterial and venous blood, vascular spaces and alveolar space. The metabolism of 1,3-BD was assumed to occur in liver, lung and rapidly perfused tissues, while that of EB was assumed to occur in liver, lung and rapidly perfused tissues via epoxide hydrolase and in liver, lung, rapidly perfused tissues and blood by GST. Metabolism of EB to DEB was not included in the model. The authors validated their model with different data sets published in the literature, which

they used to investigate the sensitivity of the model to the individual parameters. They came to the conclusion that the model was sensitive to the representation of anatomy and metabolism, and to the values of physiological and biochemical parameters. They stressed that PB-PK modelling could only be used credibly for human risk assessment if the parameters included were reliable.

Another PB-PK model was developed at CIIT on the basis of the studies conducted at that institute, and included one submodel for 1,3-BD and another for EB (Medinsky *et al*, 1994). The two models connected by the metabolism of 1,3-BD to EB in lung and liver and allowed for the metabolism of EB to DEB, the GSH conjugate, and 1,2-dihydroxy-but-3-ene in the liver. The main differences from the Johanson and Filser model were that metabolism of 1,3-BD to EB was also described in the lung compartment, that the description of the conjugation of EB with GSH used a different kinetic approach, and that no assumption was made for an intrahepatic first pass effect. In addition tissue/air partition coefficients were determined for 1,3-BD and EB independently and metabolic constants were scaled from *in vitro* data as described by Csanády *et al* (1992b). The model was validated with different sets of experimental data, and model predictions were found to compare well. The main result of the simulations was that inclusion of lung metabolism improved the fit to experimental data in mice but not rats. Assuming a 10 ppm 1,3-BD exposure, the model predicts locally generated concentrations of EB in lung tissue to be approximately 15-fold higher in lungs of mice than rats.

This model was further modified to simulate the simultaneous disposition of 1,3-BD and styrene in co-exposed mice (Leavens and Bond, 1995; see Section 7.1.3.4).

Another modification of the model of Medinsky *et al* (1994) included addition of GSH and DEB submodels and incorporation of non-enzymatic elimination of EB (Sweeney *et al*, 1995). The model was validated with data from Kreiling *et al* (1987) and from Himmelstein *et al* (1994, 1995). The simulations compared well with the experimental data with regard to GSH depletion but overestimated epoxide concentrations. According to the authors this is due to the time lag between exsanguination and tissue removal for tissues capable of epoxide biotransformation.

7.1.3.4 *Interaction Between 1,3-BD and Styrene Metabolism*

The toxicokinetic interaction between 1,3-BD and styrene was investigated in Sprague-Dawley rats (Laib *et al*, 1992). Gas-uptake studies were carried out by co-exposure of animals to a mixture of 1,3-BD between 20 and 6,000 ppm and styrene between 0 and 500 ppm. The metabolism of 1,3-BD was competitively inhibited by styrene in a concentration-dependent manner up to 90 ppm. However, higher styrene concentrations resulted in little additional inhibition. 1,3-BD had no influence on the metabolism of styrene. The results of the study were interpreted by suggesting at least two different

cytochrome P_{450} dependent monooxygenases which metabolise 1,3-BD, with only one of them inhibited by styrene.

Leavens *et al* (1995) also investigated the interaction between 1,3-BD and styrene. Male $B6C3F_1$ mice were exposed to 1,000 ppm 1,3-BD and to 0, 50, 100, 250 ppm styrene for 8 hours in a dynamic inhalation system. The rate of 1,3-BD metabolism was quantified by measuring the difference between inlet and outlet 1,3-BD concentrations. Following exposure, EB, DEB and styrene oxide were determined in the blood by GC-MS. The authors reported that with increasing styrene co-exposure, the metabolism of 1,3-BD decreased 10 to 30%. However, at all co-exposures the EB blood concentrations remained constant whereas the DEB blood concentrations decreased 35%. The authors' intepretation of the results is that styrene inhibited the oxidation of 1,3-BD to EB as well as of EB to DEB. Elimination of EB by hydrolysis or GSH depletion may also have been inhibited by styrene oxide.

The PB-PK model of Johanson and Filser (1992, 1993, 1995) was also used to investigate possible interactions between 1,3-BD metabolism with co-exposure to styrene in humans (Filser *et al*, 1993a). On the basis of published study results (Laib *et al*, 1992, Filser *et al*, 1993b, Johanson and Filser,1993, Csanády *et al*, 1994) and newly measured partition coefficients for 1,3-BD in human tissues, the model was used to simulate human exposure to atmospheric mixtures of 5 and 15 ppm 1,3-BD with 0, 20 and 50 ppm styrene. It was predicted that the presence of styrene significantly inhibits 1,3-BD metabolism in man. At exposures up to 15 ppm, the amounts of 1,3-BD metabolised were expected to be reduced to 81% and 63% with co-exposure to styrene at 20 and 50 ppm, respectively.

The PB-PK model of Medinsky *et al* (1994) was modified to simulate the simultaneous disposition of 1,3-BD and styrene in co-exposed mice (Leavens and Bond, 1995). Different approaches were tested and the conclusion was that a model with two oxidative pathways more accurately simulated the observed inhibition of 1,3-BD uptake in co-exposed mice. The first pathway shared by 1,3-BD and styrene represented a high-affinity, high-capacity metabolic pathway for 1,3-BD and a high-affinity, low-capacity pathway for styrene. Competitive inhibition between 1,3-BD and styrene was assumed for this pathway. The second pathway was not shared by the compounds and represented a lower affinity and low-capacity metabolic pathway for 1,3-BD and a low-affinity, high capacity pathway for styrene. These results support the conclusions made by other authors that more than one isoenzyme of cytochrome P_{450} is involved for metabolism of 1,3-BD and styrene, and that competition only occurs for one isoenzyme. The authors stated that further refinement of the model would be necessary.

7.1.4 Excretion

In male Sprague-Dawley rats and B6C3F$_1$ mice exposed to 0.08 to 7,100 ppm ^{14}C-labelled 1,3-BD, urine and exhaled air were the major routes of excretion of ^{14}C derived, with a concomitant increase in exhaled of $^{14}CO_2$ (Bond *et al*, 1986).

Male Sprague-Dawley rats and B6C3F$_1$ mice were exposed (nose-only) to mean concentrations of 670 and 65 ppm ^{14}C-labelled 1,3-BD for 3.4 hours (Bond *et al*, 1987). Elimination of ^{14}C from blood and tissues of both species was rapid, with 77% to 99% of the initial tissue burden being eliminated with half-lives of 2 to 10 hours, depending on the tissue.

In the gas-uptake studies described above (Bolt *et al*, 1984; Kreiling *et al*, 1986) the metabolic clearance of 1,3-BD was calculated for an "open" exposure system. For male B6C3F$_1$ mice it was 1.6 times higher than for male Sprague-Dawley rats (7,300 ml/h versus 4,500 ml/h). The exhalation rate constants were similar for both species.

The excretion of ^{14}C in monkeys exposed to 10.1 - 7,760 ppm ^{14}C-labelled 1,3-BD was investigated by Dahl *et al* (1991). At 10 ppm, slightly more ^{14}C was exhaled as $^{14}CO_2$ than excreted in urine. This ratio was reversed for 310 and 7,760 ppm exposures. ^{14}C elimination in faeces was substantially less than in urine or as $^{14}CO_2$. Other unidentified volatile metabolites were also exhaled, which made a major contribution to excretion at 7,760 ppm. Urinary excretion, which represented the major route of excretion for exposures at 10 and 310 ppm, could be described by a single negative exponential with a half-life of 9.4 hours. The other routes of excretion had more complex patterns.

Sharer and Elfarra (1992) investigated the excretion of the regio-isomers of the GSH conjugate of EB in the bile of male Sprague-Dawley rats following i.p. injection of EB (14.3 and 143 mmol/kgbw). *S*-(2-hydroxy-3-buten-1-yl)glutathione and *S*-(1-hydroxy-3-buten-2-yl)glutathione were detected in a 3:1 ratio in bile 30 minutes after injection of EB and excretion in bile was virtually complete after 120 minutes. Conjugate excretion did not exhibit saturation when the EB dose was varied between 14.3 and 286 mmol/kgbw. The total amount of EB excreted as GSH conjugates in the bile averaged only 7.6% of the doses given.

The excretion of the mercapturic acids of *S*-(2-hydroxy-3-buten-1-yl)glutathione and *S*-(1-hydroxy-3-buten-2-yl)glutathione was further investigated by Elfarra *et al* (1995). The authors synthesised five *N*-acetyl-L-cysteine *S*-conjugates of EB that might result from conjugation of GSH with EB. The five mercapturic derivatives included *S*-(4-hydroxy-2-buten-1-yl)-*N*-acetyl-L-cysteine (III) and two stereoisomers each of the regio-isomers *S*-(2-hydroxy-3-buten-1-yl)-*N*-acetyl-L-cysteine (I) and *S*-(1-

hydroxy-3-buten-2-yl)-*N*-acetyl-L-cysteine (II). Male Sprague-Dawley rats and male B6C3F$_1$ mice were injected i.p. with 71.5, 143 or 286 mmol EB/kgbw (5 - 20 mg/kgbw) and the urine was analysed for the mercapturic acids. In the rat, I and II were identified in urine and exhibited a linear relationship with the administered EB dose. The total amount of the EB dose excreted as I and II averaged 17%. All of the mercapturic acids were excreted within 8 hours after administration of EB. Excretion of II was favoured over that of I by nearly 3:1. In the mouse, I and II accounted for 25.7% of the high EB dose, and for 7.7% and 9.6% at the medium and low EB dose, respectively. In contrast to rats, mice preferentially excreted regio-isomer I over II. Mercapturic acid III was not detected in urine. In contrast to Sabourin *et al* (1992) and Bechtold *et al* (1994), the authors did not detect 1,2-dihydroxy-4-(*N*-acetyl-L-cysteinyl)-butane, which was suggested to be formed through conjugation of GSH to the epoxide hydrolase EB metabolic product, 3-butene-1,2-diol. The reason for this discrepancy was not clear.

Species differences in the urinary excretion of 1,3-BD metabolites were described by Sabourin *et al* (1992). F344 rats, Sprague-Dawley rats, B6C3F$_1$ mice, and Syrian hamsters were exposed nose-only to 8,000 ppm ^{14}C-labelled 1,3-BD for 2 h. Cynomolgus monkeys were exposed to 10, 300, or 800 ppm for 2 h. Immediately after exposure, the urine was collected for 24 hours (rats, mice, hamster) or 96 hours (monkeys). 1,2-Dihydroxy-4-(*N*-acetyl-cysteinyl)-butane (metabolite I) and S-(1-hydroxy-3-buten-2-yl)-*N*-acetylcystein) (metabolite II) were identified by GC-MS. Metabolite I is probably the result of GSH conjugation to 3-butene-1,2-diol and subsequent conversion of the conjugate to the mercapturic acid whereas metabolite II, the *N*-acetyl-cysteine conjugate of EB, is formed from the GSH conjugate of the monoepoxide. Mice excreted 3-4 times as much metabolite II as I; the hamsters and the rats produced approximately 1.5 times as much metabolite II as I; the monkeys produced primarily metabolite I. At 10 ppm, monkeys excreted only metabolite I, whereas at 300 ppm the ratio between I and II was 9:1, i.e. the same as at 8,000 ppm. Four other urinary metabolites, formed in all species in minor amounts, were not identified. The ratio of formation of metabolite I to the total formation of the two mercapturic acids correlated well with the known hepatic EH activity in the different species. These data suggest that the availability of the monoepoxide for conjugation with GSH is highest in the mouse, followed by the hamster and the rat, and lowest in the monkey.

In a follow up study, Bechtold *et al* (1994) analysed the excretion of metabolite I and metabolite II in urine from workers occupationally exposed to 1,3-BD. Employees who worked in production areas with historical atmospheric concentrations of 3 - 4 ppm 1,3-BD were compared to workers who spent variable amounts of time in low and high exposure areas (i.e. the intermediate exposure group) and workers who worked in areas with historical TWA concentrations of less than 0.1 ppm 1,3-BD (the low exposure group). In addition, urine from control persons who had no known exposures to 1,3-BD was analysed. Metabolite I, but not metabolite II, could be quantified in all urine samples. The high

exposure group had mean levels of 3,200 ng/ml of metabolite I and no measurable amounts of metabolite II. Based on the detection limit of the assay this yields a percentage of excretion of metabolite I of at least 97% relative to the sum of the two metabolites. These results support the hypothesis that humans detoxify EB mainly via the epoxide hydrolase pathway. The mean concentrations of metabolite I in the other exposure groups were about 1,200 ng/ml, 800 ng/ml and 600 ng/ml (estimated from a graph). The implications of the presence of metabolite I in urine samples from individuals with no known exposures are unknown.

In order to compare detoxification pathways in mice and rats with humans, male F344 rats and B6C3F$_1$ mice were exposed nose-only to 11.7 ppm 1,3-BD for 4 hours and the urine analysed for metabolites I and II . Excretion of metabolite I, expressed as a percentage of the sum of both metabolites, was 20% in mice, 52% in rats and, as explained above, > 97% in humans. This correlates well with the kinetic *in vitro* data reported by Csanády *et al* (1992a, 1993) who calculated the ratio of epoxide hydrolase activity to GST activity for EB in the three species as being 0.04 for mice, 0.08 for rats, and 1.8 for humans.

7.1.5 Biochemical Effect Monitoring

No validated methods for biochemical effect monitoring human exposure to 1,3-BD have been established, but method development using experimental animals has been performed to investigate the formation of blood Hb adducts after 1,3-BD exposure as a marker for previous exposures.

Sun *et al* (1989b) injected (i.p.) male Sprague-Dawley rats and male B6C3F$_1$ mice with ^{14}C-labelled 1,3-BD dissolved in corn oil. Globulin was isolated from blood samples and analysed for ^{14}C. Hb-adduct formation (measured as associated radioactivity, not characterised analytically) was linearly related to administered doses up to 100 µmol 1,3-BD/kgbw for mice and rats. Hb adducts also accumulated linearly after repeated daily administration of 100 µmol ^{14}C-labelled 1,3-BD/kgbw for 3 days. The adducts showed lifetimes of 24 to 65 days for mice and rats, respectively, which correlate with reported lifetimes for red blood cells in these species. The efficiency of Hb adduct formation in mice and rats was 0.177 and 0.407 [(pmol of ^{14}C-adducts/mg globin)/(µmol of retained ^{14}C-1,3-BD/kgbw)], respectively. This reveals that mice were approximately 2.3 times less capable than rats of converting 1,3-BD administered via i.p. injection into 1,3-BD derived Hb adducts. If the degree of 1,3-BD-induced carcinogenesis and the degree of Hb adduct formation are both due to and dependent on the extent of metabolism of 1,3-BD to reactive (alkylating) metabolites, then the amounts of Hb adducts formed in this study did not correlate with the toxicity of the compound. Hence, the authors expressed doubts about the usefulness of 1,3-BD derived Hb-adducts as indicators of the levels of

reactive chemical metabolites in blood. However, it has to be kept in mind that the adducts were measured as associated radioactivity and that administration was not by inhalation.

GC-MS has been used to determine quantitatively the formation of the adduct of EB to the *N*-terminal valine in Hb isolated from Wistar rats exposed (5 d/wk) to 0, 250, 500, and 1,000 ppm 1,3-BD for 2 weeks. In addition, urine was collected each day during exposure and in between exposures. The Hb adducts proved to be stable and were regarded as useful for dosimetry or long-term exposure. The adduct concentrations increased linearly with exposure dose up to 1,000 ppm (3 nmol/g Hb at 1,000 ppm). The amounts of mercapturic acids excreted were also linearly related to the air concentrations of 1,3-BD. Hence, the authors regarded both methods as useful for assessing occupational exposure to 1,3-BD, although the sensitivity of both methods needed improvement. In addition, the authors suggested developing methods for *in vivo* dosimetry of DEB (Osterman-Golkar *et al*, 1991).

Alkylated amino acids of Hb and serum albumin, obtained after *in vitro* reaction of EB with human blood, were characterised by HPLC (high-pressure/performance liquid chromatography) and HPLC-MS. This method is considered by the authors to be suited for further development (Müller *et al*, 1991).

Osterman-Golkar *et al* (1993) further investigated the binding of EB to the *N*-terminal valine in Hb in male B6C3F$_1$ mice and Sprague-Dawley rats. The animals were exposed (6 h/d, 5 d/wk) to 0, 2, 10, or 100 ppm 1,3-BD for 4 weeks. The adduct level increased linearly with 1,3-BD concentration in mice, whereas a deviation from linearity was observed in rats. After exposure to 100 ppm, the adduct levels were 4 times higher in mice compared to rats. Adduct levels of 1 - 3 pmol/g globin were recorded in human subjects who worked in a production area with about 1 ppm 1,3-BD. The adduct level expressed in pmol/g globin/ppm-h was about 0.5 for B6C3F$_1$ mice, 0.1 to 0.3 for Sprague-Dawley rats and 0.004 for humans (4 samples). Although preliminary, the data suggested that the adduct levels - and consequently the internal EB doses - were lower in humans than in mice and rats. The adduct levels were much lower than those seen after occupational exposures to corresponding air levels of ethylene oxide or other aliphatic epoxides.

Urine samples from workers exposed to 1,3-BD were analysed for mercapturic acids of EB, but the method lacked the sensitivity required for current 1,3-BD-exposure levels (Sorsa *et al*, 1991; Arbetsmiljöfonden, 1991). The same group has been exploring other biomonitoring methods, including GC measurement of 3-butene-1,2-diol derivatives in urine, and cytogenetic parameters such as chromosomal aberrations, SCE and micronuclei. These methods have not yet been validated (Arbetsmiljöfonden, 1991) (see Section 7.2.4.3). The approaches being developed by this group are part of the EU Environment Programme (Section 6.3).

Recently developed methods were more successful in analysing mercapturic acids of 1,3-BD metabolites in human urine (Bechtold *et al*, 1994; Section 7.1.4). However, the intention of the authors was not to establish a quantitative relationship between 1,3-BD exposure and urinary excretion of mercapturic acid. In addition, background levels of EB-derived mercapturic acid were detected also in non-exposed individuals. The implications of this finding are not clear.

A method is described for the detection of 1,3-BD-Hb adducts by the determination of *N*-(2-hydroxy-3-buten-1-yl)valine and *N*-(1-hydroxy-3-buten-2-yl)valine. the limit of detection of this method is 10-20 pmol/g globin. Administration of EB to mice and rats (up to 60 mg/kgbw i.p.) resulted in a dose-related increase in adduct formation. However, adducts were not detected in blood samples for workers exposed to 0.05-14 ppm 1,3-BD (8-h TWA) (Richardson *et al*, 1995).

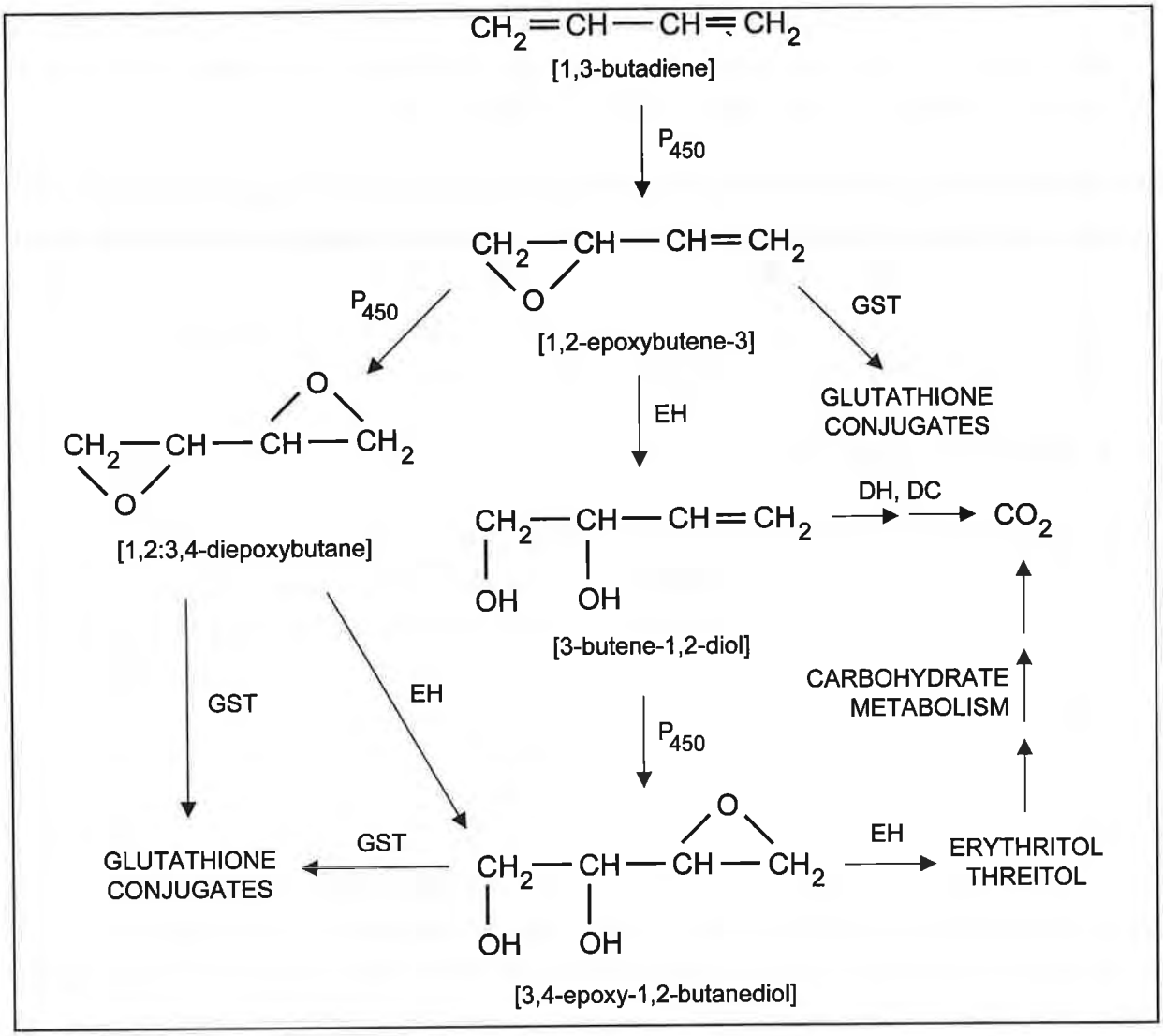
7.1.6 Summary and Evaluation

The metabolic elimination of 1,3-BD in rats and mice is linearly related to the ambient exposure concentration up to 1,000 ppm, with mice showing higher elimination rates. Above 1,000 ppm, metabolic pathways are saturated in these species. In monkeys, the metabolic elimination of 1,3-BD appears to be saturated at 300 ppm. The data presented in the previous sections support the scheme of 1,3-BD metabolism shown in Figure I.

Figure I shows that 1,3-BD is metabolised by cytochrome P_{450}-dependent monooxygenases (presumably two different isoenzymes) to the primary metabolite EB. This intermediate is subjected to further metabolism via 3 pathways, (i) hydrolysis by epoxide hydrolases to 3-butene-1,2-diol, (ii) further epoxidation by monooxygenases to yield DEB with involvement of the cytochrome P_{450} dependent isoenzymes 2E1 and 3A4 and (iii) conjugation with GSH catalysed by GST. DEB may be either conjugated with GSH or hydrolysed to 3,4-epoxy-1,2-butanediol, which also may be formed by epoxidation of 3-butene-1,2-diol. 3,4-Epoxy-1,2-butanediol may be subject to further hydrolysis yielding erythritol and threitol, or conjugated with GSH. CO_2 may be formed by dehydrogenation and decarboxylation of 3-butene-1,2-diol, or via carbohydrate metabolism of erythritol. The individual glutathione conjugates are excreted as mercapturic acids in the urine. Presumably also the diols are excreted as conjugates (glucuronides, sulphates). Identification of crotonaldehyde as a minor metabolite may indicate the involvement of the peroxidases as an additional metabolic pathway. Nauhaus *et al*, (1996) indicated that other reactive aldehydes such as 3-butenaldehyde or acrolein may be formed in small amounts in the mouse, but not in the rat. The role of reactive aldehydes in the toxicity of 1,3-BD remains to be established.

FIGURE I: Metabolism of 1,3-BD

(adapted from Malvoisin and Roberfroid, 1982)



DC decarboxylases
DH dehydrogenases
EH epoxide hydrolase
GST glutathione *S*-transferase
P_{450} cytochrome P_{450}-dependent monooxygenases

According to the *in vitro* and *in vivo* data, the biotransformation appears to be qualitatively similar in all species studied, including humans. However, owing to observed differences in both the uptake of 1,3-BD and in the metabolic kinetics, the steady-state concentrations in blood and target tissues and the resulting body burden of 1,3-BD and its individual metabolites are quantitatively different across species. The different ratios of activating/deactivating processes in the individual species have major implications with regard to the three epoxides formed by metabolic conversion of 1,3-BD.

In the past, attention has been focused on EB as the possibly ultimate carcinogenic metabolite. Of the species studied, mice are the most efficient at metabolising 1,3-BD to EB, whereas rats are less active at forming EB and primates convert even less of 1,3-BD to EB. As evidenced by data on exhalation and on blood concentrations of this epoxide, as well as by results of PB-PK modelling and other extrapolations of *in vitro* data to the *in vivo* situation, the total body burden of EB at low exposure concentrations of 1,3-BD appears to be up to 3-fold higher in the mouse compared to the rat. Monkeys also have a lower body burden of EB compared to mice. Similar conclusions can be drawn with respect to humans from *in vitro* data obtained with human lung and liver samples. If human metabolism *in vivo* follows the pattern found in monkeys and in the *in vitro* samples, one would expect that humans are closer to the rat than to the mouse with regard to the body burden of EB. This was preliminary confirmed in studies using EB-Hb adducts for EB dosimetry. Workers exposed to 1,3-BD showed at least 25-fold lower adduct levels per ppm-hour than rats, and more than 100-fold lower adduct levels than mice.

The above notwithstanding, several studies and PB-PK models suggest that the differences in EB body burden are not sufficient to explain the vast species differences between rats and mice observed in the carcinogenic response. Therefore, attention has recently been focused on DEB as the metabolite likely to play the most important role in the 1,3-BD-induced carcinogenesis. The metabolism of EB in mice proceeds mainly via further epoxidation to DEB or by conjugation with GSH. Rats are less active in forming DEB from EB but more active in conjugation of EB and its hydrolysis product butenediol with GSH. *In vivo* data from primates and *in vitro* data from human donors suggest that primates hydrolyse most of the EB formed. The extent to which primates form DEB is unknown, but can be derived from recent *in vitro* data. It has been concluded from the kinetic properties of the individual pathways that the DEB concentrations in mice must be much higher than in rats. This has been verified in *in vivo* experiments with low-level 1,3-BD exposure to mice where DEB concentrations determined in target tissues for 1,3-BD such as lung and heart were higher than the EB concentrations. In addition, DEB levels were generally much higher in mice compared to rats.

Since DEB is the most mutagenic 1,3-BD metabolite (Section 7.2.4) and is present in the target tissues of the sensitive species, the mouse, in high concentrations, but in much lower concentrations

spontaneous cytotoxicity; lymphoid organ histopathology was also evaluated. Moderate histologic changes (primarily decreased lymphoid cellularity and increased extramedullary haematopoiesis) were observed in spleens from exposed animals, but immune function was similar in control and exposed mice. These functional assays determined the ability to generate antibody-producing cells, and lymphocyte proliferation in response to mitogens and cell surface alloantigens, and spontaneous and acquired cytotoxicity (Thurmond *et al*, 1986).

No detailed data are available in other species.

7.2.2.5 *Summary and Evaluation*

No data exist for eye and skin irritation and skin sensitisation.

As 1,3-BD is a gas at room temperature, exposure to liquid material is rare. Due to its extremely low boiling point, exposure to liquid material could cause tissue damage.

The low boiling point of 1,3-BD also infers that surface accumulation is unlikely and any liquid material on the skin would evaporate rapidly. Absorption through the skin will not be significant (ECETOC, 1993b).

7.2.3 Repeated Dose Toxicity

Carpenter *et al* (1944) exposed groups of 24 rats, 12 guinea pigs, 4 rabbits, and 1 dog to 0, 600, 2,300 or 6,700 ppm 1,3-BD (7.5 h/d, 6 d/wk) for 8 months. Reduced body weight gain was noted in rats and guinea pigs at 6,700 ppm. There were no effects reported among other animals at any dose level or in rats or guinea pigs exposed to 600 or 2,300 ppm 1,3-BD.

Rats were exposed to 0, 0.45, 1.35, or 13.5 ppm for 81 days. Changes in the liver, kidney and spleen morphology, the central nervous system, and immunological status were observed in rats exposed to 0.45 ppm 1,3-BD. Morphologic changes in the nasopharynx were noted in rats exposed to 1.35 ppm. At 13.5 ppm, there were haemodynamic changes, increased permeability of the vessels, and alteration of the structure of the kidney and heart (Nikiforova *et al*, 1969). These findings are inconsistent with the results of later studies in which exposure up to 8,000 ppm was essentially without effect on those organ systems (Owen *et al*, 1987).

Groups of 40 male and 40 female Sprague-Dawley rats were exposed (6 h/d, 5 d/wk) to 0, 1,000, 2,000, 4,000 or 8,000 ppm for 13 weeks. The only effect the investigators considered to be related to 1,3-BD

exposure was a moderate increase in salivation, particularly among female rats during the last 6 to 8 weeks of exposure at the higher concentrations (Crouch *et al*, 1979).

Groups of 5 male and 5 female B6C3F_1 mice were exposed (6 h/d, 5 d/wk) to 0, 625, 1,250, 2,500, 5,000 or 8,000 ppm 1,3-BD for 15 days. No treatment-related effects were observed (NTP, 1984).

Groups of 10 male and 10 female B6C3F_1 mice were exposed (6 h/d, 5 d/wk) to 0, 625, 1,250, 2,500, 5,000 or 8,000 ppm 1,3-BD for 14 weeks. The following numbers died or were killed when moribund: 6 males and 1 female at 8,000 ppm, 6 males and 1 female at 5,000 ppm, 1 male at 2,500 ppm and another male at 1,250 ppm. Body weight gains were decreased in males at the three highest concentrations and in females at the two highest concentrations. No treatment-related histopathological effects were observed (NTP, 1984).

B6C3F_1 or NIH-Swiss mice were exposed (6 h/d, 5 d/wk) to 0 or 1,250 ppm 1,3-BD for 6 weeks. There were treatment-related effects in both strains including decreased circulating erythrocytes, total Hb and haematocrit, and increased mean corpuscular volume. The anaemia was not accompanied by a significant alteration in mean corpuscular Hb concentration, or an increase in circulating reticulocytes, or nucleated erythrocytes. These findings are consistent with a treatment-related macrocytic-megaloblastic anaemia and indicate that the bone marrow is a target organ for 1,3-BD toxicity in the mouse (Irons *et al*, 1986a,b).

B6C3F_1 male mice were exposed (6 h/d, 5 d/wk) to 0 or 1,250 ppm 1,3-BD for 6 or 30 weeks. Quantitative assessment of pluripotent stem cells was made using the colony-forming-unit spleen assay (CFU-S). The differentiation of committed myeloid cells was made by enumerating the CFU of granulocyte/macrophage (CFU-GM). The effects on haematopoiesis were assessed by long-term bone marrow culture. Neither the number of CFU-S nor CFU-GM were altered following 6 weeks' exposure to 1,3-BD, although the colonies derived from treated animals were smaller in size. Large colonies were assumed to have arisen from mature pluripotent stem cells which differentiated upon stimulus, while the smaller colonies originated from the more primitive stem cells. There were no changes in bone marrow cellularity. The incidence of both CFU-S and CFU-GM was significantly decreased after 30 weeks' exposure. There was also a significant suppression in the number of CFU-GM in long-term bone marrow cultures after 14 days in culture relative to control cultures. This alteration in the kinetics of stem cell proliferation in long-term cultures suggests a profound change in stem cell regulation, a shift in maturation or a delay in differentiation to the granulocyte/macrophage committed cell. These findings indicate that 1,3-BD causes alterations in stem cell development in mice (Leiderman *et al*, 1986).

Groups of 25 or 50 male CD-1 mice were exposed (6 h/d, 5 d/wk) to 0, 12.5 or 1250 ppm 1,3-BD for 10 weeks. Two animals died at the highest exposure level. There was no effect upon body weights of surviving animals (Anderson *et al*, 1993).

7.2.3.1 *Summary and Evaluation*

1,3-BD has a low order of repeated-dose toxicity. The target organs in the mouse are the central nervous system (CNS) and bone marrow, whereas non-specific effects were reported in the rat. The no-observed effect level (NOEL) is 2,300 ppm in the rat and 625 ppm in the mouse.

7.2.4 Genotoxicity

The genetic toxicology of 1,3-BD has been evaluated by Rosenthal (1985), De Meester (1988), Brown (1990), DECOS (1990), IARC (1992), Adler *et al* (1995) and Jacobson-Kram and Rosenthal (1995).

In general 1,3-BD is metabolised to reactive epoxide intermediates. It is converted by P_{450} dependent monooxygenases to the primary metabolite EB. EB is further oxidised to DEB. The latter is hydrolysed to 3-butene-1,2-diol which is then conjugated with glutathione.

The metabolites EB and DEB react with DNA to give alkylated products and inter-strand crosslinks. These DNA-lesions may eventually lead to damage at the gene and/or chromosomal level.

7.2.4.1 *In Vitro* (Table XVIII)

In *Salmonella typhimurium* strain TA1530 (detecting base pair substitutions), mutagenic effects were induced by 1,3-BD in the presence of S9 from rats pretreated with phenobarbital or Arochlor 1254. There was no effect on the mutational frequency in this particular strain when uninduced rat liver S9 fraction was used. 1,3-BD was mutagenic to TA1535, both with induced and non-induced rodent S9 but not mutagenic when non-induced human S9 was employed (De Meester *et al*, 1980; Arce *et al*, 1990). In a mouse lymphoma forward mutation assay, modified for testing gases and vapours, 1,3-BD was inactive both with and without metabolic activation (McGregor *et al*, 1991). A weak positive response was reported for SCE induction in Chinese hamster cells (+S9) and in human lymphocytes (+/–S9) (Sasiadek *et al*, 1991a,b). However, this effect could not be reproduced in another study in which a variety of S9 fractions were used, including those from the mouse and human (Arce *et al*, 1990).

TABLE XVIII: Genotoxicity of 1,3-BD *In Vitro*

Test system	Results without activation	Results with activation	Reference
Microbial			
Reverse gene mutation (*Salmonella typhimurium*)	–ve	+ve (strains 1530 and 1535 only)	De Meester *et al*, 1980; Arce *et al*, 1990
Modified pre-incubation *Salmonella typhimurium* (strain TA100, TA102) reverse gene mutation assay	Not tested	+ve (pre-incubation)	Hughes *et al*, 1987
Mammalian cell			
SCE (Chinese hamster cells)	–ve	+ve (weak)	Sasiadek *et al*, 1991a
SCE (human lymphocytes)	–ve +ve	–ve +ve (weak)	Arce *et al*, 1990; Sasiadek *et al*, 1991b
Gene mutation (mouse lymphoma cells)	–ve	–ve +ve	McGregor *et al*, 1991 Sernau *et al*, 1986

7.2.4.2 *In Vivo* (Table XIX)

Interaction of 1,3-BD with Macromolecules (See also Section 7.1.3.2)

The potential of 1,3-BD and its reactive metabolites to bind to DNA and proteins was studied by a number of investigators. When rats and mice were exposed in a closed system to ^{14}C-1,3-BD, radioactivity was recovered in both species from hepatic nucleoproteins and DNA (Kreiling *et al*, 1986). A similar extent of binding to DNA was observed in mice and rats but the binding to liver nucleoproteins was twice as high in mice as in rats. DNA cross-linking was evaluated in rats and mice (Vangala and Jelitto, 1989; Jelitto *et al*, 1989; Vangala *et al*, 1993). It was shown that DNA-DNA and DNA-protein cross-linking was much more pronounced in mouse lung than in liver. Interspecies comparison demonstrated results consistent with other studies: little activity in the rat compared to mice. These results are in contrast with the findings of Ristau *et al* (1990) who did not detect DNA-DNA crosslinks in livers of mice or rats following exposure (8 h/d) to 2,000 ppm for 7 days. Single strand breaks were detected in both rats and mice after inhalation exposure to 1,3-BD. Formation of these single strand breaks is probably due to the action of the reactive butene monoepoxide (EB) intermediate. Reaction products of the EB with the N-terminal valine of Hb have been identified in 1,3-BD-exposed rodents and humans (Osterman-Golkar *et al*, 1991, 1993; Recio *et al*, 1992). The data in

humans are preliminary but they suggest that the adduct level is in the order of 0.004 pmol/g globin/ppm/h for humans as compared to values of 0.5 pmol/g globin/ppm/h for mice and 0.1 pmol/g globin/ppm/h for rats. Thus the blood dose of EB/ppm/h of 1,3-BD exposure is approximately 1-2 orders of magnitude lower in humans than in experimental animals.

Genotoxic Effects In Somatic Cells

1,3-BD did not induce unscheduled DNA synthesis in the rat or mouse (Vincent *et al*, 1986; Arce *et al*, 1990). However, 1,3-BD increased SCEs and chromosomal aberrations in mouse bone marrow (Irons *et al*, 1987a; Tice *et al*, 1987). The number of micronuclei in peripheral lymphocytes and bone marrow was also increased in 1,3-BD treated mice (Cunningham *et al,* 1986; Irons *et al*, 1986a, 1987; Tice *et al*, 1987; Jauhar *et al*, 1988; Wehr *et al*, 1987; Exxon, 1990; MacGregor *et al*, 1990; Victorin *et al*, 1990; Adler *et al*, 1994; Autio *et al*, 1994). These findings could not be confirmed in the rat (Cunningham *et al*, 1986; Autio *et al*, 1994). In bone marrow micronucleus assays conducted using simultaneously exposed mice and hamsters, a 1.4-fold increase was found in the number of micronuclei in the hamster while an 11.2-fold increase was observed in the mouse (Exxon, 1990). Micronucleus induction was not found in monkeys exposed to concentrations up to 8000 ppm (2 hr), nor was there an increase in SCEs (Sun *et al*, 1989a).

A number of studies has been reported on the induction of gene mutations by 1,3-BD in somatic cells *in vivo*. With the exception of some experiments in transgenic mice (Recio *et al*, 1992) all demonstrated that in mice the substance is a somatic cell mutagen. Cochrane and Skopek (1993), and Tates *et al* (1994) reported that 1,3-BD induced HPRT mutations in splenocytes of exposed mice. The average mutational frequency in those experiments was increased up to 5-fold over control levels. Approximately half of the mutants in the HPRT locus were frameshifts while also transitions and transversions were produced at A:T base pairs. A shift in mutational spectrum was also observed in the experiments with transgenic animals (Recio *et al*, 1992, 1993, 1996; Sisk *et al*, 1994). The frequency of point mutations at A:T base pairs was significantly greater in 1,3-BD exposed mice than in air controls. In addition, there was a decrease in the frequency of G:C to A:T transitions at 5'-CpG-3' sites in 1,3-BD exposed mice.

A mouse spot test was carried out: pregnant females were exposed (6 h/d) to 500 ppm 1,3-BD for 5 days and allowed to come to term. There were no embryotoxic or teratogenic effects but coat colour spots were four times higher after 1,3-BD exposure. Negative results were obtained when 1,3-BD was tested on *Drosophila* for somatic mutation and recombination (SMART) (Victorin *et al*, 1990).

Genotoxic Effects in Germ Cells

The ability of 1,3-BD to induce heritable genetic damage was investigated in a sperm head morphology study, in a spermatid micronucleus test and in a number of dominant lethal studies in mice.

For the spermhead abnormality test, mice were exposed (6 h/d) to 1,3-BD concentrations of 200, 1,000 or 5,000 ppm for 5 days. The increase of spermhead abnormalities over control rates was small and it is unclear whether it was treatment-related (Morrissey *et al*, 1990). Animals for the spermatid micronucleus assay were exposed in a similar way (6 h/d, 5 days) to concentrations of 500 or 1,300 ppm. A statistically significant increase in micronuclei was observed 15 days after exposure. There was, however, no sign of a dose response which, as claimed by the authors, can be explained by the higher cytotoxicity at the top dose level (Xiao and Tates, 1995).

Three dominant lethal studies in the mouse have been published (Hackett *et al*, 1988a; Anderson *et al*, 1993; Adler *et al*, 1994). In the study by Hackett *et al*, dominant lethal effects were only seen in mating weeks 1 and 2 but the results were not dose-related (there was a slight effect at 200 and 1,000 ppm 1,3-BD but not at 5,000 ppm, the highest concentration tested). Results from two recent dominant lethal studies (Adler *et al* and Anderson *et al*) indicated a dominant lethal effect in animals exposed (6 h/d, 5 d/wk) to 12.5 ppm 1,3-BD for 10 weeks, whereas following a single exposure (6 h) with dose levels up to 6,250 ppm, no mutagenic effect was seen. In conclusion exposure of male mice to 1,3-BD caused dominant lethal mutations in spermatozoa and spermatids.

Recent results from inhalation studies in rats indicated that 1,3-BD does not cause dominant lethal mutations in rats, see Section 7.2.6.1 for details (BIBRA, 1996b).

Humans

Cytogenetic analyses have been carried out in peripheral blood lymphocytes of 1,3-BD exposed workers. There were no exposure-related effects in the frequencies of chromosomal aberrations, SCEs or micronuclei (Ahlberg *et al*, 1991; Sorsa *et al*, 1991, 1994, 1996; Kelsey *et al*, 1995; Au *et al*, 1995). The ambient air TWA concentration of 1,3-BD that workers were exposed to was generally below 2 ppm, occasionally exceeding 3 ppm, with short-term excursions up to 100 ppm at certain jobs.

Three studies examining gene mutation at the HPRT locus in lymphocytes of 1,3-BD workers showed conflicting results. In a pilot study by Legator *et al* (1993) an increased HPRT variant frequency (in 3 workers) was reported in a small group of 8 workers allegedly exposed to about 3 ppm. It was claimed that the variant frequency was highly correlated with the urinary 1,3-BD metabolite level.

Because of the small sample size this study has to be evaluated with care. It should also be noted that there were no historical (exposure) records of the workers and that the HPRT variant frequencies were determined employing the autoradiography method rather than the clonal assay; comparison with other studies is thus difficult. A follow up of this study showed that despite the considerable drop in 1,3-BD air levels in a 1,3-BD plant, increased HPRT variant frequencies persisted in the blood lymphocytes of the workers (Ward *et al*, 1996). Recently data have become available from studies of 1,3-BD workers in the Czech Republic (Tates *et al*, 1996) and China (Hayes *et al*, 1996). Employees (n = 100) from the Chinese and Czech plants were exposed to much higher levels of 1,3-BD than those from the Ward *et al* (1996) survey (China up to 85 ppm, Czech republic maximum of 20 ppm) but no association was found between HPRT frequency and exposure (studies in these workers were performed using the clonal assay). The discrepancy between the Ward *et al* (1996) and other studies could only be clarified by a comparative study where samples using both methodologies were analysed.

TABLE XIX: Genotoxicity of 1,3-BD *In Vivo*

Species	Results	References
Mouse		
DNA alkylation (liver)*	+ve	Jelitto *et al*, 1989; Kreiling, 1989
DNA single strand breaks (liver)*	+ve	Vangala *et al*, 1987; Walles *et al*, 1995
DNA-DNA crosslinks * - liver	+ve	Jelitto *et al*, 1989; Vangala *et al*, 1987
	–ve	Ristau *et al*, 1990
- lung	+ve	Vangala and Jelitto, 1989
DNA-adducts* (various organs)	+ve	Koivisto *et al*, 1996
DNA-damage* - single strand	+ve	Vangala *et al*, 1993
(liver and lung) - cross links	+ve	Vangala *et al*, 1993
Unscheduled DNA synthesis (UDS)	–ve	Arce *et al*, 1990; Vincent *et al*, 1986
SCE (bone marrow)	+ve	Cunningham *et al*, 1986; Tice *et al*, 1987
Micronucleus (peripheral blood)	+ve	Irons *et* al, 1986a; Tice *et* al, 1987; Jauhar *et* al, 1988; MacGregor *et al*, 1990; Wehr, 1987; Adler *et al*, 1994; Autio *et al*, 1994
Micronucleus (bone marrow)	+ve	Cunningham *et al*, 1986; Victorin *et al*, 1990; Exxon, 1990; Adler *et al*, 1994; Autio *et al*, 1994
Micronuclei (germ cells)	+ve	Xiao and Tates, 1995
Dominant lethal**	–ve	Hackett *et al*, 1988a; Adler *et al*, 1995
	+ve	Adler *et al*, 1994; Anderson *et al*, 1993, 1996a
Chromosomal aberrations (bone marrow)	+ve	Irons *et al*, 1987a; Tice *et al*, 1987
Gene mutations (lac Z) in transgenic mice		
(Mutamouse®) - liver	–ve	Recio *et al*, 1992
- bone marrow	–ve	Recio *et al*, 1992
- lung	+ve	Recio *et al*, 1992

TABLE XIX: Genotoxicity of 1,3-BD *In Vivo* (continued)

Species	Results	References
Gene mutation (HPRT) (splenocytes)	+ve	Tates *et al*, 1994; Cochrane and Skopek, 1993
Gene mutation lac I, lac Z	+ve	Recio *et al*, 1993
Mutational spectra lac I	NA	Recio *et al* , 1996; Sisk *et al*, 1994
GPA-mutations	–ve	Bigbee *et al*, 1995
Spot test	+ve	Adler *et al*, 1994
Rat		
DNA-DNA crosslinks* - liver	–ve	Jelitto *et al*, 1989; Ristau *et al*, 1990; Vangala *et al*, 1987
- lung	–ve	Vangala and Jellitto,1989
DNA single strand breaks* (liver)	+ve	Vangala *et al*, 1987
DNA-alkylation (liver)*	–ve	Jelitto *et al*, 1989; Kreiling, 1989
DNA adducts* (various organs)	+ve	Koivisto *et al*, 1996
DNA damage* - single strand breaks	+ve	Vangala *et al*, 1993
(liver and lung) - crosslinks	–ve	Vangala *et al*, 1993
UDS	–ve	Arce *et al*, 1990; Vincent *et al*, 1986
SCE (bone marrow)	–ve	Cunningham *et al*, 1986
Micronucleus (bone marrow)	–ve	Cunningham *et al*, 1986
	–ve	Autio *et al*, 1994
Dominant lethal**	–ve	Anderson *et al*, 1996
Syrian hamster		
Micronucleus (bone marrow)	±	Exxon, 1990
Primate		
Micronucleus (lymphocytes)	–ve	Sun *et al*, 1989a
SCE (lymphocytes)	–ve	Sun *et al*, 1989a
Drosophila		
Somatic mutation and recombination test (SMART)	–ve	Victorin *et al*, 1990
Sex-linked recessive lethal (SLRL)	–ve	Foureman *et al*, 1994
Human		
SCEs (lymphocytes)	–ve	Ahlberg *et al*, 1991; Sorsa *et al*, 1991, 1994, 1996; Kelsey *et al*, 1995
Micronucleus (lymphocytes)	–ve	Ahlberg *et al*, 1991, 1994, 1996
Chromosomal aberrations (lymphocytes)	–ve	Ahlberg *et al*, 1991; Sorsa *et al*, 1991, 1994, 1996; Au *et al*, 1995
Gene mutation (HPRT) (lymphocytes)	±	Legator *et al*, 1993; Ward *et al*, 1996
Gene mutation (HPRT) (lymphocytes)	–ve	Tates *et al*, 1996; Hayes *et al*, 1996

±, equivocal
* Studies also covered in Section 7.1
** Studies also covered in Section 7.2.6.3

7.2.4.3 *Metabolites*

There is a large data base on the genotoxic potential of the two major 1,3-BD metabolites (Tables XX to XXIII).

TABLE XX: Genotoxicity of EB *In Vitro*

Test system	Result without activation	Result with activation	Reference
Microbial			
Reverse gene mutation (*Salmonella typhimurium* TA 100, TA 1530, TA 1535)	+ve	Not tested	De Meester *et al*, 1978; Gervasi *et al*, 1985
Reverse gene mutation (*Escherichia coli*)	+ve	Not tested	Hemminki *et al*, 1980
Fluctuation test (*Klebsiella pneumoniae*)	+ve	Not tested	Voogd *et al*, 1981
Gene mutation $TK^{+/-}$, HPRT (human lymphoblasts)	+ve	Not tested	Cochrane and Skopek, 1994a
Mammalian cell			
UDS (rat hepatocytes)	–ve	Not applicable	Vincent *et al*, 1986 Arce *et al*, 1990
UDS (mouse hepatocytes)	–ve	Not applicable	Arce *et al*, 1990
SCE (Chinese hamster ovary cells)	+ve	+ve	Sasiadek *et al*, 1991a
SCE (human lymphocytes)	+ve	+ve	Sasiadek *et al*, 1991b
DNA-adducts (human lymphocytes)	+ve	Not tested	Leuratti and Marafante, 1992a,b
Miscellaneous			
DNA-adducts (calf-thymus DNA)	+ve	Not applicable	Leuratti and Marafante, 1992a,b
DNA-adducts (nucleosides)	+ve	Not applicable	Tretyakova *et al*, 1996

TABLE XXI: Genotoxicity of EB *In Vivo*

Test system	Result	Reference
Mouse		
SCE (bone marrow)	+ve	Sharief *et al*, 1986
SCE (splenocytes)	–ve	Kligerman *et al*, 1996
Chromosomal aberrations (bone marrow)	+ve	Sharief *et al*, 1986
Chromosomal aberrations (splenocytes)	–ve	Kligerman *et al*, 1996
DNA strandbreaks/alkaline labile sites	–ve	Kligerman *et al*, 1996
Gene mutation (splenocytes , HPRT)	+ve	Cochrane and Skopek, 1994b
Hb-adducts	+ve	Bond *et al*, 1994; Tretyakova *et al*, 1996
Rat		
SCE (splenocytes)	–ve	Kligerman *et al*, 1996
Chromosomal aberrations (splenocytes)	–ve	Kligerman *et al*, 1996
DNA strandbreaks/alkaline labile sites	–ve	Kligerman *et al*, 1996
Hb-adducts	+ve	Bond *et al*, 1994; Tretyakova *et al*, 1996

TABLE XXII: Genotoxicity of DEB *In Vitro*

Test system	Result without activation	Result with activation	Reference
Microbial			
Reverse gene mutation (*Salmonella typhimurium* TA 100, TA 1535)	+ve	+ve	McCann *et al*, 1975; Rosenkranz and Poirier, 1979
	+ve	–ve	Dunkel *et al*, 1984; Gervasi *et al*, 1985
Reverse gene mutation (*Salmonella typhimurium* TA 100)	+ve	Not tested	Wade *et al*, 1979
Reverse gene mutation (*E. coli*)	+ve	±	Glover, 1956;
	±	±	Dunkel *et al*, 1984
DNA repair (*E. coli*)	+ve	Not tested	Thielmann and Gersbach, 1978
Fluctuation test (*Klebsiella pneumoniae*)	+ve	Not tested	Voogd *et al*, 1981

TABLE XXII: Genotoxicity of DEB *In Vitro* (continued)

Test system	Result without activation	Result with activation	Reference
Yeast and fungi			
Gene conversion (*Saccharomyces cerevisiae*)	+ve	Not tested	Zimmerman, 1971; Sandhu *et al*, 1984
Mitotic recombination (crossing over) (*Saccharomyces cerevisiae*)	+ve	Not tested	Zimmerman and Vig, 1975; Simmon, 1979; Sandhu *et al*, 1984
Gene mutation (forward, reverse, mitochondrial) (*Saccharomyces cerevisiae*)	+ve	+ve	Polakowska and Putrament, 1979; Sandhu *et al*, 1984; Olzewska and Kilbey, 1975
Reverse gene mutation (*Neurospora crassa*)	+ve	Not tested	Pope *et al*, 1984; Kolmark and Kilbey, 1968
Mammalian cell systems			
DNA-DNA crosslinks (mouse liver cells)	+ve	Not tested	Ristau *et al*, 1990
UDS (rat hepatocytes)	–ve	Not applicable	Vincent *et al*, 1986
Gene mutation (mouse lymphoma cells)	+ve	Not tested	McGregor *et al*, 1988
SCE (Chinese hamster cells)	+ve	+ve	Perry and Evans, 1975; Sasiadek *et al*, 1991a
SCE (human lymphocytes)	+ve	+ve	Wiencke *et al*, 1982; Sasiadek *et al*, 1991b
Chromosomal aberrations (human lymphoblastoid cell lines)	+ve	Not tested	Cohen *et al*, 1982
Chromosomal aberrations (human bone marrow)	+ve	Not tested	Marx *et al*, 1983
Gene mutation $TK^{+/-}$, HPRT (human lymphoblasts)	+ve	Not tested	Cochrane and Skopek, 1994b
Miscellaneous			
DNA-adducts (calf thymus DNA)	+ve	Not applicable	Leuratti and Marafante, 1992a,b; Mabon and Randerath, 1996
DNA-adducts (nucleosides)	+ve	Not applicable	Tretyakova *et al*, 1996

±, equivocal

TABLE XXIII: Genotoxicity of DEB *In Vivo*

Test system	Result	Reference
Drosophila		
SMART	+ve	Graf *et al*, 1983
SLRL	+ve	Bird and Fahmy, 1953; Fahmy and Fahmy, 1970; Sankaranarayanan *et al*, 1983
Chromosome deletion	+ve	Fahmy and Fahmy, 1970
Mouse		
Host mediated assay (*Salmonella typhimurium*, TA 1530; reverse gene mutation)	+ve	Simmon *et al*, 1979
Host mediated assay (*Saccharomyces cerevisiae* D3; mitotic recombination)	–ve	Simmon *et al*, 1979
SCE (bone marrow)	+ve	Conner *et al*, 1983; Valk *et al*, 1987
SCE (alveolar macrophages)	+ve	Conner *et al*, 1983
SCE (regenerating liver cells)	+ve	Conner *et al*, 1983
SCE (splenocytes)	+ve	Kligerman *et al*, 1996
Chromosomal aberrations (bone marrow)	+ve	Valk *et al*, 1987
Chromosomal aberrations (splenocytes)	+ve	Kligerman *et al*, 1996
DNA strandbreaks/alkaline labile sites	–ve	Kligerman *et al*, 1996
Gene mutation (splenocytes, HPRT)	+ve	Cochrane and Skopek, 1994
Micronuclei (splenocytes and spermatocytes	+ve	Xiao and Tates, 1995
Chinese hamster		
SCE (bone marrow)	+ve	Valk *et al*, 1987
Chromosomal aberrations (bone marrow)	+ve	Valk *et al*, 1987
Rat		
SCE (splenocytes)	+ve	Kligerman *et al*, 1996
Chromosomal aberrations (splenocytes)	+ve	Kligerman *et al*, 1996
DNA strandbreaks/alkaline labile sites	–ve	Kligerman *et al*, 1996
Micronuclei (splenocytes/spermatocytes)	+ve	Xiao and Tates, 1995
Miscellaneous		
DNA adducts (mouse skin)	+ve	Mabon and Randerrath, 1996
Human		
SCEs (peripheral blood lymphocytes)	+ve	Kelsey *et al*, 1995

Both EB and DEB are formed *in vitro* and *in vivo* and it has been shown that they have the ability to bind covalently to DNA and other macromolecules (Leuratti and Marafante, 1992a,b; Bond *et al*, 1994; Tretyakova *et al*, 1996; Mabon and Randerrath, 1996). EB was negative in *in vitro* UDS tests (Vincent *et al*, 1986; Arce *et al*, 1990) but positive findings were obtained in microbial and mammalian gene mutation assays (De Meester *et al*, 1978; Hemminki *et al*, 1980; Voogd *et al*, 1981; Gervasi *et al*, 1985; Cochrane and Skopek, 1994b) and cytogenetic studies in mammalian cells (Sasiadek *et al*, 1991a, b). *In vivo* studies (chromosomal damage and gene mutation) in the mouse resulted in both positive and negative findings (Sharief *et al*, 1986; Kligerman *et al*, 1996) while studies in the rat (chromosomal damage) were all negative (Kligerman *et al*, 1996). With a few exceptions (UDS *in vitro*, DNA strand breaks/alkaline labile sites in the rat) all genotoxicity studies with DEB revealed the high potency of the diepoxide to induce genetic damage (see tables for references). In a comparative study by Cochrane and Skopek (1994b) the mutagenic potential of the epoxide metabolites including 3,4-epoxy-1,2-butanediol was measured in cultured human lymphoblasts (Table XXIV).

TABLE XXIV: Genotoxicity of 3,4-Epoxy-1,2-Butanediol *In Vitro*

Test system	Result	Reference
Gene mutation, $TK^{+/-}$-HPRT (human lymphoblasts)	+ve	Cochrane and Skopek, 1994b

In summary, all three metabolites were mutagenic with DEB exhibiting activity at concentrations approximately 40-100 lower than EB and 3,4-epoxy-1,2-butanediol. The higher mutagenic potential of DEB may be related to its bifunctional nature.

7.2.4.4 *Summary and Evaluation*

1,3-BD as such is not genotoxic. However, certain metabolites have the ability to interact with DNA directly to form adducts and/or crosslinks which eventually may result in gene mutations or chromosomal aberrations. The genotoxic action of 1,3-BD in various test systems depends on its biotransformation to reactive metabolites. The efficacy of this biotransformation appears to be quite different among species; mice seem to have a greater capability to transform 1,3-BD than do rats, which is exemplified by the lack of genotoxic response in the rat. Studies addressing the genotoxic potential of the main 1,3-BD metabolites have shown clearly that the diepoxide is the most potent, indicating that this intermediate may be the ultimate carcinogen.

A few cytogenetic analyses have been carried out in peripheral blood lymphocytes of 1,3-BD workers exposed to low levels (< 1 ppm). No exposure-related effects in frequencies of CA, SCEs or Mn were

observed. Inconsistent results were obtained in three studies that investigated HPRT mutational frequencies in blood lymphocytes of workers employed in the 1,3-BD industry (USA, Czech Republic, China). The one study that showed an association between elevated (HPRT) variant frequency and 1,3-BD exposure was conducted by Legator *et al*, 1993. In this study a blood sample evaluation of only 8 workers was conducted; a slight elevation of variant frequency in three of them was found. The other two studies reported negative results. More studies are needed to clarify if 1,3-BD is a true somatic cell mutagen in humans.

7.2.5 Chronic Toxicity and Carcinogenicity

The carcinogenicity of inhaled 1,3-BD was studied in Sprague-Dawley rats and in $B6C3F_1$ mice in studies undertaken by Hazleton Laboratories for the International Institute of Synthetic Rubber Producers (IISRP) and by the US National Toxicology Program (NTP).

7.2.5.1 *Inhalation Exposure of Rats*

In a 2-year study, two groups of 100 Sprague-Dawley rats of either sex were exposed (6 h/d, 5 d/wk) to 0, 1,000 or 8,000 ppm 1,3-BD (Owen, 1981a,b; Owen *et al*, 1987; Owen and Glaister, 1990). Survival was reduced in high-dose males and in low- and high-dose females (male survivors: 45/100 controls, 50/100 low-dose, 32/100 high-dose; female survivors: 46/100 controls, 32/100 low-dose, 24/100 high-dose). Increased mortality in the 8,000 ppm male group was attributed to severe nephropathy, and reduced survival in both female groups resulted from sacrifice of animals with large subcutaneous masses. There were no haematological, blood chemistry, urine or neuromuscular function effects that could be associated with treatment. Treatment did affect body-weight gain in the first 12 weeks of the study, certain organ weights and increased the incidence of common and uncommon tumours. Tumours that occurred with significantly increased incidence in males were pancreatic exocrine neoplasms and Leydig cell tumours. In females, significant increases of tumours were found in the thyroid (follicular-cell adenomas and carcinomas) and in the mammary gland (adenomas and carcinomas); the majority of these tumours were benign (Table XXV). When considered separately, the incidences of benign and malignant tumours were neither statistically significant nor dose-related. The high incidence of mammary tumours was similar to the historical incidence (90%) in concurrent control groups of Sprague-Dawley rats used at Hazleton Laboratories (Owen, 1980; Löser, 1983). Tumours with a "significantly related trend" included sarcomas of the uterus and carcinoma of the Zymbal gland although the frequencies of these tumours in treated groups were not statistically different from control. The authors conclude that under the exposure conditions used in this study 1,3-BD is a weak carcinogen in the rat and that the tumour pattern (testis, mammary gland, uterus, thyroid, Zymbal gland which are endocrine or sex-hormone sensitive organs)

is more indicative of an indirect mechanism rather than by a direct effect of reactive metabolites (Owen and Glaister, 1990).

TABLE XXV: Incidence[a] of Selected Primary Tumours in Sprague-Dawley Rats Exposed to 1,3-BD for 2 Years (from Owen and Glaister, 1990)

Tissue and tumour	Sex	Exposure concentration (ppm)		
		0	1,000	8,000
Pancreas: adenoma	M	3/100	1/100	10/100[b]
	F	2/100	0/100	0/100
Uterus: sarcoma	F	1/100	4/100	5/100
Zymbal gland: adenoma/carcinoma	M	1/100	1/100	2/100
	F	0/100	0/100	4/100
Mammary gland: adenoma/carcinoma	M	1/100	2/100	0/100
	F	50/100	79/100[b,c]	81/100[b]
Thyroid: adenoma/carcinoma	M	4/100	5/100	1/100
	F	0/100	4/100	11/100[b]
Testis: Leydig cell tumours	M	0/100	3/100	8/100[b]
Total number of tumour bearing rats	M	84/100	70/100	87/100
	F	97/100	98/100	94/100

a Number of animals with tumours/ number of animals exposed
b Statistically significant $P < 0.05$
c Not significant when compared with historical controls, therefore 1,000 ppm is a NOAEL

7.2.5.2 *Inhalation Exposure of Mice*

Two long-term inhalation exposure studies of 1,3-BD in B6C3F$_1$ mice have been reported.

In the first study (NTP I) groups of 50 male and 50 female B6C3F$_1$ mice were exposed (6 h/d, 5 d/wk) to 0, 625 or 1,250 ppm 1,3-BD for 61 weeks. The study was designed to last 103 weeks but was terminated after 60 to 61 weeks because of the high incidence of lethal neoplasms in the exposed animals (male survivors: 49/50 controls, 11/50 low-dose, 7/50 high-dose; female survivors: 46/50 controls, 14/50 low-dose, 30/50 high dose). Haemangiosarcomas originating in the heart with metastases to various organs were found in males and females. Other types of neoplasms for which the incidences were increased in animals of each sex were malignant lymphoma (T-cell lymphoma), alveolar bronchiolar adenoma or carcinoma of the lung, and papillomas or carcinomas of the forestomach. Tumours that occurred with statistically-significantly increased incidence in females only (not dose-related) included hepatocellular adenoma or carcinoma, acinar-cell carcinoma of the mammary gland and granulosa-cell tumours of the ovary (Table XXVI). Malignant lymphomas were considered the major cause of early deaths. The high incidence of haemangiosarcomas of the heart was a particularly unusual finding since these endothelial cell neoplasms have a very low spontaneous incidence rate in B6C3F$_1$ mice and are rarely induced in long-term studies. The results of this study

demonstrated that 1,3-BD is a potent multiple-organ carcinogen in the B6C3F$_1$ mouse (NTP, 1984; Huff *et al*, 1985; Melnick *et al*, 1990a,b).

TABLE XXVI: Incidence of Primary Tumours in B6C3F$_1$ Mice Exposed to 1,3-BD for 60-61 weeks (adapted from Melnick and Huff, 1992)

Tissue and tumour	Sex	Exposure concentration (ppm)		
		0	625	1,250
T-cell lymphoma	M	0/50	23/50*	29/50*
	F	1/50	10/49*	10/49*
Heart: haemangiosarcoma	M	0/50	16/49*	7/49*
	F	0/50	11/48*	18/49*
Lung: alveolar bronchiolar neoplasm	M	2/50	14/49*	15/49*
	F	3/49	12/48*	23/49*
Forestomach: squamous cell neoplasm	M	0/49	7/40*	1/44
	F	0/49	5/42*	10/49*
Mammary gland: acinar cell neoplasm	F	0/50	2/49	6/49*
Ovary: granulosa cell neoplasm	F	0/49	6/45*	12/48*
Liver: hepatocellular neoplasm	M	8/50	6/49	2/49
	F	0/50	2/47	5/49*

* Statistically significant $P < 0.05$

In the second study (NTP II) , groups of 70 to 90 male and female B6C3F$_1$ mice were exposed (6 h/d, 5 d/wk) to 0, 6.25, 20, 62.5, 200, 625 ppm of 1,3-BD for up to 2 years. Additional studies in which exposure was terminated after a limited period were also included. These studies are referred to as "stop studies". In the groups of mice exposed to 1,3-BD for 2 years survival was significantly reduced at 20 ppm and higher (terminal male survivors: 35/70 controls, 39/70 at 6.25 ppm, 24/70 at 20 ppm, 22/70 at 62.5 ppm, 3/70 at 200 ppm, 0/90 at 625 ppm; female survivors: 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, 0/90 at 625 ppm). The percentage of animals bearing malignant tumours increased from about 35% in the controls to nearly 90% in the 625 ppm exposure groups. T-cell lymphomas were the major cause of death for males and females exposed to 625 ppm. In female mice, lung neoplasms were significantly increased in all exposure groups, even when malignant and benign tumours were considered separately. Thus even at 6.25 ppm 1,3-BD was carcinogenic in mice (Table XXVII). The impact of early occurring lethal thymic lymphomas on the expression of the later developing haemangiosarcoma is illustrated by the figures on haemangiosarcomas of the heart in male mice exposed to 200 and 625 ppm. When comparing tumour rates, tumour incidences should be adjusted for early mortality. Statistical analyses of adjusted incidences showed that there was no exposure levels at which a carcinogenic response was not induced in the female mouse (Table XXII) (Melnick *et al*, 1990a,b; Melnick and Huff, 1992).

TABLE XXVII: Incidence of Primary Tumours in B6C3F$_1$ Mice exposed to 1,3-BD for 2 Years
(adapted from Melnick and Huff, 1992)

Tissue and tumour	Sex	Exposure concentration (ppm)					
		0	6.25	20	62.5	200	625
T-cell lymphoma	M	2/70	1/70	2/70	4/70	2/70	62/90*
	F	2/70	4/70	6/70	3/70	11/70*	36/90*
Heart:	M	0/70	0/70	1/70	5/70*	20/70*	6/90*
haemangiosarcoma	F	0/70	0/70	0/70	1/70	20/70*	26/90*
Lung: alveolar-	M	22/70	23/70	20/70	33/70*	42/70*	12/90*
bronchiolar neoplasm	F	4/70	15/70*	19/70*	27/70*	32/70*	25/90*
Forestomach:	M	1/70	0/70	1/70	5/70	12/70*	13/90*
squamous cell	F	2/70	2/70	3/70	4/70	7/70	28/90*
neoplasm							
Liver: hepatocellular	M	31/70	27/70	35/75	32/70	40/70*	12/90
neoplasms	F	17/70	20/70	23/70*	24/70*	20/70*	3/90
Harderian gland:	M	6/70	7/70	11/70	24/70*	33/70*	7/90*
neoplasm	F	9/70	10/70	7/70	16/70*	22/70*	7/90*
Mammary gland:	F	0/70	2/70	2/70	6/70*	13/70*	13/90*
adenocarcinoma							
Ovary: granulosa cell	F	1/70	0/70	0/70	9/70*	11/70*	6/90
neoplasm							

* Increased compared with controls (0 ppm), $P < 0.05$, after adjustment for intercurrent mortality. Methods used for analysis of tumour incidences: life-time table tests, logic regression analysis, Fisher-exact analysis and Cochran-Armitage trend test.

7.2.5.3 *Stop-Exposure Studies*

In the stop-exposure studies (Melnick and Huff, 1992a), groups of 50 male mice were exposed to 1,3-BD under one of the following regimens: 200 ppm for 40 weeks (Group A); 625 ppm for 13 weeks (Group B); 312 ppm for 52 weeks (Group C); 625 ppm for 26 weeks (Group D). After exposure was terminated animals were placed in control chambers for the remainder of the 104 week study. The total exposure to 1,3-BD was approximately equivalent for Groups A and B at 8,000 ppm/wk and approximately 16,000 ppm/wk for Groups C and D. Survival was markedly reduced in all treated groups due to the development of compound-related malignant tumours. The tumour pattern in the stop-exposure studies was similar to that in the chronic exposure studies (Table XXVIII). Lymphocytic lymphoma, haemangiosarcoma of the heart, tumours of the lung and forestomach and Harderian gland were increased even after a 13-week exposure to 625 ppm of 1,3-BD. A comparison of the incidences of lymphocytic lymphoma at similar total exposures indicates that exposure to a higher concentration for a short time results in a higher incidence of malignancies. This is evident by comparing the incidence of thymic lymphoma adjusted for early death: Group A, 19%; Group B, 47%; Group C, 15%; Group D, 84%. The concentration of 1,3-BD at concentrations of 200 ppm and above is thus a greater contributing factor to thymic lymphoma incidence than exposure duration (Bucher and Huff, 1992a).

TABLE XXVIII: Incidence of Primary Tumours in the Stop-exposure Groups of Male B6C3F$_1$ Mice[a] (adapted from Melnick and Huff, 1992a)

Tissue and tumour	Exposure concentration (ppm) and duration				
		A	B	C	D
	0	200	625	312	625
	control	40 wk	13 wk	52 wk	26 wk
T-cell lymphoma	2/70	6/50	17/50*	3/50	30/50*
	(4%)	(19%)	(47%)	(15%)	(84%)
Heart: haemangiosarcoma	0/70	15/50*	7/50*	33/50*	13/50*
	(0%)	(47%)	(31%)	(87%)	(76%)
Lung: alveolar bronchiolar	22/70	35/50*	27/50*	32/50*	18/50*
neoplasm	(46%)	(88%)	(87%)	(88%)	(89%)
Forestomach: squamous cell	1/70	6/50*	8/50*	13/50*	11/50*
neoplasm	(2%)	(20%)	(33%)	(52%)	(63%)
Harderian gland: adenoma/	6/70	27/50*	23/50*	28/50*	11/50*
adenocarcinoma	(13%)	(72%)	(82%)	(86%)	(70%)

a Percentages adjusted for intercurrent mortality
* Statistically significant $P < 0.05$

Bucher *et al* (1993) exposed mice for a single 2-hour period to concentrations of 0, 1,000, 5,000 or 1,0000 ppm 1,3-BD. Exposure conditions and methods were similar to those reported in earlier studies. After 2 years, the animals were killed and tissues and organs examined microscopically. The single exposure did not affect survival or significantly increase tumour response.

7.2.5.4 *Metabolic Aspects of Carcinogenicity*

Three different epoxides are formed by metabolic conversion of 1,3-BD. These epoxides can form DNA adducts and crosslinks which may ultimately result in genetic events. Theoretically, malignant transformation resulting from these lesions may be involved in the early stages of carcinogenicity induced by 1,3-BD. Attention had been focused on the primary metabolite, EB, and on DEB as the most mutagenic epoxide (Section 7.2.4.3). The difference in increased body burden of EB in mice compared to rats at low-exposure concentrations appears to be in the range of 2 to 5. This is supported by data on exhalation and blood and tissue concentrations of this epoxide, as well as by results of PB-PK modelling and other extrapolations of *in vitro* data to the *in vivo* situation. The large difference in carcinogenic potency of 1,3-BD between rats and mice cannot entirely be explained by the observed difference in the EB body burden. Hence, it appears likely that the body burden of other epoxides is more important. Recent *in vivo* studies demonstrated much higher DEB concentrations in mice as compared with rats. In addition, the stability and reactivity of DEB and the resulting high mutagenic activity may help to explain the species difference (DNA-crosslinks in mice, but not rats).

In summary, the available data on the toxicokinetics of 1,3-BD point to metabolic differences as a cause of the observed species differences in carcinogenic susceptibility, but do not establish a firm link between the formation of a specific metabolite (or the resulting body burden) and 1,3-BD-induced carcinogenesis. Nevertheless, the formation of EB is a prerequisite for the formation of the other two epoxides and recent data on DEB formation and reactivity indicate that this epoxide may play a major role. This renders the determination of the target dose for EB and DEB superior to the use of external exposure concentration for establishing the "biologically effective dose".

7.2.5.5 *Analysis of T-cell Lymphomas in Mice*

In a number of papers spanning several years Irons and co-workers have described their investigations into the unique susceptibility of the mouse to the development of T-cell lymphomas (Irons *et al* ,1986a-d, 1987a,b, 1989, 1993, 1996; Colagiovanni *et al*, 1993; Leiderman *et al*, 1986). The pattern of pathology in 1,3-BD treated mice (anaemia, thymic atrophy and a high incidence of thymic lymphoma/leukaemia), is also similar to that seen in mice which have received radiation treatment and is also remarkably similar to mice which have mutations leading to deficiencies in the c-Kit ligand (CKL). CKL (a mast cell growth factor or stem cell factor, SCF) is a comitogen that works together with a broad range of cytokines in the process of haematopoiesis and haematopoietic progenitor cell differentiation. Studies showed that EB suppressed a subpopulation of haematopoietic progenitor cells (HPC) that were dependant upon a stem cell factor and responsive to Interleukin 3 (IL-3).

Irons postulated that these effects caused by the metabolites of 1,3-BD were responsible for the formation of thymic lymphoma/leukaemogenesis and proposed a model for development of the disease involving secondary and tertiary effects such as a chromosomal damage and mutations by the causative agent. Although there is a great similarity between the mechanisms of haematopoiesis in mice and man, Irons argues that there are subtle differences between the two species, mainly in the cytokines needed for the cell differentiation process, SCF and the time at which cell types become differentiated to a particular cell type. A subpopulation of cells has been identified in the mouse that is uniquely susceptible to functional suppression by mouse leukaemogens. Species differences between mouse and humans in the susceptibility of primitive HPC to clonogenic suppression are reported as being six orders of magnitude suggesting that they may be important in explaining species differences in susceptibility to thymic lymphoma. The observations on the susceptibility of primitive HPC to chemical suppression has led Irons to conclude that mouse thymic lymphoma is not an appropriate biological model for hazard identification in humans.

7.2.5.6 *Summary and Evaluation*

The carcinogenic effects of 1,3-BD were studied in Sprague-Dawley rats and B6C3F$_1$ mice. The most significant observation was the greater susceptibility of mice compared with rats which was observed. 1,3-BD is a potent carcinogen in mice, producing tumours in the lungs of females at 6.25 ppm, the lowest concentration tested. At higher concentrations, 1,3-BD induced tumours at multiple sites in both sexes. Dose-related increases were found in T-cell lymphoma, hepatocellular neoplasms, squamous cell neoplasms, Harderian gland neoplasms and cardiac haemangiosarcoma, which is a very rare neoplasm in mice. In contrast, the effect of 1,3-BD in rats was substantially less pronounced; statistically significant increases in tumour incidences were observed at 1,000 and 8,000 ppm. However, at 1000 ppm, the only statistically significant increase was in mammary gland tumours, of which the majority were benign and when considered separately, there was neither a significant increase in benign nor in malignant tumours. Such a separation appears to be justified, since historical data show a high incidence of benign mammary tumours in the Sprague-Dawley rat (Löser, 1983). At 8,000 ppm the tumour pattern (endocrine and sex-hormone sensitive organs) in both sexes of the rat suggested a secondary, non-genotoxic mechanism. Finally, the stop-exposure studies indicated that in the mouse the concentration of 1,3-BD had a greater impact on thymic lymphoma incidence than the exposure duration.

7.2.6 Reproductive Toxicity

A number of toxicological investigations provide information about the potential of 1,3-BD to interfere with male and female fertility and/or with normal embryonic or foetal development (teratogenicity).

7.2.6.1 *Fertility*

Groups of 20 male CD-1 mice were exposed (6 h/d) to 0, 200, 1,000 or 5,000 ppm of 1,3-BD for 5 consecutive days in a dominant lethal study. Following exposure, the males were mated with unexposed females (2 female mice were housed with each male once a week for 8 consecutive weeks). The females were killed 12 days after the last day of cohabitation, and the uterine contents examined. There was no effect of treatment upon fertility, as demonstrated by the proportion of successful matings. Overall, the number of pregnant females, implantations per pregnancy, live implants and late deaths was unaffected by 1,3-BD exposure. There was an increase in early post-implantation loss and in the number of females with 2 or more dead implants at 200 ppm and 1,000 ppm during the first 2 weeks post-exposure, but these parameters were unaffected at subsequent time points, and were not significantly elevated at 5,000 ppm. From the data in this study, exposure of male mice to high levels of 1,3-BD has no effect on their ability to mate and impregnate

females, and produce live foetuses. The data suggest that 1,3-BD damages the spermatozoa and spermatids of mice, but the lack of potency and dose-response indicate that this is of questionable biological significance (Hackett *et al*, 1988a; Morrissey *et al*, 1990).

Groups of 20 male B6C3F_1 mice were exposed (6 h/d) to 0, 200, 1,000 or 5,000 ppm of 1,3-BD for 5 consecutive days. During the 5th week following exposure the mice were killed, examined for gross lesions of the reproductive tract, and the sperm examined for abnormaliies. No gross lesions were detected. The mean percentage of normal sperm heads per total examined was 98.4, 98.0, 97.2 and 96.3 for the 0, 200, 1000 and 5000 ppm groups respectively (Hackett *et al*, 1988b; Morrissey *et al*, 1990). Although the decreases were statistically significant for the 1000 and 5000 ppm groups, these 1 to 2% reductions in normal sperm heads lacked biological significance.

Groups of 20 male (102/E1 × C3H/E1)F_1 mice, 10-12 wk old, were exposed (6 h/d) to 0 or 1,300 ppm 1,3-BD for 5 days. Each male was mated 4 hours after exposure with two untreated virgin females for 4 weeks. Mated females were removed and replaced. Females were killed at gestation day 14-16. Exposure did not affect the numbers of pregnancies, implantations, live implants, or dead implants. The percentage of dead implants was increased at weeks 1 and 2 after cessation of exposure, but this was only significant at week 2. Dominant lethals were only significantly increased at 2 and 3 weeks, but a major influence on this trend was an increase in the number of live implants per female in the control group (Adler *et al*, 1994).

Groups of 50 male C3H/E1 mice, 10-12 wk old, were exposed (6 h/d) to 0 or 1300 ppm 1,3-BD for 5 days. Each male was mated 8 days after exposure with two untreated virgin 102/E1 females for 1 week. Females were allowed to litter. Exposure did not affect fertility or significantly induce dominant lethals, as determined by the numbers of litters, and litter size at birth and weaning (Adler *et al*, 1995).

Male CD-1 mice, in groups of 25 (control, low dose) or 50 (high dose), were acutely or subchronically exposed to 1,3-BD prior to mating with untreated virgin females (two females per male). One of the females was killed on gestation day 17, whilst the other was allowed to litter. In the acute study, males were exposed to 0, 1,250 or 6,250 ppm 1,3-BD for 6 hours and rested for 5 days before mating. Treatment did not affect fertility, as determined by the percentage of successful matings in each group. There was no effect upon reproductive parameters, except for a decrease in implantation frequency at 1,250 ppm. In the subchronic study, males were exposed (6 h/d, 5 d/wk) to 0, 12.5 or 1,250 ppm 1,3-BD for 10 weeks prior to mating. Treatment did not affect the percentage of successful matings in each group, although implantation frequency was decreased, and early post-implantation losses were increased at 1,250 ppm compared to the control group (Anderson *et al*, 1993). However, the

frequency of these effects fell within control ranges for CD-1 mice in another laboratory (Christian, 1996). In a follow-up study, male CD-1 mice were exposed (6 h/d, 5 d/wk) to 0, 12.5, 65 or 130 ppm 1,3-BD for 4 weeks, and mated to untreated virgin females 4 days later. There was an increase in the incidence of early post-implantation losses at 65 and 130 ppm, but treatment had no effect upon other reproductive parameters (period to coition, mating and pregnancy frequencies, number of impantations, and incidence of late deaths and dead foetuses; BIBRA, 1996a).

Male Sprague-Dawley rats (25 animals per group, 50 in room control group) were exposed (6 h/d, 5 d/wk) to 0, 65, 400 or 1,250 ppm 1,3-BD for 10 weeks. There was no effect upon pregnancy rate, mating frequency, period to coition, or pre- and post-implantation losses. Although there was a significant decrease in implantation frequency at 65 ppm, this was not considered to be a genetic effect because there was no significant increase in post-implantation loss, and no reduction at higher concentrations (BIBRA, 1996b).

Rats (12/sex/group), guinea pigs (6/sex/group), and rabbits (2/sex/group) were exposed (7.5 h/d, 6 d/wk) to 0, 600, 2,300 or 6,700 ppm 1,3-BD for up to 8 months. It is unclear from the information provided when mating occurred; male and female rats, guinea pigs and rabbits may have been continuously cohabitating throughout the study. There was a reduction in body weight gain of the rats and male guinea pigs and there was a slight decrease in litter frequency in exposed rats, but this response was not dose-dependent and mean litter size was not affected (although all unborn foetuses were counted as births). Reproductive parameters were apparently unaffected in guinea pigs or rabbits, but only data for total pups per group were reported. These data indicate that long-term, high level exposure to 1,3-BD did not affect reproductive performance of rats, rabbits and guinea pigs (Carpenter *et al*, 1944).

7.2.6.2 *Other Effects on Reproductive Organs*

Groups of 110 male and 110 female Sprague-Dawley rats were exposed (6 h/d, 5 d/wk) to 0, 1,000 or 8,000 ppm 1,3-BD for 2 years. A pathological examination of the reproductive organs revealed no evidence for treatment-related effects. Ovarian atrophy was not observed in females exposed to 8,000 ppm 1,3-BD (4.2% incidence versus 4.3% in the controls). Likewise, testicular atrophy was not observed in males exposed to 8,000 ppm 1,3-BD (19.3% incidence versus 31% in controls) (Owen, 1981a,b).

Groups of 50 male and 50 female B6C3F_1 mice were exposed (6 h/d, 5 d/wk) to 0, 625 or 1,250 ppm 1,3-BD for 61 weeks. The study was originally designed to run for 104 weeks but was terminated after 61 weeks because of high mortality. Ovarian and testicular atrophy and uterine involution (probably a

secondary response to ovarian atrophy) were increased in the mice at both exposure levels (4%, 89% & 83% and 0%, 40% and 23% for ovarian and testicular atrophy for control, 625 ppm and 1250 ppm exposure groups respectively (NTP, 1984).

Groups of 70 male and 70 female B6C3F_1 mice were exposed (6 h/d, 5 d/wk) to 0, 6.25, 20, 62.5, 200 or 625 ppm 1,3-BD for up to 104 weeks (90 animals of each sex in the top concentration group). Ten animals of each sex were examined from each group at 39 and 65 week interim periods. Absolute and relative testis weight was reduced in a concentration-dependent manner after both 39 and 65 weeks of exposure (significant at 200 and 625 ppm), whilst testicular atrophy was only observed at 625 ppm. Ovarian atrophy was noted in female mice, and occurred in a concentration- and time-dependent manner. After 39 weeks atrophy was noted only at 200 ppm and above, but after 65 weeks the lowest effective concentration was 62.5 ppm, and after 104 weeks atrophy was observed in all treatment groups (although this coincided with the onset of reproductive senescence). Uterine atrophy was also noted in animals exposed to 625 ppm 1,3-BD for 104 weeks, and again is probably related to the degenerative changes occurring in the ovary (NTP, 1993).

Male (102/E1 $\times$ C3H/E1)F_1 mice (2-6 animals per group) were exposed (6 h/d) to 0, 200, 500 or 1300 ppm 1,3-BD for 5 days, and then maintained for a further 2-15 days prior to termination. Testis weight was reduced to 75 - 90% of control 11-15 days after cessation of exposure at 1,300 ppm 1,3-BD, but there was no effect at 200 ppm; effects at 500 ppm were inconsistent (a reduction similar to that caused by 1300 ppm in one experiment, no effect in the replication experiment.) Spermatid micronuclei were analysed by the suspension method. The incidence of micronuclei was increased in all treatment groups after 15 days' exposure, but not at 2 or 5 days, indicating that the premeiotic germ cells may be a target for 1,3-BD. There was no obvious dose-response relationship, which was explained by the authors as arising from cytotoxicity at the highest exposure concentration (Xiao and Tates, 1995; Section 7.2.4.2).

Rats (12/sex/group), guinea pigs (6/sex/group), and rabbits (2/sex/group) were exposed (7.5 h/d, 6 d/wk) to 0, 600, 2,300 or 6,700 ppm 1,3-BD for up to 8 months. No evidence of testicular degeneration was found at the histological examination (Carpenter *et al*, 1944).

7.2.6.3 *Developmental Toxicity*

Female Sprague-Dawley rats (40 animals in the control group, 24 per treatment group) were exposed to 0, 200, 1,000 or 8,000 ppm 1,3-BD during gestation days 6 through 15. Maternal body weight gain during the exposure period was significantly reduced at all exposure concentrations, with weight loss in the 8,000 ppm group. The adjusted maternal body-weight gain (maternal body weight minus the

weight of the gravid uterus) for the entire gestation period was significantly reduced in the 1,000 and 8,000 ppm groups. Uterine implantation parameters were unaffected. At 8,000 ppm, there was a significant reduction in foetal body weights; increased foetal and/or litter incidences in delays in ossification of the ribs (wavy ribs) and thoracic centra, and incomplete ossification of the sternum. These effects are consistent with growth retardation associated with maternal toxicity. There were no teratogenic effects that were statistically significant or outside the historical control range for this rat strain. The NOEL for maternal toxicity was 200 ppm and the NOEL for developmental effects 1,000 ppm (Irvine, 1981, 1982).

Groups of 24 to 28 pregnant female Sprague-Dawley rats were exposed (6 h/d) to 0, 40, 200 or 1,000 ppm 1,3-BD on days 6 through 15 of gestation. At 1,000 ppm 1,3-BD, the adjusted maternal body weight gain was significantly decreased. There were no significant differences between groups in the uterine implantation parameters, foetal body weights, and in the incidences of foetal malformation and variations. The NOEL for maternal toxicity was thus 200 ppm and the NOEL for developmental toxicity was greater than 1,000 ppm 1,3-BD (Hackett *et al*, 1987a; Morrissey *et al*, 1990).

Groups of 18 to 22 pregnant female CD-1 mice were exposed to 0, 40, 200 or 1,000 ppm 1,3-BD on days 6 through 15 of gestation. 1,3-BD concentrations of 200 and 1,000 ppm significantly reduced adjusted maternal body weight gains (by 18% and 22% respectively), reduced foetal and placental weights, and increased the number of supernumerary ribs. Exposure to 1000 ppm 1,3-BD also caused retarded sternal ossification. There were no embryo-foetal deaths or malformations. The authors also reported an effect on male foetal body weight at 40 ppm, but the statistical analysis used to make this conclusion was inappropriate (Christensen, 1996). The NOEL for both maternal and developmental (foetal) toxicity was 40 ppm 1,3-BD (Hackett *et al*, 1987b; Morrissey *et al*, 1990). Thus, foetal retardation occurred only in conjunction with maternal toxicity, of which it is judged to be a consequence. There was no evidence of any changes that would be classified as teratogenic.

Groups of 25 (control, low dose) or 50 (high dose) male CD-1 mice were exposed to 0, 1,250 or 6,250 ppm 1,3-BD for 6 hours and rested for 5 days before being mated with two untreated virgin females for one week. One female was killed on gestation day 17, whilst the other was allowed to litter. Offspring from the control and high dose P generation were maintained for 37 weeks post-partum. There was no significant effect upon tumour incidence in the F_1 generation (Anderson *et al*, 1996).

Groups of 25 (control, low dose) or 50 (high dose) male CD-1 mice were exposed (6 h/d, 5 d/wk) to 0, 12.5 or 1,250 ppm 1,3-BD for 10 weeks. After exposure they were each immediately mated with two untreated virgin females. One female was killed on gestation day 17, whilst the other was allowed to

litter. The incidence of late post-implantation losses and abnormalities was increased at 12.5 ppm, but not at 1,250 ppm, compared to historical controls for this strain of mouse at the laboratory where the study was performed (there were no late post-implantation losses or abnormalities in the study control group), although it fell within control ranges for CD-1 mice in another laboratory (Christian, 1996.) The incidence of foetal death in the 12.5 ppm group may be in part due to the greater number of implants per litter in this group (12.75 compared to 12.09 in controls). In the CD-1 mouse more than 14 implants per litter is associated with proportionately higher foetal death frequencies, due to increased competition for maternal resources in the uterus. The frequency of litters with > 14 implants was 1/23, 7/24 and 0/38 for control, 12.5 ppm and 1,250 ppm groups respectively, and the incidence of late deaths in the 12.5 ppm group was 24% (4/17) and 43% (3/7) for females with ≤14 and > 14 implants respectively. Of the 7 abnormalities seen in this treatment group, 3 (all exencephaly) were seen in one of the litters, and may therefore be a chance effect. The F_1 offspring were maintained for 75 weeks post-partum. There was no increase in earlier onset of tumours, but an apparent increased incidence of liver tumours in the male offspring of exposed animals. However no histological examination of tumour and non-tumour tissues was carried out, and there was no statistical analysis of the data. The authors concluded that there was no treatment-related difference in tumour incidence between offspring of 1,3-BD-exposed and control males (Anderson *et al*, 1993, 1996; BIBRA, 1995). In a follow-up study, exposure of male CD-1 mice to 12.5, 65 and 130 ppm 1,3-BD for 4 weeks had no effect upon the incidence of foetal malformations (BIBRA, 1996a).

When male Sprague-Dawley rats (25 animals per group, 50 in room control group) were exposed (6 h/d, 5 d/wk) to 0, 65, 400 or 1,250 ppm 1,3-BD for 10 weeks, there was no effect upon foetal viability or frequency of abnormalities (BIBRA, 1996b).

7.2.6.4 *Metabolic Aspects of Reproductive Toxicity*

The role of the metabolites EB and DEB in the aetiology of 1,3-BD mutagenicity and carcinogenicity has been well established in mice (Section 7.2.4.3 and 7.2.5.4). In contrast, there is little direct evidence for a possible role of metabolism in the reproductive toxicity of 1,3-BD, although limited studies have demonstrated a link between oxidation metabolites of 1,3-BD and toxicity to the gonads in rodents.

Female $B6C3F_1$ mice and Sprague-Dawley rats (10 animals per group) were administered EB or DEB (0.005-1.43 mmol/kgbw, i.p.) daily for 30 days, after which time the ovaries and uterus were removed, weighed and preantral follicles counted. EB reduced organ weights and follicular count in mice but not rats. DEB was ovotoxic and reduced uterine weight in both species, but mice were apparently more sensitive. In another study in mice, analogues with two unsaturated sites, which can therefore be

oxidised to the respective diepoxides (e.g. isoprene, vinylcyclohexene and its mono- and diepoxides) were also ovotoxic, whereas analogues which could not form diepoxides (e.g. epoxybutane, vinylcyclohexane, ethylcyclohexene) were not. In both studies, DEB was considerably more potent than EB. These studies demonstrate the requirement for complete oxidation of 1,3-BD to DEB as a prerequisite for rat ovarian toxicity (Doerr *et al*, 1995,1996).

Male (102/E1 × C3H/E1)F_1 mice and Lewis rats (3-6 animals per group) were administered single doses of EB (0.57 or 1.14 mmol/kgbw, i.p.) or DEB (0.18 or 0.35 mmol/kgbw, i.p. for mice; 0.24 - 0.47 mmol/kgbw for rats). Testes were removed 1-25 days later and spermatid micronuclei were analysed by the suspension method. EB reduced absolute and relative testis weights at 1.14 mmol/kgbw in both species. DEB reduced absolute testis weight at 0.35 mmol/kgbw in mice and at 0.47 mmol/kgbw in rats. Spermatid micronuclei were observed in the testes of rats and mice after exposure to both EB and DEB. The temporal correlation of clastogenicity led the authors to conclude that premeiotic germ cells, specifically the preleptotene and late spermatocytes, are a target for EB and DEB in both species (Xiao and Tates, 1995). Again, this study demonstrates that 1,3-BD metabolites are toxic to the gonads, and that DEB is more potent than EB but, unlike the work of Doerr and co-workers, it does not conclusively demonstrate the requirement for complete oxidation of 1,3-BD to DEB for expression of toxicity.

7.2.6.5 *Summary and Evaluation*

Studies in guinea pigs, rabbits and rats have shown no adverse effects on fertility at exposure concentrations as high as 6,700 ppm 1,3-BD for up to 8 months. Studies in mice suggest that subacute/subchronic exposure to high concentrations of 1,3-BD may increase the incidence of post-implantation losses, but the lack of a demonstrable dose-response relationship for this effect suggests that it is of questionable biological significance. One dominant lethal study included an eight-week post-exposure serial mating protocol, which demonstrated significant reduction in implantations only at 1-2 weeks post-exposure, suggesting an effect of treatment upon mature germ cell populations, but not upon premeiotic stages. This is in apparent contrast with the demonstration of clastogenic effects in the spermatids of mice exposed to 1,3-BD, which were temporally correlated with exposure of premeiotic cells, and is indicative that premeiotic mutations do not persist to a biologically significant degree (Zeiger, 1994).

In developmental toxicity studies conducted with 1,3-BD in rats and mice, no toxicity to the developing foetus was seen at exposure concentrations below those which caused maternal toxicity. One study reported an increased incidence of abnormalities in the offspring of male mice exposed to 1,3-BD, but the outcome of data analysis will have been affected by the absence of any malformations in control

offspring. Indeed, the incidence of malformations from exposed groups was within the historical range for this strain, and a subsequent study at the same laboratory failed to replicate the finding. Therefore, it is unlikely that this observation was a result of toxic insult to the foetus. Similar effects were not seen in rats.

Structural abnormalities have been detected in the ovaries and testes of mice exposed to 200 ppm 1,3-BD, and in older females exposed to concentrations as low as 6.25 ppm. Such effects were not observed in rats exposed to up to 8,000 ppm. The effects in female mice at concentrations lower than 200 ppm are not regarded to be of toxicological significance as they occurred after the normal reproductive period in the mouse, and may be related to reproductive senescence.

The role of metabolism in the aetiology of reproductive toxicity has not been rigorously demonstrated. Indirect evidence for DEB as the ultimate toxicant in ovarian and testicular atrophy has been presented, but its role in the aetiology of other toxic effects, such as dominant lethality, is largely a matter of speculation.

Overall, these studies again demonstrate the unique susceptibility of the mouse to 1,3-BD, and give further support for the involvement of 1,3-BD metabolism in the expression of toxicity.

7.3 EFFECTS ON HUMANS

Data on the human health effects of 1,3-BD are sparse. Most of the information is derived from studies on workers in synthetic rubber plants, where co-exposure to other chemicals, notably styrene, α-methyl styrene, toluene and benzene occurred (Illing and Shilker, 1985). Few of the studies, particularly those in Bulgaria or the USSR contain information on the atmospheric concentration or duration of exposure, and control data are not generally provided (IARC, 1992).

7.3.1 Short-term Exposure

7.3.1.1 *Inhalation*

Humans exposed for ≥ 6 hours to 1,3-BD at ≥ 2,000 ppm complained of slight smarting of the eyes and difficulty in focusing; however, no other subjective symptoms, including narcosis, were seen at concentrations up to 8,000 ppm (Carpenter *et al*, 1944).

Checkoway and Williams (1982) reported minimal changes in haematological indices among eight workers exposed to about 20 ppm 1,3-BD, 14 ppm styrene and 0.03 ppm benzene, relative to those among 145 workers exposed to less than 2 ppm 1,3-BD and styrene and less than 0.1 ppm benzene. IARC (1992) considered that these changes could not be interpreted as an effect on the bone marrow.

7.3.1.2 *Oral*

Ingestion is highly unlikely. Cold (freeze) burns would be the probable outcome.

7.3.1.3 *Dermal*

Liquid splashes will chill the skin and cause cold burns.

7.3.2 Irritation, Sensitisation and Immunotoxicity

7.3.2.1 *Skin*

Dermal contact with liquid 1,3-BD causes cold burns and frostbite.

7.3.2.2 *Eye*

Humans exposed to 2,000 ppm 1,3-BD for 7 hours and 4,000 ppm 1,3-BD for 6 hours complained of slight smarting of the eyes (Carpenter *et al*, 1944).

7.3.2.3 *Sensitisation*

No data are available.

7.3.3 Long-term Exposure

Several studies have been reported on the effects of occupational exposure to 1,3-BD, mainly from the USSR and Bulgaria. None are substantiated by sufficient details on the atmospheric concentration or duration of exposure, and control data are not generally provided. The effects reported include haematological disorders, kidney malfunctions, laryngotracheitis, upper-respiratory-tract irritation, conjunctivitis, gastritis, various skin disorders and a variety of neurasthenic symptoms, as well as hypertension and neurological disorders (IARC, 1992).

7.4 EPIDEMIOLOGY

The epidemiological literature on 1,3-BD is dominated by reports of two large retrospective cohort mortality studies; one of employees engaged in the styrene-1,3-BD rubber industry (SBR) (Delzell *et al*, 1995, 1996a; Macaluso *et al*, 1996) and one of employees in monomer production (Divine and Hartman, 1996). The study by Delzell *et al* (1995) includes most of the workers previously studied by other investigators (Meinhardt *et al*, 1978, 1982; Lemen *et al*, 1990; Matanoski and Schwartz, 1987; Matanoski *et al*, 1988, 1990). In addition to these studies, there have been two other smaller studies of monomer workers (Cowles *et al*, 1994; Ward *et al*, 1995). Apart from a slight excess of cancers in the cohort of 1,3-BD monomer workers studied by Ward *et al* (1995), all of the studies have reported deficits of mortality from all causes, all cancers, or any other broad category of disease, such as those of the cardiovascular or respiratory systems.

The first of the studies (Meinhardt *et al*, 1978, 1982) was prompted by concerns surrounding a cluster of cases of leukaemia. This, in conjunction with the later finding of leukaemia (T-cell lymphoma, Section 7.2.5.5) in mice exposed to 1,3-BD, led to attention being focused on the incidence of mortality from lymphohaematopoietic cancer (LHC) in the above-mentioned cohort studies. No statistically significant excess of cancers other than LHC has been reported in any of these studies.

7.4.1 SBR Workers

In January 1976, two men who had been employed at adjacent SBR facilities in Port Neches, Texas, died of leukaemia. In response, the National Institute for Occupational Safety and Health (NIOSH) mounted a retrospective cohort mortality study at the two SBR facilities (described as A and B) (Meinhardt *et al*, 1978, 1982). The cohort comprised white male workers employed for more than 6 months in plant A between 1943 and 1976, or in plant B between 1950 and 1976. The Standard Mortality Ratio (SMR, expressed as the ratio of observed over expected deaths) from all causes of death at plant A was 80 based on 246 deaths in a cohort of 1,656, and at plant B it was 66 based on 80 deaths in a cohort of 1,094. The all cancers SMRs were 78 and 53 respectively. A non-significant excess of cases of LHC was seen at plant A (9 observed versus 5.79 expected), but there was no such excess at plant B (2 observed versus 2.55 expected). All the cases at plant A were concentrated among those workers first employed before 1945, the date by which the process had been modified from batch to continuous feed operation. The authors commented that length of employment and time since initial employment, did not demonstrate any discernible pattern with the incidence of LHC. The length of employment and time from start of employment to death was extremely short for some cases. Two of the five cases at plant A and the case at plant B worked for 18 months or less at the plants and died within four years of starting employment at the plants. Exposure estimates were only

available after 1976. Mean exposures (8-h TWA) of 1.24 ppm (s.d. 1.20 ppm) and 13.5 ppm (s.d. 29.9 ppm) were reported for plants A and B. This study has been updated, but not reported in full (Lemen *et al*, 1990). The results have not altered substantially. More recently, workers from the two plants, subsequently combined into one plant, have been included as one of the 8 plants in the study by Delzell *et al* (1995, 1996b) described below.

The second group of SBR workers to be studied comprised employees at 7 facilities in the USA and 1 in Canada (Matanoski and Schwartz, 1987; Matanoski *et al*, 1988, 1990). The dates at which the personnel records were complete varied from one plant to another. The cohort was, therefore, defined as all men who had been employed for at least 1 year between 1943, or whenever the personnel records were complete, and 1976. In the 1987 report, the all causes SMR was 81, based on 1,995 deaths in a cohort of 13,920. The all cancers SMR was 84, and the LHC SMR was 85, based on 40 deaths. There were no significant findings for the cohort as a whole but there were results, described as suggestive, for sub-groups of employees, particularly circulatory disease in black employees. However, the latter finding requires cautious interpretation as SMRs for black workers were inflated. Race was unknown for 15% of the cohort and subjects with unknown race were classified using death certificate information if deceased, but were classified as white. The cohort was redefined for the 1990 report after reassessment of the dates when the records could be considered complete, and to facilitate follow-up at the Canadian plant. The principal results were unchanged. The all causes SMR was 81, based on 2,441 deaths in a cohort numbering 12,110, the all cancers SMR was 85, and the SMR for LHC was 97 based on 55 deaths. For specific LHCs, mortality was also similar to expected; leukaemia (22 observed, 22.9 expected), lymphosarcoma (7 observed, 11.5 expected) and other lymphatic cancers (17 observed, 15.3 expected).

To investigate these 55 cases of LHC in more detail, the employees were divided into 4 occupational groups (Production, Utilities, Maintenance, and Other), and LHC was sub-divided into several narrower categories of disease. Increased mortality was reported for leukaemia in black production workers (3 observed versus 0.5 expected), but not in white production workers (4 observed versus 4.8 expected), and a category of tumours described as 'other lymphatic' was in excess in both racial groups. Maintenance workers had fewer LHC deaths than expected for both blacks and whites. This study does not demonstrate any significant excess of cancer for the cohort as a whole. From the limited information provided on exposure, the results of the sub-cohort analyses do not show any pattern that can be related to exposure.

LHC in this cohort was further investigated in a nested case-control study (Santos-Burgoa, 1988; Santos-Burgoa *et al*, 1992). No exposure data in the form of industrial hygiene monitoring were utilised. Instead, all jobs, for all time periods, were assessed by 5 experts who allocated scores for

exposure to 1,3-BD, and to styrene, on a scale from 0 to 10. Analyses were then based on division of cases and controls into categories such as exposed and non-exposed, or semi-quantitative estimates based on the 0-10 scale, such as mean cumulative logarithmic exposure score. For each of the 59 cases of LHC available for study, up to 4 controls were selected, matched on plant, age, years of hire, duration of employment and survival until the death of the case; 193 controls were selected from those who met all these matching criteria. Numerous analyses were performed for various subsets of clinical diagnosis within LHC, and for various estimates of exposure. Multivariate analyses were also performed to enable the effect of 1,3-BD to be estimated after correction for styrene exposure and matching factors. The significant findings are largely concentrated on the diagnostic group 'all leukaemia', for which relative risks in the range 7-10 are reported for exposure to 1,3-BD versus no exposure, and for 'high' exposure versus 'low' exposure. These relative risks are independent of styrene exposure, for which no elevated risk for leukaemia was found after correcting for 1,3-BD exposure. In contrast, the diagnostic group 'other lymphatic' cancer is associated with styrene exposure and other factors beside 1,3-BD exposure. No association between 1,3-BD exposure and lymphosarcoma was observed. The interpretation of the leukaemia results from this nested case-control study is, however, not obvious (Acquavella, 1989). The leukaemia findings (Odds Ratio = 9.4, exposure prevalence 60%) conflict markedly with the results of the base cohort study, which found no leukaemia excess (Observed/Expected = 22/22.9) (Cole *et al*, 1993). This apparent discrepancy may be due to bias in the selection of controls or exposure cut point, or a low incidence of leukaemia in the control population (Cole *et al*, 1993). The relative risk for leukaemia in the case-control study has been shown to depend upon the way in which exposure is dichotomised or sub-divided into groups (Cole *et al*, 1993). Indeed, results based on some commonly used subdivisions, e.g. mean 1,3-BD score, show a relative risk of less than 1 for the higher exposed group.

The largest, most comprehensive study of 1,3-BD exposed workers was recently completed by Delzell *et al* (1995, 1996b) It includes 7 of the 8 plants studied by Matanoski *et al* (1988,1990) and the two plants, subsequently combined into one plant, studied by Meinhardt *et al*, and extends mortality follow-up through 1991. The study population was restricted to men who as of January 1, 1992 had worked at the subject SBR plants for at least 1 year. Follow up started in 1943 at four plants and between 1950 and 1965 at the remaining four plants. Based on these criteria, 15,649 SBR workers were included in this study. At the one Canadian facility, which was the only plant with substantial non-1,3-BD related manufacturing operations, another 2,315 men were enumerated who worked in non-SBR departments or in unspecified parts of the plant. These men were included as SBR workers by Matanoski *et al*, but were not included as such by Delzell *et al* (1995). However, these non-SBR workers were included as unexposed workers (to 1,3-BD) in some internal analyses reported by Delzell *et al* (1995).

For the total cohort, there were 3976 deaths, including 950 cancer deaths. The SMRs for the categories all causes of death and all cancers were 87 (95% CI 85-90) and 93 (95% CI 87-99), respectively, indicating lower mortality rates for workers than for the general USA population. Mortality from lung cancer was similar to general population rates (SMR = 101, 95% CI 91-112). SMRs for LHC indicated a slightly elevated rate for leukaemia (SMR =131, 95% CI 97-132), but not for lymphosarcoma (SMR = 80, 95% CI 40-144) and other lymphatic cancers (SMR = 97, 95% CI 70-132). For leukaemia, the SMR increased substantially when restricted to longer term employees (SMR = 201, 95% CI 134-288), but the same pattern was not seen for lymphosarcoma (SMR = 110, 95% CI 36-257) and other lymphatic cancers (SMR = 98, 95% CI 58-155). The same general pattern, with slightly higher SMRs for leukaemia, was seen when the analyses were restricted to hourly workers. Leukaemia mortality was not elevated for workers employed less than 10 years (SMR = 95), but was elevated for workers employed 10-19 years (SMR = 170) and 20 or more years (SMR = 204). The majority of the leukaemia excess occurred among workers hired in the 1950s and the elevated mortality was most manifest in the 1985-91 time period. Twenty four of the 48 leukaemia deaths occurred subsequent to the previous studies by Meinhardt *et al* (1978, 1982) and Matanoski *et al* (1988, 1990).

Cell type analysis of leukaemia mortality indicated an elevated mortality from chronic lymphocytic leukaemia (SMR = 249), chronic myelogenous leukaemia (SMR = 213), but not from acute myelogenous leukaemia (SMR = 87). However, these cell type analyses were hindered by the fact that 31% of the leukaemia death certificates did not specify cell type, (a percentage similar to that seen for the USA population). There was also an excess of acute unspecified leukaemia (SMR = 240), a finding of uncertain implication without an attribution of these deaths to the lymphocytic or myelogenous cell types.

The relationships between mortality rates and exposure to 1,3-BD and styrene were evaluated more specifically by analyses of relatively homogeneous exposed groups (called process groups) and through analyses which included quantitative estimates of monomer exposures. Workers were assigned to one of five process groups based on their complete work histories, and mortality patterns were evaluated for these groups. The five groups were: rubber production, maintenance, labour, laboratories, and other operations, but most workers were included in more than one process group category because of frequent job changes. Nonetheless, the analyses by process groups afforded an opportunity to relate mortality both to monomer exposure and to aspects of specific jobs in the plants, although most analyses were restricted to the six plants which had detailed work history information.

An increase in leukaemia mortality was seen in production workers (n=21, SMR = 169; 95% CI 104-258), laboratory workers (n=10, SMR = 431; 95% CI 207-793) and labourers, (n=16, SMR = 220; 95%

CI 126-357). There was only a slight elevation in leukaemia mortality in maintenance workers (12 observed versus 10.9 expected deaths). Lymphosarcoma mortality was close to expected for three of the process groups, but a moderate elevation was observed in maintenance workers (n=8, SMR = 192; 95% CI 83-379). Only a small percentage of the work force could be included in analyses of mutually exclusive process groups. These analyses identified laboratory workers and maintenance labourers as having the clearest excesses of leukaemia.

Retrospective quantitative estimates of exposure to 1,3-BD, styrene and benzene were developed for use in regression analyses of mortality rates. The exposure assessment was totally theoretical, viz. there were no actual exposure data available. The quantitative estimation of exposure to 1,3-BD and styrene was done by estimating the sum of background workplace exposures and exposures related to the conduct of specific tasks. Two measures of exposure were derived for each job: an 8-hour time weighted average (TWA) exposure and the number of 15 minute exposures over 100 ppm per year. The latter was used to assess the effect of peak exposures, as experimental studies with mice have found exposure intensity to be more important in carcinogenesis than exposure duration. The exposure estimation study was uniquely comprehensive and thorough, but it was not possible to validate exposure estimates for the first four decades of the industry. Exposure monitoring was not conducted prior to the early 1970s and the early monitoring data (see Fajen *et al*, 1990; Lemen *et al*, 1990; McGraw, 1990) is probably not reliable (Macaluso *et al*, 1996).

Median estimated exposure levels for 1,3-BD and styrene for various subgroups of deceased workers provide some insight about the exposure patterns of various decedent subgroups. Median exposures for all decedents were 19.0 part per million years (ppm-years) for 1,3-BD and 9.7 ppm-years for styrene. For LHC decedents, only leukaemia decedents had higher median exposures to both 1,3-BD and styrene; their values of 36.4 for 1,3-BD and 22.4 for styrene were roughly twice the values for all decedents. Also noteworthy were the low exposure values for 1,3-BD and styrene for Hodgkin's disease decedents and the higher exposure to styrene, but not 1,3-BD, for non-Hodgkin's lymphoma decedents.

Poisson regression analysis (PRA) models were used to control for the potential effects of confounding factors (age, calendar period, years since hire, race and co-monomer exposure). Table XXIX presents rate ratios (RRs) from these analyses and SMRs for the different cumulative exposure categories. Both SMRs and RRs show a trend of increasing leukaemia mortality with increasing cumulative 1,3-BD exposure. Elevated mortality is apparent in the 20-99 ppm-year category (RR = 2.0), and in the higher exposure categories of 100-199 ppm-years (RR = 2.1), and 200+ ppm-years (RR = 3.6). For 1,3-BD, the PRA and the simpler SMR analysis gave similar results. However, Delzell *et al* (1995) present a series of analyses based on different cut-off points which indicate that the trend

is not uniform. Some of these analyses do not show a steadily increasing relative risk with 1,3-BD exposure as observed in Table XXVIII. One analysis based on cut-off points of 25, 50 and 150 ppm-years shows barely increased relative risks for 0.1-24 ppm-years (RR = 1.2, 19 leukaemias) and 50-149 ppm-years (RR = 1.3, 10 leukaemias). The highest relative risk was in the 150+ ppm-years (RR 3.8, 11 leukaemias) and the relative risk was also elevated in the 25-49 ppm-years category.

TABLE XXIX: Leukaemia Mortality Results By Cumulative Exposure To 1,3-BD and Styrene - Workers From 6 Plants with Detailed Work History Records

Exposure (ppm-years)		Observed/Expected	SMR	Poisson Model RR[a]
Range	Median			
1,3-BD				
0	0	7/8.1	86 (35-178)	1.0
0.1-19	5.4	14/13.6	103 (56-172)	1.1
20-99	42.7	18/8.9	202 (120-320)	2.0
100-199	134.1	7/3.3	213 (85-438)	2.1
200+	311.9	5/1.5	331 (107-773)	3.6
Styrene				
0	0	6/6.2	97 (36-212)	1.0
0.1-19	4.8	22/18.9	116 (73-176)	1.0
20-39	28.4	7/4.2	166 (67-342)	1.2
40-59	48.7	6/2.2	271 (100-591)	1.8
60+	81.2	10/3.9	254 (122-468)	1.3

[a] Adjusted for age, years since hire, calendar year and race, and styrene or 1,3-BD exposure

Leukaemia SMRs also increase with increasing styrene exposure, but this apparent trend was not seen in the PRA. Analysis of the effect of peak exposures, defined as the number of times a worker exceeded 100 parts per million for 15 minutes, gave inconsistent results. Some increase in risk was seen in the intermediate exposure category, but none in the highest category of peak exposure. These findings appear to conflict with the results of the process group analysis which showed the clearest leukaemia associations for laboratory workers and maintenance labourers - jobs known to have opportunity for the highest peak exposures in this industry. However, Delzell *et al* (1995) note that there may have been substantial misclassification of subjects with respect to peak exposures, and this may have obscured a real trend of increasing risk with increasing peak exposure level. In addition, the highest peak exposures were considered to have only lasted for a few seconds and therefore would have been omitted from this peak exposure analysis.

Additional analyses of data on monomer peaks and employment in certain work areas have recently been reported by Delzell *et al* (1996b). New indices of 1,3-BD peak exposure were developed including BD100 peaks (exposure to 100+ ppm 1,3-BD for any period of time during the performance of a task), BD500 peaks (exposure to 500+ ppm 1,3-BD for any period of time during the performance

of a task) and the duration of employment in work areas entailing definite or possible exposure to 1,3-BD peaks. For each job, the average annual frequency of peaks was calculated (100+ ppm and 500+ ppm) and used to calculate the cumulative average annual frequency of BD100 peaks (BD100 peak-years) and BD500 peaks (BD500 peak-years). A similar measure of styrene peak exposure was derived based on exposure to 50+ ppm styrene for any period of time during the performance of a task.

Poisson regression analyses indicated that the leukaemia RR increased weakly, but consistently, with increasing BD100 peak-years: RRs were 1.0, 2.3 (95% CI 1.2-4.6) and 3.1 (95% CI 1.5-6.2) for 0, > 0-< 3288 and 3288+ BD100 peak years, respectively. The association between BD500 peak-years and leukaemia was weaker and did not display a consistent dose-response pattern. However, controlling for BD100 peak-years considerably weakened the trend of increasing leukaemia mortality with increasing 1,3-BD exposure. Table XXX shows the effect of controlling for BD100 peak-years BD500 peak-years and years of definite exposure to 1,3-BD peaks on the RR for leukaemia in Poisson regression models. An important feature of these analyses is that there was no indication of increased relative risk of leukaemia in workers exposed to less than 100 ppm-years who never experienced peaks of 100 ppm or more. In addition, the RR was only slightly elevated (RR = 1.3) in workers exposed to 100-199 ppm-years who never experienced peaks of 100 ppm or more. However, care should be taken not to over-interpret the results of the analysis given the potential correlation between the peak and cumulative exposure measures.

TABLE XXX: Poisson Regression RRs for Leukaemia by Cumulative Exposure Controlled for BD100 Peak-Years, BD500 Peak-Years and Years of Definite Exposure to Peaks

Cumulative exposure (ppm-years)	Exposure variables controlled in model							
	None[b]		BD100 peak-years		BD500 peak-years		Definite peak-years	
	RR[a]	95% CI	RR[a]	95% CI	RR[a]	95% CI	RR[a]	95% CI
0	1.0		1.0		1.0		1.0	
> 0 - 19	1.1	0.5-2.6	0.7	0.2-1.9	0.8	0.3-2.1	0.7	0.3-2.0
20 - 99	2.0	0.9-4.6	1.0	0.3-3.1	1.5	0.6-4.1	1.2	0.4-3.3
100 - 199	2.4	0.9-6.5	1.3	0.3-5.0	1.9	0.6-6.3	1.4	0.4-4.8
200+	4.6	1.6-13.3	2.5	0.6-10.6	3.7	1.1-12.7	2.6	0.7-9.8

[a] Adjusted for age, years since hire, calendar year and race
[b] No adjustment for styrene exposure as in Table XXIX

In summary, with the exception of leukaemia, the study by Delzell *et al* (1995) showed SBR worker mortality rates to be similar to or less than general population rates over a 48 year study period. In particular, mortalities from LHC other than leukaemia were not in excess in this cohort and were not related meaningfully to estimates of exposure to 1,3-BD and styrene. The most important finding from

this study is the clear indication that leukaemia mortality is elevated among long term SBR workers. This finding is based primarily on increased leukaemia mortality over the last ten years of the study period. The leukaemia excess is particularly apparent for laboratory workers and maintenance labourers, and, to a lesser extent, for certain production workers. Leukaemia mortality shows a clear trend of moderately increasing mortality with increasing estimated cumulative 1,3-BD exposure, although it remains unclear which leukaemia cell types are related to occupational exposures, due to the limitations of diagnostic information of death certificates. The importance of peak exposures could not be resolved by Delzell *et al* (1995), but later analyses reported by Delzell *et al* (1996a) indicated that there was no increased risk of leukaemia in workers exposed to less than 100 ppm-years who never experienced exposure to peaks of 100 ppm or more.

7.4.2 Monomer Workers

7.4.2.1 *Port Neches Study*

Three studies of the mortality experience of 1,3-BD monomer workers have been published. The largest and most informative is based on the production facility that supplied raw material to the SBR plant at Port Neches, Texas (Divine and Hartman, 1996). As noted earlier, the SBR workers at Port Neches have been the subject of another investigation (Meinhardt *et al*, 1978, 1982; Lemen *et al*, 1990) and were included in the cohort studied by Delzell *et al* (1995). Some subjects from the cohort of monomer workers also worked in the SBR facilities and were included in both investigations. The cohort of monomer workers investigated by Divine and Hartman (1996), was the subject of three earlier reports (Downs *et al*, 1987; Divine, 1990; Divine *et al*, 1993) which covered the period from the opening of the plant in 1942 through 1990. Earlier reports found an excess of deaths from lymphosarcoma among persons first employed before 1946, and among short-term, but not long term workers with potential for daily exposure to 1,3-BD. No excess of leukaemia was seen in the group with the potential for daily exposure to 1,3-BD or for the cohort overall, but there was a non-significant elevation for the group potentially exposed to 1,3-BD on an intermittent basis.

The most recent update extended the follow-up period to 1994 and included additional workers first employed between 1990 and 1994, giving a cohort of 2,795 male workers employed for at least six months between 1942 and 1994. Exposure classifications were not based on industrial hygiene data. Instead, a cumulative exposure score was derived which was based on two factors: calendar time and work exposure class. Each job was assigned to one of six exposure classes (0, 1, 2, 3, 4, 5) based on its potential for 1,3-BD exposure, both in terms of frequency and intensity. In order to weight the exposure estimate by calendar time, the follow-up period was divided into five time periods and weights were assigned which ranged from 10 for the earliest period to 1 for the most recent time

period. In addition, the exposure classes and the results of industrial hygiene sampling were used to group jobs with similar potential for exposure into three categories (background, low and varied exposure). Workers were assigned to three groups. The background exposure group consisted of those who were only ever employed in background jobs. The low exposure and varied exposure groups included persons who held jobs in these categories, but who may have had employment time in more than one exposure group.

The SMR for all causes of death was 88 based on 1,222 deaths, and the SMR for all cancers was 92 (282 deaths). The SMR for LHC was 147 and was significantly increased (42 observed versus 28.6 expected), including 9 observed versus 4.7 expected for lymphosarcoma (SMR = 191, 95% CI = 87-364), 13 observed versus 11.5 expected for leukaemia (SMR = 113, 95% CI = 60-193), and 15 observed versus 9.9 expected from cancer of other lymphatic tissue (SMR = 152, 95% CI = 85-250). Interest has focused on lymphosarcoma because of earlier mortality investigations of this group of workers. However, the diagnosis of lymphosarcoma is no longer routinely used, the more common diagnosis being lymphoma. Non-Hodgkin's lymphoma (NHL) is a more appropriate grouping for analysis and Divine and Hartman (1996) reported 17 observed deaths versus an estimated 9.7 expected deaths due to NHL (SMR = 176, 95% CI = 103-282). No other SMRs for NHL were reported, as the category of NHL was not included in the standard analyses of mortality. However, it was reported that sixteen decedents were first employed before 1950, 6 were employed for more than 5 years and 12 were in the varied exposure group.

Analysis by duration of employment and time first employed showed that the excess of lymphosarcoma was found primarily in those employed fewer than five years (6 of the 9 cases, SMR = 261) and that 7 of the cases of lymphosarcoma were first employed during World War II (SMR = 241). There was no evidence of an elevated rate of lymphosarcoma in workers first employed in 1946 or later, and four of the seven lymphosarcoma decedents first employed during the war, worked for less than 5 years. No excess risk of LHC was observed in the group of workers first employed after 1950 (3 observed versus 3.7 expected deaths), but Divine and Hartman (1996) reported that extremely high peak exposures still occurred well into the 1950s and 1960s. Analysis by exposure group showed that there was a small excess of LHC in the background exposure group (6 observed versus 3.9 expected deaths), no excess in the low exposure group (11 observed versus 11.0 expected deaths) but a significant excess in the varied exposure group (31 observed deaths; SMR = 172, 95% CI = 117-244). The highest SMR in the varied exposure group was for lymphosarcoma (SMR = 249, 7 observed deaths), but there was no elevation in workers employed for more than 10 years (1 observed versus 1.0 expected death). Mortality due to leukaemia was also slightly elevated in the varied exposure group (11 observed versus 7.1 expected deaths), but this increase was only observed in shorter

duration workers (< 10 years), and there were only 3 leukaemia deaths in workers employed for more than ten years (versus 2.6 expected).

Three different methods of survival analysis were used to model the data for five groups of cancers: all LHC, leukaemia, lymphosarcoma, NHL, multiple myeloma. None of the modelling approaches provided any indication that the estimate of cumulative exposure to 1,3-BD was significantly associated with an increased risk of cancer (RR is between 0.99 and 1.0).

7.4.2.2 *Kanawha Valley Study*

The mortality experience of a second cohort of workers involved in the manufacture of 1,3-BD was reported by Ward *et al* (1995). The study population was identified from records of a larger cohort of 23,139 chemical workers at three petrochemical facilities in the Kanawha Valley, West Virginia, whose mortality experience has been reported by Rinsky *et al* (1988). A cohort of 364 workers was identified. The majority (277) worked in 1,3-BD production at a US government plant (the Rubber Reserve Unit) which operated from 1943 to 1946 and which produced 1,3-BD monomer indirectly from ethanol. The remainder worked in units which produced 1,3-BD from olefin cracking. Both of these processes were different from that used in the Port Neches plant (Section 7.4.2.1). The Ward *et al* (1995) cohort was followed through 1990.

The SMR for deaths from all causes in the 1,3-BD production cohort was 91 (185 deaths) and 105 for all cancers (48 deaths). LHC deaths were elevated (7 observed versus 4.0 expected), but this was entirely due to a significantly elevated SMR for lymphosarcoma deaths (n = 4; SMR = 577; 95% CI = 1.57-14.8). Deaths from leukaemia (2 observed versus 1.6 expected), and other lymphatic cancer (1 observed versus 1.3 expected), were close to expected. Three of the lymphosarcoma decedents worked in the Rubber Reserve Unit; the longest period of employment was 39 months. Furthermore, there was a high potential for confounding exposure as noted by Ward *et al* (1995). Rinsky *et al* (1988) also reported an excess of lymphosarcoma deaths (48 observed versus 34.0 expected) in the larger cohort from which the 1,3-BD production workers were taken, although the follow-up period was shorter (through 1978). Ott *et al* (1989) conducted nested case-control studies of NHL, multiple myeloma and leukaemia within the cohort studied by Rinsky *et al* (1988) and found no associations with 1,3-BD exposure. The NHL cases in the case-control study mainly consisted of lymphosarcoma decedents (48/52). Ott *et al* (1989) reported a statistically significant increased odds ratios for NHL for alkyl sulphates, metal salts, and a number of work areas, but the odds ratio for 1,3-BD exposure was < 1.

7.4.4 Summary and Evaluation

The two most informative epidemiological studies are the Port Neches study of monomer workers by Divine and Hartman (1996) and the study of SBR workers by Delzell *et al* (1995, 1996b). These studies provide a basis for determining levels of 1,3-BD exposure that may be leukemogenic and levels which appear to have no excess risk. In addition to these studies, there have been two other smaller studies of monomer workers (Cowles *et al*, 1994; Ward *et al*, 1995). Apart from a slight excess of cancers in the cohort of 1,3-BD monomer workers studied by Ward *et al* (1995), all studies reported a lower than expected mortality from all causes, all cancers, or from any other broad category of disease (such as those of the cardiovascular or respiratory systems). Furthermore, no statistically significant excess of cancers other than LHC were reported in any of these studies.

The assessment of human risk from 1,3-BD exposure has been performed by examining separately the mortality from leukaemia and lymphosarcoma (or NHL where possible) in workers with exposure either from 1,3-BD manufacture or SBR production. Cole *et al* (1993) argue persuasively that it is possible that each of the malignant neoplasms of lymphatic and haematopoietic tissues has a different aetiology and that "the observation of an excess of leukaemia in one group of workers, and of NHL in a second group, is not evidence of a common aetiologic agent affecting both groups, or of a consistency between the two studies." Nevertheless, it can be argued that the evaluation of consistency across studies should be based on the broader category of LHC because of difficulties in distinguishing clinically between carcinomas within this group and the potential for progression from one type of LHC to another during the course of disease in an individual (OSHA 1990). Table XXXI summarises mortality from leukaemia, lymphosarcoma and other LHCs in SBR workers and the three cohorts of 1,3-BD monomer workers. It illustrates the different pattern of LHC mortality seen in two cohorts of monomer workers (Divine and Hartman, 1996; Ward *et al*, 1995) compared to that in SBR workers (Delzell *et al* , 1995).

The Divine and Hartman (1996) study had high quality cohort enumeration, vital status follow-up, and statistical analyses, and a non-quantitative, exposure assessment. The results of this study showed low or null mortality rates for most specific causes of death compared with US rates. Leukaemia mortality was not elevated for this cohort and there was no relationship between leukaemia mortality and exposure to 1,3-BD. The only potential 1,3-BD-related effect was an approximate 50% excess of non-Hodgkin's lymphoma (combining data for lymphosarcoma and other lymphatic cancers). Mortality from NHL, however, was not elevated among long-term workers and did not increase with increasing 1,3-BD exposure in analyses which employed a wide variety of exposure metrics. An excess of lymphosarcoma and reticulosarcoma was also reported in a small cohort of 1,3-BD production workers studied by Ward *et al* (1995).

TABLE XXXI: Number of Deaths and SMRs for Lymphatic and Haematopoietic Cancer (LHC) in Studies of SBR and Monomer Workers

Study	Leukaemia			Lymphosarcoma			Other LHC			All LHC		
	Obs/Exp[a]	SMR	95% CI	Obs/Exp	SMR	95% CI	Obs/Exp	SMR	95% CI	Obs/Exp	SMR	95% CI
SBR workers												
Delzell *et al* (1995)												
Overall	48/36.6	131	97-174	11/13.7	80	40-144	42/43.1	97	70-132	101/93.4	108	88-131
10+ years worked and 20+ years since hire	29/14.5	201	134-288	5/4.5	110	36-257	18/18.4	98	58-155	52/37.3	139	104-183
Monomer workers												
Divine and Hartman (1996)	13/11.5	113	60-193	9/4.7	191	87-364	19/12.3	154	93-241	41/28.5[b]	144	103-195
Ward *et al* (1995)	2/1.6	123	15-444	4/0.7	577	157-1480	1/1.64	61	1-339	7/4.0	175	70-361
Cowles *et al* (1994)	0/-	-	-	0/-	-	-	0/-	-	-	0/1.2	-	-

[a] Number of deaths observed/expected

[b] Deaths coded to ICD 209 (8th revision) are excluded from table.

There is an interesting similarity between the lymphosarcoma decedents in the cohorts studied by Ward *et al* (1995) and Divine and Hartman (1996). In both cohorts the excess is concentrated in workers first employed during the World War II and employed for less than five years in monomer production. High exposure to 1,3-BD during the early years of monomer production might explain the increased risk for lymphosarcoma in workers employed during this period. However, the survival analyses reported by Divine and Hartman (1996) do not support the hypothesis in the Port Neches cohort. The war time workers at Port Neches were unusual in that many were experienced refinery workers lent by five local oil companies to work in the plant for short periods of time. In the Kanahwa cohort studied by Ward *et al* (1995), the role of 1,3-BD exposure is difficult to clarify given the relatively short and poorly characterised periods of exposure of the lymphosarcoma decedents - in particular the three who worked in the Rubber Reserve Unit, the high potential for confounding exposure noted by the authors, and the high background level of lymphosarcoma mortality in the much larger cohort of chemical workers from which this small group was drawn. On balance, the evidence would suggest that NHL is not related to 1,3-BD exposure. The lack of excess NHL in the SBR workers study supports this non-causal interpretation.

The Divine and Hartman (1996) study has several advantages for the assessment of human risk from 1,3-BD exposure. It is a fairly large study, though expected deaths are only 31% of those in the Delzell *et al* (1995) study. The study has a long study period of 52 years providing ample time for the manifestation of 1,3-BD related health effects. The exposure scenario for monomer workers has, in general, fewer confounding factors compared with exposures in the SBR industry. Therefore, the lack of a leukaemia excess for these workers suggests that exposures characteristic of the monomer industry carry minimal leukaemia risk. Unfortunately, no quantitative exposure data are available for this study, providing, at present, no guidance as to the putative NOEL.

The Delzell *et al* (1995) study of SBR workers is unparalleled among studies of 1,3-BD-exposed workers in terms of its size, range of analyses, and attention to detail in exposure assessment. The findings indicate worker mortality rates are similar to, or lower than, general population rates for most causes of death - findings which mirror those of 1,3-BD monomer workers. For leukaemia, the results of a broad range of analyses indicate elevated mortality among long-term workers in jobs which entail 1,3-BD and styrene exposures. The leukaemia excess is most pronounced for jobs which have opportunity for intermittent high 1,3-BD exposures. Leukaemia mortality increased with increasing estimated exposure for categories of 20-99 ppm-years and higher. The exposure metrics used in the various analyses performed by Delzell *et al* (1995) did not adequately represent the repeated short-term high level exposures characteristic of the process groups which had leukaemia excesses. Thus, the potential importance of intermittent peak versus average exposures could not be evaluated effectively. Further analyses by Delzell *et al* (1996a) which incorporate new indices of 1,3-BD peak exposure, suggest that either average 1,3-BD exposures were underestimated in the original study

due to incomplete consideration of peak exposures or that peak exposures may be independently related to leukemia. These analyses indicate that workers who never performed tasks which involved exposure to 1,3-BD of 100 ppm or more for any period of time, show no elevated risk of leukaemia for cumulative 1,3-BD exposure up to 100 ppm-years.

The data from this study provide evidence of a leukaemogenic excess in the SBR industry. The trend of increasing leukaemia mortality with increasing estimated 1,3-BD exposure supports the hypothesis that 1,3-BD is a human leukaemogen. However, other possible explanations are that the leukaemia excess is related to combined exposure to 1,3-BD and styrene or to some other unidentified substance present during SBR manufacture which is correlated with 1,3-BD exposure. These two explanations, while speculative, would be consistent with the lack of a leukaemia excess among monomer workers. Preußman *et al* (1989) and Lohwasser (1996), for example, pointed to changes in the intermediates used to accelerate SBR vulcanisation. Bird (1996) noting the lack of an excess of leukaemia in SBR workers only exposed before 1950, speculates that the excess of leukaemia may have resulted from a change in the SBR process during the 1950's to a low temperature emulsion polymerisation process involving a hydroperoxide iron system (Brown *et al*, 1954; Blackley, 1975). This process includes the generation of active intermediates and oligomers. These alternate causations are supported by the absence of excess leukaemia in monomer studies (Divine and Hartman,1996) and in monomer workers within the updated SBR cohort (Delzell *et al* , 1996b).

It is also possible that monomer workers had lower exposure than those associated with excess leukaemia for SBR workers, in particular, less opportunity for exposures to peaks of 100 ppm or more. However, no data exist at present to evaluate this possibility. The 'peaks' analysis of Delzell *et al* (1996a) suggests that peak exposures may explain some of the inconsistencies between the SBR and monomer studies and also provide an explanation for the high leukaemia SMR seen in polymerisation, maintenance labour and laboratory workers. No increased risk of leukaemia was apparent for workers who experienced cumulative exposure less than 100 ppm-years and never experienced peaks of 100 ppm or more. The median exposure of workers in the 20-99 ppm-years exposure category was 42.7 ppm-years which would suggest that a cumulative exposure of 40 ppm-years is a conservative working lifetime safe level.

and urine are being measured in mouse and rat with the aim of understanding their roles in species differences. The relative mutagenicity and mutational spectra in human and rodent cells from the 1,3-BD monoepoxide and the diepoxide are being determined to assess their role in mediating *in vivo* genotoxicity. To do this, the frequency and types of mutational spectra in HPRT from rodent lymphocytes and human cell lines are being established.

9.3 GENOTOXICITY

While some studies have demonstrated the absence of mutagenic responses in workers exposed occupationally to 1,3-BD at concentrations ranging from 1 to 10 ppm (Sorsa *et al*, 1996; Hayes *et al*, 1996) others indicate a possible mutagenic response correlated with urinary metabolites (Ward *et al*, 1996). Resolution of these findings, which may be due to the different methodologies employed, is anticipated in biomonitoring studies under consideration (HEI, 1996). These investigations will utilise sensitive markers of metabolism and of genetic changes in workers occupationally exposed to 1,3-BD.

9.4 GENETIC POLYMORPHISM

The research described in Section 9.3 will include parameters to examine inter-individual variation in response due to genetic polymorphism. Such polymorphism might be mediated through epoxide hydrolase (no specific data available for 1,3-BD epoxides) or through polymorphic GST isoenzymes (GSTM1 and GSTT1) which have a known influence on SCE and chromosomal aberrations induced by 1,3-BD epoxides (Wiencke and Kelsey, 1993;Sorsa *et al*, 1994).

9.5 CARCINOGENICITY

To test the hypothesis that the diepoxide metabolites caused the lung neoplasms seen in mice but not rats (because of greater formation of the diepoxide in mice), a cancer study of the 1,3-BD diepoxide is ongoing in these species. In addition to determining the carcinogenicity of the diepoxide, the study will also examine specific mutations in the K ras gene occuring in the lung tissue of exposed animals, since previous 1,3-BD exposed mice studies had indicated potential activation of this oncogene (Henderson/HEI, 1996). Quantification of DNA adducts in rodent tissues is also in progress (Swenberg/CMA, 1996; Tretyakova *et al*, 1996) with the aim of identifying possible associations with tumorigenicity.

The T-cell leukaemia observed in mice from 1,3-BD exposure is now known to be due to a specific effect of 1,3-BD monoepoxide on a bone marrow stem cell population; this interaction is not seen in rat or man. The chronic leukaemia observed in the recent epidemiology study of SBR workers (Delzell *et al*, 1995,

1996a) has no counterpart in previous animal cancer bioassays. Studies are ongoing to determine if 1,3-BD metabolites affect chronic lymphocytic leukaemia (CLL) specific chromosome aberrations and transcription factors in isolated human bone cells. Other chemicals present in the SBR process will also be examined for direct or confounding activities (Irons/CMA, 1996; Irons *et al*, 1996).

9.6 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Observations of reproductive and developmental effects of 1,3-BD exposed mice as well as studies of the epoxide metabolites of 4-vinyl cyclohexene, provide evidence that the presence of diepoxide is critical for ovarian toxicity and that species differences are related to pharmacokinetic differences. The observed ovarian toxicity may be specific to the mouse and not applicable to man. Research is continuing on the comparison of the effect and metabolism of the epoxide metabolites in rodent and human ovarian tissue (Sipes/CMA, 1996; Doerr *et al*, 1996).

9.7 EPIDEMIOLOGY

Recent findings by Delzell *et al* (1995, 1996a) indicating a relationship between 1,3-BD exposure and leukaemia provides valuable insight for hazard identification (causality) and indicated an association with increased exposure. Although the quantitative exposure estimate for every worker exposed to 1,3-BD, styrene and benzene was available for every member of the cohort, some assumptions made about emission rates, air dispersion model and direction of task in these estimates need to be tested and refined. These data are being reanalysed to examine the differences in mortality findings between the SBR and monomer industry studies and within the various SBR studies. Areas of focus for further evaluation include the process changes in the SBR industry in the 1950s from the hot emulsion (persulphate process) to cold emulsion (redox-based technology) and the possible formation of biologically reactive by-products in this redox process. The findings of Delzell *et al* give some support for such a hypothesis as they surprisingly reported no excess of leukaemias (17 observed versus 16.3 expected) in hourly workers hired before 1950 and only 5 observed deaths versus 8.5 expected in those who worked for less than 10 years (Delzell *et al* , 1996a).

9.8 CONCLUSION

Existing data needs for hazard evaluation have been at least partially fulfilled with further explanation for the observed species differences. New needs, particularly for risk assessment purposes have arisen. These are subject of ongoing research programmes which will contribute to appropriate risk assessment and standard setting.

10. REVIEW OF EXISTING CANCER RISK ASSESSMENTS

Numerous (mathematical) risk assessments have been carried out for 1,3-BD. Two of these were prepared by the US Environmental Protection Agency (EPA, 1985a,b) in anticipation of the exposure limits proposed by the US Occupational Safety and Health Administration (OSHA, 1990). Other risk assessments prepared pursuant to the proposed OSHA limit were developed by ICF/Clement (1986) under contract to OSHA and by Environ (1986) under contract to the US Chemical Manufacturing Association. NIOSH prepared two risk assessments, the first in co-operation with Hattis and Wasson (1987) at the Center for Technology, Policy and Industrial Development at the Massachusetts Institute of Technology and the second by Dankovic *et al* (1991) from NIOSH. An alternative to the Dankovic *et al* risk assessment has been presented by Shell Oil (1990). Finally, DECOS (1990) published a document setting a health-based recommended occupational exposure limit based on a risk assessment. All of these risk assessments are based on the animal data for quantifying carcinogenic risk (rat study: Owen *et al*, 1987; mouse studies: NTP, 1984 [NTP I] and Melnick *et al*, 1990a,b; Melnick and Huff, 1992 [NTP II]). Some have been reviewed by Grossman and Martonik (1990) and by OSHA (1990).

EPA's Office of Toxic Substances (OTS) based their risk assessment on the mouse data (NTP I), using a quantal multi-stage model (EPA, 1985a). Because of the early termination of this experiment the dose was adjusted by a factor: (study duration/lifetime)^3; absorption of 1,3-BD was assumed to be 100%. Models were fitted for total haemangiosarcoma and all tumours. A two-stage model was used because the study included two exposure groups. However, OTS stated that the second stage coefficient was negligible, effectively yielding a one-stage model at low exposure concentrations. Estimates for additional risk at an occupational exposure of 100 ppm varied from 3,298 to 8,843 in 10,000 exposed workers.

The EPA Carcinogen Assessment Group (CAG) used both the rat and mouse bioassay for the quantitative risk assessment (EPA, 1985b). The mouse study (NTP I) was considered to be the primary data set; several deficiencies were noted in the rat study at that time. Based on a preliminary report by Lovelace Inhalation Toxicology Research Institute (Bond *et al*, 1986) the dose was expressed as (mg 1,3-BD absorbed)/kgbw/d; 54% absorption was assumed regardless of the dose. A two-stage quantal model was used for the mouse data as this model did not fit the female rat data. The high-dose group data were rejected and a one-stage model equivalent to the one-hit model was used. CAG adjusted the risk estimate (rather than the dose as OTS did) from the mouse study by the factor: (study duration/lifetime)^3 to compensate for the fact that exposure duration was less than the lifetime of the animals. The assumption was made that a human exposed to any given concentration experiences the same lifetime risk as the mouse, which implies that a unit dose risk estimate for mice can be applied directly to humans. Assuming an occupational exposure of 100 ppm, risk estimates derived from rat data were 208 in 10,000 and 4,595 in 10,000, depending on the model used. The high risk estimate was

obtained using the one-hit model and the lower estimate was extracted from a multi-stage model. Based on the pooled male and female mouse tumour data an additional risk estimate of 7,930 in 10,000 was calculated for a lifetime 8-hour daily exposure of 100 ppm.

A risk assessment carried out under contract to OSHA (ICF/Clement, 1986) was similar to the CAG risk assessment. The mouse bioassay (NTP I) was used for fitting the model; absorption in humans was presumed to vary with dose at the same rate as it did in mice, i.e. the percentage absorption was assumed to increase as the concentration decreased (Bond *et al*, 1986). The quantal multi-stage model was applied to the pooled male and female mouse tumour data (papillomas of the forestomach were excluded), and the high-dose group was dropped for the male mice, resulting effectively in a one-hit model. By fitting the multi-stage model to the individual tumour sites, it was noted noted that dose-response parameter estimates could be derived for tumour types not associated with possible immune suppression or viral activation mechanisms. ICF made a similar adjustment as CAG in the final risk estimate for the less than lifetime duration of exposure. It was also assumed that lifetime human risk associated with exposure was equal to the mouse risk at the same concentration. Based on the male mouse bioassay, the maximum likelihood estimate of the lifetime excess risk associated with occupational exposure (8 h/d) to 1 ppm 1,3-BD for 240 d/y during 47 of 74 years was 245 per 1,000. The corresponding risk estimate based on the female mice was 76 per 1,000. At 100 ppm, the risk estimate was 10,000 in 10,000 based on the male and female mouse data (ICF/Clement, 1986).

A risk assessment performed under contract to the US Chemical Manufacturers Association (Environ, 1986) focused primarily on the rat bioassay. Environ used the CAG approach of absorbed dose, expressing dose as mg/kgbw. The quantal multi-stage, Weibull and Mantel-Bryan models were used to fit the pooled rat tumour incidence data and the Hartley-Sielkin time to tumour model was fitted to the male mouse data. Female mouse data were excluded because the data on the absorbed dose in mice were based exclusively on males and also because male mouse data yielded a higher risk. Unlike CAG and ICF, Environ calculated a unit risk estimate on the basis of mg/kgbw/d and ventilation rate, and assumed that human risk was similar to the animal risk. This assumption lowered the estimated human risk compared to those of CAG and ICF which assumed equivalence on a ppm basis. The Environ risk estimate from the male mouse data resulted in a lifetime excess risk of 4.65 per 1,000 following occupational exposure to 1 ppm 1,3-BD. Risk estimates derived from the rat data are up to 45-fold lower than the mouse-based estimates. At 100 ppm, the risk estimate based on male mouse data was 3,730 in 10,000; based on the male rat data it was between 154 and 559 in 10,000; based on female rat data, the additional risk was between 560 and 730 in 10,000.

Hattis and Wasson (1987) carried out a risk assessment under co-operative agreement with NIOSH. A PB-PK model was created to predict the amount of 1,3-BD metabolised as a result of exposure to various airborne concentrations. However, no tissue-specific estimates were made, and the model did

not attempt to estimate EB or DEB concentrations. Unfortunately, some of the key data for the construction of a PB-PK model were not available and the authors were forced to estimate certain values, e.g. blood:air and tissue:blood partition coefficients. No experiments were carried out to validate the model. The risk estimates were several fold lower than the CAG risk estimate.

The risk assessment of Dankovic *et al* (NIOSH) is based on preliminary NTP II data since the pathological evaluations were not complete. This study includes exposures at a concentration of 6.25 ppm. Dankovic *et al* used data provided in the published report and received additional information on the time of death and tumour status of each individual mouse (used for model fitting). The metric dose used for model fitting was the external exposure concentration. 1,3-BD metabolism appears to be linear up to 200 ppm and somewhat sublinear as the external concentration was raised to 625 ppm (Laib *et al*, 1988). In addition, tumour formation at 625 ppm was strictly non-linear, quantal tumour responses dropping as the exposure was raised from 200 ppm to 625 ppm. In contrast, lymphocytic lymphoma increased disproportionately, especially in male mice (Melnick *et al*, 1990a,b). These data suggest that many of the non-linear metabolism and non-linear tumour responses could be avoided by dropping the 625 ppm dose group from the analysis. Models were fitted both with and without data from the 625 ppm dose group, but the models without the 625 ppm group were considered to represent a better estimate of the low dose tumour response. The tumour onset models evaluated were the one-stage, two-stage and three-stage Weibull time-to-tumour models. Preference was given to the following: (i) the model having the least number of stages, unless the model with a larger number of stages resulted in a better fit; (ii) models in which lymphomas and haemangiosarcomas were treated as rapidly fatal and all other tumour types as incidental; (iii) analyses where the 625 ppm group was dropped. The 1,3-BD doses to which the mice were exposed were extrapolated to humans by assuming that the equivalent human dose (in mg) for any given mouse dose of 1,3-BD is larger than the mouse dose by a factor: $(\text{human mass/mouse mass})^{0.75}$. For the purpose of extrapolation, this means that the mouse dose of 1,3-BD was multiplied by 1.49 to estimate the human dose. The site yielding the largest extrapolated risks at low exposure concentrations, i.e. the most sensitive site, was the female mouse lung. Based on this site, the projected excess risk for a person occupationally exposed to 2 ppm 1,3-BD, for an entire working lifetime, was estimated to be 597 cases of cancer per 10,000, or approximately 6 per 100.

In an updated appraisal of Dankovic *et al* (1993) these estimates were attenuated. Taking into account interspecies differences in EB formation and detoxification and applying the adjustment for body weight using the 0.75 power scaling factor, the risk estimate was reduced to 2 per 100. Taking into account differences in metabolism and using only mg/kgbw scaling the risk estimate was reduced to 1 in 100.

In the risk assessment by Shell Oil (1990), calculations were based on unaudited data from NTP II (Melnick *et al*, 1990a,b) and on the rat data (Owen *et al*, 1987). In addition, use was made of published and corrected data relating to retention data in rats and mice (Bond *et al*, 1986), pharmacokinetic

behaviour of 1,3-BD in mice, rats and monkeys (Dahl *et al*, 1991) and relative enzyme activity across species. Both quantal and time-to-tumour models were applied in this analysis. The risk estimate, based on pooled malignant tumours in the NTP II study using 1,3-BD retained as the dose measure and the Weibull multi-stage time-to-tumour model, is 2.5 in 10,000 assuming workers are exposed (8 h/d, 250 d/y) to 2 ppm 45 years out of the assumed 74 year life). Using the epoxide metabolite directly in the modelling resulted in a similar risk of 2 in 10,000. The quantal multi-stage model indicates somewhat higher risks whether 1 or 2 stages are fitted, with risks ranging from 5 to 10 in 10,000. Risk estimates based on haemangiosarcoma were much lower because in the NTP II study there were no such malignancies at either 6.25 ppm or 20 ppm and only a single response at 62.5 ppm. This resulted in a risk estimate of less than 1 in 1,000,000. The highest risk obtained in rats was 8 in 10,000. This result was found using the female rat tumour data, including mammary fibro-adenomas and the retained 1,3-BD as the dose measure. Using the epoxide metabolite as the measure of biologically effective dose yielded risk estimates less than 3 in 100,000. Overall, this analysis predicted an occupational risk of below 1 in 1,000, maybe lower than 1 in 1,000,000, following exposure to 1,3-BD at 2 ppm for 45 years.

DECOS (1990) used the rat study to calculate the risk for exposed workers adopting the linear non-threshold model as the basis for risk estimation and extrapolating to low levels of the dose-response relationship. According to the authors the approach was conservative, representing an upper limit for risk, i.e. the true risk is not likely to be higher than the estimate, but could be lower. Species differences were not taken into account but it was assumed that using the rat tumour data for extrapolation would probably further over-estimate the risk for humans. Tumours produced with a dose-related increase in rats were pooled, which resulted in a tumour incidence at the low dose of 21% and at the high dose of 38%. Only the data from the low dose group were used because of the non-linear relationship. Assuming a 40-years exposure (8 h/d) time and a life expectancy of 75 years, the concentration calculated to give a risk of 4 in 1,000 was 47.4 mg/m^3 (21.5 ppm). When a risk of 1 in 10,000 (40-years exposure) was considered, the exposure level was 1.18 mg/m^3 (0.5 ppm).

Hallenbeck (1992) compared estimates of environmental and occupational risks of 1,3-BD using both non-PB-PK and PB-PK models. Data from the NTP I study were used to calculate the cancer risk factor employing a linear interpolation method for extrapolation from high to low doses. Hallenbeck's non-PB-PK model accounts for exposure concentration, intake rate, time of exposure, life expectancy, latency and body weight in the estimation of dose. His PB-PK model accounts for alveolar ventilation, cardiac output, blood flow in tissues, V_{max}, K, KF, partition coefficients, body weight and tissue volumes. Assuming an intake of 10 m^3 air/day which contains 2 ppm 1,3-BD over a period of 45 years (250 days/year), the calculated risk using the non-PB-PK approach was 30 in 1,000 and 20 in 1,000 using the PB-PK-based model. According to the author, both non-PB-PK and PB-PK models should be considered with caution until they can be validated by epidemiological studies.

Fielder (1996) provided the rationale for an ambient air quality standard in the UK, based on the arguments that (i) 1,3-BD is tumorigenic in rat and mouse and (ii) occupational exposure to 1,3-BD has been shown to be associated with an increased incidence of malignancies of the lymphatic/lymphopoietic system. Therefore 1,3-BD should be regarded as a potent genotoxic animal carcinogen and a probable human carcinogen. Although the carcinogenic potency may be greater in mice than in man, it is prudent to assume the absence of a threshold. For carcinogenic compounds a pragmatic approach should be followed based essentially on the assessment of the exposure at which detection of increased risk is not possible, and followed by application of a safety factor. The starting point in the derivation of a recommended standard was the identification of 1,000 ppb as the "no detectable effect level" in populations occupationally exposed to 1,3-BD. This was divided by 10 to take into account the difference between a working lifetime and a chronological lifetime and by a further 10 to account for intra-individual differences in sensitivity, to give a value of 10 ppb. However in the UK ambient levels of 1,3-BD in urban air do not exceed 1 ppb as a running annual average. Therefore the Expert Panel on Air Quality Standards of DOE agreed to recommend 1 ppb as the air quality standard for 1,3-BD as a running annual average.

The risk estimates of some models can be excluded because they are inconsistent with the epidemiology data, e.g. Dankovic *et al* (1993). Turnbull *et al* (1990) examined the conflict between risk predicted from the animal and epidemiological data. They found that if average human exposure in the cohort studied by Matanoski *et al* 1990) was > 1 ppm, which is likely, the CAG risk estimates based on either the mouse NTP I study or the rat bioassay over-predict the observed incidence in humans.

An alternative approach to dose-response modelling on 1,3-BD uses the data from the continuous and stop-exposure mouse studies (NTP II) to describe the relation between exposure duration, time to death and tumour related mortality. This type of approach is often used to analyse epidemiological data, but its use with animal data is novel. Mortality incidence and specific tumour rate (e.g. lung adenocarcinoma and heart angiosarcoma) appeared to be related to $(\text{exposure concentration})^3$ and $(\text{exposure duration})^2$ respectively. The specific tumour rate of malignant lymphoma appears to be related to $(\text{exposure concentration})^5$ $(\text{exposure duration})^{1.6}$. This suggests that the relation between exposure level / duration and specific tumour rate is non-linear. Consequently, the use of cumulative exposure as a dose measure is not appropriate. In particular with long periods of exposure at low dose levels it will result in an over-estimation of actual risk (Ten Berge, 1996).

IARC (1992) evaluated the strength of evidence for carcinogenicity of 1,3-BD arising from human and experimental data. It was concluded that, while there was sufficient evidence in experimental animals, the evidence in humans was limited. It was noted that *in vitro* studies suggested that the metabolism of 1,3-BD is qualitatively similar in experimental animals and humans.

10.1 SUMMARY AND EVALUATION

This above overview clearly shows the complex nature of a quantification of risk to man associated with 1,3-BD exposure (see also ECETOC, 1996b). There is a substantial difference in the risks predicted by the various models. The models are highly dependent on the choice made with regard to the animal system (mouse or rat), tumour data, dose (external/internal) and mathematical approach (one-hit, multiple-stage, time-to-tumour). There are different views on the predictive value of the various models for human risk assessment and also on the type of data that should be used in the models. The reasons for this include a lack of understanding of the mechanism of action of 1,3-BD in different species and continuing scepticism concerning the value of the results of carcinogenicity studies in rodents as a basis for predicting human risk. The metabolism studies (Section 7.1.4) suggest that the rat is a more appropriate species than the mouse for human risk assessment. The risk estimates based on data from the rat include the following: CAG: 208 to 4,595/10,000 at 100 ppm (life-time exposure), Environ: 154 to 559/10,000 at 100 ppm (life-time exposure), Shell Oil: less than 1/1,000 at 2 ppm,(45-years exposure) and DECOS: 4/1,000 at 21.5 ppm (40-years exposure).

11. EXISTING OCCUPATIONAL EXPOSURE LIMITS

Existing national occupational exposure limits (OELs) range from 1 to 100 ppm 1,3-BD expressed as an 8-hour TWA concetration (Table XXXII). The supporting documentation indicates that most OELs are based on the finding of multiple organ cancer in rats or mice. Techniques used to extrapolate an OEL from animal data involved either the use of adjustment/safety factors or quantitative risk assessment models. The Netherlands, for example, chose the less sensitive rat model as the basis for an OEL of 50 ppm and concluded that this should not lead to an underestimation of the risk for humans.

Some countries do not base the OEL on animal or human data. In Germany, for example, OELs for genotoxic carcinogens are based on technical feasibility (TRK value), since it is considered that a safe exposure limit cannot be identified scientifically. The US American Conference of Governmental and Industrial Hygienists (ACGIH) and the UK Health and Safety Executive (HSE) based the OELS, in part, on data which indicate that average workplace exposures below 10 ppm are achievable.

TABLE XXXII: National OELs

Country	TWA[a] (mg/m^3)	STEL[a] (mg/m^3)	TWA (ppm)	STEL (ppm)	Reference
EU					
Austria	-	-	-[b]	-	DFG, 1996
Belgium	22	-	10	-	Moniteur Belge, 1995
Denmark	22	-	10	-	NLI, 1994
Finland	110	-	50	-	HTP-Värden (1993)
France	-	-	-	-	ILO, 1991
Germany	-	-	-[b]	-	DFG, 1996
Greece	-	-	-	-	ACGIH, 1995
Ireland	-	-	-	-	Irish HSE, 1994
Italy	4.4	-	2	-	ACGIH, 1995
Luxembourg	-	-	-	-	
Netherlands	110	-	50	-	Arbeidsinspectie, 1995
Portugal	22	-	10	-	Norma Portugesa, 1990
Spain	22	-	-	-	ILO, 1991
Sweden	1	10	0.5	5	AFS, 1996
UK	22[c]	-	10	-	HSE, 1996
OECD					
Australia	22	-	10	-	ILO, 1991
Canada	22	-	10	-	ILO, 1991
Japan	-	-	-	-	JSIH, 1994
Norway	2.2	-	1	2	Arbeidstilsynet, 1995
Switzerland	11	-	5	-	SUVA, 1994
USA - ACGIH	4.4	-	2	-	ACGIH, 1995
- OSHA	2.2	-	1[d]	5	OSHA, 1996
- NIOSH	-	-	-	-	

TWA Time-weighted average concentration (8-h working period)
STEL Short-term exposure limit (15 min, unless specified)
a Official values; some countries use different conversion factors and/or other ambient temperature (e.g. USA at 25 °C)
b Germany and Austria: TRK values: 15 ppm (34 mg/m^3) after polymerisation and loading, 5 ppm (11 mg/m^3) for other applications
c Maximum Expousre Limit (MEL)
d Permissible Exposure Limit (PEL)

12. SUMMARY EVALUATION AND RECOMMENDATION FOR A SCIENTIFICALLY BASED OCCUPATIONAL EXPOSURE LIMIT

12.1 SUBSTANCE IDENTIFICATION

Common name:	1,3-butadiene
CAS registry No:	106-99-0
EEC No:	601-013-00-X, nota D
EEC classification:	F+ ; R 12 / Carc. Cat. 2; R 45
EEC labelling:	R: 45-12 / S: 53-9-16-33
EINECS name:	buta-1,3-diene
EINECS No:	203-450-8
Formula:	C_4H_6
Structure:	CH_2=CH–CH=CH_2
Molecular mass:	54.09

12.2 OCCURRENCE AND USE

12.2.1 Chemical and Physical Properties

1,3-butadiene (1,3-BD) is a colourless, non-corrosive gas (boiling temperature: –4.4 °C) with a mildly aromatic or gasoline-like odour (threshold concentration 1.0 to 4.0 mg/m^3). 1,3-BD has a high vapour pressure: 2,477 hPa at 20 °C. It is soluble in organic solvents, but only slightly in water. 1,3-BD is a highly reactive material which can dimerise to 4-vinylcyclohexene. It polymerises readily, especially in the presence of oxygen. 1,3-BD in air can form acrolein and explosive peroxides.

The technical product is transported as a liquefied gas under pressure with an inhibitor such as aliphatic mercaptans, *o*-dihydroxybenzene or *p-tert*-butyl catechol to prevent polymerisation and/or peroxide formation.

Conversion factors for 1,3-BD concentrations in air, calculated at 20 °C and 1,013 hPa are:

1 mg/m^3 = 0.445 ppm
1 ppm = 2.249 mg/m^3

12.2.2 Occurrence and Use

1,3-BD does not occur as a natural product.

Industrial emissions arise during (i) production of crude 1,3-BD and petroleum refining, (ii) 1,3-BD monomer extraction, (iii) production of 1,3-BD containing polymers and derivatives, and (v) plastic products manufacturing.

1,3-BD has also been identified in automobile exhaust, cigarette smoke, gasoline formulations and liquefied petroleum gas (LPG), and small amounts are released by the burning of plastics or rubber.

12.2.3 Exposure Levels at the Workplace

There is considerable amount of information on the measured exposures to 1,3-BD covering the petrochemical, 1,3-BD producing and 1,3-BD consuming industries. The most recent data from the European 1,3-BD producing industry indicates that the vast majority of workplace exposures are below 1 ppm although there are a number of job categories that appear to be associated with higher exposures. These are most often those jobs which involve either breaking into lines, such as maintenance or sampling, or filling operations. This is also reflected by the available short-term exposure data. Exposure during use of 1,3-BD in the production of various polymers in Europe indicate that 71.1% of all measurements did not exceed a level of 1 ppm and 93.3% did not exceed 5 ppm expressed as a time-weighted average (TWA) concentration for an 8-hour working period. However, higher exposure may occur during some tasks.

12.2.4 Exposure Levels in the Environment

The average reported environmental air concentration of 1,3-BD varies from 0.04 ppb (measured in rural areas of the UK) to 100 ppb measured in the vicinity of a petrochemical complex in the USA (DoE, 1994 as quoted in DoE, 1996 and Texas Control Board, 1990 as quoted in ATSDR, 1992).

Typical average concentrations in urban areas of 0.5 ppb are reported. However, due to heavy traffic and very cold weather conditions, considerably higher day average concentrations have been observed, for example 9.7 ppb during the winter of 1991 in London (DoE, 1994 as quoted in DoE, 1996).

Indoor air concentrations reported are between 5 and 9 ppb, probably originating from cigarette smoke. These are significantly higher than the outdoor concentrations of 0.45 ppb reported in the same study (Löfroth *et al*, 1989 as quoted in DoE, 1996).

The only measured concentration reported for surface waters is 2 µg/l (Ewing *et al*, 1977 as quoted in DoE, 1996 and HSDB, 1992). Estimates of surface water concentrations based on plant effluent monitoring, taking into account dilution on-site and in the receiving environment, indicate levels between 0 and 7 ng/l (CEFIC, 1996).

There are no reported soil concentrations of 1,3-BD. Given the physical nature of 1,3-BD, it is not expected to be present in the soil compartment (DoE, 1996).

1,3-BD (8-9 µg/kg) has been detected in olive oil that was packed in 1,3-BD rubber-modified acrylonitrilic bottles (McNeal and Breder, 1987 as quoted in ATSDR, 1992 and HSDB, 1992). Other surveys of food packaging containing 1,3-BD monomer did not show any levels of 1,3-BD in the food itself (Startin and Gilbert, 1984 as quoted in IARC, 1992 and ATSDR, 1992).

1,3-BD has been detected in drinking water but at such low levels that quantification has not been possible (EPA, 1978; Kraybill, 1980; both as quoted in IARC, 1992 and ATSDR, 1992).

12.2.5 Measuring Methods

Almost all methods for the sampling of 1,3-BD in air involve the collection of a large volume of contaminated air and concentration of the volatile components, including 1,3-BD (e.g., by adsorption on to charcoal and desorption by methylene chloride). This solution is then separated, and compounds identified and analysed by GC-FID or electron capture device (ECD). These methods allow for the detection of very low concentrations, e.g. in the background workplace or ambient air (down to ppt levels) (HSE, 1986, 1989; CONCAWE method [Bianchi and Cook, 1988]; NIOSH method [NIOSH, 1987; Lunsford and Gagnon, 1987; as quoted in ATSDR, 1991]; Eller, 1978; Locke *et al*, 1987, both as quoted in IARC, 1992; Gentry and Walsh, 1987 as quoted in DECOS, 1990).

For personal monitoring in the workplace, gas detector tubes are used (Saltzman and Harman, 1989 as quoted in IARC, 1992).

Methods in use by the 1,3-BD monomer production facilities and styrene-butadiene rubber (SBR) plants in the EU are currently being investigated by CEFIC (European Chemical Industry Council) and ESRA (European Synthetic Rubber Association) in a joint Task Force. This study involves a comparison of sampling and analytical techniques.

12.3 HEALTH SIGNIFICANCE

From the substantial database on 1,3-BD there is clear evidence of a substantial difference in sensitivity to toxicological effects between the mouse and all other species studied. The toxicokinetic data suggest that this is related to species differences in the degree of biotransformation of 1,3-BD to reactive metabolites.

Inhaled 1,3-BD is metabolised by cytochrome P_{450}-dependent mono-oxygenases to the primary metabolite 1,2-diepoxybutene-3 (EB). This intermediate is subjected to further metabolism via 3 pathways, (i) hydrolysis by epoxide hydrolases to 3-butene-1,2-diol, (ii) further epoxidation by mono-oxygenases to 1,2:3,4-diepoxybutane (DEB), and (iii) conjugation with glutathione (GSH) catalysed by GSH *S*-transferases (GST) (Malvoisin *et al*, 1979; Malvoisin and Roberfroid, 1982).

The biotransformation appears to be qualitatively similar across species, including humans (Kreuzer *et al*, 1991; Csanády *et al*, 1992a, 1993; Sabourin *et al*, 1992). However, owing to observed differences both in the uptake of 1,3-BD and in the kinetics of the metabolism of 1,3-BD, the steady-state concentrations in blood and target tissues, and the resulting body burden of 1,3-BD and its individual metabolites, are quantitatively different across species. For EB, the body burden in the mouse appears to be up to 3-fold higher than for the rat (Kreiling *et al*, 1986, 1987; Bond *et al*, 1986; Dahl *et al*, 1991). *In vivo* data on primates and *in vitro* data with human tissue samples suggest that humans and other primates are closer to the rat than the mouse with regard to the metabolism and resulting body burden of EB (Sabourin *et al*, 1992). This conclusion was supported by a biomonitoring study measuring EB-Hb adducts. Adduct levels per ppm-hour in 1,3-BD exposed workers were calculated to be 25-fold lower than in the rat and more than 100-fold lower than in mice (Osterman-Golkar *et al*, 1993). Even greater species differences exist with regard to the extent of formation of DEB (Csanády *et al*, 1992a, 1993; Himmelstein *et al*, 1994, 1995; Thornton-Manning *et al*, 1996). As compared with human data, the differences in DEB concentration in blood and tissues ranged from 40-fold in the rat to 160-fold in the mouse and were especially noteworthy in the target tissues for 1,3-BD-induced carcinogenicity, such as the mouse lung. Specifically DEB was not detected in rat lungs (Thornton-Manning *et al*, 1996). On the basis of *in vitro* data it is expected that DEB levels in humans would be even lower than those found in rats (Seaton *et al*, 1995). However, due to the high inter-individual variability observed for the 1,3-BD and EB *in vitro* metabolism in human donor samples, a broad range of variation has to be assumed with regard to the body burden of the 1,3-BD derived epoxides in human populations.

1,3-BD has a low acute and subchronic toxicity. The target organs in the mouse are the central nervous system (CNS) and bone marrow, whereas non-specific effects were reported in the rat. The no-observed effect level (NOEL) is 2,300 ppm in the rat (Carpenter *et al*, 1944) and 625 ppm in the mouse (NTP, 1984).

1,3-BD as such is not genotoxic. However, certain metabolites have the ability to interact with DNA directly to form adducts and/or crosslinks which eventually may result in gene mutations or chromosomal aberrations. The genotoxic action of 1,3-BD in various test systems depends on its biotransformation to reactive metabolites. The efficacy of this biotransformation appears to be quite different among species; mice seem to have a greater capability to transform 1,3-BD than do rats, which is exemplified by the lack of genotoxic response in rats (for references, see Table XVIII to XXIV). Studies addressing the genotoxic potential of the main 1,3-BD metabolites have shown clearly that the diepoxide is the most potent, indicating that this intermediate may be the ultimate carcinogen.

A few cytogenetic analyses have been carried out in peripheral blood lymphocytes of 1,3-BD workers exposed to low levels (< 1 ppm). No exposure-related effects in frequencies of chromosome aberration, sister chromatid exchange (SCE) or micronuclei were observed. Inconsistent results were obtained in three studies that investigated HPRT mutational frequencies in blood lymphocytes of workers employed in the 1,3-BD industry in the USA, Czech Republic and China (Hayes *et al* , 1996; Tates *et al* , 1996; Ward *et al* , 1996). The one study that showed an association of elevated (HPRT) variant frequency and 1,3-BD exposure (up to 3 ppm) was conducted by Legator and co-workers. In this study a blood sample evaluation of only 8 workers was conducted; a slight elevation of variant frequency in three of them was found. The other two studies (with high exposure levels up to 85 ppm) reported negative results. More studies are needed to clarify this matter.

The carcinogenic effects of 1,3-BD were studied in Sprague-Dawley rats (Owen, 1981a,b; Owen *et al*, 1987) and in B6C3F$_1$ mice (NTP, 1984; Melnick and Huff, 1992). Striking species differences between mice and rats were also observed in these studies. 1,3-BD is a potent carcinogen in mice, with tumours found in lungs of females at 6.25 ppm, the lowest concentration tested. At higher concentrations, 1,3-BD produced a dose-related incidence of multiple types of tumours in both sexes of the mouse, including T-cell lymphoma, haemangiosarcoma, alveolar bronchiolar neoplasm, squamous cell neoplasm, hepatocellular neoplasm, and Harderian gland neoplasm. Liver tumours were marginally increased in females at 20 ppm and higher and in males at 200 ppm, but it is questionable whether this increase is related to treatment, given the high liver tumour incidence in this hybrid mouse strain (Maronpot *et al*, 1987). In contrast, 1,3-BD is a less potent carcinogen in rats than in mice. At 1,000 ppm the only statistically significant increase was in the incidence of mammary gland tumours, the majority of which were benign. When considered separately, there was neither a significant increase of benign nor of malignant tumours. Such a separation appears to be justified, since historical data show a high incidence of benign mammary tumours in the Sprague-Dawley rat (Löser, 1983). The tumour pattern in both sexes of the rat at 1,000 and 8,000 ppm involved only endocrine sensitive organs. This suggests the involvement of a secondary mechanism in rats but not in mice.

Studies in guinea pigs, rabbits and rats have not shown adverse effects on fertility at exposure concentrations as high as 6,700 ppm 1,3-BD for up to 8 months (Carpenter *et al*, 1944). Studies in mice suggest that repeated exposure to high concentrations of 1,3-BD may increase the incidence of post-implantation losses, but the lack of a demonstrable dose-response relationship for this effect suggests that it is of questionable biological significance. One dominant lethal study included an eight-week post-exposure serial mating protocol, which demonstrated significant reduction in implantations only at 1-2 weeks post-exposure, suggesting an effect of treatment upon mature germ cell populations, but not upon premeiotic stages (Hackett *et al*, 1988a; Morrissey *et al*, 1990). This is in apparent contrast with the demonstration of clastogenic effects in the spermatids of mice exposed to 1,3-BD (Xiao and Tates, 1995), which were temporally correlated with exposure of premeiotic cells, and is indicative that premeiotic mutations do not persist to a biologically significant degree (Zeiger, 1994).

In developmental toxicity studies conducted with 1,3-BD in rats and mice, no toxicity to the developing foetus was seen at exposure concentrations below those which caused maternal toxicity (Hackett *et al*, 1987a,b; Irvine, 1981, 1982; Morrissey *et al*, 1990). One study reported an increased incidence of abnormalities in the offspring of male mice exposed to 1,3-BD, but the outcome of data analysis will have been affected by the absence of any malformations in control offspring (Anderson *et al*, 1993; BIBRA, 1995). Indeed, the incidence of malformations from exposed groups was within the historical range for this strain. A subsequent study at the same laboratory failed to replicate the finding (BIBRA, 1996a). It is thus unlikely that this observation was a result of a toxic effect on the foetus. No similar effects were seen in rats (BIBRA, 1996b).

Structural abnormalities have been detected in the ovaries and testes of mice exposed to 200 ppm 1,3-BD, and in older females exposed to concentrations as low as 6.25 ppm (NTP, 1984, 1993; Melnick and Huff, 1992). Such effects were not observed in rats exposed to up to 8,000 ppm (Owen, 1981a,b). The effects in female mice at concentrations lower than 200 ppm were not regarded as of toxicological significance as they occurred after the normal reproductive period in the mouse, and were likely to be related to reproductive senescence.

The two most informative epidemiological studies are those of monomer workers by Divine and Hartman (1996) and of SBR workers by Delzell *et al* (1995, 1996b). However, the findings from these studies are potentially in conflict. The study by Delzell *et al* (1995) provides strong evidence of a leukaemogenic effect in the SBR industry as increasing leukaemia mortality increased with increasing estimated 1,3-BD exposure. In contrast, there was no increase in leukaemia mortality in the cohort of monomer workers studied by Divine and Hartman (1996) and no relationship between leukaemia mortality and 1,3-BD exposure. The latter study was smaller than that of Delzell *et al* (1995) and expected deaths were only 31% of those in the SBR workers cohort. Nevertheless, the monomer workers study had more than

adequate power to detect an excess in leukaemia and it had the advantage of an exposure scenario which, in general, had fewer confounding factors compared with exposures in the SBR industry. Unfortunately, the exposure assessment in the monomer study was not quantitative. Comparisons of occupational exposure to 1,3-BD in the monomer and SBR industries (e.g. JACA Corp., 1987 as quoted by IARC, 1992) suggest that mean levels of exposure to 1,3-BD in the cohorts of monomer and SBR workers would have been similar.

The study by Delzell *et al* (1995) indicated elevated leukaemia mortality among long-term workers in jobs which entailed 1,3-BD and styrene exposures. Leukaemia mortality increased with increasing estimated exposure for categories of 20-99 ppm-years and higher. The leukaemia excess was most pronounced for jobs with opportunity for intermittent high exposures. The potential importance of intermittent peak versus average exposures was not evaluated adequately by Delzell *et al* (1995), but further analyses by Delzell *et al* (1996a) suggest that either average 1,3-BD exposures were underestimated in the original study due to incomplete considerations of peak exposures or that peak exposures may be independently related to leukaemia. This may account for the differences seen between the monomer and SBR workers. Other possible explanations for the differences are that the leukaemia excess is related to combined exposures to 1,3-BD and styrene, or to exposure to an unidentified substance which is correlated with 1,3-BD exposure.

The only possible 1,3-BD related effect in the monomer workers was an approximate 50% excess of non-Hodgkin's lymphoma (NHL). Mortality from NHL, however, was not elevated among long-term workers and did not increase with increasing 1,3-BD exposure in analyses which employed a wide variety of exposure metrics. Overall, the evidence would suggest that NHL is not related to 1,3-BD exposure and the lack of excess NHL in the SBR workers support this non-causal interpretation.

Overall, these two epidemiological studies suggest that exposures characteristic of the monomer industry carry minimal leukaemia risk, and the SBR workers' findings indicate no increase risk of leukaemia in workers who never performed tasks which involved exposure to 1,3-BD of 100 ppm or more for any period of time and whose cumulative exposure did not exceed 100 ppm-years (Delzell *et al,* 1996a; Divine and Hartman,1996).

12.4 FINAL EVALUATION AND RECOMMENDATION

12.4.1 Hazard Identification

Based on considerable evidence from both animal and epidemiological studies it is clear that the critical health effect related to exposure to 1,3-BD is carcinogenicity. It follows that protection against carcinogenic hazard will protect for all other possible health effects of 1,3-BD.

Since 1,3-BD is a gas at room temperature, skin absorption is not a concern and a skin notation is not warranted (ECETOC, 1993b).

12.4.2 Risk Assessment

12.4.2.1 *Animal Data*

1,3-BD is transformed in the body by both activation and deactivation processes. Oxidation by cytochrome P_{450} to the mono and diepoxides may be followed by hydrolysis to the diols or conjugation with GSH. Studies in rodents show that the balance between activation and deactivation in the mouse favours the formation of free epoxides in tissues and blood. The levels of these metabolites are much lower in rats than in mice as this balance is markedly different.

Genotoxic activity has been demonstrated clearly in the mouse and equivocally in the hamster, but not in other non-human species. Consequently it is clear that the potency of 1,3-BD to induce genotoxic effects in mice is higher than in other species. Nevertheless, the metabolites of 1,3-BD thought to be responsible for the genotoxic action in mice are formed in other mammals, although the rates of formation and detoxification differ considerably.

1,3-BD is a multi-organ carcinogen in both the rat and the mouse. In a two-year bioassay 1,3-BD produced lung tumours in female mice at concentrations as low as 6.25 ppm, the lowest concentration tested. Rats are very much less susceptible to the effects of 1,3-BD with significant increases of treatment-related tumours observed only following exposures to 8,000 ppm. As the tumour types, incidences and concentrations eliciting tumour responses in rats and mice are so different it is probable that the mechanisms of action of 1,3-BD are different in the two species. 1,3-BD is a genotoxic carcinogen in mice, whereas in rats, tumours are seen only in endocrine-sensitive organs suggesting a non-genotoxic process. To further complicate the analysis, T-cell lymphomas in mice are also known to be derived from non-genotoxic mechanisms resulting from a cytotoxic effect of the monoepoxide on a sub-population of haematopoetic stem cells in the mouse. This effect is unique to the mouse.

This species difference is evident in relation to repeated dose toxicity, reproductive toxicity and genotoxicity, as well as carcinogenicity. Mechanistic data indicate that differences in metabolism, both in the formation and removal of the epoxides, are in part responsible for this difference in susceptibility. Limited *in vivo* data in primates and *in vitro* data obtained from human tissue samples suggest that in humans and primates, exposure to 1,3-BD results in a lower body burden of the reactive epoxides of 1,3-BD than in mice. Since it is the epoxides, and in particular the diepoxide, that are considered to be responsible for the toxicity of 1,3-BD, humans and primates would be expected to be less sensitive to the carcinogenic effects of 1,3-BD than mice. Indeed, if humans were as sensitive as the mouse to the effects of 1,3-BD and the model of Dankovic *et al* (1993) was true, then a dramatic increase in cancer incidence would have been noted in 1,3-BD exposed cohorts.

The rat data also appear to be inappropriate as a basis for human risk assessment, although the rationale is not as compelling as that for the mouse data.

The metabolism data obtained in human tissue studies are too variable to provide a firm basis for comparison of the relative body burden of metabolites in humans and rats. Additionally, the tumour type associated with 1,3-BD exposure in humans (see next section) is different from those found in rats. Thus, the rat data cannot be confidently related to the human experience either on a mechanistic or a dose- response basis.

In conclusion, neither rat nor mouse data provide an appropriate basis for setting an OEL.

12.4.2.2 *Human Data*

The metabolism of 1,3-BD in humans is qualitatively similar to rodents. However, there are quantitative differences in the resulting body burden of the reactive epoxides arising from exposure to 1,3-BD. *In vivo* data from primates, and *in vitro* data with human tissue samples, suggest that the metabolism of 1,3-BD and body burden of EB in humans and other primates are more like that of the rat than the mouse. In addition, the urinary conjugate indicative of diepoxide formation has not been detected in human urinary biomonitoring experiments. However, due to the inter-individual variability for 1,3-BD and EB metabolism observed in human donor samples *in vitro*, a range of body burdens for 1,3-BD epoxides must be assumed to exist in exposed human populations. Even so, genetic polymorphism with regard to the GSH activity is not considered to have a major impact on the overall detoxification rates of 1,3-BD epoxide metabolites.

A number of investigators have done biomonitoring on humans occupationally exposed to 1,3-BD including such endpoints as HPRT, chromosomal damage, Hb adducts and urinary metabolites. The overall assessment is that no chromosomal damage nor Hb adducts has been consistently demonstrated.

There is a reported association of 1,3-BD exposure and increased variant HPRT frequency at exposure to 3 ppm 1,3-BD. However, there are conflicting results from the other HPRT assays using differing methodologies and populations. Thus these human data require corroboration before much weight can be placed upon them.

The epidemiological data on worker populations exposed to 1,3-BD leave a number of questions unresolved. There is a statistically significant association between 1,3-BD exposure and chronic leukaemia frequency in SBR workers, but no such association has been observed in monomer workers. The reason for the inconsistencies between the findings of the study of SBR workers (Delzell *et al*, 1995) and that of monomer workers (Divine and Hartman, 1996) remains unclear. In addition, the importance of intermittent high exposures has not been evaluated adequately. Nevertheless, the epidemiological data should be given precedence for the purposes of deriving a risk assessment for man. As noted in the previous section, the available rodent data are at variance with observations in humans. For example, lung tumours are seen at very low doses in the mouse but are not seen in any epidemiological studies in association with 1,3-BD exposure. Conversely, no increase in any leukaemia type has been reported in any of the various animal bioassays. The animal studies also conflict in that T-cell lymphomas are seen in mice and endocrine tumours in rats. Thus, there appears to be no good animal model for 1,3-BD induced tumours which is of direct relevance to man.

The SBR workers study by Delzell *et al* (1995) is of most value for risk assessment as it has a uniquely comprehensive exposure estimation and covers a large cohort drawn from a large number of locations. However, it does not preclude the use of the monomer workers study of Divine and Hartman (1996). This is also a large study with a long follow-up period and the exposure scenario for monomer workers has, in general, markedly fewer confounding factors compared with exposures in the SBR industry. Unfortunately, the exposure assessment for this study was not quantitative, providing, at present, no guidance as to the putative NOEL.

The study of Delzell *et al* (1995) provides strong evidence of a leukaemogenic exposure in the SBR industry. In contrast, there was no increase in leukaemia mortality in the cohort of monomer workers studied by Divine and Hartman (1996), nor any relationship between leukaemia mortality and estimates of 1,3-BD exposure. Conversely, the excess of NHL seen in monomer workers was not corroborated by the SBR study and did not demonstrate a relationship with any of the 1,3-BD exposure metrics studied by Divine and Hartman (1996). Thus, leukaemia is the critical effect for risk assessment.

The form of leukaemia associated with exposure to 1,3-BD in the SBR industry is unclear. The largest elevation in mortality was reported for chronic leukaemias, both lymphocytic and myelogenous. However, the form of leukaemia was unspecified for a number of leukaemia deaths. Poisson regression analyses for chronic myelogenous leukaemia showed no evidence of an association with 1,3-BD

exposure and the relationship between chronic lymphocytic leukaemia and 1,3-BD exposure was much weaker than that observed between all leukaemias and 1,3-BD exposure (Delzell *et al*, 1995).

Taken at face value, the SBR workers' findings indicate a lowest effect level in the 20-99 ppm-years category and no increased risk for the 1-19 ppm-years category. However, Delzell *et al* (1995) present a series of Poisson regression analyses based on different cut-off points and a variety of structural forms for relative risk including linear, multiplicative, power and polynomial, which indicate that the trend is not uniform or well defined. Some of these analyses do not show a steadily increasing relative risk with 1,3-BD exposure as observed in Table XXIX. One analysis based on cut-off points of 25, 50 and 150 ppm-years shows barely increased relative risks for 0.1-24 ppm-years (RR = 1.2, 19 leukaemias) and 50-149 ppm-years (RR = 1.3, 10 leukaemias). The highest relative risk was in the 150+ ppm-years (RR = 3.8, 11 leukaemias) and the relative risk was also elevated in the 25-49 ppm-years category. Analyses which incorporate nine exposure categories show a random, but increased, pattern of relative risk in the six exposure categories between 5 and 200 ppm years. These analyses suggest that some caution is required when attempting to derive a working lifetime safe level.

The dose response relationship between leukaemia and cumulative exposure in the SBR study is poorly defined. Cumulative exposure to 1,3-BD was only just significant at a 5% level in analyses using a range of models which incorporated a single term for 1,3-BD exposure. One explanation for this poor relationship is that the cumulative exposure metric is an inappropriate starting point for calculating a numerical value for a working lifetime safe level. The groups exhibiting elevated leukaemia rates in the SBR workers study are those that experienced intermittent high exposures. This suggests that high level intermittent exposures may be more important for the critical effect than long periods of low exposure. Support for this concept is also provided by the data from stop exposure studies in the mouse.

Analyses of the SBR workers data that also take into account exposure to peaks of greater than 100 ppm, indicated that the highest relative risks were observed for workers with exposure to peaks and cumulative exposures of over 200 ppm years, but that there was no increased relative risk in workers exposed to less than 100 ppm years who never experienced exposure to peaks of 100 ppm or more (Delzell *et al*, 1996a). The median exposure of workers in the 20-99 ppm-years exposure category was 42.7 ppm-years which would suggest that a cumulative exposure of 40 ppm-years is a conservative working lifetime safe level.

A number of other considerations suggest that the working lifetime safe level is at least as high as 40 ppm-years. The quantitative exposure assessment for SBR workers, while remarkable in methods and scope, had a number of conventions that might underestimate exposure. In addition, exposures characteristic of the monomer industry from 1943-1994 were not related to excess leukaemia risk.

12.4.3 Derivation of Occupational Exposure Limit Value

The following factors provide the basis for deriving an OEL for 1,3-BD.

- Leukaemia is the critical health effect from epidemiological studies.
- The dose-response relationship between leukaemia and cumulative exposure in the SBR study is poorly defined at cumulative exposure levels below 150 ppm-years. Consequently it is difficult to define a working lifetime safe level using only information about cumulative exposure.
- A recent analysis of the SBR study data incorporating information about peak exposure has demonstrated the significance of peak exposures in man. The data from stop-exposure studies in the mouse suggest that the cumulative exposure metric alone is an imperfect starting point for calculating a numerical value for an OEL because short periods of high exposure may be more important than long periods of low exposure.
- Analyses of the SBR workers data that also take into account exposure to peaks of greater than 100 ppm, indicated that the highest relative risks were observed for workers with exposure to peaks and cumulative exposures of over 200 ppm-years. There was no increased relative risk in workers assigned to the 20-99 ppm-years exposure group who never experienced exposure to peaks of 100 ppm or more. Consequently this concentration (100 ppm) can be used to define a working lifetime safe level.
- A conservative approach would be to take the median exposure of workers in this group, i.e. 42.7 ppm-years as an effective working lifetime safe level.
- Additional margins of safety are built into the above argument as there is little evidence that there was increased risk in workers exposed to 1,3-BD below 200 ppm-years and that exposures may have been underestimated in the SBR workers study.
- The above arguments indicate that an OEL of 1 ppm would protect workers against non-neoplastic and neoplastic effects.
- Exposures characteristic of the monomer industry from 1943-1994 were not related to excess leukaemia risk although it is likely that exposures to 1,3-BD would have been well in excess of 1 ppm.

12.4.4 Recommendation

An OEL of 1 ppm (8-h TWA) is recommended for 1,3-BD.

Peak exposures are central to the epidemiological data and consequently a STEL is required to protect against potential effects. However, it is not possible to define a STEL on a scientific basis. Thus, a STEL of 10 ppm (15-min TWA) is recommended on a pragmatic basis, being 10 times the recommended OEL.

No skin notation is warranted.

At present no method for biological monitoring can be recommended.

A number of suitable methods are available for short-term, long-term and continuous sampling measurements of 1,3-BD at the recommended OEL of 1 ppm.

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- Abstract superseded by publication
- Abstract only, without further (detailed) information
- Internal report, superseded by scientific paper
- Irrelevant data reported
- No exposure data reported
- No formal protocol reported
- No new data reported
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13.3 DATABASES CONSULTED

BIOSIS, Chem. Abs. , CHEMLIST, CSNB, EMBASE, GIABS, HSDB, MEDLINE, TOXALL, ULIDAT

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