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1,3-Butadiene
Criteria Document

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No. 4

1,3-BUTADIENE CRITERIA DOCUMENT

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European Centre for Ecotoxicology
and Toxicology of Chemicals

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PREFACE

This report has been prepared by ECETOC for use by the Commission of the EC DG V and its Scientific Expert Group. It contains an original review and assessment of toxicological data and quantitative risk assessments (chapters 7 to 10) to provide a scientific basis for an occupational exposure limit for 1,3-butadiene (chapter 12). Information on occurrence, production and use, exposure and uptake, and measurement techniques (chapters 3-6) has been drawn largely from existing reviews.

SPECIAL ABBREVIATIONS

1,3-BD	1,3-butadiene
CFU-S	colony-forming assay of stem cells
CFU-GM	CFU of granulocyte/macrophage
CAG	Carcinogen Risk Assessment Group (US EPA)
DEB	1,2:3,4-diepoxybutane
EB	1,2-epoxybutene-3
eMuLV	ecotropic MuLV retrovirus
GSH	glutathione
HLC	haematopoietic and lymphatic cancer
HPLC	high-pressure/performance liquid chromotography
LOEL	lowest-observed effect level
MS	mass spectrometry
MuLV	murine leukemia virus
NOEL	No-observed effect level
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, 1992a), see references not cited: NTP, 1992
PB-PK	physiologically-based pharmacokinetic (model)
SBR	styrene-butadiene rubber
SMR	standard mortality ratio
SCE	sister-chromatid exchange
SLRL	sex-linked lethal
SMART	somatic mutation and recombination test
STEL	short-term exposure limit (15 min, unless specified)
TWA	time-weighted average (concentration) for an 8 h working period
UDS	unscheduled DNA synthesis

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SUMMARY AND CONCLUSIONS

1,3-Butadiene (1,3-BD) is a colourless, non-corrosive gas with a mildly aromatic or gasoline-like odour. 1,3-BD polymerises readily, especially in the presence of oxygen. The technical product is shipped as a liquified gas under pressure with an inhibitor to prevent polymerisation and/or peroxide formation, such as *p-tert*-butyl catechol.

1,3-BD is not known to occur as a natural product. Industrial emissions arise during (i) production of crude 1,3-BD and petroleum refining, (ii) 1,3-BD monomer production, (iii) transfer of 1,3-BD, (iv) production of 1,3-BD containing polymers, derivatives, rubber and plastic products manufacturing. 1,3-BD has also been identified in automobile exhaust, cigarette smoke, and gasoline formulations, and small amounts are released by the burning of plastics or rubber.

Exposure Levels and Daily Intake

There is limited information on occupational levels exposure in Europe (see below). The Conseil Européen de l'Industrie Chimique (CEFIC), the International Institute of Synthetic Rubber Producers (IISRP) and the Association of Plastics Manufacturers in Europe (APME) have started to collect European exposure data.

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 exposures to 1,3-BD in most process areas were less than 10 ppm; however, maximum 8-h time-weighted average exposures (8-h TWA) were frequently between 10 and 125 ppm (in one case as high as 374 ppm) in operations involving decontamination and maintenance of process equipment, sampling and analysing of quality control samples, and loading or unloading tank trucks or rail cars.

Based on data used to underpin the German TRK value, personal exposure levels (8-h TWA) are approximately 5 ppm, with maxima of 30 ppm during the manufacturing and purification of 1,3-BD in petroleum refineries and extraction facilities. Data from the USA show that many job categories have exposures below or around 5 ppm, the great majority of levels lying below 10 ppm, with the exception of maintenance and distribution jobs. Exposure levels (8-h TWA) associated with manufacturing and use of gasoline are generally very low.

High exposures (5 to 50 ppm, 8-h TWA, max. 500 ppm) occur during the connection of pipes for transfer of 1,3-BD (reported in Germany).

Workplace 8-h TWA concentrations during the manufacturing of 1,3-BD based polymers in Germany were between 10 and 20 ppm (mixture of personal and background measurements), with a maximum (peak) concentration of 50 ppm. Data from 5 polymer plants in the USA showed personal exposure levels generally below 0.5 ppm, with two exceptions at approximately 5 ppm. In two other surveys

of the North American synthetic rubber producers, the majority of exposures was below 10 ppm. The latter picture is confirmed by data collected during health surveys or epidemiological studies. These exposures should not be regarded as representative of conditions in the 1940's, when exposures were higher.

No 1,3-BD could be detected during the manufacturing of tyres from synthetic rubber. The evaporation of 1,3-BD from other plastic products should not constitute a significant source for exposure at end-use.

1,3-BD has been detected in urban air in the USA at ppt to ppb levels. 1,3-BD may also be present in indoor air, e.g. due to cigarette smoking and in drinking water. No residual 1,3-BD could be detected in foodstuffs packaged in materials made from 1,3-BD.

The non-occupational daily intake has been calculated to be 2.62 µg/person, assuming a mean urban air concentration of 0.29 ppb/day (USA data, section 5.3.1) and human air intake of 20 m^3/day.

Measurement

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 onto charcoal and desorption by methylene chloride). This solution is then separated, and the compounds identified and analysed by gas-chromatography (GC) equipped with a flame ionisation device (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, CONCAWE and NIOSH methods).

For personal monitoring at the workplace, gas detector tubes are used.

Toxicity

There is an extensive data base on the toxic effects of 1,3-BD. Toxicological studies have revealed a remarkable difference in sensitivity to 1,3-BD between the mouse and all other species investigated.

The metabolic elimination of 1,3-BD is linearly related to the ambient exposure concentration up to about 1000 ppm in rats and mice, with mice showing higher elimination rates. Above 1000 ppm, metabolic pathways are approaching saturation in these species. In monkeys the metabolic elimination of 1,3-BD appears to be saturated at about 300 ppm. The biotransformation appears to be qualitatively similar across species, including humans. However, differences in uptake and kinetics of 1,3-BD result in quantitative difference in body burden of 1,3-BD and its individual metabolites across species. For the metabolite 1,2-epoxybutene (EB) the body burden in the mouse appears to be threefold higher than for the rat. *In vivo* data on primates and *in vitro* data on human samples suggest that humans are

closer to the rat than the mouse with regard to metabolism and resulting body burden of EB.

Upon inhalation, 1,3-BD has a low acute and subchronic toxicity. The target organs in the mouse are the central nervous system (CNS) and the bone marrow, whereas in the rat non-specific effects were reported.

1,3-BD itself is not genotoxic. The genotoxic action of 1,3-BD in various test systems depends on its biotransformation to reactive metabolites. Some of these metabolites apparently have the ability to directly interact with DNA and cause gene-mutations and chromosomal aberrations. When comparing the results of *in vivo* tests performed with 1,3-BD, its genotoxic activity has been demonstrated clearly in the mouse and equivocally in the hamster, but not in other species.

The carcinogenic effects after (life-time) inhalation of 1,3-BD were studied in Sprague-Dawley rats and in B6C3F1 mice. The 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 tumours were found at multiple sites. In contrast, 1,3-BD is less potent in rats, where statistically significant increases in tumour incidences were observed at 1000 ppm and 8000 ppm. The tumour pattern in both sexes of the rat suggest that a hormone-related mechanism is involved. The only tumours seen at 1000 ppm with statistically significant increases were mammary gland tumours in the female. The majority of these tumours were benign. There was neither a significant increase of benign nor of malignant tumours when considered separately. Based on this information 1000 ppm is a NOEL for the rat.

Special studies designed to assess fertility did not show adverse effects in guinea pigs, rabbits and rats. Developmental toxicity studies conducted with 1,3-BD show that there was no toxicity to the developing foetus at exposure concentrations below those which caused maternal toxicity. Overall these studies show again the unique susceptibility of the mouse to 1,3-BD.

With regard to the epidemiological studies, some authors recognised a qualitative association between 1,3-BD exposure and haemotopoietic and lymphatic cancer, while others see no causal relationship. The available studies, however, are inappropriate for quantitative risk assessment since, in the absence of measured concentrations, the exposure data were only qualitative.

Quantitative Risk Assessment (Models)

Numerous quantitative risk assessments with regard to the carcinogenicity of 1,3-BD have been carried out. The range of risk values determined using the mouse bioassay are incompatible with findings of the epidemiological studies. Values for extrapolation on the rat bioassay also show some variation for the best estimated lifetime risk. The predictive value of mathematical models used for extrapolation of animal bioassay data to low human exposure is questionable because the models (i) are not validated, (ii) are derived from mathematical assumptions rather than

knowledge of biochemical mechanisms, (iii) demonstrate a wide variety of risk estimates depending on the models used, and (iv) give the impression to be precise which cannot be justified from the approximations and assumptions upon which they were based. Until these concerns are adequately addressed, this type of quantitative risk assessment in unsuitable as a basis for setting an exposure limit.

Final Evaluation and Recommendation

With regard to the effects of 1,3-BD on experimental animals, it is obvious that the mouse is more sensitive than all other species investigated. This holds true for subchronic toxicity, reproductive toxicity, genotoxicity and carcinogenicity. Based on the results of *in vivo* genotoxicity tests performed with 1,3-BD, it has to be assumed that the potency of 1,3-BD to induce genotoxic effects in mice is higher than in other species. 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. Based on the toxicokinetic data available for a comparison of species including humans, the rat appears to be an acceptable conservative model on which to base an exposure limit value for humans.

The carcinogenic potential of 1,3-BD is clearly the dominant concern of health effects related to 1,3-BD exposure. There is some doubt whether genotoxic action is the critical mechanism for induction of tumours in the rat. This is substantiated by the tumour pattern observed in the rat which is more indicative of an indirect mechanism mediated through the endocrine system. However, resolution of this issue is not possible on the basis of the available information.

The uncertainties discussed above make it difficult to derive a scientifically sound occupational limit. The lowest occupational exposure limit used in EC member states today is 5 ppm (German TRK value for certain applications; based on technical feasibility). This concentration is 200-fold lower than the identified NOEL in the rat. In addition, with all the reservations expressed above considered, the quantitative risk assessments based on the rat bioassay suggest that the risk of additional cancer deaths at 5 ppm is low. Most important perhaps, epidemiological data generally do not demonstrate any excess mortality from all causes, all cancers or any other broad category of disease for past exposure concentrations which were most likely higher than the current exposure concentrations. The controversy with regard to the possible association between 1,3-BD exposure and haematopoietic and lymphatic cancer, which has been proposed by some authors and rejected by others, still has to be resolved.

In view of all the available evidence, it is concluded that an occupational exposure limit (OEL) of 5 ppm should protect workers against non-neoplastic and neoplastic effects.

The ongoing research programme will add significantly to the understanding of the mechanism and toxicokinetics of 1,3-BD-induced carcinogenesis, and provide information on exposure-based epidemiology. Thus, the OEL should be re-

evaluated after this new information will have been incorporated into the database. This work should be completed by 1995.

Since skin absorption of 1,3-BD is not a concern, no skin notation is suggested.

There is no evidence to suggest that it is critical to determine a short-term exposure limit (STEL). However, because of the uncertainty about the biological relevance of high short-term exposures to 1,3-BD, a STEL of 100 ppm (15 min TWA) is recommended as a complimentary control to the OEL of 5 ppm.

At present, no method for biological monitoring can be recommended.

A number of suitable methods are available for carrying out short-term, long-term and continuous sampling measurements of 1,3-BD at the recommended OEL of 5 ppm (section 6.1).

1. SUBSTANCE IDENTIFICATION

1.1 Identity

Common name:	1,3-butadiene
CAS name:	1,3-butadiene
CAS registry N°:	106-99-0
EEC N°:	601-013-00-X, nota D
EEC classification:	F+ ; R 13 / Carc. Cat. 2; R 45
EEC labelling:	R: 45-13 S: 53-9-16-33
RTECS N°:	EI 9275000
IUPAC name:	1,3-butadiene
EINECS name:	buta-1,3-diene
EINECS N°:	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 α,γ-butadiene *trans*-butadiene diethylene divinyl erythrene NCI-C50602 pyrrolylene vinylethylene
ES:	1,3-butadieno
FR:	1,3-butadiène
IT:	1,3-butadiene

NL: PT:	1,3-butadieen 1,3-butadieno
Chemical group:	unsaturated hydrocarbons
Formula:	C_4H_6
Structure:	$CH_2{=}CH{-}CH{=}CH_2$
Molecular mass:	54.09 (Weast *et al*, 1988)
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 or 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. 1,3-BD in air 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 1.9	Verschueren, 1983 Sax, 1991
Threshold odour concentration, mg/m^3 (odour: mildly aromatic)	1.0-3.5 4.0	Amoore and Hautala, 1983; DECOS, 1990 Sax, 1991
Solubility in water, g/kg at 20°C	0.735	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]; Jow and Hansch, n.d.[b]; Banerjee and Howard, 1988[c]
Flash point, closed cup, °C	< -76	DECOS, 1990
Explosion limits in air[d], %	2.0-11.5	Sax, 1991
Auto-flammability, ignition temp., °C	429 420	DECOS, 1990 Sax, 1991

a As quoted in Hazardous Substances Database (HSDB, 1992)
b As quoted in Sax (1991)
c As quoted in DECOS (1990)
d Temperature range not specified

The technical product is shipped as a liquified gas under pressure with an inhibitor to prevent polymerisation and/or peroxide formation, such as aliphatic mercaptans or *o*-dihydroxybenzene (Windholz *et al*, 1976) or *p-tert*-butyl catechol (ICI, 1992). 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 is not known to occur as a natural product (Santodonato, 1985 as quoted in IARC, 1992).

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

According to a 1984 survey by the US EPA, atmospheric emissions of 1,3-BD from facilities which produce or process 1,3-BD were approximately 10 million pounds/year or approximately 5,000 tonnes/year; 70% of these emissions were attributed to equipment leaks and 30% to process venting (Mullins, 1990).

1,3-BD has been identified in automobile exhaust (Miller *et al*, 1978 as quoted in ATSDR, 1991; US EPA, 1990 as quoted in IARC, 1992) and sidestream cigarette smoke (0.4 mg/cigarette) (Löfroth *et al*, 1989, as quoted in ATSDR, 1991 and IARC, 1992). It is present as a contaminant in some gasoline formulations (Sigsby *et al*, 1987; Stump *et al*, 1989, both as quoted in ATSDR, 1991; section 5.1.1). Liquified petroleum gas (LPG) also contains a small percentage of 1,3-BD (CONCAWE, 1992).

Small amounts of 1,3-BD may be produced by thermal degradation of polyurethane-coated wire during electrical overload (Rigby, 1981 as quoted in ATSDR, 1991) and by the burning of other 1,3-BD based-plastics or rubbers (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).

3.2 Occurrence at the Workplace

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 reactions with photochemically produced hydroxyl radicals, nitrate radicals, ozone and molecular oxygen

(Guicherit and Schulting, 1985; ATSDR, 1991). 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, 1991). Very low-level exposures may occur by ingestion of contaminated food and drinking water, and inhalation of gasoline vapours, automobile exhaust or cigarette smoke (section 5.3.2-5.3.6). Quantification is not possible for lack of data (ATSDR, 1991).

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 catalytic steam cracking of petroleum fractions (light oil and naphtha) for the manufacture of ethylene. It is also obtained by catalytic dehydrogenation of butene or butane-butene mixtures, or alcohol. Other production methods are available (Windholz *et al*, 1976; Melnick and Huff, 1992a).

Statistics on the annual quantities of 1,3-BD produced in Western Europe are in Table II.

TABLE II

Annual Production* of 1,3-BD in Western Europe
(APPE, 1992)

Country or area	1985	1986	1987	1988	1989	1990	1991
Benelux	352	369	405	421	384	439	387
Germany	513	466	498	539	533	547	534
France	288	292	307	334	329	284	286
United Kingdom	183	177	176	186	178	146	164
Italy, Spain, Portugal, Finland, Austria	332	314	359	405	457	489	442
Total Western Europe	1,668	1,618	1,745	1,885	1,881	1,905	1,813

* Quantities produced in kt/y. Production capacities are, in kt/y: 370 (Netherlands), 620 (Germany), 360 (France), 350 (UK), 360 (Italy), Spain (132), 42 (Portugal), 20 (Finland) and 47 (Austria) (SRI International, 1991).

4.2 Use

The major uses of 1,3-BD are in the manufacture of synthetic rubber such as styrene-butadiene rubber (SBR) or polybutadiene rubber used in tyres and tyre products, thermoplastic resins such as acrylonitrile-butadiene-styrene (ABS) used in automotive parts and business machines, and styrene-butadiene latex suspensions used in paints and carpet backing. 1,3-BD is used as a chemical intermediate in the production of neoprene (for industrial and automotive

rubber goods) and adiponitrile (a nylon precursor) (Windholz *et al*, 1976; Verschueren, 1983; IARC, 1986, 1992; Melnick and Huff, 1992a).

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. It is possible that dermal contact with the liquified gas may occur during loading and unloading of tanks, or by the accidental rupture of tanks. However, no such accidents have been reported (ATSDR, 1991).

Limited information on the European exposure situation is available (see below). The Conseil Européen de l'Industrie Chimique (CEFIC), the International Institute of Synthetic Rubber Producers (IISRP) and the Association of Plastics Manufacturers in Europe (APME) have initiated a collection of European exposure data.

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 exposures to 1,3-BD in most process areas were less than 10 ppm; however, maximum 8-h TWA exposures were frequently between 10 and 125 ppm (in one case as high as 374 ppm) in operations involving decontamination and maintenance of process equipment, sampling and analysing of quality control samples, and loading or unloading tank trucks or rail cars (Fajen *et al*, 1990).

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

Exposure levels during the manufacturing of 1,3-BD in German petroleum refineries (crackers) are mostly below 5 ppm. Levels up to 30 ppm were measured at a few places. These levels were reported as mean values from 93 shifts and consisted of a mixture of personal samples and background concentration measurements (Deutscher Ausschuß für Gefahrstoffe, n.d.). Data from other European Countries are not available.

During investigations at petroleum refineries and petrochemical facilities in the USA producing crude 1,3-BD (usually a C_4 stream obtained as a by-product of ethylene manufacturing), the following exposure levels were identified (Table III).

TABLE III

Exposure of Workers Involved in Petroleum Refining and Production of Crude 1,3-BD since 1984
(Heiden Associates, 1987 as quoted in IARC, 1992)

Job category	N° of facilities	Concentration (ppm) Mean*, 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

* Mean 8-h TWA concentration levels, weighted by number of exposed workers [at each facility]

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

TABLE IV

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

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

* Not detected

5.1.2 1,3-BD Monomer Production

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

Information on purified 1,3-BD monomer facilities in the USA is reported by IARC (1992) as follows.

> "Detailed industrial hygiene surveys were conducted by the US National Institute of Occupational Safety and Health in 1985 in four of 10 US facilities where 1,3-butadiene was produced by solvent extraction of C_4 fractions originating as ethylene co-product streams (Krishnan *et al*, 1987). Levels of 1,3-butadiene to which workers in various job categories were exposed are summarised in Table V. 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. Geometric means of full-shift exposure levels for other job categories were below 1 ppm. Short-term samples showed that such activities as open-loop sampling and cylinder voiding were associated with peak exposures of 100 ppm. Full-shift area samples indicated that ambient concentrations of 1,3-butadiene were greatest in the railcar terminals (geometric mean, 1.77 ppm) and in the tank storage farm (2.12 ppm)."

TABLE V

Full-shift 1,3-BD TWA Exposure Levels* at Four US 1,3-BD Monomer Production Facilities (1985)
(Krishnan *et al*, 1987 as quoted in IARC, 1992)

Job category	N° of samples	Exposure level (ppm)		
		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

* Personal breathing zone samples

"In 1984, the US Chemical Manufacturers' Association obtained data on personal exposure to 1,3-butadiene before 1984 from 13 monomer-producing companies, categorized broadly by job type (Table VI). These data were collected by an older method and provide a historical perspective on the data reported in Table V. The highest exposures were in the maintenance and distribution jobs. 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)."

TABLE VI

Occupational Exposure to 1,3-BD in the Monomer Industry*
(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 (79.4)	111 (19.7)	5 (0.9)			
Maintenance (%)	329	247 (75.1)		47 (14.3)	35 (10.6)		
Supervisory (%)	64	60 (93.8)	4 (6.2)				
Distribution (%)	206	60 (29.1)	121 (58.7)	16 (7.8)	5 (2.4)	2 (1.0)	2 (1.0)
Laboratory (%)	126	58 (46.0)	68 (54.0)				
Total (%)	1,287	871 (67.8)	304 (23.6)	68 (5.3)	40 (3.1)	2 (0.1)	2 (0.1)

* Based on data obtained before 1984 from 13 US monomer production plants

Additional European monitoring data are available from a Finnish plant producing purified 1,3-BD, where 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 of 1,3-BD ranged from < 0.1 to 447 ppm (mean 11.5 ppm, median < 0.1 ppm; 46 samples, mean sampling time 2.5 h). The highest concentrations were measured during sample collection. The workers used protective clothing and respirators 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).

5.1.3 Transfer of 1,3-BD

1,3-BD is transported in large quantities, either in the form of a C4 fraction or as pure 1,3-BD. High exposure concentrations occur during the connection of filling pipes. Data from such operations in Germany (173 mean values from personal shift-samples) show exposure levels between 5 and 50 ppm;

concentrations reached 500 ppm in some cases (Deutscher Ausschuß für Gefahrstoffe, n.d.).

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

Concentrations at the workplace of German facilities for the manufacture of 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 gas chromatography (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, n.d.).

Information on the polymer and derivatives industry in the USA is reported in IARC (1992) as follows.

> "Detailed industrial hygiene surveys were conducted in 1986 in five of 17 US facilities where 1,3-butadiene was used to produce styrene-butadiene rubber, nitrile-butadiene rubber, poly-butadiene rubber, neoprene and adiponitrile (Fajen, 1988). Levels of 1,3-butadiene to which workers in various job categories have been exposed are summarized in Table VII. 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 to more than 100 ppm. Full-shift area sampling indicated that geometric mean ambient concentrations of 1,3-butadiene were less than 0.5 ppm and usually less than 0.1 ppm in all locations at the five plants."

TABLE VII

Full-shift 1,3-BD TWA Exposure Levels* 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

* Personal breathing zone samples

"Eight-hour time-weighed average (TWA) exposures to 1,3-butadiene in the polymer industry were obtained by personal sampling in 11 North American synthetic rubber plants in 1978-84 and reported by the International Institute of Synthetic Rubber Producers in 1984 (JACA Corp., 1987) (Table VIII). The highest exposures were found for tank car loaders (15% of exposures, >10 ppm), reactor operators (18% of exposures, >10 ppm) and laboratory technicians (6% of exposures, >10 ppm). Sampling and analytical techniques and job descriptions were not available."

TABLE VIII

Occupational Exposure to 1,3-BD in the Polymer Industry*
(JACA Corp., 1987 as quoted in IARC, 1992)

Occupational group	N° of samples	8-h TWA 1,3-BD exposure (ppm)							
		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	500.00-1000.00
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)					
Warehouseman (%)	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)

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

A review of monitoring data from more than 800 exposures obtained during site visits to two SBR plants in the USA in October 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 IX).

TABLE IX

1,3-BD Exposure Data from Two SBR Plants* (1981-1987).
(Tozzi, 1988)

Job Category**	<0.50 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/ 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%

* Percentage within job category
** < 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, quoted from IARC (1992) as follows.

> "In a US styrene-butadiene rubber manufacturing plant in 1979, the only two departments in which levels were greater than 10 ppm were tank farm (53.4 ppm) and maintenance (20.7 ppm) (Checkoway and Williams, 1982). In samples taken at one of two Us styrene-butadiene rubber plants 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). Overall mean 8-h TWA exposure levels differed considerably between the two plants, however: 1.24 ppm in one plant and 13.5 ppm in the other (Meinhardt *et al*, 1982)."

No data are available on levels of exposure to 1,3-butadiene before the 1970s, when different processes and working conditions (e.g. during the Second World War) would have resulted in exposure conditions different from those now prevalent in developed countries (IARC, 1992).

Data from synthetic rubber and plastics manufacturing in the USA are discussed in IARC (1992) as follows.

> "Unreacted 1,3-butadiene was detected as only a trace (0.04-0.2 ng/mg) in 15 of 37 bulk samples of polymers and other chemicals synthetized from 1,3-butadiene and analysed in 1985-86. Only two samples contained measurable amounts of 1,3-butadiene: tetrahydrophtalic anhydride (53 ng/mg) and vinyl pyridine latex (16.5 ng/mg) (JACA Corp., 1987)."

> "Detailed industrial hygiene surveys were conducted in 1984-87 in a US rubber tyre plant and a US industrial hose plant where styrene-butadiene rubber, polybutadiene and acrylonitrile-butadiene rubber were processed. No 1,3-butadiene was detected in any of a total of 124 personal full-shift samples from workers in the following job categories, which were identified as involving potential exposure to 1,3-butadiene: Banbury operators, mill operators, extruder operators, curing operators, conveyer operators, calendering operators, wire winders, tube machine operators, tyre builders and tyre repair and buffer workers (Fajen *et al*, 1990)."

> "Measurements taken in 1978 and 1979 in personal 8-h samples in companies where acrylonitrile-butadiene-styrene moulding operations were conducted showed levels of <0.05-1.9 mg/m^3 (Burroughs, 1979; Belanger & Elesh, 1980; Ruhe & Jannerfeldt, 1980). In a polybutadiene rubber warehouse, levels of 0.003 ppm were found in area samples; area and personal samples taken in tyre plants conained 0.007-0.05 ppm (Rubber Manufacturers' Association, 1984). 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-h sample) (Ropert, 1976)."

5.2 Biological Monitoring

No methods for biomonitoring of human exposure have been established and data on levels in humant tissues are not available. Methods are currently being developed under the EC-STEP programme and at the US Chemical Industry Institute of Toxicology (CIIT) (section 7.1.5 and 9.3).

5.3.3 Environmental Levels

5.3.1 Ambient Air

From a literature survey, 1,3-BD concentrations of 1-5 ng/m^3 were reported in municipal surroundings and 66 µg/m^3 in an industrial area with 1,3-BD producing industry (year and location of measurements not specified) (De Jong *et al*, 1983 as quoted in DECOS, 1990). In 1980, low levels of 1,3-BD (≤ 1 ppb) were found in urban and suburban areas of the Netherlands (Guicherit and Schulting, 1985).

No other European data are readily available.

1,3-BD has been detected in urban air in the USA (Table X and XI). A compilation of air monitoring data for the USA (1970-1987) showed that median concentrations of 1,3-BD are 0.29 ppb in urban areas (196 data points), 0.32 ppb in suburaban areas (385 data points) and 0.10 ppb (only 2 data points) in rural areas (Shah and Heyerdahl, 1988 as quoted in ATSDR, 1991). In remote areas in the USA, 1,3-BD was measured in 1981-82 at concentrations of (number of samples, mean, range): 3, 0.74, ND-0.22 ppb; 3,0.37, ND-0.15 ppb; 3,0.19, ND-0.45 ppb (Seila *et al*, 1984 as quoted in ATSDR, 1991).

TABLE X

Levels of 1,3-BD in Urban Air
(adapted from IARC, 1992)

Location	Year of sampling	Concentration (ppb)	Reference*
Urban air	Unknown	1-10	Neligan, 1962; Cote and Bayard, 1990
Tulsa, OK	1978	5.9-24.4**	Arnts and Meeks, 1981
Houston, TX	1973-74	0-19	Siddiqi and Worley, 1977
Denver, CO	Unknown	2	Hunt *et al*, 1984
Texas	Unknown	<0.4-12.5	Hunt *et al*, 1984
Los Angeles/ Riverside, CA	Unknown	0.09	Parsons and Wilkins, 1976

* Quoted in IARC, 1992
** Combined with 2-butene

TABLE XI

Environmental Levels
(adapted from ATSDR, 1991)

Location	Year of sampling	No of samples	Concentration (ppb)		Reference**
			Mean	Range	
Houston, TX	1973 1974 1974	9 7 4	33.4[a] 27.2 3.0	ND-150[a] 8.0-57 ND-8.4	Lonneman *et al*, 1979
Los Angeles, CA	1968	-	12.4[a]	-	Kopczynski *et al*, 1972
Riverside, CA	1965-66	8	-	ND-2.0	Stephens and Burleston, 1967
Los Angeles, CA	1960	16	3.1	ND-9	Neligan, 1962
Boone, NC - downtown - outskirts	1981-82	3 3 3	0.11 4.2 0.15	ND-0.34[a] 0.34-5.0 0.11-0.22	Seila *et al*, 1984

** Quoted in ATSDR, 1991
[a] Data reported in ppbC (parts per billion carbon).
ND below detection limits

Low levels of 1,3-BD (0.5 to 10 ppb) were detected in ambient air at urban locations in the USA; however, levels as high as 2 ppm were detected in community air at the perimeter of the industrial complex in Port Neches (TX) where 1,3-BD and styrene-butadiene rubber are produced (Durchin, 1990).

In the vicinity of a petrochemical complex in Allendale (TX), 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, 1991).

5.3.2 Indoor Air

1,3-BD concentrations in the indoor air of a tavern were 4.98 ppb and 8.60 ppb (2 studies), probably from cigarette smoke when compared to the concentration of 0.45 ppb 1,3-BD in the outdoor air (Lofroth *et al*, 1989 as quoted in ATSDR, 1991). The concentration of 1,3-BD in a public building in California in 1965 was 9.0 ppb; the source was not specified (Stephens and Burleson, 1967 as quoted in ATSDR, 1991).

5.3.3 Soil

No data are available.

5.3.4 Water

In a survey of 14 heavily industrialised river basins in the USA in 1975-76, 2 ppb 1,3-BD was found at one out of 204 sites. Waste water from synthetic rubber manufacturers did not contain 1,3-BD (Ewing *et al*, 1977 as quoted in ATSDR, 1991 and HSDB, 1992).

5.3.5 Levels in Food and Drinking Water

Olive oil bottled in 1,3-BD rubber-modified acrylonitrilic bottles contained 8-9 µg/kg (6 samples; 3 of 3 brands), the bottles themselves containing residues as high as 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) nor in yoghurt packaged in polystyrene with 1,3-BD rubber-modified polystyrene lids (2 samples); the detection limit was 1 µg/kg. Chewing gum based on 1,3-BD rubber did not contain residues of the monomer (McNeal and Breder, 1987 as quoted in ATSDR, 1991 and HSDB, 1992).

Plastic tubs containing margarine (5 major brands in the UK) contained <5-310 µg/kg 1,3-BD, but the monomer was not found in the margarine samples themselves (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 these containers (detection limit 1 μg/kg) (Startin and Gilbert, 1984 as quoted in IARC, 1992 and ATSDR, 1991).

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, 1991).

5.3.6 Hobbies and Lifestyle

1,3-BD levels in smoky (from cigarette smoking) indoor environments were typically 10-20 mg/m^3 (Löfroth *et al*, 1989 as quoted in IARC, 1992). Other sources are mentioned in section 3.3.

6. MEASUREMENT TECHNIQUES AND ANALYTICAL METHODS

6.1 At the Workplace

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

TABLE XII

Methods for the analysis of 1,3-BD in air
(adapted from IARC, 1992)

Sample preparation	Assay**	Limit of detection	Reference
Collect on solid sorbent tube; desorb with dichloromethane; chill in ice	GC/FID	0.044 mg/m^3	Eller, 1987*
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]*
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 $\mu\ell/\ell$)	Locke *et al*, 1987*
Collect air sample; assay directly	FT-IR	5 ppm (10 mg/m^3)	Harman, 1987*
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]

* As quoted in IARC, 1992

** FT-IR, Fourier transform-infrared absorption spectroscopy; GC/FID, gas chromatography/flame-ionisation detection

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 gas chromatograph (GC) equipped with a suitable detector or combination of GC and mass spectrometry (MS) (ATSDR, 1991).

The determination of 1,3-BD in personal air can be obtained using the procedure outlined in NIOSH Method 1024 (NIOSH, 1987), which has been described by ATSDR (1991) as follows.

> "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 flame ionization detector (FID) separates 1,3-BD from any interfering compounds that may be present. The choice of chromatography column for this determination is not crucial, as long as it cleanly separates 1,3-BD from other compounds. The estimated detection limit of this method is 0.02 $\mu g/m\ell$, 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, quantisation 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)."

The methods used by the Swedish National Occupational Board of Occupational Safety and Health have been described by Lundberg (1986) as follows.

> "A gas-chromatographic method has been evaluated for air concentrations of butadiene in the range 1,065 to 4,590 mg/m^3 [474 to 2,043 ppm]. The butadiene is adsorbed on carbon in a tube through which the air is pumped, desorbed with carbon disulfide, and the desorbate analyzed with gas chromatography (NIOSH, 1977; Swedish National Board of

Occupational Safety and Health, 1979). 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)."

"Infrared spectrophotometry can be used for continuous monitoring of 1,3-butadiene in air. Disturbance from other sources can usually be minimized by suitable choice of wavelength."

A method for the monitoring of 1,3-BD in air has been developed at the Finnish Institute of Occupational Health. This method involves sampling by means of carbon tubes and analysis by GC with flame ionisation detection (FID). The detection limit is 0.8 µg/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 µg/m^3 with approximately 90% recovery, using GC with ECD (electron capture device) (Ahlberg *et al*, 1991).

The following method is recommended by CONCAWE (modified 8/86). This method 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 onto a 50 m BP-1 methylsilicone (chemically bonded) capillary column held at -35°C. Separation of 1,3-BD from other C4 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 (10-50mℓ/min) onto a Perkin Elmer ATD 50 sorbent tube packed with 900 mg of Molecular Sieve 13X. The tube is thermally desorbed onto a cold trap held at -30°C. The trapped components are then re-injected by ballistic heating onto 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 Monitoring

The Dutch Expert Committee for Occupational Standards has reviewed methods for environmental 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%. Butadiene is analyzed gaschromatographically, equipped with 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-butadiene (NIOSH, 1987). Checkoway and Williams (1982) were able to detect with this method concentrations as low as 0.03 ppm (0.066 mg/m^3) of BD."

> "Stephens and Burleson (1967) 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 µg/m^3). A long-term (8 h) indicator tube is commercially available. It is specific for butadiene and the detection range is 0.067-5.836 mg (Gentry and Walsh, 1987)."

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).

6.3 Biological Tissues

A standardised method for assaying 1,3-BD or its metabolites in biological tissues is not available. Methods are currently being developed under the EC-STEP programme and at the US Chemical Industry Institute of Toxicology (CIIT) (section 7.1.5 and 9.3).

7. TOXICOLOGY

7.1 Toxicokinetics

The relevant route for exposure to 1,3-BD is inhalation. Accordingly, studies on toxicokinetics after oral or dermal exposure of experimental animals have not been conducted. Human toxicokinetic data are not available, with the exception of some *in vitro* data obtained using fractions of liver and lung.

7.1.1 Uptake

The distribution 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 obtained uptake data 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 from the gas phase was 10,280 mℓ/h for the mouse and 5,750 mℓ/h for the rat. The data show, in principle, that 1,3-BD is metabolised by mice at about twice the rate of rats.

Rats and mice were exposed to ^{14}C-labelled 1,3-BD for 6 h in a nose-only device. Concentrations were 0.08 ppm to 1,000 ppm for mice or 0.08 ppm to 7,100 ppm for rats. The amount of ^{14}C retained at 6 h ranged from 1.5% (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 concentration-related decrease in the percentage of inhaled 1,3-BD retained with increasing exposure concentration for both rats and mice. When the total amount of ^{14}C retained at 6 h 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 nose-only inhalation for 2 h to concentrations ranging from 10 to 7,760 ppm ^{14}C-labelled 1,3-BD (Sun *et al*, 1989a; Dahl *et al*, 1991). The uptake of 1,3-BD in the monkey was calculated from the total 1,3-BD metabolites formed and excreted within the 96 h period after the exposure. Residual ^{14}C retained in the monkeys at the end of the 96 h post-exposure 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-h post-exposure collection period. The uptake rates calculated by this procedure were 0.13, 0.07 and 0.05 nmol/(min x kg 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 kg 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 h to ^{14}C-labelled 1,3-BD. The exposure concentrations were 670 ppm for rats and 65 ppm for mice and were selected because previous studies (Bond *et al*, 1986) had demonstrated that these concentrations result in similar amounts of 1,3-BD and its metabolites being retained on a mg/kg body weight basis. 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 were no apparent differences between the amount of ^{14}C derived from 1,3-BD deposited in the tissue of rats (exposed to 670 ppm) and mice (exposed to 65 ppm), but tissues of mice attained significantly greater concentrations of ^{14}C than rats when expressed as the amount/µmol of 1,3-BD inhaled.

Data are currently being generated for primates (section 9.1).

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. Phenobarbital pretreatment of the rats induced 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 mono-oxygenases in the metabolism of 1,3-BD.

These results were corroborated by Bolt *et al* (1983) who found also 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 rats (Sprague-Dawley), mice (NMRI and B6C3F$_1$), 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 mono-oxygenase activity. The authors also investigated homogenates from lung tissue. Only tissues from mice and rats produced measurable epoxide concentrations.

Pretreatment of male Sprague-Dawley rats or male B6C3F$_1$ mice with 1,3-BD (740 and 7,600 ppm, nose only, 6 h per day, 5 d) 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, benzo(a)pyrene induced mice, and of humans. In rat microsomes, 29% R- and 71% S-

enantiomer were formed, a ratio that was not affected by phenobarbital pretreatment. In mouse microsomes, 46% R- and 54% S-enantiomer were formed. Benzo(a)pyrene pretreatment did not affect this ratio, but phenobarbital pretreatment changed the ration 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 significant differences in the ratio of optical isomers formed between species.

Using mouse liver microsomes (male $B6C3F_1$) Elfarra *et al* (1991) confirmed the cytochrome P-450-mediated formation of EB as the primary metabolite 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.

The conjugation of EB to 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 GSH S-transferase.

The metabolism of EB was also investigated by Kreuzer *et al* (1991) in liver fractions from mice (male NMRI), rats (male Sprague-Dawley), and humans (one sample). In microsomes, only hydrolysis of EB was observed, NADPH-dependent metabolism of the epoxide with subsequent formation of DEB was not detected. In the concentration range examined, metabolism in liver microsomes of mice was strikingly lower than that of rats and humans. The apparent Km-values for the epoxide hydrolase activity were 1.5, 0.7 and 0.5 mmol 1,3-BD monoxide/ℓ incubate for mice, rats and humans respectively; the corresponding Vmax-values were 19, 17 and 14 nmol 1,3-BD monoxide/(mg protein x min) for mice, rats and humans. Glutathione S-transferase catalysed conjugation of EB to GSH in cytosolic fractions, revealing first order kinetics in the measured range. The derived ratios Vmax/Km were 15, 11 and 8 [nmol 1,3-BD monoxide x ℓ/(mg protein x min x nmol of 1,3-BD monoxide)] for mice, rats, and humans respectively. On the basis of these *in vitro* results, the investigators attempted to estimate the relative importance of the epoxide hydrolase and glutathione conjugation for the *in vivo* metabolism of EB for the 3 species. Standardised to 1 kg body weight and for a 1,3-BD monoxide body burden in the liver of 0.5 mmol/ℓ tissue, the authors estimated that the total 1,3-BD monoxide metabolism in humans would be between 2 and 7 times less than in rats, whereas mice metabolise the epoxide 1.3 times faster than rats. The ratios between the epoxide hydrolase and the GSH transferase pathways were estimated to be 0.21, 0.38, and 0.6 for mouse, rat and humans, respectively.

Filser *et al* (1992) determined enzyme specific kinetics of 1,3-BD in liver microsomes from mice, rats and one human sample. From the *in vitro* data

they extrapolated maximum rates for the 1,3-BD metabolism to EB *in vivo*, resulting in 243, 157, and 99 μmol/h/kg body weight 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).

The species differences between mice, rats, and humans were also investigated by Csanády *et al* (1992a) using liver and lung fractions obtained from male Sprague-Dawley rats, male B6C3F1 mice, and humans (12 liver samples, 5 lung samples). Maximum rates for 1,3-BD oxidation to EB (Vmax) were highest for mouse liver microsomes (2.6 nmol/mg protein/min) compared to humans (1.2) and rats (0.6). The Vmax for 1,3-BD oxidation by mouse lung microsomes was similar to that of mouse liver but about ten-fold higher than the reaction in human or rat lung microsomes. The Km-values were 5.14, 2.0, and 3.75 μmol/ℓ for the reaction in human, mouse, and rat microsomes. The Vmax/Km 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-450IIE1 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). Only mouse liver microsomes displayed rates of EB oxidation to the 1,3-BD 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 mass spectrometric analysis. The Vmax for this reaction was 0.2 nmol/mg protein/min with a Vmax/Km ratio of 13. Human liver microsomes displayed the highest rate of EB hydrolysis. The Vmax for this reaction ranged from 9 to 58 nmol/mg protein/min and was at least 2-fold higher than the Vmax observed in mouse and rat liver microsomes. The median Km-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 Vmax/Km ratios of about 32, 3.6, and 9.5 for the reaction in human, mouse and rat microsomes. The kinetic constants for conjugation of the 1,3-BD monoepoxide with glutathione in hepatic cytosolic fractions were: Vmax 45 (human), 500 (mouse), 241 (rat) [(nmol/(mg protein x min)]; Km 10.4 (human), 35.3 (mouse), 13.8 (rat) mmol/ℓ. These results demonstrated that in general the Km's for the detoxification reactions were about 1,000-fold higher than the Km's 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/detoxication ratio revealed that mice have a significantly higher ratio of activation/deactivation (72) than rats (5.8) and humans (6.3). 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).

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, both under conditions of low and high exposure concentrations.

With the same methods the inhalation toxicokinetics of EB was 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 x kg vs. 13,400 mℓ/h x kg). 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 Vmax was 350 µmol/h/kg in mice and > 2,600 µmol/h/kg in rats. Thus, with increasing exposure concentration the metabolic capacity for EB became 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 h of exposure to 1,3-BD. From about 12 h onward mice showed signs of acute toxicity and hepatic non-protein sulphydryl content of the animals was virtually depleted. In rats using the same protocol the hepatic non-protein sulphydryl content showed no major depletion and no toxicity was observed (Kreiling *et al*, 1988). From their results the authors concluded that the EB metabolism in mice is predominantly via the GSH-transferase pathway when compared with the epoxide hydrolase pathway.

The distribution of ^{14}C in blood of male Sprague-Dawley rats and male B6C3F$_1$ mice was investigated after inhalation of 7, 70, and 1,000 ppm ^{14}C-labelled 1,3-BD for 6 h (Bond *et al*, 1986). Samples of blood were analysed by vacuum line-cryogenic distillation at 2, 4, and 6 h 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 nonvolatile 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 based only on co-distillation with authentic standards. In addition, the authors noted that significantly higher concentrations of $^{14}CO_2$ were found in the blood of the rats than that of the mice.

The effects of different exposure concentrations of 1,3-BD on the cellular non-protein sulphydryl (NPSH) 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 h (Deutschmann and Laib, 1989). A dose 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 starts at an exposure concentration of about 250 ppm. 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.

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 1,3-BD metabolism was increasingly inhibited by styrene via a competitive mechanism up to 90 ppm. Higher styrene concentrations resulted in a small additional inhibition only. 1,3-BD had no influence on the metabolism of styrene. The K_i-value of 0.23 µmol/ℓ tissue calculated for styrene differed remarkably from its apparent Michaelis-Menten constant of 40 µmol/ℓ tissue (Schwegler *et al*, 1990). This was interpreted as suggestive of at least two different cytochrome P450 dependent monooxygenases which metabolise 1,3-BD, with only one of them inhibited by styrene (Laib *et al*, 1992).

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 h (Sun *et al*, 1989a; Dahl *et al*, 1991). Exhaled air and excreta were collected up to 96 h after the end of the exposure. The exhaled air was led through -45, -95, -160, and -

195°C traps. These traps were calibrated with CO_2 (trapped at -195°C), 1,3-BD (-160°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 to either of the 2 lower concentrations were nonvolatile. This was not the case for the highest exposure concentration from which 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 nonvolatile radioactive metabolites in the blood of monkeys with that of rats and mice after a 2 h 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, the conclusion was made that for equivalent inhalation exposures the concentrations of total 1,3-BD derived metabolites in the blood were 5 or 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 at this time point steady state conditions were met. (The Task Force is aware of inconsistencies reported by Dahl et al, 1991; an erratum is to be published in Toxicology and Applied Pharmacology).

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

Indirect evidence for the *in vivo* formation of DEB can be derived from DNA adducts 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). 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 tentatively 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-(2-hydroxy-3-butene-1-yl)guanine as one of the expected reaction products of EB were detected in mouse liver DNA hydrolysates but not in rat liver DNA hydrolysates.

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, cell nuclei of liver and lung tissues 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 bifunctionally-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 to 2,000 ppm 1,3-BD for 8 h/d for 7 d. The authors used cesium trifluoroacetate density-gradient centrifugation for the detection and quantification and were able to demonstrate that DNA-DNA crosslinks were formed after *in vitro* incubation of DNA with DEB.

On the basis of the kinetic data accumulated by Filser and his coworkers over the last decade, Johanson and Filser (1992) developed a 12-compartment PB-PK model for 1,3-BD, EB, and glutathione. The model describes the uptake, distribution and elimination of 1,3-BD together with the formation, distribution and elimination of EB via the 3 possible pathways. In order to describe GSH conjugation, a compartment for production and non-epoxybutene dependent elimination of GSH was included. The 1,3-BD and EB compartments were linked via a common intrahepatic compartment describing also 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 stated that the predicted concentration-time curves agreed well with the experimental data. Their model suggests 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. This difference increases to 3-fold at exposure concentrations higher than 1,000 ppm.

Further refinement of this PB-PK model is being undertaken at Filser's laboratory. In addition, two other models are being developed in the USA at the Chemical Industry Institute of Toxicology (CIIT) to include human *in vitro* data (Csanády *et al*, 1992b; Bond *et al*, 1992) and at the Inhalation Toxicology Research Institute (AZ) (Shyr *et al*, 1992) (section 9.1).

7.1.4 Excretion

In male Sprague-Dawley rats and male B6C3F$_1$ mice, urine and exhaled air were the major routes of excretion of ^{14}C derived from exposure to 0.08 to 7,100 ppm ^{14}C-labelled 1,3-BD, with a concomitant increase in exhalation of $^{14}CO_2$.

Male Sprague-Dawley rats and B6C3F$_1$ mice were exposed nose only for 3.4 h to mean concentrations of 670 and 65 ppm ^{14}C-labelled 1,3-BD (Bond *et al*, 1987). For both rats and mice, elimination of ^{14}C from blood and tissues was rapid, with 77% to 99% of the initial tissue burden being eliminated with half lives of 2 to 10 h, 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 about 1.5 times higher than for male Sprague-Dawley rats (7,300 mℓ/h vs. 4,500 mℓ/h). The exhalation rate constants were similar for both species.

The excretion of ^{14}C in monkeys exposed to 10.1 to 7,760 ppm ^{14}C-labelled 1,3-BD was investigated by Dahl *et al* (1991). At 10 ppm, slightly more of the inhaled ^{14}C was exhaled as CO_2 than was excreted in the 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 CO_2. Other unidentified volatile metabolites were also exhaled, these latter metabolites being a major route of excretion at 7,760 ppm. Urinary excretion could be described by a single negative exponential with a half life of 9.4 h. The other routes of excretion had more complex patterns. Urine was the largest pathway of excretion for the exposures at 10 and 310 ppm. (The Task Force is aware of inconsistencies reported by Dahl *et al*, 1991; an erratum is to be published in Toxicology and Applied Pharmacology).

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 the exposure, the urine was collected for 24 h (rats, mice, hamster) or 96 h (monkeys). 1,2-Dihydroxy-4-(N-acetyl-cysteinyl) butane (metabolite I) and 1-hydroxy-2-(N-acetylcysteinyl)-3-butene (metabolite II) were identified by GC-MS methods. 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 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.

7.1.5 Biological Monitoring

No validated methods for biomonitoring human exposure to 1,3-BD have been established, but method development using experimental animals has been

performed to investigate the formation of blood haemoglobin (Hb) adducts after 1,3-BD exposure as a marker for previous exposures.

Sun *et al* (1989b) treated male Sprague-Dawley rats and male B6C3F$_1$ mice with ^{14}C-labelled 1,3-BD in corn oil (i.p. injection). 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 per kg body weight for mice and rats. Hb adducts also accumulated linearly after repeated daily administration of 100 µmol ^{14}C-labelled 1,3-BD per kg body weight 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/kg body weight)], 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 the route of 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 to 0, 250, 500, and 1,000 ppm 1,3-BD 5 d/wk over 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 needs improvement. In addition, the authors suggested developing methods for *in vivo* dosimetry of DEB (Osterman-Golkar *et al*, 1991).

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

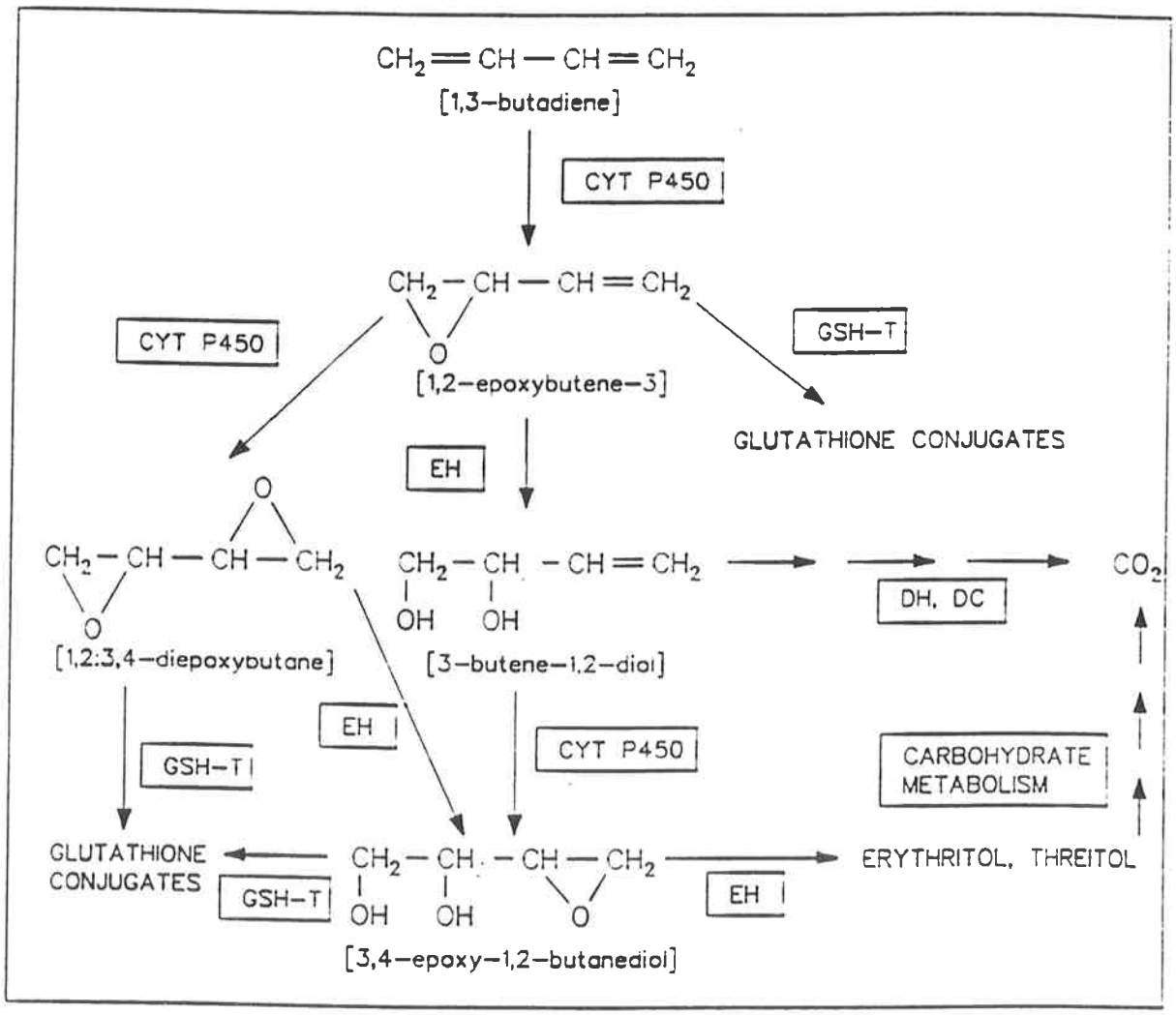
Urine samples from workers exposed to 1,3-BD were analysed for mercapturic acids of EB, but the method lacked the sensitivity required for current 1,3-BD-exposure levels (Sorsa *et al*, 1991; Arbetsmiljöfonden, 1991). The same group has been exploring other biomonitoring methods, including GC measurement of 3-butene-1,2-diol derivates in urine, and cytogenetic parameters such as chromosomal aberrations, SCE and micronuclei. These methods have not yet been validated (Arbetsmiljöfonden, 1991) (cf. section 7.2.4.3). The approaches being developed by this group are part of the EC-STEP programme (section 9.3).

7.1.6 Summary and Evaluation

The metabolic elimination of 1,3-BD is linearly related to the ambient exposure concentration up to about 1,000 ppm in rats and mice, 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 metabolism shown in Figure I. 1,3-BD is metabolised by cytochrome P-450 dependent mono-oxygenases (presumably 2 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 mono-oxygenases to yield DEB and (iii) conjugation to GSH catalysed by GSH S-transferases. DEB either may be conjugated to GSH or may be 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 subjected to furth- hydrolysis yielding erythritol and threitol, or may be conjugated to GSH. CO_2 may be formed by action of dehydrogenases and decarboxylases on 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.

FIGURE I

Metabolism of 1,3-BD
(adapted from Malvoisin and Roberfroid, 1982)



CYT P450	cytochrome P450-dependent monooxygenases
DH, DC	dehydrogenases, decarboxylases
EH	epoxide hydrolases
GSH-T	GSH-S-transferase

According to the *in vitro* and *in vivo* data, the biotransformation appears to be qualitatively similar across species, 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. Although it has not been clarified which metabolite is the ultimate carcinogenic metabolite, attention has been focused on the primary metabolite formed, 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 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. Even greater species differences may exist with regard to the epoxides formed by EB metabolism. In terms of assessing the 'biologically effective dose', the area under the concentration-time curve for EB appears to be superior to the measurement of external exposure concentration.

7.2 Toxicodynamics

This section describes the toxicodynamics of 1,3-BD in experimental animals and its effects on *in vitro* systems.

7.2.1 Acute Toxicity

7.2.1.1 Inhalation

A 2-h LC_{50} was determined for the mouse at 270 mg/ℓ or 122,000 ppm; a 4-h LC_{50} in rats was 285 mg/ℓ or 129,000 ppm (Shugaev, 1969).

Exposure of rabbits to 250,000 ppm (25%) 1,3-BD for 2 min induced light anaesthesia, while exposure for 8 to 10 min induced deep anaesthesia. Death due to respiratory paralysis occurred after 25 to 35 min exposure to this high concentration of 1,3-BD (Carpenter *et al*, 1944).

7.2.1.2 Oral

An LD_{50} for the rat is 5,480 mg/kg; and for the mouse 3,210 mg/kg (Ripp, 1968).

7.2.1.3 Dermal

No data are available.

7.2.2 Irritation, Sensitisation and Immunotoxicity

7.2.2.1 Skin Irritation

No data are available.

7.2.2.2 Eye Irritation

No eye irritation studies are available.

No eye irritation was reported in chronic bioassay studies in rats and mice exposed by inhalation to 1,250 ppm and 8,000 ppm 1,3-BD respectively (NTP, 1984; Owen *et al*, 1987).

7.2.2.3 Sensitisation

No data are available.

7.2.2.4 Immunotoxicity

B6C3F$_1$ mice were exposed by inhalation to 0 or 1,250 ppm 1,3-BD (6 h/day, 5 days/week) for 6 or 12 weeks. Immune function assays were selected to evaluate specific humoral and cell-mediated immunity and 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 comparable between 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.

7.2.3 Subchronic 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/day, 6 days/week) for 8 months. Reduced body weight gain was noted in rats and guinea pigs at 6,700 ppm. There were no effects reported among animals exposed to 600 or 2,300 ppm 1,3-BD.

In a Russian study, 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 immunologic 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, rats exhibited haemodynamic changes, increased permeability of the vessels, and alteration of the structure of the kidney and heart (Nikiforova *et al*, 1969 as quoted in Chemical Abstracts). This finding could not be confirmed in later studies with doses as high as 8,000 ppm (Owen *et al*, 1987).

Groups of 40 male and female Sprague-Dawley rats were exposed to 0, 1,000, 2,000, 4,000 or 8,000 ppm 6 h/day, 5 days/week 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. Bone-marrow was not examined (Crouch *et al*, 1979).

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

Groups of 10 male and female $B6C3F_1$ mice were exposed to 0, 625, 1,250, 2,500, 5,000 or 8,000 ppm 1,3-BD (6 h/day, 5 days/week) for 14 weeks. The following numbers died or were killed when moribund: 6 males and 1 female exposed to 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 histopathologic effects were observed (NTP, 1984).

$B6C3F_1$ or NIH-Swiss mice were exposed to 0 or 1,250 ppm 1,3-BD (6 h/day, 5 days/week) for 6 weeks. Treatment-related changes in both strains included decreased circulating erythrocytes, total haemoglobin and haematocrit, and increased mean corpuscular volume. The anaemia was not accompanied by a significant alteration in mean corpuscular haemoglobin concentration, nor an increase in circulating reticulocytes, nor an increase in circulating 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 to either 0 or 1,250 ppm 1,3-BD (6 h/day, 5 days/week) for either 6 or 30 weeks. Quantitative assessment of pluripotent stem cells was made using the spleen colony-forming assay (CFU-S). The differentiation of committed myeloid cells was made by enumerating the CFU of granulocyte/macrophage (CFU-GM) and 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 of exposure to 1,3-BD, although the colonies derived from treated animals were small 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 after 6 weeks of exposure. The number of both CFU-S and CFU-GM were significantly decreased after 30 weeks of 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).

7.2.3.1 Summary and Evaluation

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 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 reviewed by Rosenthal (1985), De Meester (1988), Brown (1990), the Dutch Expert Committee on Occupational Standards (DECOS, 1990) and IARC (1992).

In general 1,3-BD is metabolised to reactive epoxide intermediates. It is converted to EB, and further oxidised to DEB or hydrolysed by epoxidehydrolase to 3-butene-1,2-diol. The latter product is then metabolised to 3,4-epoxyde-1,2-butanediol by oxidation (section 7.1.3).

The metabolites EB and DEB react with DNA to give alkylated products (reaction of EB and DNA) and interstrand crosslinks (section 7.1.3). This type of adduct may eventually lead to damage at the gene and/or chromosomal level.

7.2.4.1 *In Vitro* (Table XIII)

In *Salmonella* 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 mutation frequency in this particular strain when uninduced rat liver S9 fraction was used. 1,3-BD was mutagenic to TA1535, both with induced and uninduced rodent S9 but not mutagenic when uninduced 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 XIII

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	McGregor *et al*, 1991

7.2.4.2 *In Vivo* (Table XIV)

When Wistar rats and B6C3F$_1$ mice were exposed in a closed system to ^{14}C-BD, radioactivity was recovered in both species from hepatic nucleoproteins and DNA (Kreiling *et al*, 1986). Defined alkylating products could only be isolated from livers of mice (Jelitto *et al*, 1989). In the same study the occurrence of protein-DNA and DNA-DNA cross-links (probably a biological effect of the bifunctional alkylating metabolite DEB) was shown in alkaline elution profiles from the livers of mice at dose levels of 250 ppm and higher. No such crosslinks were detected in the rat (Jelitto *et al*, 1989). However, Ristau *et al* (1990) did not detect DNA-DNA crosslinks in livers of B6C3F$_1$ mice or Sprague-Dawley rats exposed to 2,000 ppm 1,3-BD for 7 days, 8 h/day, but,

interstrand crosslinks were formed in purified liver DNA after incubation with DEB. 1,3-BD did not induce unscheduled DNA synthesis in the rat or mouse after exposure to 10,000 ppm (Arce *et al*, 1990). However, 1,3-BD increased sister chromatid exchanges (SCE) and chromosomal aberrations in mouse bone marrow (Cunningham *et al*, 1986; 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; Tice *et al*, 1987; Jauhar *et al*, 1988; Victorin *et al*, 1990). These effects could not be confirmed in the rat (Cunningham *et al*, 1986). 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). Exposure of male mice up to 5000 ppm (6 h/d on 5 consecutive days) of 1,3-BD had no effect on their ability to mate and impregnate females, and produce live fetuses (dominant lethal study; Hackett *et al*, 1988a). A transgenic mouse (lac Z target gene, Mutamouse®) was used in a pilot study to determine the induction of 1,3-BD mutations in mouse tissues. The mutant frequency in bone-marrow and liver samples did not exhibit a significant increase above background while in lung there was a 2-fold increase (Recio *et al*, 1992). Micronucleus induction was not found in monkeys exposed to concentrations of 1,3-BD up to 8,000 ppm for 2 hours, nor was there an increase in frequency of SCE (Sun *et al*, 1988). Negative results were obtained when 1,3-BD was tested on *Drosophila* for somatic mutation and recombination (SMART) (Victorin *et al*, 1990).

7.2.4.3 Humans (Table XIV)

In a study of workers employed at a Finnish facility which produces 1,3-BD, cytogenetic analyses revealed no increase in sister chromatid exchanges, chromosomal aberrations or micronuclei in peripheral blood. The ambient air concentrations of 1,3-BD were generally below 1 ppm and the workers used protective clothing and respirators (Ahlberg *et al*, 1991; Sorsa *et al*, 1991).

TABLE XIV

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
DNA-DNA crosslinks		
liver	+ve	Jelitto *et al*, 1989; Vangala *et al*, 1987
	-ve	Ristau *et al*, 1990
lung	+ve	Vangala and Jelitto, 1989
Unscheduled DNA synthesis (UDS)	-ve	Arce *et al*, 1990; Vincent *et al*, 1986
Sister chromatid exchanges (SCE) (bone marrow)	+ve	Cunningham *et al*, 1986; Tice *et al*, 1987
Micronucleus (peripheral blood)	+ve	Tice *et al*, 1987; Jauhar *et al*, 1988; MacGregor *et al*, 1990; Wehr *et al*, 1987
Micronucleus (bone marrow)	+ve	Cunningham *et al*, 1986; Victorin *et al*, 1990; Exxon, 1990
Dominant lethal	-ve	Hackett *et al*, 1988a
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	
lung	+ve	
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	+ve	Vangala *et al*, 1987
DNA-alkylation (liver)	-ve	Jelitto *et al*, 1989; Kreiling, 1989
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
Syrian hamster		
Micronucleus	±	Exxon, 1990
Primate		
Micronucleus	-ve	Sun *et al*, 1989a
SCE	-ve	Sun *et al*, 1989a
Drosophila		
Somatic mutation and recombination test (SMART)	-ve	Victorin *et al*, 1990
Human		
SCE's (lymphocytes)	-ve	Ahlberg *et al*, 1991; Sorsa *et al*, 1991
Micronucleus (lymphocytes)	-ve	Ahlberg *et al*, 1991; Sorsa *et al*, 1991
Chromosomal aberrations (lymphocytes)	-ve	Ahlberg *et al*, 1991; Sorsa *et al*, 1991

±, equivocal

7.2.4.4 Metabolites (Tables XV to XVIII)

Two major metabolites, EB and DEB, are formed *in vitro* and *in vivo* and both bind covalently to DNA (Leuratti and Marafante, 1992a,b). EB was reported to be genotoxic in *Salmonella*, *E. coli* and *Klebsiella* (De Meester *et al*, 1978; Hemminki *et al*, 1980; Voogd *et al*, 1981; Gervasi *et al*, 1985). It did not induce unscheduled DNA synthesis in rat and mouse hepatocytes (Arce *et al*, 1990). Chromosome damage was induced by EB *in vitro* (without metabolic activation) and *in vivo* (Sharief *et al*, 1986; Sasiadek *et al*, 1991a,b).

DEB produced increases in mutations, gene conversions, mitotic recombination, SCE's and chromosomal aberrations in *in vitro* short term tests in the absence of an S9 fraction (references in Table XVII). However, DEB did not induce unscheduled DNA synthesis in rat or mouse hepatocytes (Arce *et al*, 1990). In a mouse host-mediated assay with *Salmonella* and *Saccharomyces*, reverse gene mutations were induced but no mitotic recombination occurred (Simmon *et al*, 1979). Chromosome aberrations and SCE's were increased in a dose-related way in DEB-treated mice and Chinese hamsters.

TABLE XV

Genotoxicity of EB *In Vitro*

Test system	Results without activation	Results 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
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

TABLE XVI

Genotoxicity of EB *In Vivo*

Test system	Results	Reference
Mouse		
SCE (bone marrow)	+ve	Sharief *et al*, 1986
Chromosomal aberrations (bone marrow)	+ve	Sharief *et al*, 1986

TABLE XVII

Genotoxicity of DEB *In Vitro*

Test system	Results without activation	Results with activation	Reference
Microbial			
Reverse gene mutation (*Salmonella typhimurum* 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 (*E. coli*)	+ve	±	Glover, 1956; Dunkel *et al*, 1984
DNA repair (*E. coli*)	±	±	Thielmann and Gersbach, 1978
Fluctuation test (*Klebsiella pneumoniae*)	+ve	Not tested	Voogd *et al*, 1981
	+ve	Not tested	
Yeast and fungus			
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
Reverse gene mutation (*Neurospora crassa*)	+ve	Not tested	Pope *et al*, 1984
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
Miscellaneous			
DNA-adducts (calf thymus DNA)	+ve	Not applicable	Leuratti and Marafante, 1992a,b

±, equivocal

TABLE XVIII

Genotoxicity of DEB *In Vivo*

Test system	Results	Reference
Drosophila		
SMART	+ve	Graf *et al*, 1983
SLRL	+ve	Bird and Fahmy, 1953; Fahmy and Fahmy, 1970; Sankaranarayanan *et al*, 1989
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; Walk *et al*, 1987
SCE (alveolar macrophages)	+ve	Conner *et al*, 1983
SCE (regenerating liver cells)	+ve	Conner *et al*, 1983
Chromosomal aberrations (bone marrow)	+ve	Valk *et al*, 1987
Chinese hamster		
SCE (bone marrow)	+ve	Valk *et al*, 1987
Chromosomal aberrations (bone marrow)	+ve	Valk *et al*, 1987

7.2.4.5 Summary and Evaluation

1,3-BD itself is not genotoxic. However, its metabolites can interact with DNA directly to form adducts and crosslinks which eventually may result in gene mutations and 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; the mouse is clearly more sensitive to 1,3-BD induced genetic alterations than other species.

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. These studies were undertaken by Hazleton Laboratories for

the International Institute of Synthetic Rubber Producers (IISRP) and by the US National Toxicology Program (NTP) to determine if there is a potential health hazard to exposed humans.

7.2.5.1 Inhalation Exposure of Rats

In a 2-year study, 100 Sprague-Dawley rats/sex were exposed to 0, 1,000 or 8,000 ppm for 6 h/d, 5 d/wk (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 effects on haematology, blood chemistry, urine parameters and neuromuscular function that could be associated with treatment. Treatment did affect body weight gain (in the first 12 weeks of the study), certain organ weights and incidence of common and uncommon tumours. Tumours that occurred with significantly increased incidences in males were pancreatic exocrine neoplasms and Leydig cell tumours. In females, significantly increased incidences 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. When considered separately, the incidences of benign and malignant tumours were neither statistically significant nor dose-related. The high incidence of mammary tumours is consistent with the historical 90% incidence 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 (Table XX). The authors concluded 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 mediated through the endocrine system rather than by a direct effect through the production of reactive metabolites (Owen and Glaister, 1990).

TABLE XIX

Incidence of Selected Primary Tumours in Sprague-Dawley Rats[a,b]
(from Owen and Glaister, 1990)

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

a Exposed to 1,3-BD for 2 years
b Numbers of rats examined were 100/group/sex
* Statistically significant $P < 0.05$

7.2.5.2 Inhalation Exposure of Mice

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

In the first study (NTP I) groups of 50 male and female B6C3F$_1$ mice were exposed to 625 or 1,250 ppm 1,3-BD for 6 h/day, 5 days/week 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 neoplasia 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 metastasis 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 significant 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 XX). Malignant lymphomas were considered the major cause of early deaths. The high incidence of haemangiosarcoma of the heart was a particularly unusual finding since these endothelial cell neoplasms are uncommon in B6C3F$_1$ mice. These normally occur at a very low spontaneous rate and are rarely induced in long-term studies. The results of this study demonstrate that 1,3-BD under the treatment conditions of this assay is a potent multiple organ carcinogen in the B6C3F$_1$ mouse (NTP, 1984; Huff *et al*, 1985; Melnick *et al*, 1990a,b).

TABLE XX

Incidence of Primary Tumours in B6C3F$_1$ Mice[a]
(adapted from Melnick and Huff, 1992a)

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

a Exposed to 1,3-BD for 60-61 weeks
* Statistically significant $P < 0.05$

In the second study (NTP II) lower dose levels were selected for testing to better characterise exposure response relationships. Groups of 70-90 male and female B6C3F$_1$ mice were exposed to 0, 6.25, 20, 62.5, 200, 625 ppm of 1,3-BD 6 h/d, 5 d/wk up to 2 years. Additional studies in which exposure was stopped 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, incidences of lung neoplasms were significantly increased in all exposure groups, including malignant and benign tumours considered separately. Thus even at 6.25 ppm 1,3-BD was carcinogenic in mice (Table XXI). 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 this should be accounted for by adjusting tumour incidences for early mortality. Statistical analyses of adjusted incidences showed that there was no exposure level at which a carcinogenic response was not induced in the female mouse (Table XXI) (Melnick *et al*, 1990a,b; Melnick and Huff, 1992a).

TABLE XXI

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

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

* 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, groups of 50 male mice were exposed to 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 XXII). 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%. Obviously the concentration of 1,3-BD at concentrations of 200 ppm and above is a greater contributing factor to thymic lymphoma incidence than exposure duration.

TABLE XXII

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

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

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 be converted to genetic lesions. Theoretically, these lesions may be involved in the early stages of carcinogenicity induced by 1,3-BD. Attention had been focused mainly on the primary metabolite formed, EB (section 7.1.3.2). The difference in increased body burden of EB of mice compared to rats at low exposure concentrations appears to be in the range of 2 to 5 (Bond *et al*, 1986). This is supported by data on exhalation and blood concentration of this epoxide, as

well as by results of PB-PK modelling and other extrapolations of *in vitro* data to the *in vivo* situation (Johanson and Filser, 1992). Only a part of the large difference in carcinogenic potency of 1,3-BD between rats and mice can 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. Csánady *et al* (1992) estimated that the body burden of both epoxides (EB and DEB) in mice was 12.4 fold higher that in rats. In addition, the formation of the diepoxide (DEB) and its subsequent reaction with DNA may help to explain the species difference (DNA-crosslinks in mice, but not rats; higher formation rates in mouse microsomes than in rat microsomes).

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 2 epoxides. This renders the determination of the target dose for EB superior to the use of external exposure concentration for establishing the "biologically effective dose".

7.2.5.5 Summary and Evaluation

The carcinogenic effects of 1,3-BD were studied in Sprague-Dawley rats and B6C3F$_1$ mice. As in other toxicological studies with 1,3-BD, the greater susceptibility of mice compared with rats was also observed here. 1,3-BD is a potent carcinogen in mice, with tumours found 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 of the mouse. Dose-related increases in neoplasms included 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 is less pronounced, where 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 was benign. 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). The tumour pattern (endocrine and sex-hormone sensitive organs) in both sexes of the rat suggests a hormone-related mechanism is involved. Finally, the stop-exposure studies indicate that in the mouse the concentration of 1,3-BD has a greater impact on tumour incidence than the exposure duration.

7.2.6 The Role of the MuLV Retrovirus in 1,3-BD-induced Leukemogenesis

Irons *et al* (1987b) found that chronic exposure to 1,3-BD (1,250 ppm, 6 h/d, 5 d/wk for 3 to 21 weeks) markedly increased the quantity of the ecotropic (capable of infecting mouse cells) MuLV retrovirus recoverable from the bone marrow, thymus and spleen of the $B6C3F_1$ mouse. Expression of other endogenous retroviruses was not enhanced. Age-matched controls not exposed to 1,3-BD either yielded no ecotropic virus or very small numbers of virus-producing cells.

Irons *et al* (1989) subsequently conducted a comparative study in the $B6C3F_1$ hybrid mouse and the NIH-Swiss mouse. The latter strain only rarely expresses any type of endogenous retrovirus (Chattapadhyay, 1980; Jenkins *et al*, 1982).

$B6C3F_1$ mice and NIH-Swiss mice were chronically exposed to 1,3-BD (1,250 ppm, 6 h/d, 5 d/wk for 1 year). The incidence of thymic lymphomas in the $B6C3F_1$ mouse was 57% at the end of one year. These lymphomas were all of T-cell origin and exhibited elevated expression of the endogenous ecotropic retrovirus (eMuLV). Leukemogenesis was preceded by anaemia, bone marrow cytogenetic abnormalities and an increase in the amount of eMuLV recoverable from the bone marrow, thymus and spleen. In contrast, NIH-Swiss mice similarly exposed to 1,250 ppm 1,3-BD resulted in a 14% incidence of thymic lymphoma, with no increase in eMuLV. However, the same haematologic and cytogenetic abnormalities observed with the $B6C3F_1$ mouse also occurred with the NIH-Swiss mouse indicating that it is the eMuLV background that influences the susceptibility to 1,3-BD induced leukemogenesis in the mouse.

The above comparative data also indicate that the presence of the ecotropic retrovirus is not an absolute requirement for thymic lymphoma development following 1,3-BD exposure, since some increased incidence of thymic lymphoma was also observed in the NIH-Swiss mouse. However, the retrovirus could well account for the marked difference in the thymic lymphoma incidence between these strains and possibly between species. In addition, the bone marrow damage also appears to be a prerequisite for the enhanced murine leukaemia.

7.2.7 Reproductive Toxicity

A number of toxicological investigations, mainly conducted over the last 10 years, 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).

7.2.7.1 Fertility

Rats (12/sex/group), guinea pigs (6/sex/group), and rabbits (2/sex/group) were exposed 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. Toxic levels of 1,3-BD were given based on a reduction in body weight gain of the rats and guinea pigs. The authors reported a slight decrease in litter frequency in rats, but this response was not dose-dependent and mean litter size was not affected. The mean rat litter size in exposed groups was equal to or greater than controls. No reproductive parameters were affected in guinea pigs or rabbits. 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).

Groups of 20 male CD-1 mice were exposed to 0, 200, 1,000 or 5,000 ppm for 6 h/d on 5 consecutive days in a dominant lethal study. Following exposure, the males were mated with unexposed females (2 female mice were mated with each male mouse per week) for 8 consecutive weeks. The females were killed 12 days after the last day of cohabitation, and the uterine contents examined. Although the authors reported slight effects (i.e. an increase in the number of dead implants/total implants, female with $\geq$ 2 dead implants, number of dead implants/pregnancy) during the first 2 weeks post-exposure, evaluation of the data showed that these statistically significant observations were neither dose-dependent nor biologically important. Overall, the number of pregnant females, number of implantations per litter, number of live foetuses, number of dead implantations per total implantations, and the number of resorptions were unaffected by 1,3-BD exposure. From the data in this study, exposure of male mice to high levels of 1,3-BD have no effect on their ability to mate and impregnate females, and produce live foetuses (Hackett *et al*, 1988a).

Groups of 20 male B6C3F$_1$ mice were exposed to 0, 200, 1,000 or 5,000 ppm 1,3-BD for 6 h/d on 5 consecutive days. During the 5th post-exposure week the mice were killed, examined for gross lesions of the reproductive tract, and the sperm examined. No gross lesions were detected. The mean percentage of normal sperm heads per total examined was 98, 98, 97 and 96 for the 0, 200, 1,000 and 5,000 ppm groups, respectively. These values were 99.7, 98.8 and 97.9% of the control group value for the 200, 1,000 and 5,000 ppm group respectively (Hackett *et al*, 1988b). Although the decreases are statistically significant for the 1,000 and 5,000 ppm groups, these 1 to 2% reductions in normal sperm heads lack biological significance.

7.2.7.2 Other Effects on Reproductive Organs

Additional information about effects of 1,3-BD on reproductive organs was found in studies designed for other purposes.

Groups of 110 male and 110 female Sprague-Dawley rats were exposed to 0, 1,000 or 8,000 ppm 1,3-BD for 6 h/d, 5 d/wk for 2 years. Ovarian atrophy was not observed in females exposed to 8,000 ppm 1,3-BD (4.2% incidence vs. 4.3% in the controls). Likewise, testicular atrophy was not observed in males exposed to 8,000 ppm 1,3-BD (19.3% incidence vs. 31% in controls) (Owen, 1981a,b).

Groups of 50 male and 50 female $B6C3F_1$ mice were exposed to 0, 625 or 1,250 ppm 1,3-BD for 6 h/d, 5 d/wk for 61 weeks. The study was originally designed to run for 104 weeks but was terminated after 61 weeks because of high mortality. Ovarian atrophy and testicular atrophy were increased in the mice at both exposure levels (NTP, 1984).

Groups of 50 male and 50 female $B6C3F_1$ mice were exposed to 0, 6.25, 20, 62.5, 200 or 625 ppm 1,3-BD for 6 h/d, 5 d/wk for 104 weeks. Ovarian atrophy was noted in female mice at 65 weeks of exposure, and after completion of the reproductive life of this species at 6.25 ppm and 20 ppm (65 or more weeks of exposure). Testicular atrophy occurred after 65 weeks in the male mice at 625 ppm, with reduced testicular weights at 200 and 625 ppm 1,3-BD (Melnick and Huff, 1992a).

7.2.7.3 Developmental Toxicity

Three studies were performed in which the developmental toxicity of 1,3-BD was assessed. The results were negative for concentrations at which there was no maternal toxicity.

Female Sprague-Dawley rats were exposed to 0, 200, 1,000 or 8,000 ppm 1,3-BD during gestational days 6 through 15. Maternal body weight gain during the exposure period was significantly reduced at all exposure concentrations, with weight loss for 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 for 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 of ossification of the ribs (wavy ribs), delays in ossification of the thoracic centra and incomplete ossification of the sternum. There were no teratogenic effects that were statistically significant or outside the historical control range for this rat strain. These effects are consistent with growth retardation associated with maternal toxicity. The NOEL for maternal toxicity is 200 ppm and the NOEL for developmental effects is 1,000 ppm (Irvine, 1981, 1982).

Groups of 24 to 28 pregnant female Sprague-Dawley rats were exposed to 0, 40, 200 or 1,000 ppm 1,3-BD for 6 h/d on days 6 through 15 of gestation. Maternal body weight gain was significantly decreased by 1,000 ppm 1,3-BD.

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 200 ppm and the NOEL for developmental toxicity was greater than 1,000 ppm (Hackett *et al*, 1987a).

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 maternal body weight gains, reduced foetal and placental weights, and increased the number of supernumerary ribs. Exposure to 1,000 ppm 1,3-BD also caused retarded sternal ossification. There were no embryo-foetal deaths or malformations. The study authors also reported an effect on male foetal body-weight at 40 ppm, but the statistical analysis used to make this conclusion was inappropriate. The NOEL for both maternal and developmental (foetal) toxicity is 40 ppm (Hackett *et al*, 1987b). Thus, the foetal retardation is judged to be a consequence of maternal toxicity. There was no evidence of any changes that would be classified as teratogenic.

7.2.7.4 Summary and Evaluation

Studies specifically focusing on assessment of fertility did not show adverse effects in guinea pigs, rabbits and rats at exposure concentrations as high as 6,700 ppm for up to 8 months. 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. Overall, these studies show again the unique susceptibility of the mouse to 1,3-BD.

In bioassays (NTP 1984; Melnick and Huff, 1992a) structural abnormalities have been detected in the testes and ovaries of mice exposed to 1,3-BD concentrations as low as 6.25 ppm. Such effects were not observed in the bioassay with rats exposed to up to 8,000 ppm (Owen, 1981a,b). The effects in mice are not regarded as of any toxicological significance as they occurred after the normal reproductive period in the mouse.

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 occurs (Illing and Shillaker, 1984). Few of the studies, particularly those in Bulgaria or the USSR are substantiated by details 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 h 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 8000 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 considered that these changes cannot be interpreted as an effect on the bone marrow (IARC, 1992).

7.3.1.2 Oral

Ingestion is highly unlikely. Freeze burns are the probable outcome.

7.3.1.3 Dermal

Liquid splashes will chill the skin and may cause irritation.

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 h and 4,000 ppm 1,3-BD for 6 h 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. Few are substantiated by 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 three large retrospective cohort mortality studies, one of employees in monomer production (Downs *et al*, 1987; Devine, 1990), and two of employees engaged in the styrene-1,3-BD rubber industry (SBR) (Meinhardt *et al*, 1978, 1982; Matanoski and Schwartz, 1987; Matanoski *et al*, 1988, 1990).

None of these studies has shown any excess of mortality from all causes, all cancers, or any other broad category of disease, such as those of the cardiovascular or respiratory system.

The first of the studies (Meinhardt *et al*, 1978) was prompted by concerns surrounding a cluster of cases of leukaemia. This, in conjunction with the laboratory finding of leukaemia (T-cell lymphoma, section 7.2.4) in mice exposed to 1,3-BD, led to a detailed analysis of the incidence of mortality from haematopoietic and lymphatic cancer (HLC) in the above-mentioned three cohort studies. No statistically significant excess of cancers other than HLC has been reported in any of these studies.

The only other studies that could be considered to have a bearing on the human carcinogenicity of 1,3-BD are studies of the rubber industry, some of which report excesses of sub-categories of HLC (Andjelkovic *et al*, 1976, 1977; McMichael *et al*, 1976; Monson and Nakano, 1976; Monson and Fine, 1978). As none of them mentions exposure to 1,3-BD as a risk factor, they are unsuitable for risk assessment purposes.

Epidemiological investigations of 1,3-BD were first instigated by reports of a cluster of cases of leukaemia at Port Neches, USA. To study the cluster, the National Institute of Occupational Safety and Health (NIOSH) mounted a retrospective cohort mortality study at 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 Standardised 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. An non-significant excess of cases of HLC 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 there was no discernible pattern of increasing incidence of HLC with increasing duration of exposure. Indeed 2 of the 5 cases of leukaemia at plant A were diagnosed within 3 years of commencement of employment, as was the case in plant B. Exposure estimates were only available after 1976. These showed that the time weighted average exposure to 1,3-BD at plant B (mean 13.5 ppm, s.d. 29.9) was about 10 times that at plant A (mean 1.24 ppm, s.d. 1.20). This study has been updated, but not reported in full (Lemen *et al*, 1990). The results have not altered substantially.

The available exposure data suggest that the incidence of HLC is lower at the plant with higher exposure levels of 1,3-BD. The cases of HLC at plant A were all first employed at the plant prior to 1945 when the operating conditions were markedly different from those at the time the study was conducted. Considerations of latency and duration of exposure make it improbable that the cases of HLC at plant A were occupationally related (Acquavella, 1989; Cole *et al*, 1992).

The retrospective cohort mortality study of monomer workers (Downs *et al*, 1987) was based on the production facility that supplied raw material to the SBR facilities at Port Neches, and indeed some subjects are included in both cohorts. The study included all men employed for 6 months or more between 1943 and 1979. No exposure data were reported. Instead, qualitative exposure categories were allocated by consideration of job and duration. The 4 exposure categories were described as 'low' (not normally exposed to 1,3-BD), routine (exposed to 1,3-BD on a daily basis), 'non-routine' (intermittent exposure to 1,3-BD, with the possibility that peak exposure concentrations may be higher than among those with routine exposure) or 'unknown' (because employee promoted and previous job not known). The overall SMR was 80, based on 603 deaths in a cohort of 2,586, and that for all cancers was 84. The SMR for HLC was increased, but not significantly so. Based on 21 deaths, the SMR for HLC was quoted as either 169 or 138, depending on assumptions about the ethnic composition of the cohort. The SMR was lower when local comparison rates were used instead of national ones. A breakdown of the HLC cancers revealed an excess of lymphosarcoma and reticulosarcoma (8 observed versus 3.4 expected) that was common to all 4 exposure groups, and a smaller and more erratic excess for leukaemia (7 observed versus 4.5 expected) (Downs *et al*, 1987). When updated (Divine, 1990), it was reported that the recalculated lymphosarcoma and reticulosarcoma excess (9 observed versus 3.9 expected) was most apparent in those first employed prior to 1946 and for short duration, that is, less than 10 years. The leukaemia cases were mostly employees with 'routine' exposure for less than 5 years prior to 1946, while lymphopoietic cancer was not elevated in workers with long-term exposure.

The other study in the SBR industry involved employees at 7 facilities in the USA and 1 in Canada (Matanoski and Schwartz, 1978; 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 HLC 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. 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 HLC SMR was 97 based on 55 deaths. To investigate these 55 cases of HLC in more detail, the employees were divided into 4 occupational groups (Production, Utilities, Maintenance, and Other), and HLC was sub-divided into several narrower categories of disease. Increased mortality was reported for leukaemia in black production employees (3 observed versus 0.5 expected), but not in white production employees (4 observed versus 4.8 expected), and a category of tumours described as 'other lymphatic' was in excess in all groups. 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.

This cohort was further investigated in a nested case-control study (Santos-Burgoa, 1988; Santos-Burgoa *et al*, 1992). No dose data in the form of industrial hygiene monitoring were reported. Instead, all jobs, for all time periods, were assessed by 5 experts, and allocated scores for exposure to 1,3-BD, and to styrene, on a scale from 0 to 10. Analyses were then based on divisions of cases and controls into categories such as exposed and non-exposed, or semi-quantitative estimates based on the 0-10 scale, such as cumulative logarithmic exposure score. For each of the 59 cases of HLC 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 HLC, 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 other confounding 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. In contrast, the diagnostic group 'other lymphatic' cancer is

associated with styrene exposure and other factors beside 1,3-BD exposure. The interpretation of 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*, 1992). This apparent discrepancy may be due to bias in the selection of controls or exposure scores, or a low incidence of leukaemia in the control population (Ott, 1990). The relative risk for leukemia 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*, 1992). Indeed, some subdivisions show a relative risk of less then 1 for the higher exposed group. To resolve this discrepancy an updated cohort study is under way (Acquavella, 1992, section 9).

7.4.1 Summary and Evaluation

None of these studies has shown any excess of mortality from all causes, all cancers, or any other broad category of disease. In addition, these three cohort studies do not provide an aggregation of data on which a risk assessment can be calculated by a meta-analysis. Overall, they do not show evidence of a raised risk for all causes, all cancers or for the *a priori* hypothesis of HLC (Cole *et al*, 1992). In total they show 36 cases of leukaemia versus 34 cases expected, and 54 cases of HLC other than leukaemia versus 50 cases expected. Such excesses as are reported for the studies are for either sub-classifications of diagnosis, sub-groups of employees, or both. These excesses are seen by some to present a coherent picture (Melnick and Huff, 1992b), but equally they could conceivably be nothing more than the predictable outcome of multiple analyses.

Some authors recognised a qualitative association between 1,3-BD exposure and HLC (Matanoski *et al*, 1990; Landrigan, 1990), while others see no casual relationship (Acquavella, 1989, 1992; Cole *et al*, 1992). Regardless, these studies are inappropriate for quantitative risk assessment because of the absence of exposure data for the individual workers in the cohorts. This may be quantitatively resolved by the ongoing updated cohort study in which 1,3-BD exposure estimates will be reassessed and analysed (Acquavella, 1992). In the meantime, it is reasonable to assume that the exposure levels for the workers investigated in the cohort studies must have been higher than levels reported in the 1980's (section 5.1) due to less advanced control technologies (Mullins, 1990).

8. GROUPS AT EXTRA RISK

There is no convincing evidence that any particular group is at extra risk.

One of the epidemiological studies has indicated a raised risk for arteriosclerotic heart disease in all black workers and for the leukaemia in black production workers exposed to 1,3-BD (Matanoski *et al*, 1990). These excesses could be explained to some extent by the assumption that live workers of unknown race were white, whereas the race of all dead workers was known. Otherwise the significance of this observation would be easier to assess if there was knowledge of associated polymorphisms related to the toxicokinetics of 1,3-BD.

9. GAPS IN KNOWLEDGE AND ONGOING RESEARCH

It is evident from the previous discussion in chapter 7 that 1,3-BD induces different responses across the spectrum of species tested. 1,3-BD produces a potent carcinogenic response in the mouse at multiple sites (Melnick and Huff, 1992a), while in the rat, a weak response occurs at 1,3-BD concentrations nearly three orders of magnitude higher than in the mouse (Owen *et al*, 1987). In humans, the response is at present equivocal. Genotoxicity data parallels this species-dependent finding. Clear cytogenetic changes occur in the mouse (Tice *et al*, 1987); these are borderline in the hamster (Exxon, 1990), but absent in the rat (Arce, 1990) and primate (Sun *et al*, 1989a).

Such quantitative differences in response compromise any direct extrapolation between species. Existing disposition and metabolism data in the mouse (Kreiling *et al*, 1986; Bond *et al*, 1986), rat (Kreiling *et al*, 1986; Bond *et al*, 1986; Bolt *et al*, 1984) and primate (Dahl *et al*, 1991) indicate that these quantitative differences are at least in part due to the differences in activation and deactivation of two mutagenic metabolites of 1,3-BD; 1,3-BD monoxide and DEB.

A number of research projects detailed below have been initiated to provide explanations for the species differences. New data arising from these projects expected during 1992-94 will reduce the uncertainty factors used for the extrapolation to humans and improve upon existing risk assessments.

9.1 Toxicokinetics

Comparative *in vivo* toxicokinetic data in mouse, rat and primate for the validation of a PB-PK model are being developed by Henderson and co-workers at the Inhalation Toxicology Research Institute at Lovelace (AZ). This will extend the original work of this group (Bond *et al*, 1986, 1988; Dahl *et al*, 1990, 1991; Sabourin *et al*, 1992) by including repeated exposures at a range of 1,3-BD concentrations. In addition, the US Chemical Industry Institute of Toxicology (CIIT) is developing a PB-PK model (Bond *et al*, 1992; Csanády *et al*, 1992b).

9.2 Haematopoietic and Bone-Marrow Effects

In view of the increase in lymphoma seen in the mouse (Melnick and Huff, 1992a) and the controversial leukaemia findings in humans (Acquavella, 1989), studies are under way at the University of Colorado (Irons and coworkers) to characterise the metabolism and fate of 1,3-BD metabolites in bone marrow cells in the mouse and in human marrow and lymph cells. These studies

should show whether 1,3-BD elicits responses in human bone marrow cells which are likely to lead to particular types of leukaemia.

9.3 Genotoxicity

Highly specific and sensitive tests are being developed at the University of North Carolina (Swenberg and coworkers) for measuring the type and amount of 1,3-BD-DNA interactions in target and non-target tissues in rodents, and to measure specific mutations using mouse, rat, and human cells in culture. These studies will test the feasibility of detecting biologically relevant levels of 1,3-BD-DNA adducts and will characterise the DNA sequence changes in the living animal. The objective of this work is to provide information as to which metabolite(s) might be the key contributor(s) to DNA damage and to identify the specific DNA sequence alterations so that critical genes can be followed for mutations. At present, the causal agent is unknown although the mutagenic epoxide metabolites are implicated. Quantification of DNA adducts in target tissues will help to explain quantitative differences in species sensitivity.

This work ties in with ongoing work on biomarker research conducted at the Chemical Industry Institute of Toxicology as well as a study of the method development of biomonitoring of human exposure to styrene and 1,3-BD (Sorsa, 1992) which forms part of EC-STEP program.

9.4 Epidemiology

The mortality of over 17,000 workers employed in the synthetic rubber industry or in a 1,3-BD monomer producing facility has been examined in three large retrospective cohort studies (Meinhardt *et al*, 1982: Matanoski *et al*, 1990; Divine, 1990). Detailed analyses within individual cohorts and among employment subgroups indicate some discrepant findings (Ott, 1990). The interpretation of a case-control study nested within the largest of these cohort studies remains a controversial issue (Cole *et al*, 1992), but in any case the exposure information was categorised in such a way that a risk assessment is not possible.

An update of the largest of the cohorts (SBR workers) is currently under way (IISRP/University of Alabama, 1991) to monitor trends in lymphopoietic cancer rates and to detect if any elevated rates are seen for any of the long latency cancers. The cohort study update is exposure based and will assess exposures to 1,3-BD for all individuals in the cohort, and assess the magnitude of the possibly confounding exposures of styrene and benzene (Acquavella, 1992). The update will also include a careful categorisation of all HLC cases into distinct etiologic entities so that excesses of vague diagnostic groups, such as lymphopoietic cancer, are clarified (Acquavella, 1990). The study period is

1942-1991 involving a study population of more than 20,000 workers; process analysis, job analysis, and exposure estimation will be included. These have been weak or deficient aspects of the previous epidemiological studies. By 1994, the resulting data of the observed disease incidence associated with a particular exposure interval, together with the available toxicokinetic and genotoxic mechanistic work, should better define the human cancer risk from 1,3-BD exposure.

9.5 Summary

There is considerable research being carried out at major centres in Europe and the US to investigate the basis for the species difference in species susceptibility to the carcinogenic effects of 1,3-BD, which is the major endpoint of concern. This should provide a better understanding of mechanisms and species differences. It is anticipated that by 1994-1995 there will be a better scientific basis for setting an Indicative Limit Value for 1,3-BD.

10. REVIEW OF EXISTING CANCER RISK ASSESSMENTS

Numerous 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 performed by ICF/Clement (1986) under contract to OSHA itself, and by Environ (1986) under contract to the US Chemical Manufacturing Association. The US National Institute of Occupational Safety and Health (NIOSH) recently submitted two risk assessments, the first was performed by 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 the Dutch Expert Committee for Occupational Standards (DECOS) submitted 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 bioassay data (rat study: Owen *et al*, 1987; mouse studies: NTP, 1984 [NTP I] and Melnick *et al*, 1990a,b; Melnick and Huff, 1992a [NTP II]) for quantifying carcinogenic risk. Some of these risk assessments 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 OTS adjusted the dose by the factor: $(\text{study duration/lifetime})^3$. OTS assumed absorption of 1,3-BD to be 100%. Models were fitted for both 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. CAG applied the quantal multi-stage model (EPA, 1985b). They considered the mouse study (NTP I) to be the primary data set, and noted several deficiencies of the rat study at that time. The dose was expressed as mg/kg absorbed (regardless of dose a 54% absorption was assumed) per day based on a preliminary report by Lovelace Inhalation Toxicology Research Institute (Bond *et al*, 1986). A two-stage quantal model was used for the mouse data. This model was found not to fit the female rat data. Therefore, the high dose group data were dropped and a one-stage model equivalent to the one hit model was used for the female rat data. CAG adjusted the risk estimate (rather than the dose as OTS did) from the mouse study by the factor: $(\text{study duration/lifetime})^3$ to compensate for the less than lifetime length of exposure. CAG made the assumption that a human

exposed to any given concentration experiences the same lifetime risk as does the mouse, which implies that a unit dose risk estimate for mice can be applied directly to humans. Risk estimates (assuming an occupational exposure of 100 ppm) derived from rat data were 208 in 10,000 and 4,595 in 10,000, depending on the model used for calculating these risks. 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.

The ICF/Clement risk assessment (ICF/Clement, 1986), carried out under contract to OSHA, was similar to the CAG risk assessment. The mouse bioassay (NTP I) was used for fitting the model and it was presumed that absorption in humans varied with dose at the same rate it varied with dose in mice, which means that 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 mouse and pooled 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, ICF noted that dose response parameter estimates can be derived for tumour types not associated with possible immune suppression or viral activation mechanisms. ICF adjusted the final risk estimate for less than lifetime exposure exactly as CAG. It was also assumed by ICF that lifetime human risk associated with exposure was equal to the mouse risk at the same concentration. Based on the male mouse bioassay, the ICF maximum likelihood estimate of the lifetime excess risk associated with occupational exposure to 1 ppm 1,3-BD, for 8 h/day, 240 days a year for 47 of 74 years is 245 per 1,000. The corresponding risk estimate based on the female mice is 76 per 1,000. At 100 ppm, the risk estimate is 10,000 in 10,000 based on the male and female mouse data (ICF/Clement, 1986).

The Environ risk assessment (Environ, 1986), performed under contract to the US Chemical Manufacturers Association, focused primarily on the rat bioassay. Environ used the CAG approach of absorbed dose, expressing dose as mg/kg. 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 were 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 x day) and ventilation rate, and assumed that human risk was similar to the animal risk. This assumption has the effect of lowering the estimated human risk compared to those (CAG, ICF) assuming equivalency on a ppm basis. The Environ risk estimate from the male mouse bioassay 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 the male mouse data is 3,730 in 10,000; based on the male rat data it is between 154 and 559 in 10,000; based on female rat data, the additional risk is between 560 and 730 in 10,000.

Hattis and Wasson (1987) carried out a risk assessment under cooperative 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 1,3-BD mono-epoxide or di-epoxide concentrations. Unfortunately, some of the key data for the construction of a PB-PK model were not available. Therefore, the authors were forced to estimate parameters like blood:air and tissue:blood partition coefficients. No experiments were carried out to validate the model. The Hattis and Wasson risk estimates are several fold lower than the CAG risk estimate.

The risk assessment of Dankovic *et al* (NIOSH) is based on the second NTP bioassay in mice (NTP II). The data used are preliminary since the pathologic evaluations had not been completed. 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 (which were 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 the factor: $(\text{human mass}/\text{mouse mass})^{3/4}$. For the purpose of extrapolation, this means that the mouse dose of 1,3-BD is 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, is 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, is estimated to be 597 cases of cancer per 10,000, or approximately 6 per 100.

In the risk assessment by Shell Oil (1990), calculations were based on unaudited data from the second NTP mouse bioassay (NTP II, Melnick *et al*, 1990a,b) and on rat data from the IISRP study (Hazleton, 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 estimates 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 to 2 ppm (250 days a year, 8 hours a day for 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.

The Dutch Expert Committee for Occupational Standards (DECOS) used the rat study to calculate the risk for exposed workers. The linear non-threshold model was adopted by this expert group as the basis for risk estimation, using an extrapolation to low levels of the dose-response relationship. According to the authors of the report, this approach should be regarded as 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 lead to an over-estimate of 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 year, 8 h/day exposure (life expectancy of 75 years) the concentration calculate to give a risk of 2.5 in 10,000 is 21.5 ppm. When a risk of 1 in 10,000 is considered, the exposure level is 0.5 ppm (DECOS, 1990).

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, Vmax, K, KF, partition coefficients, body weight and tissue volumes. Assumimg 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.

10.1 Summary and Evaluation

This overview clearly shows the complex nature of a quantification of risk associated with 1,3-BD exposure. There is a substantial difference in the risks predicted by the various models, which is highly dependent on the choice made with regard to the animal system (mouse or rat), tumour data, definition of dose (external/internal) and type of mathematical approach (one-hit, multiple-stage, time-to-tumour). Clearly 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 debate include our lack of understanding of the mechanism of action of 1,3-BD in different species and continuing scepticism concerning the value of quantitative risk assessment in predicting human risk. However, metabolism studies (section 7.1.4) support that the rat is the more appropriate species for human risk assessment. The risk estimates based on data from the rat oncogenicity study include the following: CAG: 208/10,000 at 100 ppm, Environ: 154/10,000 at 100 ppm, Shell Oil: less than 1/1,000 at 2 ppm, and DECOS: 1/4,000 at 21.5 ppm.

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 Matanovski study cohort 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.

11. EXISTING OCCUPATIONAL EXPOSURE LIMITS

The existing national occupational exposure limits for 1,3-BD cover a range of time-weighted averages (TWA) between 5 ppm and 100 ppm (Table XXIII).

The available documentation supporting these limit values (Table XXIII) indicates that many are based on the finding of multiple organ cancer in rats or mice. DECOS in The Netherlands have chosen the less sensitive rat model as the basis for their exposure limit and concluded that this should not lead to an underestimation of the risk for humans. Techniques used to extrapolate an occupational limit value from animal data involved either the use of adjustment/safety factors or quantitative risk assessment models.

Other authorities elected to not base their limit value on animal or human data. German policy is such that a limit for genotoxic carcinogens should be based on technical feasibility (TRK value), since 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 their limit values, in part, on data which indicate that average workplace exposures are achievable below 10 ppm.

TABLE XXIII

National Occupational Exposure Limits

Country	TWA[a] (mg/m^3)	STEL at 20°C[b]	TWA (ppm)	STEL	Reference
EC					
Belgium	22	-	10	-	ILO, 1991
Denmark	22	-	10	-	ILO, 1991
France	-	-	-	-	
Germany	-	-	-[c]	-	DFG, 1984, 1991
Greece	-	-	-	-	Bayer, 1992
Ireland	-	-	-	-	
Italy	22	-	10	-	ACGIH, 1991
Luxembourg	-	-	-	-	
Netherlands	110	-	50	-	DECOS, 1990; Arbeidsinspectie, 1991 Bayer, 1992
Portugal	22	-	10	-	Bayer, 1992
Spain	22	-	-	-	HSE, 1991
UK	22	-	10	-	
OECD					
Austria	-	-	-[c]	-	DFG, 1984, 1991
Australia	22	-	10	-	ILO, 1991
Canada	22	-	10	-	Bayer, 1992
Finland	73	-	50	-	ILO, 1991
Japan	-	-	-	-	Bayer, 1992
Norway[d]	2.2	-	1	2	Arbeidstilsynet, 1990
New Zealand	22	-	10	-	Bayer, 1992
Sweden	20	40	10	20	AFS, 1990; ILO, 1991
Switzerland	11	-	5	-	ILO, 1991
USA - ACGIH	22	-	10	-	ACGIH, 1986, 1991
- OSHA	2210	-	1000[e]	-	OSHA, 1989
- NIOSH	-	-	-	-	
Other Countries					
Czechoslovakia	20	40	-	-	ILO, 1991
Hungary	-	10[f]	-	-	ILO, 1991
Poland	100	-	-	-	ILO, 1991
Singapore	22	-	10	-	Bayer, 1992
USSR	-	100	-	-	ILO, 1991

TWA Time-weighted average concentration (8h-working period)
STEL Short-term exposure limit (15 min, unless specified)

a Official values; some countries use different converion factors and/or other ambient temperature

b Official values; some values apply at 25°C (e.g. USA)

c Germany and Austria: TRK values: 15 ppm after polymerisation and loading, 5 ppm for other applications

d No production facility

e PEL, permissible exposure limit. OSHA has proposed a reduction to 2 ppm with a STEL of 10 ppm (OSHA, 1990)

f Ceiling value (may not be exceeded)

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

12.1 Substance Identification

Common name:	1,3-butadiene
CAS registry N°:	106-99-0
EEC N°:	601-013-00-X, nota D
EEC classification:	F+ ; R 13 / Carc. Cat. 2; R 45
EEC labelling:	R: 45-13 S: 53-9-16-33
EINECS name:	buta-1,3-diene
EINECS N°:	203-450-8
Formula:	C_4H_6
Structure:	$CH_2{=}CH{-}CH{=}CH_2$
Molecular mass:	54.09 (Weast *et al*, 1988)

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 (Amoore and Hautala, 1983; Verschueren, 1983; Weast *et al*, 1988; Sax, 1991).

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

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 is not known to occur as a natural product.

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

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

12.2.3 Exposure Levels at the Workplace

The predominant route of occupational exposure to 1,3-BD is by inhalation.

Limited information on the European exposure situation is available (see below). The Conseil Européen de l'Industrie Chimique (CEFIC), the International Institute of Synthetic Rubber Producers (IISRP) and the Association of Plastics Manufacturers in Europe (APME) have initiated a collection of European exposure data.

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 exposures to 1,3-BD in most process areas were less than 10 ppm; however, maximum 8-h time-weighted average exposures (8-h TWA) were frequently between 10 and 125 ppm (in one case as high as 374 ppm) in operations involving decontamination and maintenance of process equipment, sampling and analysing of quality control samples, and loading or unloading tank trucks or rail cars (Fajen *et al*, 1990).

Based on data used to underpin the German TRK value, personal exposure levels (8-h TWA) are approximately 5 ppm, with maxima of 30 ppm during the manufacturing and purification of 1,3-BD in petroleum refineries and extraction facilities (Deutscher Ausschuß für Gefahrstoffe, n.d.). Data from the USA show that many job categories have exposures below or around 5 ppm, the great majority of levels lying below 10 ppm, with the exception of maintenance and distribution jobs (Heiden Associates, 1987; Krishnan *et al*, 1987; JACA Corp., 1987, all as quoted in IARC, 1992). Exposure levels (8-h TWA) associated with

manufacturing and use of gasoline are generally very low (CONCAWE, 1987 as quoted in IARC, 1992).

High exposures (5 to 50 ppm, 8-h TWA, max. 500 ppm) occur during the connection of pipes for transfer of 1,3-BD in Germany (Deutscher Ausschuß für Gefahrstoffe, n.d.).

Workplace 8-h TWA concentrations during the manufacturing of 1,3-BD based polymers in Germany were between 10 and 20 ppm (mixture of personal and background measurements), with a maximum of 50 ppm (Deutscher Ausschuß für Gefahrstoffe, n.d.). Data from 5 polymer plants in the USA showed personal exposure levels generally below 0.5 ppm, with two exceptions at approximately 5 ppm (Fajen, 1988 as quoted in IARC, 1992). In two other surveys of the North American synthetic rubber producers, the majority of exposures was below 10 ppm (JACA Corp., 1987 as quoted in IARC, 1992; Tozzi, 1988). The latter picture is confirmed by data collected during health surveys or epidemiological studies (Checkoway and Williams, 1982; Meinhardt *et al*, 1982, both as quoted in IARC, 1992). These exposures should not be regarded as representative of conditions in the 1940's (IARC, 1992), when exposures were higher (Mullins, 1990).

No 1,3-BD could be detected during the manufacturing of tyres from synthetic rubber (Fajen *et al*, 1990; Rubber Manufacturer's Association, 1984, both as quoted in IARC, 1992). The evaporation of 1,3-BD from other plastic products should not constitute a significant source for exposure at end-use (JACA Corp., 1987 as quoted in IARC, 1992).

12.2.4 Exposure Levels in the Environment

1,3-BD has been detected in urban air in the USA at ppt to ppb levels. 1,3-BD may also be present in indoor air, e.g. due to cigarette smoking (Lofroth *et al*, as quoted in ATSDR, 1991) and in drinking water (Kraybill, 1980 as quoted in IARC, 1992 and ATSDR, 1991). No residual 1,3-BD could be detected in foodstuffs packaged in materials made from 1,3-BD (McNeal and Breder, 1987 as quoted in ATSDR, 1991; Startin and Gilbert, 1984 as quoted in IARC, 1992 and HSDB, 1992).

The non-occupational daily intake has been calculated to be 2.62 µg/person, assuming a mean urban air concentration of 0.29 ppb/day (USA data, section 5.3.1) and human air intake of 20 m^3/day (ATSDR, 1991).

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 onto charcoal and desorption by methylene

chloride). This solution is then separated, and the compounds identified and analysed by gas-chromatography (GC) equipped with a flame ionisation device (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 at the workplace, gas detector tubes are used (Saltzman and Harman, 1989 as quoted in IARC, 1992).

12.3 Health Significance

The database on the adverse effects of 1,3-BD is extensive. An obvious feature of this database is the substantial difference in sensitivity between the mouse and all other species studied. The toxicokinetic data suggest that this sensitivity difference is at least in part related to species differences in the biotransformation of 1,3-BD to reactive metabolites.

The predominant route of occupational exposure is by inhalation. The metabolic elimination of 1,3-BD is linearly related to the ambient exposure concentration up to about 1,000 ppm in rats and mice, with mice showing higher elimination rates. Above 1,000 ppm, metabolic pathways are approaching saturation in these species (Kreiling *et al*, 1986, 1987). In monkeys, the metabolic elimination of 1,3-BD appears to be saturated at about 300 ppm (Sabourin *et al*, 1992). 1,3-BD is metabolised by cytochrome P-450 dependent mono-oxygenases to the primary metabolite 1,2-epoxybutene-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 to GSH catalysed by GSH S-transferases (Malvoisin *et al*, 1979; Malvoisin and Roberfroid, 1982). According to the *in vitro* and *in vivo* data, the biotransformation appears to be qualitatively similar across species, including humans (Kreuzer *et al*, 1991; Csanády *et al*, 1992a; 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 three-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 resultant body burden of EB (Sabourin *et al*, 1992). Even greater species differences may exist with regard to other reactive epoxides formed by EB metabolism (Csanády *et al*, 1992a).

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 NOEL is 2,300 ppm in the rat (Carpenter *et al*, 1944) and 625 ppm in the mouse (NTP, 1984).

1,3-BD *itself* 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 and 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 (for key references, see Tables XIII to XVIII).

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, 1992a). The 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 only marginally increased in females at 20 ppm and higher and in males at 200 ppm. It is questioned whether this increase in hepatocellular neoplasms is related to chemical treatment, given the high liver tumour incidence in this hybrid mouse strain (Maronpot *et al*, 1987). In contrast, 1,3-BD is less potent in rats, with a statistically significant increase observed only in the incidence of mammary gland tumours at 1,000 ppm, the majority of these tumours being 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 involved only endocrine and sex-hormone sensitive organs. This suggests the involvement of a hormone-related mechanism.

Studies specifically designed to assess fertility did not show adverse effects in guinea pigs, rabbits and rats at exposure concentrations as high as 6,700 ppm for up to 8 months (Carpenter *et al*, 1944). Developmental toxicity studies conducted with 1,3-BD show that there was no toxicity to the developing foetus at exposure concentrations below those which caused maternal toxicity (Irvine, 1981, 1982; Hackett *et al*, 1987a,b). Overall, these studies show again the unique susceptibility of the mouse to 1,3-BD and demonstrate the absence of foetal effects below maternally toxic concentrations.

In bioassays with mice (NTP 1984; Melnick and Huff, 1992a), structural abnormalities have been detected in the testes and ovaries following exposure

to 1,3-BD concentrations as low as 6.25 ppm. Such effects were not observed in the bioassay with rats exposed to up to 8000 ppm (Owen, 1981a,b). The effects in mice are not regarded as toxicologically significant since they occurred after the normal reproductive period of the mouse.

Several studies demonstrated that an endogenous, ecotropic retrovirus (murine leukaemia virus, MuLV) is involved in the thymic lymphoma development following 1,3-BD exposure in the $B6C3F_1$ mouse (Irons *et al*, 1987a,b, 1989). NIH-Swiss mice do not carry an active retrovirus and are less susceptible to the induction of thymic lymphomas by 1,3-BD. In rats, an increase in the incidence of thymic lymphomas was not observed, again demonstrating species differences in the susceptibility to adverse effects induced by 1,3-BD. This murine retrovirus is not known to exist in humans.

Few studies are available to assess the acute effects of 1,3-BD in man. At concentrations > 2000 ppm for > 6 h 1,3-BD caused slight smarting of the eye (Carpenter *et al*, 1944). In workers exposed to a mixture of chemicals, including 1,3-BD, minimal changes in haematological indices were observed, but these changes can not be interpreted as an effect of 1,3-BD (Checkoway and Williams, 1982). Genotoxic effects were not observed in workers of a 1,3-BD production facility, with 1,3-BD concentrations below 1 ppm (Sorsa *et al*, 1991).

With regard to epidemiological studies (Downs *et al*, 1987; Divine, 1990; Meinhardt *et al*, 1982; Matanoski *et al*, 1990), some authors recognised a qualitative association between 1,3-BD exposure and haematopoietic and lymphatic cancer (HLC) (Matanoski *et al*, 1990; Landrigan, 1990; Santos-Burgoa *et al*, 1992), while others see no causal relationship (Acquavella, 1989, 1992; Cole *et al*, 1992). This conflict may be resolved by updating of one cohort study in which 1,3-BD exposure estimates will be reassessed and analysed (Acquavella, 1992). In the meantime, the available studies are inappropriate for quantitative risk assessment, since, in the absence of measured concentrations, the exposure data were only qualitative. Nevertheless, it is reasonable to assume that the exposure levels for the workers investigated in the cohort studies must have been higher than current levels due to less advanced control technologies (Mullins, 1990).

Numerous quantitative risk assessments with regard to the carcinogenicity of 1,3-BD have been carried out (EPA, 1985a,b; ICF/Clement, 1986; Environ, 1986; Dankovic *et al*, 1991; Shell Oil, 1990; DECOS, 1990). Depending on the choice of species, definition of dose, choice of tumour data and the applied mathematical extrapolation method, a wide range of best estimated lifetime risks result. The range of risk values determined using the mouse bioassays are incompatible with findings of the epidemiological studies. Many more human cancer deaths would be expected in the cohort studies if the mouse extrapolations gave accurate predictions (Turnbull *et al*, 1990). Values for the

extrapolation based on the rat bioassay also show some variation for the best estimated lifetime risk. Again, the range of values for mouse and rat reflect the uncertainty of the current risk assessment procedures for 1,3-BD.

12.4 Final Evaluation and Recommendation

12.4.1 Hazard Identification

1,3-BD induces neoplasms at multiple sites in both sexes of rats and mice. 1,3-BD is not genotoxic itself, but some of its metabolites (epoxides) interact with DNA to form adducts and crosslinks which eventually may result in gene mutations and/or chromosomal damage. In contrast to most other toxicological end-points, the predominant hypothesis states that no threshold dose can be defined for genotoxic carcinogens. Consequently, the carcinogenic potential of 1,3-BD is clearly the dominant concern of health effects related to 1,3-BD exposure. Previous evaluations of limit values for 1,3-BD identified carcinogenicity as the critical health hazard associated with 1,3-BD exposure.

12.4.2 Risk Assessment

Despite the current controversy over one epidemiological case-control study, the epidemiological data generally do not show evidence of an increased risk for cancer at past exposure levels. These historical levels are generally thought to be well in excess of the lowest current health-based limit value used in EC member states (10 ppm). However, the absence of adequate exposure data for the cohorts involved makes these studies unsuitable for determining an acceptable exposure level for workers. A major epidemiological study is currently ongoing, involving more than 20,000 workers exposed in styrene-butadiene rubber (SBR) plants in North America over the period 1942 to 1991. It will include retrospective estimates of exposure for a range of job functions. Completion of the study in 1994 should enable the first realistic estimate of potential cancer risk to workers at defined exposure levels, thus offering a better basis for setting an occupational exposure limit for 1,3-BD than currently exists.

Although mathematical models are used for extrapolation of animal bioassay data to low human exposure, the predictive value of such models is questionable because the models: (i) are not validated, (ii) are derived from mathematical assumptions rather than knowledge of biochemical mechanisms, (iii) demonstrate a wide variety of risk estimates depending on the models used, and (iv) give an impression of precision which cannot be justified from the approximations and assumptions upon which they are based. Until these concerns are more adequately addressed, this type of quantitative risk assessment is unsuitable as a basis for setting an occupational exposure limit.

With regard to the effects of 1,3-BD on experimental animals, it is obvious that the mouse is more sensitive to 1,3-BD than all other species investigated. This holds true for subchronic toxicity, reproductive toxicity, genotoxicity and 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 on primates and *in vitro* data obtained from human tissue samples suggest that both humans and other primates form less reactive epoxides of 1,3-BD than rodents, especially the $B6C3F_1$ mouse. For this reason, humans and primates are expected to be less sensitive to the carcinogenic effects of 1,3-BD. The epidemiological data support this view: if humans are nearly as sensitive as the mouse, the cohort studies would have resulted in a clear-cut increase in cancer incidence associated with 1,3-BD exposure. In addition, the incidence of thymic lymphoma seen in the $B6C3F_1$ mouse can be attributed, in part, to the presence of a murine retrovirus. This may explain why this type of tumour was not seen in the rat and was present with a markedly lower incidence in the NIH-Swiss mouse, which does not carry this retrovirus. These considerations lead to the conclusion that data from mouse studies do not provide an appropriate basis for setting an occupational exposure limit. Although the primate is the preferred animal model, the lack of sufficient data precludes its use. Based on the toxicokinetic data, the rat appears to be an acceptably conservative model on which to base an exposure limit value for humans.

The chronic study in Sprague-Dawley rats produced good survivability, thus allowing for complete expression of tumours. The only tumours seen at 1000 ppm with statistically significant increases were mammary gland tumours in the female. The majority of these tumours were benign. There was neither a significant increase of benign nor of malignant tumours when considered separately. Separation of tumour types is reasonable since historical data show a high incidence of benign mammary tumours in the Sprague-Dawley rat. Based on this information, 1000 ppm is a NOEL for the rat.

When comparing the results of *in vivo* genotoxicity tests performed with 1,3-BD, its genotoxic activity has been demonstrated clearly in the mouse and equivocally in the hamster, but not in other species. Consequently, it has to be assumed 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 are formed in all mammals, albeit at a different rate. This leads to some doubt whether genotoxic action is the critical mechanism for induction of tumours in the rat, the only other species tested in a long-term bioassay. This is substantiated by the tumour pattern observed in the rat, which is more indicative of an indirect mechanism mediated through the endocrine system rather than by a direct genotoxic effect through the production of reactive metabolites. However, resolution of this issue is not possible on the basis of the available information.

The uncertainties discussed above make it difficult to derive a scientifically sound occupational exposure limit. The lowest occupational exposure limit used in EC member states today is 5 ppm (German TRK value for certain applications; based on technical feasibility). This concentration is 200-fold lower than the identified NOEL in the rat. In addition, with all the reservations expressed above considered, the quantitative risk assessments based on the rat bioassay suggest that the risk of additional cancer deaths at 5 ppm is low (section 10.1). Most important perhaps, the epidemiological studies conducted so far did not demonstrate any excess mortality from all causes, all cancers, or any other broad category of disease for exposure concentrations which were most likely higher than the current exposure concentrations. The controversy with regard to the possible association between 1,3-BD exposure and haematopoietic and lymphatic cancer, which has been proposed by some authors and rejected by others, still has to be resolved.

In view of all the available evidence, it is concluded that an occupational exposure limit of 5 ppm should protect workers against non-neoplastic and neoplastic effects.

12.4.3 Recommendations

An occupational exposure limit value (OEL) of 5 ppm is recommended for 1,3-BD.

The current lowest limit in EC member states is 10 ppm, based on potential health effects. In Germany, a TRK value of 5 ppm, based on technical feasibility, exists for certain applications. The experience of many years of monitoring worker health indicates that these existing limit values provide adequate protection, limiting any health-related risk to workers.

The ongoing research programme will add significantly to the understanding of the mechanism and toxicokinetics of 1,3-BD-induced carcinogenesis, and provide information on exposure-based epidemiology. Thus, the OEL should be re-evaluated after this new information will have been incorporated into the database. This work should be completed by 1995.

Since skin absorption of 1,3-BD is not a concern, no skin notation is suggested.

There is no evidence to suggest that it is critical to determine a short-term exposure limit (STEL). However, because of the uncertainty about the biological relevance of high short-term exposures to 1,3-BD, a STEL of 100 ppm (15 min TWA) is recommended as a complimentary control to the OEL of 5 ppm.

At present, no method for biological monitoring can be recommended.

A number of suitable methods are available for carrying out short-term, long-term and continuous sampling measurements of 1,3-BD at the recommended OEL of 5 ppm (section 6.1).

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