ECHA Scientific report
for evaluation of limit values for 1,3-butadiene at the workplace

Prepared by the European Chemicals Agency

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List of abbreviations

Abbreviation	Definition
ACGIH	American Conference of Governmental Industrial Hygienists
ACSH	Advisory Committee for Safety and Health at Work
AGS	Ausschuss für Gefahrstoffe (German Committee on Hazardous Substances)
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
ANSES	Agence Nationale de SEcurité Sanitaire de l'alimentation, de l'environnement et du travail (French Agency for Food, Environmental and Occupational Health & Safety)
ATSDR	Agency for Toxic Substances and Disease Registry (USA)
BAR	Biologische Arbeitsstoff-Referenzwerte (Biological reference value; corresponds to the background level present concurrently, in a reference population of persons of working age who are not occupationally exposed to this substance).
BAuA	Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (German Federal Institute for Occupational Safety and Health)
BCM	B-cell malignancy
1,3-BD	1,3-Butadiene
BDE	Butadiene diolepoxide
BGV	Biological Guidance Value
BLV	Biological Limit Value
BLW	Biologische Leit-Wert
BMD	Benchmark dose
BMO	Butadiene monoepoxide
BOEL(s)	Binding Occupational Exposure Limit(s)
bw	Body weight
CA	Chromosome aberration
CAD	Chemical Agents Directive 98/24/EC
CAS RN	CAS Registry Number (unique identifier providing an unambiguous means to distinguish chemical substances or molecular structures when there are many possible systematic, generic, proprietary or otherwise trivial names)
CI	Confidence Interval
CLP	Regulation EC No 1272/2008 on the Classification, Labelling and Packaging of substances and mixtures (CLP Regulation)
CMD / CMRD	Carcinogens and Mutagens Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work. The amendment of the CMD, Directive 2022/431/EU also brought reprotoxic substances within the scope of the directive, changing the original title on the protection of workers from the risks related to exposure to carcinogens or mutagens at work to the protection of workers from the risks related to exposure to carcinogens, mutagens or reprotoxic substances at work (CMRD).
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CVD	Cardiovascular disease
DEB	1,2:3,4-Diepoxybutane

Abbreviation	Definition
DECOS	Dutch Expert Committee for Occupational Standards
DFG	Deutsche Forschungsgemeinschaft (German Research Foundation)
DHBMA	3,4-dihydroxylbutyl mercapturic acid
DMDTC	dimethyldithiocarbamate
EB	1,2-epoxy-3-butene
EBD	3,4-epoxy-1,2-butanediol
EC	European Commission
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
ERR	Exposure-risk relationship
EU	European Union
FA	Fanconi anemia
FID	Flame ionization detector
GC	Gas chromatography
GESTIS Substance Database	GEfahrSToffInformationsSystem (German information system for the safe handling of hazardous substances and other chemical substances at work) Substance Database
HB	Haemoglobin
HPRT	Hypoxanthine–guanine–phosphoribosyltransferase
HR	Hazard ratio
HSGC	Headspace gas chromatography
IARC	International Agency for Research on Cancer (World Health Organization)
ICD-10	International Classification of Diseases 10th Revision
INRS	Institut national de recherche et de sécurité (National Research and Safety Institute for the Prevention of Occupational Accidents and Diseases)
INSST	Instituto Nacional de Seguridad y Salud en el Trabajo (Spain: National Institute for Safety and Health at work)
IOELV(s)	Indicative Occupational Exposure Limit Value(s)
ISO	International Organization for Standardization
IQR	Interquartile range
LOAEC	Lowest observed adverse effect concentration
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOQ	Limit of quantification
MHBMA	2-hydroxy-3-butenyl mercapturic acid
MM	Multiple myeloma
MN	Micronucleus
MoA	Mode of action
MS	Member State
NHL	Non-Hodgkin lymphoma
NIOSH	National Institute for Occupational Safety and Health (USA)
NOAEC	No-observed adverse effect concentration
NOAEL	No-observed adverse effect level
NTP	National Toxicology Program (USA)
OECD	Organisation for Economic Co-operation and Development
OECD TG	OECD Test Guidelines for the testing of chemicals

Abbreviation	Definition
OEHHA	Office of Environmental Health Hazard Assessment (USA)
OEL(s)	Occupational exposure limit(s)
OR	Odds ratio
OSHA	Occupational Safety and Health Administration (USA)
PBL	Peripheral Blood Lymphocyte
PBPK	Physiologically Based Pharmacokinetic
PoD	Point of Departure
ppb	Parts per billion
ppm	Parts per million
RAC	Risk Assessment Committee
RAR	Risk Assessment Report
REACH	Regulation (EC) No 1907/2006 of the European Union concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals
RR	Relative Risk
SBR	Styrene-butadiene rubber
SCE	Sister-Chromatid Exchange
SCOEL	Scientific Committee on Occupational Exposure Limits (former committee of the European Commission)
SLA	Service Level Agreement
SMR	Standardised mortality ratio
STEL	Short term exposure limit
TCEQ	Texas Commission on Environmental Quality
TRGS	Technische Regeln für GefahrStoffe (German Technical regulations for hazardous substances)
TWA	8h time-weighted average
VLB	Valeur limite biologique (Biological Limit Value)
VOC	Volatile organic compounds
WHO	World Health Organisation

Procedure and outcome

I. European Commission request

The European Commission is responsible for preparing the proposals for amendment of Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens, mutagens or reprotoxic substances at work (CMRD).
In line with the 2017 Commission Communication '*Safer and Healthier Work for All' - Modernisation of the EU Occupational Safety and Health Legislation and Policy*[1], it asked the advice of ECHA's Committee for Risk Assessment (RAC) to assess the scientific relevance of occupational exposure limits for some carcinogenic chemical substances.

Therefore, the Commission requested ECHA on 23 February 2023, in accordance with the Service Level Agreement (SLA) (Ares(2023)942305), to evaluate the following substance: 1,3-butadiene (EC number 203-450-8), in accordance with the CMRD.

In support of the Commission's request, ECHA has prepared a scientific report concerning occupational limit values for 1,3-butadiene (EC number 203-450-8) at the workplace.

In the preparatory phase of making this report, a call for evidence was started on 17 March 2023 to invite interested parties to submit comments and evidence by 15 June 2022.

This scientific report was made available at: Occupational exposure limits-Consultations on OEL recommendation on **21 September 2023** and interested parties were invited to submit comments by **20 November 2023**.

The Committee for Risk Assessment (RAC) will develop its opinion on the basis of the scientific report submitted by ECHA.

II. Literature search & data collection

This report is based on international assessments such as (EU RAR, 2002, Health Canada, 2000, US EPA, 2002, SCOEL, 2007, AGS, 2010, ANSES, 2011, ANSES, 2022, IARC, 2012, ATSDR, 2012, DECOS, 2013, OEHHA, 2013, TCEQ, 2015).
A literature search of published papers (literature database PubMed[2]) from the last ten years completed the source of information (date of last literature search: 05/2022).[3]
Databases used were last accessed: 05/2022.

III. ECHA evaluation and recommendation

The tables below present the outcome of the scientific evaluation to derive limit values for 1,3-butadiene.

Table 1: Outcome of the scientific evaluation

Derived Limit Values	Value
OEL as 8-hour TWA	None proposed
STEL	None proposed
BLV	None proposed
BGV	None proposed

Notations	Value
Skin	None proposed

[1] http://ec.europa.eu/social/main.jsp?langId=en&catId=148&newsId=2709&furtherNews=yes
[2] https://pubmed.ncbi.nlm.nih.gov/
[3] All references are listed at the end of the report

Notations	Value

Table 2: Cancer exposure-risk relationship*

Air concentration of 1,3-BD (mg/m^3)	Excess life-time cancer risk (cases per 100 000 exposed)
0.07	4
0.68	40
6.8	400
68	4000

In the future, the aim will be to set limit values for non-threshold substances between the predetermined "upper risk level" and the "lower risk level". It is agreed that the upper risk is 4:1 000 (corresponding to 4 predicted cancer cases in 1 000 employees) and the lower risk level is 4:100 000. This assumes exposure occurs over 8 hours per day, 5 days a week over a 40-year working life period (ACSH, 2022).

1. Chemical agent identification and physico-chemical properties

The chemical identifiers and main physico-chemical properties of 1,3-butadiene are listed in Table 3 and Table 4.

Table 3: Chemical Identification

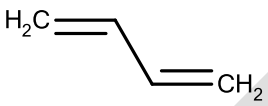
Identifier	
IUPAC Name	buta-1,3-diene
Synonyms	1,3-butadiene
EC/ List No	203-450-8
CAS RN	106-99-0
Chemical structure	
Molecular formula	C4H6
Molecular weight	54.09 g/mol

Table 4: Physico-chemical properties[4]

Property	
Appearance	Colorless gas (at 20°C and 1013 hPa), with a mild gasoline-like odor *
Boiling point	-4.41°C (at 1013 hPa)
Density	0.6149 g/cm^3 (at 25°C)
Vapour pressure	217 kPa (at 17°C) 255 kPa (at 22°C)
Partition coefficient (log Pow)	1.99 (at 20°C)
Water solubility	735 mg/L (at 20°C)
Viscosity	Not applicable
Conversion factor	1 ppm = 2.25 mg/m^3 (at 20°C)[5] 1 mg/m^3 = 0.44 ppm (at 20°C)

* (ATSDR, 2012, SCOEL, 2007)

[4] Values obtained from registration data published on www.echa.europa.eu

[5] $concentration\left[\frac{mg}{m^3}\right] = 147.43\ \frac{g}{mol} \cdot \frac{1.013 \cdot 10^5 Pa \cdot 1m^3}{8.314 \cdot \frac{Pa \cdot m^3}{mol \cdot K} 293.15K} \cdot 10^{-3} \cdot concentration[ppm]$

2. EU harmonised classification and labelling- CLP (EC) 1272/2008

The harmonised classification for 1,3-butadiene (1,3-BD) is presented in Table 5.

Table 5: EU classification: Summary of existing classifications

Index No	International chemical ID	EC number	CAS RN	Annex VI of CLP hazard class and category	Hazard statement code
601-013-00-X	1,3-butadiene, buta-1,3-diene	203-450-8	106-99-0	Press. Gas Flam. Gas 1 Muta. 1B Carc. 1A	/ H220 H340 H350

3. Chemical Agent and Scope of Legislation – Regulated uses in the EU

Table 6: Scope of legislation

Legislation	Applicable	Comment
Directive 98/24/EC (CAD)	Not listed	/
Directive 2004/37/EU (CMRD)	Listed	Binding
Regulation (EC) No 1907/2006 (REACH) – Annex XIV (Authorisation)	Not listed	/
Regulation (EC) No 1907/2006 (REACH) – Annex XVII (Restriction)	Not listed	/
Regulation (EU) 528/2012 – Biocidal Products	Not listed	/
Regulation (EC) 1107/2009 - Plant Protection Product	Not listed	/
Directives 2001/83/EC – Human Medicinal Products	Not listed	/
Directives 2004/28/EC – Veterinary Medicinal Products	Not listed	/
Directive 2008/50/EC – Ambient Air Quality Directive	Listed	Annex X (Measurements of ozone precursor substances), list of volatile organic compounds recommended for measurement contains 1,3-butadiene
Regulation (EC) No 282/2008 – Recycled plastic materials and articles intended to come into contact with foods	Listed	Annex I (Authorised Use), the substance (1,3-butadiene, styrene, methyl methacrylate, butyl acrylate) copolymer cross-linked with divinylbenzene or 1,3-butanediol dimethacrylate is intended to be used up to 12% w/w as impact modifier in rigid (PVC). Finished articles are intended to be used in contact with all types of foodstuffs for long term storage at room temperature.
Regulation (EC) No 1223/2009 – Cosmetic Products Regulation	Listed	Annex II (List of substances prohibited in cosmetic products); petroleum, coal, tar and natural gas and their derivates generated using distillation and/or other processing methods if they contain=0.1% w/w 1,3-butadiene.
Directive 2008/68/EC – Inland Transport of Dangerous Goods	Listed	Annex II, III

3.1 REACH Registrations

Table 7: REACH Registrations and tonnage

Substance(s)		Tonnage (tonnes/annum)	
Name	**EC number**	**Full registration**	**Intermediate uses**
1,3-butadiene (buta-1,3-diene)	203-450-8	> 1 000 000 tpa 209 active registrants)	Rubber production and processing; Polymer production and processing

1,3-BD was assessed by the German competent authority under substance evaluation (Article 46 of REACH). The registrants proposed a $DMEL_{long\text{-}term,\ inhalation,\ systemic}$ of 1 ppm (2.21 mg/m^3) which was taken in the risk assessment for workers. The report concluded that *"Based on the registrants' DMEL of 1 ppm, the reported exposure values do not exceed this DMEL in general."*

4. Existing Occupational Exposure Limit values

4.1 Occupational Exposure Limits (OEL)

A binding OEL of 1 ppm (2.2 mg/m^3) is set in the EU. Some EU- and non-EU countries have established OEL and short-term limit values (STEL) values for 1,3-BD (Table 8). The list should not be considered as exhaustive.

Table 8: Existing Occupational Exposure Limits (OELs)

Country	TWA (8 hrs)		STEL (15 min)		Remarks / Notations
	ppm	mg/m^3	ppm	mg/m^3	
EU countries					
European Union	1	2.2			
Austria	1 (1)	2.2 (1)	8 (1)(2)	17 (1)(2)	(1) TRK value (based on technical feasibility) (2) 15 minutes average value
Belgium	1 (1)	2.2 (1)			(1) Additional indication "C" means that the agent falls within the scope of Title 2 concerning carcinogenic, mutagenic and reprotoxic agents of Book VI of the Codex on well-being at work.
Denmark	1 (1)	2.2 (1)	2 (1)(2)	4.4 (1)(2)	(1) Carcinogen (2) 15 minutes average value
Finland	1	2.2			
France	1	2.2			
Germany (AGS)	2 (1) 0.2 (2)	5 (1) 0.5 (2)	16 (1)(3)	40 (1)(3)	(1) Workplace exposure concentration corresponding to the proposed tolerable cancer risk. (see background document: AGS) (2) Workplace exposure concentration corresponding to the proposed preliminary acceptable cancer risk. (see background document: AGS) (3) 15 minutes average value
Hungary	1	2.2			
Ireland	1	2.2			
Italy	1	2.2			
Latvia	1	2.2			
Netherlands		2			
Norway	1	2.2			

Poland	1	2.2			
Romania	1	2.2			
Spain	1	2.2			
Sweden	0.5	1	5 (1)	10 (1)	(1) 15 minutes average value
Non-EU countries					
Australia	10	22			
Canada-Ontario	2				
Canada-Québec	2	4.4			
China		5			
New Zealand	0.05	0.1			
Singapore	2	4.4			
South Africa	4				
South Africa Mining	2	4			
South Korea	2		10 (1)		(1) 15 minutes average value
Switzerland	2	4.4			
United Kingdom	1	2.2			
USA-OSHA	1		5 (1)		(1) 15 minutes average value

Notes: TWA: Time-Weighted-Average; STEL: Short term exposure limit; TRK: Technical Guidance Concentrations; OSHA: Occupational Safety and Health Administration.
Source: GESTIS Substance Database; European Risk Observatory Report: Exploratory Survey of Occupational Exposure Limits for Carcinogens, Mutagens and Reprotoxic Substances at EU Member States Level (EU-OSHA); last accessed March 2023.

4.2 Biological Limit Values (BLV) and Biological Guidance Values (BGV)

4.2.1 Biological Limit Values (BLV)

Two EU Member States have established BLVs for metabolites of 1,3-BD in the urine (Table 9).

Table 9: Existing Biological Limit Values (BLV)

Country	Metabolites of 1,3-butadiene in urine	Specifications	Reference
Germany	DHBMA (correlation of air concentration of 1,3-butadiene to internal exposure of DHBMA): 0.45 mg/m³ - 600 µg/g creatinine 1.1 mg/m³ - 1000 µg/g creatinine 2.3 mg/m³ - 1600 µg/g creatinine 4.5 mg/m³ - 2900 µg/g creatinine 6.8 mg/m³ - 4200 µg/g creatinine	EKA value (correlation between external and internal exposure) Sampling time: end of exposure or end of shift; for long-term exposures: at the end of the shift after several previous shifts	(DFG, 2021)
	MHBMA (correlation of air concentration of 1,3-butadiene to internal exposure of DHBMA): 0.45 mg/m³ - 10 µg/g creatinine 1.1 mg/m³ - 20 µg/g creatinine 2.3 mg/m³ - 40 µg/g creatinine 4.5 mg/m³ - 80 µg/g creatinine 6.8 mg/m³ - 120 µg/g creatinine		
Spain	DHBMA: 2.5 mg/L (Scheduled revision in 2024)	VLB: Valor límite biológico Sampling time: end of exposure or end of shift	(INSST, 2023)

	Haemoglobin adducts of MHBMA in blood 2.5 pmol/g Hb (Scheduled revision in 2024)	Sampling time: not critical	(INSST, 2023)

Notes: DHBMA: 3,4-dihydroxybutyl mercapturic acid; EKA: exposure equivalents for carcinogenic agents; MHBMA: 2-hydroxy-3-butenyl mercapturic acid; VLB: Valor límite biológico.
Source: BIOTOX, last accessed March 2023.

4.2.2 Biological Guidance Values (BGV)

Two EU Member States have proposed BGVs for metabolites of 1,3-BD in the urine (Table 10).

Table 10: Existing Biological Guidance Values (BGV) for the general population

Country	Metabolites of 1,3-butadiene in urine	Specifications	Reference
Germany	400 µg DHBMA/g creatinine (non-smokers) < 2 µg MHBMA/g creatinine (non-smokers)	BAR value: Sampling time: at the end of exposure or the end of the shift, for long-term exposures: at the end of the shift after several shifts	(DFG, 2021)
France	DHBMA: 750 µg/L or 550 µg/g creatinine (non-smokers) 1100 µg/L or 750 µg/g creatinine (smokers) 3-MHBMA: 20 µg/L or 15 µg/g creatinine (non-smokers) 120 µg/L or 110 µg/g creatinine (smokers)	VBI value	(ANSES, 2020)

Notes: DHBMA: 3,4-dihydroxybutyl mercapturic acid, MHBMA: 2-hydroxy-3-butenyl mercapturic acid; 3-MHBMA: N-acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine.
Source: BIOTOX, last accessed March 2023.

5. Occurrence, uses and occupational exposure

5.1 Occurrence

1,3-BD is not known to occur widely as a natural product. However, forest fires caused by lightning strikes or due to ignition by volcanic lava or ash are considered to be a natural source of 1,3-BD in the air.

Large amounts of 1,3-BD are released into the air by industrial sources primarily in the production of synthetic rubbers and polymers. Industrial releases to water and soil are relatively low, and the 1,3-BD will likely evaporate quickly into the air based on its physical and chemical properties. Automobile exhaust is a constant source of 1,3-BD release into the air. Other sources include cigarette smoke and the smoke of wood fires (ATSDR, 2012).

1,3-BD has also been measured at very low levels in plastic or rubber of food containers, but it has not been found often in food samples. Exposure through ingestion of food and drinking water is expected to be very low compared to exposure through breathing contaminated air.

5.2 Production and uses

5.2.1 Manufacturing processes

1,3-BD was first produced in the late nineteenth century by pyrolysis of petroleum hydrocarbons (Kirshenbaum, 1978, as cited in (IARC, 1999)). Commercial production started in the 1930s. Butadiene is manufactured primarily as a coproduct of steam cracking of hydrocarbon streams to produce ethylene in the United States, western Europe and Japan. However, in certain parts of the world (e.g., China, India, Poland and Russia) it is still produced from ethanol. The earlier manufacturing processes of dehydrogenation of n-butane and oxyhydrogenation of n-butenes have significantly declined in importance and output.
Efforts have been made to make 1,3-BD from other feedstocks such as other hydrocarbons, coal, shale oil and renewable sources such as animal and vegetable oil, cellulose, hemicellulose and lignin, but in the United States none of these has moved beyond the research and development stage (Müller & Löser, 1985; Sun & Wristers, 1992, as cited in (IARC, 1999)).

Steam cracking is a complex, highly endothermic pyrolysis reaction. During the reaction, a hydrocarbon feedstock is heated to approximately 800°C and 34 kPa for less than one second, during which carbon–carbon and carbon–hydrogen bonds are broken. As a result, a mixture of olefins, aromatics, tar and gases is formed. These products are cooled and separated into specific boiling-range cuts of C1, C2, C3 and C4 compounds. The C4 fraction contains 1,3-BD, isobutene, 1-butene, 2-butene and some other minor hydrocarbons. The overall process yields of 1,3-BD depend on both the process parameters and the composition of feedstocks. Generally, heavier steam-cracking feedstocks produce greater amounts of butadiene. Separation and purification of butadiene from other components is carried out mainly by an extractive distillation process. The most commonly used solvents are acetonitrile and dimethylformamide; dimethylacetamide, furfural and N-methyl-2-pyrrolidinone also have been used for this separation. Another commercial process to separate 1,3-BD from other hydrocarbons uses a solution containing cuprous ammonium acetate, which forms a weak copper(I) complex with 1,3-BD (Müller & Löser, 1985; Sun & Wristers, 1992, as cited in (IARC, 1999)).

The most widely used production method for 1,3-BD is recovery from a by-product stream during the production of ethylene. The crude C4 component stream isolated from the steam cracking process is fed to butadiene extraction units, where butadiene is separated from the other C4s by extractive distillation. These manufacturing processes occur using dedicated facilities/equipment and closed systems, where exposure is unlikely or occasional and controlled. The total production capacity in the EU was reported in 2002 to be between 1.2 and 5.0 million tonnes per year (EU RAR, 2002). The latest REACH registration data indicates that 1,3-BD is manufactured in and/or imported into the European Economic Area, between 1 and 10 million tonnes per year.

5.2.2 Uses

1,3-BD is mainly used in closed systems as an intermediate for polymerisation and copolymerisation. The major use is in the production of synthetic rubber, thermoplastic resins and latex. More information can be found in the EU RAR (EU RAR, 2002).

1,3-BD is used primarily in the production of synthetic rubbers, including styrene–butadiene rubber (SBR), polybutadiene rubber (BR), styrene–butadiene latex (SBL), chloroprene rubber (CR) and nitrile rubber (NR). Important plastics containing 1,3-BD as a monomeric component are shock-resistant polystyrene, a two-phase system consisting of polystyrene and polybutadiene; ABS polymers consisting of acrylonitrile, butadiene and styrene; and a copolymer of methyl methacrylate, butadiene and styrene (MBS), which is used as a modifier for poly(vinyl chloride). It is also used as an intermediate in the production of chloroprene, adiponitrile and other basic petrochemicals (United States National Library of Medicine, 1997, as cited in (IARC, 1999)).

The EU RAR (2002) reports that the US EPA published a study into the releases of 1,3-BD from production and use (Buchanan, 1989). It was based mainly on data submitted by US industry in

1984 to derive emission factors for 1,3-BD, and covers five possible types of release: process vent discharges, equipment leaks, emission from secondary sources (e.g. wastewater), storage-related emissions, and emergency or accidental release. Losses during handling are possible but should be low for safety reasons (due to the explosion hazard).

This fits with the REACH registration data which indicates that the majority of the manufacture and use processes occur using dedicated facilities/equipment and closed systems, while there remain some activities, especially post-production such as calendaring and extrusion processes where release/exposure is possible. The REACH registration data also confirms that the majority of the tonnage continues to be used in polymerisation processes, and particularly in rubber production. Additional uses, in much smaller tonnages, reported in the vast majority of the 207 active registrants include formulation and distribution, use as a fuel, and use as a laboratory reagent (solvent). In terms of formulation and distribution 1,3-BD is used to make gas mixtures together with other hydrocarbons (with nitrogen or helium as excipient), which are supplied in pressurised gas cylinders as standards for laboratories. The registration data also shows that application of the substance and the polymers manufactured occur across a very wide variety of products: lubricants and greases, adhesives and sealants, anti-freeze products, coating products, fillers, putties, plasters, modelling clay, finger paints, non-metal-surface treatment products, inks and toners, leather treatment products, polishes and waxes, polymers and textile treatment products and dyes.

The REACH registration also describes potential releases to the environment from various sources, which are not directly relevant to occupational exposure, but may contribute to background exposure. This includes minimal potential for release to the environment from the use of 1,3-BD indoors in closed systems (e.g. cooling liquids in refrigerators, oil-based electric heaters). It is also used outdoors in closed systems with minimal potential for release (e.g. hydraulic liquids in automotive suspension, lubricants in motor oil and brake fluids). There is also potential for release to the environment from the use of 1,3-BD in mixtures used by professionals such as machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners and outdoor use as processing aid. 1,3-BD can also be found in complex articles, with no release intended, such as vehicles. It can be found in products with material based on: rubber (e.g. tyres, shoes, toys) and plastic (e.g. food packaging and storage, toys, mobile phones) (ECHA, 2023).

5.3 Occupational exposure

Potential exposure to 1,3-BD can primarily occur in the following industrial activities:
- petroleum refining and related operations (production of C4 fractions containing 1,3-BD, and production and distribution of gasoline),
- production of purified 1,3-BD monomer,
- production of various butadiene-based rubber and plastics polymers and other derivatives, and
- manufacture of rubber and plastics products (tyres, hoses and a variety of moulded objects).

Workers in the production of rubber, plastics, and resins are likely exposed to higher levels of 1,3-BD (ATSDR, 2012).

Some studies have been identified conducted in different EU Member States that monitor occupational exposure to 1,3-BD:
- Monitoring in a Finnish butadiene monomer production plant in 1991, generally indicated ambient air levels of less than 10 ppm (22 mg/m^3) at different sites (33 samples; mean sampling time, 5.3 h). In personal samples for 16 process workers, the concentrations ranged from <0.1 ppm to 477 ppm (<0.22–1050 mg/m^3) (mean, 11.5 ppm (25 mg/m^3); median, <0.1 ppm (<0.22 mg/m^3); 46 samples; mean sampling time, 2.5 h). The highest concentrations were measured during sample collection. Protective clothing and respirators were used during this operation (IARC, 1999).

- In the Swedish petroleum refinery industry, a study of personal exposure to 1,3-BD was

conducted. Personal exposure measurements were taken between 2009 and 2011. The arithmetic mean (AM) of personal exposure to 1,3-BD of process technicians was 5.4 µg/m3 (95% CI 3.1–9.5 µg/m3) and 1.8 µg/m3 (95% CI 1.1–2.9 µg/m3). Process technicians working mainly outdoors had a statistically significant higher exposure to 1,3-BD compared with process technicians working mainly indoors (7.2 versus 0.7 µg/m3, $p < 0.05$). There was no statistically significant difference between process technicians working in the process area and process technicians working in the tank park. There was no significant difference in 1,3-BD levels between process technicians working outdoors at the two refineries. One group (four persons) working with loading of LPG on to railroad tank cars and drainage of tanks in the tank park had an increased exposure to 1,3-BD compared with the rest of the exposure groups. The increased exposure was associated with the loading of LPG on to railroad tank cars, performed during 50–100% of the work time.
The AM of personal exposure to 1,3-BD during these work shifts (N = 13 samples) was 15.6 µg/m3 (95% CI 7.4–33.1 µg/m3), while the exposure levels were below the LOD during work shifts when tanks were being drained (N = 3). For process technicians working in the process area at Refinery 2, the within-worker variability and the between-worker variability were similar (53 and 47%, respectively). For the other occupational exposure groups—outdoor and indoor maintenance workers, laboratory workers, engineers, inspectors, safety and emergency workers, and administration personnel at Refinery 1 (in total 216 samples), and outdoor maintenance workers, laboratory workers, engineers, and inspectors at Refinery 2 (in total 78 samples)—the average 1,3-BD exposure was low, with only a minor fraction of samples above the LOD. The study concluded that refinery workers in the Swedish petroleum refinery industry have a low average personal exposure to 1,3-BD. Mean exposure was less than 1% of the Swedish OEL (Akerstrom et al., 2016).

- All exposures to 1,3-BD collected in the Italian Information System on Occupational Exposure to Carcinogens (SIREP) and recorded in the period 1996–2015 were considered in the study 'Appraisals of levels and patterns of occupational exposure to 1,3-butadiene'. Overall, the AM of exposure to 1,3-BD was 0.12 mg/m^3 (SD=0.37, GM=0.03 mg/m^3 GSD=6.73 M=0.03 mg/m3, IQR=0.007–0.097), and was higher among men (0.12 mg/m^3, 97% of exposed workers) than among women (0.05 mg/m^3, 3%).
  The sector most at risk for higher levels of exposure was rubber and plastic products manufacturing (GM=0.17 mg/m^3), even if most of the exposures were gathered in the manufacture of chemicals (N=18 744) where, also, the few 1,3-BD -exposed women were employed. The occupational group with the highest level for 1,3-BD exposure, regardless of the economic sector, was "building structure cleaners" (GM=0.11 mg/m^3), even if with a low number of measurements (N=145). Regarding job tasks, the work activity suffering higher levels of exposure was the moulding activity among machine operators of plastic products (GM=0.28 mg/m^3, 0.5% of exposed workers of which were 33% women)(Scarselli et al., 2017).

The exposure estimates in the REACH registration dossiers are based on modelling, with the usual caveats on the representativeness of the models (and the reliability, conservativeness etc. of the assumptions). However, the exposure levels reported are somewhat in line with the Italian Information System on Occupational Exposure to Carcinogens (SIREP), ranging from 0.02 mg/m³ for those activities occurring in closed systems up to 2 mg/m³ for waste handling activities. Higher exposures close to 2 mg/m³ were also estimated/reported for manual transfer activities.

5.3.1 Routes of exposure

The main route of occupational exposure to 1,3-BD, a gas, is by inhalation. The potential for oral or dermal exposure cannot be entirely excluded but is considered to represent a very minor route of exposure in comparison to inhalation. Dermal exposure to a gas is only likely to occur if it condenses on to the worker's skin.

5.4 General population exposure

General population exposure is through inhalation, dermal exposure and oral exposure via drinking water, food and smoking tobacco.

Releases of 1,3-BD into the air occur from:
- vehicle exhaust
- tobacco smoke
- wood burning
- burning of rubber and plastic
- forest fires
- accidental or intentional release at manufacturing plants

According to the EU RAR (2002), long-term monitoring programs for 1,3-BD have been carried out in some countries. Typical levels of 1,3-BD measured in urban areas are around 1.5 µg/m^3 and vehicle emissions are thought to contribute significantly to these levels. For example, the measured urban levels in the UK (pre-Brexit) were up to 1.5 µg/m^3 but higher levels have been noted under certain conditions, for example, heavy traffic and cold weather combined producing an episodic concentration of 22 µg/m^3 (0.01 ppm). For the purposes of risk assessment for the EU RAR, the concentrations calculated from industry release information were used, that is 222 µg/m^3 (0.1 ppm) for emissions from local industry dominated sources and 1.5 µg/m^3 (0.0007 ppm) for a regional background level. This corresponds to the average amount of 1,3-BD in the air reported in the USA to be between 0.04 and 0.9 ppb (parts of 1,3-BD per billion parts of air) in cities and suburban areas (ATSDR, 2012).

There are few polymers that are considered 'direct food additives'; the one relevant to 1,3-BD is styrene-butadiene rubber (SBR as mentioned above). These products are used as components of chewing gum and are considered intentional additives in this 'food product'. Potential releases in this application deliver monomers directly to the oral cavity of the gum consumer and, given the short pathway from product use/monomer release to human contact, the health risk assessments for these applications are straightforward (Leber, 2001).

1,3-BD has been measured at very low levels in plastic or rubber of food containers, but it has not been found often in food samples. Exposure to 1,3-BD through ingestion of food and drinking water is expected to be very low compared to exposure through breathing contaminated air. People may be exposed to small amounts of 1,3-BD if gasoline gets on their skin or by breathing air that contains gasoline fumes (ATSDR, 2012).

Other polymer applications are known that lead to potential human exposures to monomers. These include toys that are articles posing skin and oral contact potentials to children. Polymeric furniture and wall/ floor coverings are commonly known to elevate airborne levels of monomers in living spaces and workspaces. Various medical devices exist that pose a range of exposure contact potentials to humans. High-level contact applications are those in which polymeric devices are implanted into the body for protracted periods of time. In the other extreme are those products that represent a transient or 'remote' opportunity for contact, such as bandage adhesives or crutch arm pads. Such uses would be expected to contribute negligible risks associated with monomer migrations (Leber, 2001).

5.5 Summary

Significant amounts of 1,3-BD can be released into the air from industrial sources primarily in the production of 1,3-BD in the order of millions of tonnes/year, and its use in the production of synthetic rubbers and polymers. Due to technological advances and organisational improvements (better awareness and training related to risk management) occupational exposures have reduced over the last few decades while the tonnage manufactured and used in the EU/EEA has increased. Those activities that report higher occupational exposures seem to be related to the manual manipulation of polymers and plastics (moulding, extrusion, calendaring etc.), and to waste handling and manual transfers. The general population may also

be exposed to 1,3-BD because it is a ubiquitous environmental pollutant (from vehicle exhausts, tobacco smoke and industrial releases). Exposure to 1,3-BD through ingestion of food and drinking water is expected to be very low compared to exposure through breathing contaminated air.

6. Monitoring exposure

6.1 Monitoring methods (external exposure)

Several validated methods are available for measuring 1,3-BD. However, the limit of quantification indicated in these methods may not be low enough for the OEL derived later. Thus, also peer review articles have been considered to assess the possibilities of measuring lower concentrations of 1,3-BD at the workplace.

The principle of these methods is as follows: air sampling is performed by passing air actively through a sorbent tube or by using diffusive sampling. The retained 1,3-BD is then extracted for analysis by either thermal desorption or desorption with a solvent, followed by analysis via gas chromatography with different detectors.

Table 11 shows some of the available methods for measurement of 1,3-BD in air. It is possible to measure 1,3 BD in air in the range of the µg/m^3 or ppb.

Table 11: Overview of sampling and analytical methods for air monitoring at the workplace

Method	Analytical technique	LOQ Sampling volume Time	Sampling methods/ desorption	Comments
(DFG, 2012)	HSGC/ FID (1)	1.1 mg/m^3 Flow rate: 0.03 l/min 16L (8 hours)	Activated carbon tubes (active) Methyl acetate/ DMA desorption	
(OSHA, 1985)	GC/ FID (2)	0.343 mg/m^3 Flow rate: 0.05 l/min 3L (1 hour)	Cleaned charcoal coated with 4-tert-butylcatechnol (active) CS_2 desorption	
(NIOSH, 1994)	GC/ FID (2)	0.04 mg/m^3 Flow rate: 0.01-0.5 l/min 25 (1 hour at max flow rate)	Coconut charcoal tubes (active) methylene chloride desorption	
(INRS, 2021)	GC/ FID (2)	0,002 mg/m^3 (validation range 0.008 to 0.160 mg/m^3. Flow rate: 0.01 l/min 4.8L (8 hours)	Carbopack X (active) Thermal desorption	
(INRS, 2022)	GC/ FID (2)	0,27mg/m^3 Flow rate: 0.05 L/min 24L (for 8 hours)	Activated carbon tubes (active) CS_2 desorption	The method lacks sensitivity re. the new French OEL, and is only recommended for concentrations higher than 2 * OEL
Reported by (Akerstrom et al., 2016) following (Strandberg et al., 2014)	GC/MS (3)	0.001 mg/m^3 8 hours	Carbopack X (Passive) Thermal desorption	

(1) HSGC/ FID: head space gas chromatography with flame ionisation detector
(2) GC/ FID: gas chromatography with flame ionisation detector
(3) GC/MS: gas chromatography / mass spectrometry

6.2 Biomonitoring of exposure (internal exposure)

(DFG, 2021) and (ANSES, 2020) considered different biomarkers of exposure to 1,3-BD (see details on metabolism of 1,3-BD in section 7.1).

The biomarkers considered are:

- Excretion of mercapturic acids (3,4-Dihydroxybutyl mercapturic acid (DHBMA) and 2-Hydroxy-3-butenyl mercapturic acid (MHBMA) in urine
- 1,3-BD in exhaled air, urine or blood
- Haemoglobin adducts in blood 1- and 2-hydroxy-3-butenyl valine (MHBVal) and THBVal (N-(2,3,4-trihydroxybutyl)valine)
- DNA adducts in blood

1,3-BD was discarded as a biomarker despite its specificity because of its rapid elimination after the end of the exposure in the case of exhaled air and the poor correlations and scarcity of data in other biological media.

The haemoglobin adducts identified (MHBVal and THBVal) could be an indicator of cumulative exposure (circa 18 weeks). However, they were not proposed by either (DFG, 2021) or (ANSES, 2020) because of scarcity of data, analytical difficulties, and indication of high background concentrations for THBVal of unknown origin.

The DNA adducts were also not considered adequate for routine because of limited data and lack of routine techniques available for the occupational monitoring.

Consequently, DHBMA and MHBMA are considered the most suitable biomarkers for the exposure to 1,3-BD.

It should be noted that MHBMA is considered as a mixture of isomers: 1-MHBMA (N-acetyl-S-(1-hydroxymethyl-2-propenyl)-L-cysteine) and 2-MHBMAN-acetyl-S-(2-hydroxymethyl-3-propenyl)-L-cysteine. Recently a new isomer has been identified 3-MHBMA (ANSES, 2020).

Both ANSES and DFG propose to DHBMA and MHBMA for the biological monitoring proposed. They seem complementary as DHBMA shows high sensitivity but is not a specific biomarker while MHBMA shows high specificity (DFG, 2021). However, DFG considers MHBMA (isomer mixture) while (ANSES, 2020) chooses the 3-MHBMA because this is the isomer the majority MHBMA isomer.

6.2.1 Background levels for general population, i.e. non-occupationally exposed

1,3-BD is not known to occur as a natural product (ATSDR, 2012). However,1,3-BD is almost always present in the air at low levels due to its emission from motor vehicles. The primary route of potential exposure to 1,3-BD for the general population is inhalation. Some exposure may occur through ingestion of contaminated food or water or dermal contact. However, these routes of exposure are unlikely under most circumstances.

1,3-BD is not a common contaminant of water supplies. Although some food packaging contains residual 1,3-BD, the available data indicate that it does not usually migrate to the food (NTP, 2016).

Elevated concentrations may occur in the vicinity of point sources, such as municipal structural fires, wood and brush fires; cigarette smoking; vehicle emissions and gasoline volatilization (IARC, 2012).

(DFG, 2021) and (ANSES, 2020) report several studies on non-occupationally exposed populations. A more recent survey of the US population (NHANES 2011-2016) reported by (Nieto et al., 2021) also looks at urine metabolites of 1,3 BD in the general population. All the studies differentiate between smokers and no smokers as 1,3 BD is a complement of tobacco smoke

(NHANES) and significant difference in the levels of the urine metabolites of 1,3 BD in urine have been found in the studies.

The NHANES survey measured several mercapturic acids in urine resulting from the metabolism of 1,3 BD and looked for (possible) correlations based on smoking/ non-smoking, sex, race, body mass index and diet. The biomarkers used included MHBMA and DHBMA and two additional mercapturic acids: N-acetyl-S-(1-hydroxymethyl-2-propenyl)-L-cysteine (1HMPeMA or 1MHBMA) and N-acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine (4HBeMA or 3-MHBMA). The study found that 1HMPeMA and MHBMA were detected only in 0.66% and 9.84% of the samples respectively and correlations were not attempted for them. Full data including exposure percentiles are reported by CDC (CDC, 2019a, CDC, 2019b) (Table 12).

Table 12 summarizes the concentrations of biomarkers of 1,3 BD in urine of persons of the general population (i.e. not occupationally exposed). The values are given as mean value ± standard deviation, unless stated otherwise. Some of the publications also reported data for 1- and 2-MHBMA but those were often below the LOQ, in particular for non-smokers and have not been reported in the table below.

Table 12: Concentrations of biomarkers of 1,3 BD in urine of the general population (references below are cited in (DFG, 2021) and (ANSES, 2020))

Country	Year	Group	n	DHBMA in urine	MHBMA in urine (MHMBA isomer if relevant)	References
USA	2005	NS	7	255/– (42.8–766) µg/l [a]	6.1/– (3.7–11.1) µg/l [a]	Sapkota et al. (2006)
		NS	7	244/– (46.3–513) µg/l [a]	4.7/– (2.2–16.1) µg/l [a]	
USA	2007	NS	25	–	0.09 ± 0.10 µg/g creatinine	Sarkar et al. (2008)
		NS	20	–	0.06 ± 0.10 µg/g creatinine	
		S	25	–	2.70 ± 1.59 µg/g creatinine	
		S	20	–	3.64 ± 3.12 µg/g creatinine	
USA	2013	S			2.55± 0.1.72 µg/g creatinine	Sarkar et al. (2013)
USA	2008	NS	1077	239 µg/g creatinine [b]	0.18 µg/g creatinine [b]	Roethig et al. (2009)
		S	3585	327 µg/g creatinine [b]	2.10 µg/g creatinine [b]	
USA	2008	NS	59	105/– (< LOD–582 µg/g creatinine [a]	21/– (< LOD–122 µg/g creatinine) [a]	Ding et al. (2009)
		S	61	510/– (166–1092 µg/g creatinine) [a]	10/– (< LOD–59.7 µg/g creatinine) [a]	
USA	2008	NS	17	97.7 ± 36.6 µg/g creatinine [b]	0.50 ± 0.33 µg/g creatinine [b]	Carmella et al. (2009)
		S	17	153 ± 75.9 µg/g creatinine [b]	9.06 ± 9.51 µg/g creatinine [b]	
USA	2013-2014	NS +S	1703 - 1783	283/ 617µg/g creatinine (260-307/555-696)[d]	**3-MHBMA** 6.85/54.5 µg/g creatinine (6.09-7.70/46.0-66.4)[c]	CDC, 2019
		S	884-905	336/768 µg/g creatinine (332-403/713-889)[d]	**3-MHBMA** 26.2/110 µg/g creatinine (21-32.6/88.6-141)[c]	
		NS		267/565 µg/g creatinine (245-290/496-670)[d]	**3-MHBMA** 4.96/16.5 µg/g	

Country	Year	Group	n	DHBMA in urine	MHBMA in urine (MHMBA isomer if relevant)	References
					creatinine (4.39-5.61/13.3-26.3)[c]	
USA	2016	NS/S	488		**3-MHBMA** Mean: 6.9 12,1 µg/L, 75TH percentile)	Boyle et al. (2016)
USA	2014	S	36	631± 452 µg/g creatinine	11± 12 µg/g creatinine	Kotapati (2014)
USA		NS	1203	331±279 µg/l	**3-MHBMA** 6.40±10 µg/l	Alwis (2012
		S	347	440±311 µg/l	**3-MHBMA** 36±34 µg/l	
Thailand	2006	NS	24	–	51.1/ (18.9–107) µg/gcreatinine [a]	Arayasiri et al. (2010)
Italy	2008	NS	33	166/–(16–599) µg/l [c]	–	Carricri et al. (2009)
Germany	2003	NS	10	270 ± 42(123–528) µg/g creatinine	7.4 ± 0.6 (4.1–10.6) µg/g creatinine	Urban et al. (2003)
		S	10	379 ± 53(68–638) µg/g creatinine	50.8 ± 8.2 (8.9–85.4) µg/g creatinine	
Germany	2008	NS	73	289/760(19.4–2500) µg/l [a]	< 2/< 2(< 2–2.5) µg/l [a]	Schettgen et al. (2009)
		S	81	398/1079(15.4–1959) µg/l [a]	< 2/8.6(< 2–17.5) µg/l [a]	
Germany	2010	NS	54	159/329(60.2–797) µg/g creatinine [a]	< 5/< 5(< 5–< 5) µg/g creatinine [a]	Eckert et al. (2011)
		S	40	211/417(107–432) µg/g creatinine [a]	< 5/9.5 (< 5–11.9) µg/g creatinine [a]	
Germany	2015	NS	25	76.2 (47.4-349) µg/g creatinine		Pluym et al (2015)
		S (<10 cig/day)	<25	112 (65.5-243) µg/g creatinine		
		S (>10 cig/day)	<25	122 (52.9-244) µg/g creatinine		

[a] Median/95th percentile (range)
[b] Values calculated on the basis of creatinine excretion of 1.7g/day; mean values and range are given
c Mean value / 95th percentile (range or 95%CI)
[d] Median (95% CI)
NS: Non-smoker; S: Smoker

6.2.2 Correlations between internal and external exposure

Many studies have followed the correlation between air concentration of 1,3 BD and internal concentration of the biomarkers. This section focuses on the studies that correlate air concentration and urine concentration of the urine biomarkers identified as most suitable in the introduction to this section (MHBMA, 3- MHBMA and DHBMA). Table 13 provides an overview of most of the literature considered by (DFG, 2021) and (ANSES, 2020) when considering the correlation between 1,3 BD in air and urinary mercapturic acids.

Table 13: Overview of studies showing correlations between 1,3 BD and mercapturic acids in urine (see (DFG, 2021) and (ANSES, 2020))

Air 1,3 BD (ppm)	n	DHBMA in urine	MHBMA in urine	Reference
–	10	630 ± 190 µg/l	–	Bechtold et al. (1994)
occasionally	3	1390 ± 550 g/l	–	
3–4	7	3200 ± 1600 µg/l	–	
–	6	580 ± 191 µg/g creatinine	–	Wardet al. (1996)
0.03[a]	5	355 ± 250 µg/g creatinine	–	
3.5 [a]	8	1690 ± 201 µg/g creatinine	–	
0.12	8	684 ± 176 µg/g creatinine	–	Wardet al. (1996)
0.21	7	596 ± 155 µg/g creatinine	–	
0.30	7	761 ± 245 µg/g creatinine	–	
0.3	19	694 ± 365 µg/l	–	Hallberg et al. (1997)
2.4	24	2429 ± 1877 µg/l	–	
1.0 [a]	7	600 µg/g creatinine	–	Hayeset al. (2000)
1.1 [a]	6	1500 µg/g creatinine	–	
3.5 [a]	3	700 µg/g creatinine	–	
45 [a]	9	8700 µg/g creatinine	–	
0.012	16	669 µg/l [b]	4.2 µg/l [b]	vanSittert et al. (2000)
4.3	5	2719 µg/l [b]	97 µg/l [b]	
0.01[a/b]	22	355 µg/l [b]	1.6 µg/l [b]	vanSittert et al. (2000)
0.17 [a/b]	23	508 µg/l [b]	3.6 µg/l [b]	
0.49 [a/b]	30	1479 µg/l [b]	20 µg/l [b]	
0.01 [a]	25	353 ± 157 µg/l	1.7 ± 1.5 µg/l	Albertini et al. (2001)
0.28 [a]	24	764 ± 728 µg/l	9.4 ± 13.0 µg/l	
0.77 [a]	33	4647 ± 6630 µg/l	120.2 ± 228.2 µg/l	
0.15	23	585 µg/g creatinine	–	Ammenheuser et al. (2001)
1.48	24	2046 µg/g creatinine	–	
–	10	1610 ± 600 µg/g creatinine	–	Fustinoni et al. (2002)
0.024	30	1800 ± 940 µg/g creatinine	–	
0.0004	43	602 ± 207 µg/l	7.5 ± 7.0 µg/l	Fustinoni et al. (2004)
0.005	42	605 ± 409 µg/l	10.5 ± 13.7 µg/l	
0.003	25 ♂	513 ± 272 µg/l	8.3 ± 10.1 µg/l	Albertini et al. (2007)
0.004	26 ♀	332 ± 285 µg/l	14.9 ± 10.3 µg/l	
0.176	23 ♀	508 ± 597 µg/l	19.2 ± 27.5 µg/l	
0.359	30 ♂	854 ± 567 µg/l	47.9 ± 44.3 µg/l	
0.14±0.15	16♀	716,1 ± 830,7 µg/L	8.3± 8,1 µg/l	Kotapati et al. (2015)
0.30±0.18	16♂	3136,1 ± 2560,3 µg/L	95,9 ± 111 µg/L	

(DFG, 2021) developed EKA correlation for both DHBMA and MHBMA:

- For DHBMA it considered that the most suitable study to use to propose a correlation is that of (Ammenheuser et al., 2001). Indeed the air and urine samples were taken the same day, which is important as 1,3-BD had a short half-life). Also the urine values were corrected with creatinine excretion. Consequently, the following equation was used to develop the EKA correlation: C_{DHMBA} (µg/g creatinine) =1300 * C_{BD} (ppm) +300

- For MHBMA, it considered the study by (Albertini et al., 2001) which identified that MHBMA is formed at a 32-fold lower rate than DHBMA. Consequently the EKA correlation was derived applying such correction to the slope of the correlation found for DHMBA (see above).

(ANSES, 2020) considered that there were enough data to develop correlations for MHBMA and DHBMA in urine based on the studies of (van Sittert et al., 2000), (Albertini et al., 2001) and (Kotapati et al., 2011). However, no VLB was proposed because low concentrations (0.008 mg/m^3 and 0.0008 mg/m3, corresponding to risk of 10^{-5} and 10^{-6} extrapolation would be needed. It should be noted that air correlation intended for the ANSES extrapolation are much lower than those considered by DFG.

It is not proposed to set a BLV or a BGV for 1,3 BD:

- Although the data available on correlations between air and urine exposure would allow the derivation of a BLV, the value derived will be an extrapolation carrying out high uncertainty, if the final OEL proposed is very low.
- As 1,3 BD does not bioaccumulate and there is no indication of dermal uptake, the occupational exposure can be well controlled via an OEL.

6.2.3 Biomonitoring analytical methods

Several analytical methods are available for the determination of the biomarkers described at the beginning of section 6.2.
Table 14 provides an (non-exhaustive) overview of the methods available to measure the urinary biomarkers for 1,3 BD.

Table 14: Analytical methods for 1,3 BD B metabolites in urine

Biomarker	Method	Analytical technique	LOQ
DHBMA in urine	(DFG, 2007)	LC–MS/MS(1)	75.9 µg/L
DHBMA in urine	Albertini et al. 2003	GC-NECI-MS-MS(2)	5 µg/L
MHBMA in urine	(DFG, 2007)	LC–MS/MS(1)	2.73 µg/L
MHBMA in urine	Albertini et al. 2003	GC-NECI-MS-MS(2)	0.1 µg/L
3-MHBMA in urine	Alwis et al 2012	LC-ESI-MS/MS(3)	0,6 µg/L

(1) Liquid Chromatography with tandem mass spectrometry
(2) Gas Chromatography with negative electron-capture ionization and tandem mass spectrometry
(3) Liquid Chromatography-Electrospray Ionization- tandem mass spectrometry

6.2.4 Data which may impact the results interpretation

Exposure to chloroprene, chlorinated derivate from 1,3-BD, was described as leading to the formation of MHBMA et DHBMA (Eckert et al. 2013), without specifying which quantitative influence this concomitant exposure may have on the urinary levels of DHBMA and MHBMA. Also competitive inhibition of 1,3-BD metabolism by styrene was described in (Laib et al. 1992) without specifying which quantitative influence this concomitant exposure may have on the urinary levels of DHBMA and MHBMA. Concerning MHBMA, genetic polymorphism of GST and epoxide hydrolase may also have an influence (Albertini et al. 2007).

7. Health Effects

7.1 Toxicokinetics (ADME- absorption, distribution, metabolism and excretion)

7.1.1 Human data

There are limited human data on the toxicokinetics of 1,3-BD and its metabolites.
In workers, exposed by inhalation to 3-4 ppm of 1,3-BD, metabolism to epoxybutene (EB) with subsequent hydrolysis to butenediol (B-diol) occurs. The mercapturic acid (glutathione) conjugate of B-diol has been identified as a urinary metabolite although no detectable levels of the EB-mercapturate were found. This suggests that detoxification of EB by hydrolysis to B-diol, with subsequent conjugation (EU RAR, 2002) (see also Figure 1).

7.1.1.1 1,3-BD

- A study by Lin et al. (2001) set up a human inhalation study to explore influential physiologic factors that determine the respiratory uptake of 1,3-BD. 133 healthy volunteers in Boston, Massachusetts (USA) were exposed to 2 ppm (4.42 mg/m^3) of 1,3-BD for 20 minutes, followed by purified air for another40 minutes (under an approved human subjects protocol). Five exhaled breath samples collected during exposure were used to determine the respiratory uptake of 1,3-BD, which was defined as absorbed 1,3-BD (micrograms) per kilogram of bodyweight during exposure. Although subjects were given identical administered doses (40 ppm/min), there was a wide range of uptake (0.6–4.9 µg/kg). Of the studied physiologic factors, the blood air partition coefficient and alveolar ventilation were most significant in determining the respiratory uptake ($p< 0.001$ for each). In the multiple regression analysis, females had significantly higher respiratory uptake of 1,3-BD than males on a weight basis. For all subjects, increasing age and cigarette smoking led to significantly decreased respiratory uptake of 1,3-BD.

7.1.1.2 Metabolites of 1,3-BD

- Albertini et al. (2007) found that females appeared to absorb less 1,3-BD per unit of exposure, as reflected by urine metabolite concentrations. No difference has been seen between genders in the pattern of 1,3-BD detoxification, as evidenced by urinary metabolite levels [1,2-dihydroxy-4-acetyl) butane and 1-dihydroxy-2-(N-acetylcysteinyl)-3-butene].
- Urinary excretion rates of the metabolites DHBMA and MHBMA are reported to be > 97% and < 3% respectively but the fraction of the inhaled dose is not known (INRS, 2023). In smokers, the relative excretion of mercapturic acids is distributed as follows: 93% for DHBMA, 5% for THBMA and 2% for MHBMA (Kotapati et al., 2014).
- Haemoglobin adducts from various metabolites of 1,3-BD have been identified and measured in humans. Elevated levels of the haemoglobin adduct EB have been reported in the blood of 1,3-BD occupationally exposed workers (in EU RAR (2002)).
- Analytical techniques have recently been developed to measure the haemoglobin adduct (diepoxide metabolite of 1,3-BD) N, N-(2,3-dihydroxy-1,4-butadiyl) valine (pyr-Val). Swenberg et al. (2007) reported that the pyr-Val adduct was not quantifiable in human blood samples from workers with cumulative occupational exposures of up to 6.3 ppm-weeks. In a subsequent study in which improvements were made to the technique to improve the sensitivity, quantifiable amounts of pyr-Val were found in the blood of occupationally exposed workers. At exposures between 0.1 and 1.0 ppm humans form approximately 10% of the quantities of the pyr-Val adduct formed by rats (Georgieva et al., 2010).
- *In vitro* studies, using human tissue, pointed out that metabolism of 1,3-BD to EB occurs in human liver, lung and bone marrow. Further metabolism of the monoepoxide to diepoxybutane (DEB) was studied, in liver and lung tissue, no detectable levels of the diepoxide were measured. Human liver tissue had greater capacity for metabolism to EB compared with lung tissue. However, the results for lung tissue must be treated with some caution as diseased tissue was used. There is evidence for considerable inter-individual variation in the capacity of human liver tissue to metabolise 1,3-butadiene to EB, with some human liver tissue samples showing capacity for metabolism comparable to, or exceeding, that in the mouse. The involvement of specific cytochromes P450 in the metabolism of 1,3-BD to the monoepoxide has been demonstrated, and raises the possibility that differences in expression of cytochromes P450 may explain some of the intra-individual variability that has been seen *in vitro* (reported in EU RAR (2002)).

7.1.2 Animal data

7.1.2.1 Absorption

Studies in rodents and non-human primates have shown that 1,3-BD is absorbed through the lungs ((EU RAR, 2002); (SCOEL, 2007); (ATSDR, 2012)). The model simulates concentrations of 1,3-butadiene and 3,4-epoxy-1-butene in lung, liver, and kidney, all target tissues for 1,3-butadiene metabolite intoxication, as well as in blood, fat, gastrointestinal tract, and lumped

compartments for muscle and viscera (Kohn and Melnick 1993, 1996, 2000, 2001; as cited in (ATSDR, 2012)).

Uptake and metabolism obey simple first order kinetics (EU RAR, 2002).
In close-chamber studies, the uptake of inhaled 1,3-BD in mice and rats was linear to 2,000 and 1,000 ppm, respectively, above which metabolism is saturated (Kohn and Melnick 2001). Absorption of 1,3-bd was demonstrated by measurements of the metabolite, 1,2-epoxy-3-butene (EB), in the test chamber (due to exhaled air) and measurement of 1,3-BD metabolites in blood of male Sprague- Dawley rats exposed 1–10,000 ppm and male B6C3F1 mice exposed to 1–6,000 ppm for 6–8 hours in closed chambers (Filser et al. 2007). In rats, EB concentrations in the test chamber reached a plateau at all exposure concentrations. In mice, chamber concentrations of EB were higher than for rats. EB levels reached a plateau at exposure concentrations up to 1,000 ppm, but no plateau was observed at exposure concentrations of 2,000–6,000 ppm. The study authors suggest that this concentration-dependence is due to "breakdown" of hepatic glutathione-S-transferase mediated 1,3-BD conjugation (as cited in (ATSDR, 2012)).

EU RAR (2002) reports quantitative species differences in the toxicokinetics of 1,3-BD. In comparison with the rat, the mouse absorbs and retains approximately 4-7-fold higher concentrations of 1,3-BD per kg bodyweight. At equivalent exposures, the mouse also produces approximately 2-20-fold higher concentrations of EB, than the rat.
Very low concentrations of the diepoxide metabolite (tentatively identified in the blood of monkeys, *in vivo)* have been detected in the blood and various tissues of rats and mice at relatively high 1,3-BD exposure. Again, where measurements are available, tissue levels of diepoxybutane (DEB) are generally higher in mice compared with rats, by up to 163-fold.

7.1.2.2 Distribution

1,3-BD is distributed widely in the body ((SCOEL, 2007); (EU RAR, 2002)).

In rats, partition coefficients were highest for fat (21.9), similar for liver, kidney, muscle, and spleen (0.87–0.94), and lowest in brain (0.43) (Johanson and Filser 1993, as cited in (ATSDR, 2012)).

7.1.2.3 Metabolism

1,3-BD is rapidly metabolised by cytochrome P450-dependent mono-oxygenases: the primary metabolite of BD is 1,2-epoxy-3-butene (EB), which is further metabolised by three pathways: (i) hydrolysis by epoxide hydrolases to 3-butene-1,2-diol (ii) further epoxidation to 1,2,3,4-diepoxybutane, and (iii) conjugation with glutathione (Malvoisin *et al*., 1979; Malvoisin and Roberfroid, 1982, as cited in SCOEL (2007)) (see Figure 1).

EB may be oxidised to 1,2:3,4-diepoxybutane (DEB) or hydrolysed to 1,2-dihydroxy-3-butene (DHB). Hydrolysis of DEB yields 3,4-epoxy-1,2-butanediol (EBD), which may also be formed from DHB by epoxidation of the double bond. The epoxides (EB, DEB, and EBD) have the potential to react with DNA and proteins, such as haemoglobin (Hb). Alternatively, they may be inactivated via hydrolysis or conjugation with glutathione (GSH).
In both rats and mice, (Boogaard et al., 2001) found that major metabolites derived from EB were: 1,2-dihydroxybutyl mercapturic acid (DHBMA, also referred to as MI), formed by hydrolysis of EB to DHB followed by subsequent metabolism to DHBMA, and an isomeric mixture of 1- and 2-monohydroxy- 3-butenyl mercapturic acid (MHBMA, also referred to as MII), formed via direct conjugation of EB with GSH. In the blood, EB may react with the *N*-terminal valine of Hb which leads to the formation of MHBVal, a stable Hb adduct.

According to both *in vitro* and *in vivo* data, the biotransformation appears to be qualitatively similar across species, including humans (Kreuzer *et al*., 1991; Csanády *et al.,* 1992; Sabourin *et al*., 1992, as cited in SCOEL (2007)). However, because of differences in uptake and kinetics, the steady-state blood and tissues levels are quantitatively different.

Metabolic elimination of 1,3-BD is linearly related to ambient exposure concentration up to about 1000 ppm (2250 mg/m^3) in rats and mice, with mice showing higher elimination rates. The metabolic pathways appear to be saturated above 1000 ppm (2250 mg/m^3) in rats and mice, and above 300 ppm (675 mg/m^3) in monkeys (Kreiling *et al*., 1986, 1987; Sabourin *et al*., 1992). The body burden for 1,2-epoxy-3-butene appears to be up to three times higher for mice than for rats (Kreiling *et al*., 1986, 1987; Bond *et al*., 1986; Dahl *et al*., 1991) (as cited in SCOEL (2007)).
Kreiling et al. (1986) investigated the pharmacokinetics of 1,3-BD in mice after inhalation exposure of 10 to 5000 ppm (22-11063 mg/m^3) in a closed system and compared it with that of rats. Linear pharmacokinetics applied in both species at exposure concentrations below 1000 ppm, saturation of metabolism was observed at concentrations of about 2000 ppm. Metabolic clearance in the lower concentration range where first order metabolism applies was 7300 mL/h (rat) and 4500 mL/h (mice). Maximal metabolic elimination rate (Vmax) in mouse was 400 pmol/h/kg compared with 220 pmol/h/kg in rats. The results show that the higher rate of 1,3-BD metabolism in mice when compared to rats may only in part be responsible for the considerable difference in the susceptibility of both species to 1,3-BD-induced carcinogenesis.

Finally *in vivo* data on primates and *in vitro* data on human tissues suggest that humans and other primates are closer to rats than mice with regard to the metabolism of 1,3-BD and resultant body burden of 1,2-epoxy-3-butene (Sabourin *et al*., 1992) (as cited in SCOEL (2007)).

However, the data showed a much higher rate of hydrolytic metabolism of 1,3-BD in humans compared to animals, which was reflected in a much higher DHBMA/(MHBMA+DHBMA) ratio, and in much lower levels of MHBVal in humans, confirming *in vitro* results. Assuming a genotoxic mechanism, the data of this study coupled with recent data on DNA and Hb binding in rodents, suggest that the cancer risk for humans from exposure to 1,3-BD will be less than for the rat, and much less than for the mouse (Boogaard et al., 2001).

As per Figure 1, 1,3-BD is metabolized to at least three genotoxic metabolites: a monoepoxide (1,2-epoxy-3-butene, EB), a diepoxide (1,2:3,4-diepoxybutane, DEB), and an epoxydiol (3,4-epoxy-1,2-butanediol, EBD) (Himmelstein et al., 1997, Melnick and Kohn, 1995).

Himmelstein et al. (1994) (as cited in ECHA (2023)) investigated species differences in 1,3-BD metabolism at exposure of 62.5, 625 and 1250 ppm (equivalent to 140, 1406 or 2812 mg/m^3): the *in vivo* blood concentrations of 1,3-BD, butadiene monoxide and butadiene diepoxide in rats (5-12 males per exposure) and mice (13-19 males per exposure) were measured during and following 6-hr exposures to inhaled 1,3-BD.

- Collection of blood: During exposure, blood samples for the measurement of 1,3-BD and butadiene monoxide were collected at 2, 3, 4 and 6 hours of exposure. Blood samples for the measurement of butadiene diepoxide were collected at 3 and 6 h of exposure. After exposure, blood samples for all of these were collected at 2-10 min intervals up to 30 min post-exposure. A minimum of three blood samples per time point were collected.
- Absorption: 1,3-BD concentrations in blood were at steady-state 2 hours after the start of exposure and remained constant during exposure to 62.5, 625 and 1250 ppm. The concentration in mice blood was 2-fold that of rats. 1,3-BD levels in blood were not proportional to the inhaled concentrations suggesting saturable uptake in both species. Levels declined rapidly after exposure, such that by 30 min they were 1-12% of the steady state concentration.
- Metabolites: Butadiene monoxide was detected in blood of rats and mice but levels in mice were 4-8 times higher than in rats. Levels declined rapidly after exposure, such that by 20 min they were 10-40% of the steady state concentration. Butadiene diepoxide was only detected in the blood of mice.

These data suggest that the greater sensitivity of mice to 1,3-BD-induced toxicity and carcinogenicity compared to rats, may be partially explained by the increased metabolism resulting in higher concentrations of the mono- and di-epoxides.

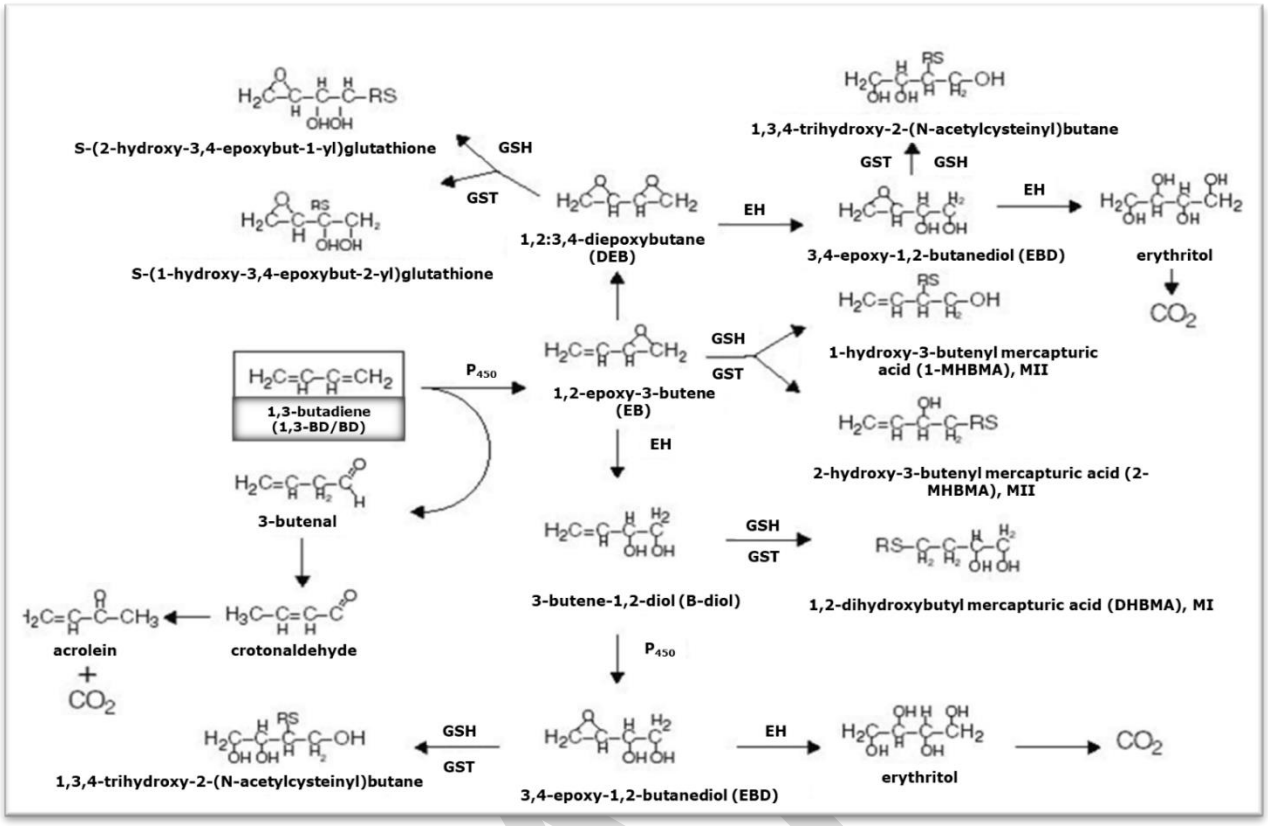


Figure 1: Metabolism of 1,3-BD, adapted from (Health Canada, 2000)

More recent studies have confirmed that mice form greater quantities of the diepoxide metabolite than rats. Studies using 1,3-BD exposures at 1 ppm for 4 weeks (which are more occupationally relevant) showed that the concentration of the haemoglobin adduct of the diepoxide metabolite (pyr-Val) was greater than 30-fold in the blood of mice compared to that in rats (Swenberg et al. 2007, as cited in ECHA (2023)). Georgieva et al (2010) (as cited in ECHA (2023)) also exposed rats and mice to 1,3-BD at 0.1 to 625 ppm for 10 or 20 days and showed that mice formed 10- to 60-fold more of the haemoglobin adduct compared to rats at similar exposures.

7.1.2.4 Excretion

Metabolic elimination of 1,3-BD is linearly related to ambient exposure concentration up to about 1000 ppm (2250 mg/m^3) in rats and mice, with mice showing higher elimination rates (SCOEL, 2007).
About half of inhaled 1,3-BD is metabolised and exhaled, while the other half is excreted in the urine, within 10 hours (ATSDR, 2012).

7.1.3 *In vitro* data

In vitro studies indicate that in the mouse, lung and liver tissue have similar capacity for 1,3-BD metabolism while in rats and humans, liver tissue has a greater capacity for metabolism than does lung tissue, although some metabolism does take place in lung tissue (ECHA, 2023).

Detoxification pathways are kinetically favoured over activation pathways in rodent and human tissue, although the ratio of activation: detoxification is highest in mouse tissue compared with rat or human tissue:

- In mouse liver and lung tissue, detoxification of EB appears to be mainly by conjugation with glutathione, with hydrolysis to B-diol, a relatively minor pathway.
- In human liver and lung, in comparison, detoxification of EB is primarily by hydrolysis, with only some glutathione conjugation. This finding from *in vitro* studies supports the *in vivo* human metabolism data.
- Formation of the diepoxide has been demonstrated in mouse liver tissue exposed to 1,3-BD

in vitro, but not in rat or human tissue, although formation of DEB has been demonstrated in cDNA-expressed human liver microsomes exposed to EB.

A recent study (Filser et al, 2010, as cited in ECHA (2023)) showed a qualitative species difference in the metabolism of 1,3-BD in isolated perfused livers from rats and mice: predominantly EB and B-diol were found in both species but DEB was only detected in mouse livers.

7.1.4 Summary

There are limited human data on toxicokinetics of 1,3-BD showing that gender and smoking status may affect its respiratory uptake. Urinary concentrations of 1,3-BD metabolites reflect the process of 1,3-BD excretion.

In vivo, 1,3-BD is well-absorbed through the lungs and widely distributed through the body. It is then rapidly metabolised by cytochrome P450-dependent mono-oxygenases, and further conjugation occurs. Uptake and metabolism obey simple first order kinetics.

According to both *in vitro* and *in vivo* data, the biotransformation appears to be qualitatively similar across species, including humans. However, quantitatively, *in vivo* data on primates and *in vitro* data on human tissues suggest that humans and other primates are closer to rats than mice. Further the data showed a much higher rate of hydrolytic metabolism of 1,3-BD in humans compared to animals, which was reflected in a much higher DHBMA/(MHBMA+DHBMA) ratio, and in much lower levels of MHBVal in humans, confirming *in vitro* results.

From the limited comparative information available from *in vitro* and *in vivo* studies, it appears that in relation to the formation of epoxide metabolites, the metabolism of 1,3-BD in humans is quantitatively more similar to that of the rat, rather than the metabolism in mouse. However, *in vitro* studies have demonstrated considerable inter-individual variability in the oxidative metabolism of 1,3-BD (ECHA (2023)).

7.2 Acute toxicity

7.2.1 Human data

There is little information available on the human effects of single exposure to 1,3-BD and the data are of poor quality.

EU RAR (2002) reviewed available literature and reported these findings:

- A slight increase in pulse rate was noted as a result of breathing 10,000 ppm 1,3-BD for 5 minutes (Larionov et al. 1934; as cited in EU RAR (2002)). Blood pressure and respiration were apparently not markedly affected. In volunteers exposed to 1,3-BD, unsteadiness was noted after exposure at 4000 ppm for 6 h and the odour was described as objectionable, but there was little effect at 8000 ppm (17,701 mg/m3) for 8h (Carpenter et al, 1944). Other human studies described in the EU RAR (2002) are stated to be "unreliable and of doubtful significance" and are not described here.
- In separate studies with 4 volunteers per test, sensitivity of the eye to light was reported to be altered following exposure to 1.7 ppm 1,3-BD and an electrocortical conditioned response (light stimulated desynchronisation of α-rhythm of the brain) occurred at 1.6 ppm (Ripp, 1965, 1967; as cited in EU RAR (2002)). No effect levels were 1.6 ppm and 1.4 ppm, respectively. According to EU RAR (2002)), *"these studies are unconventional and unreliable and the results are considered of doubtful significance"*.

7.2.2 Animal data

In studies of limited quality, *in vivo* studies have shown a low toxicity for 1,3-BD.

7.2.2.1 Acute oral toxicity

Oral LD_{50} values of 5,480 mg/kg and 3,210 mg/kg have been reported for the rat and mouse

respectively (Ripp, 1969, as cited in EU RAR (2002).

7.2.2.2 Acute inhalation toxicity

Values of LC_{50}> 100 000 ppm were observed in mice after inhalation exposure up to 4 hours. The main clinical signs were respiratory irritation and narcosis (ANSES, 2022).

Overall, the quality of data on the acute inhalation toxicity of 1,3 -BD is poor. In the rat the LC_{50} value after a 4h exposure was 285,000 mg/m^3 (128,803 ppm) and in the mouse was 270,000 mg/m^3 (122,024 ppm) after 2h exposure. Rapid onset of narcosis was reported in both species (Shugaev, 1969). Similar results from other low-quality studies are described in the EU RAR (2002).

7.2.3 Summary

Limited human data on acute 1,3-BD toxicity suggest that humans can tolerate an exposure concentration of 17,702 mg/m^3 (8000 ppm) for 8 hours without adverse symptoms.

There are only limited, poor quality data on the single exposure toxicity of 1,3-BD. The lowest LC_{50} is 270,000 mg/m^3 (122,024 ppm) for 2h in mice. It can be concluded that 1,3-BD is of low acute toxicity following single inhalation or oral exposure.

In conclusion, 1,3-BD has low inhalation acute toxicity in both humans and animals (EU RAR (2002)).

7.3 Specific target organ toxicity/Repeated dose

7.3.1 Human data

In humans, associations between for 1,3-BD exposure were observed for the following effects (further described separately in the below sections):

- Cardiovascular effects
- Neurological effects
- Pulmonary functions
- Diabetes
- Childhood diseases

7.3.1.1 Cardiovascular effects

The association between cardiovascular disease (CVD) and 1,3-BD was observed in the following studies:

- Matanoski et al. (1990) examined mortality patterns in 12110 (1195 black and 10915 white) male workers employed 1 or more years in eight styrene-butadiene polymer (SBR) manufacturing plants in the United States and Canada between 1943-1982. No attempt has been made to assess individual exposures. Authors divided the population into major work activities (production, warehouse and shipping, maintenance, laboratory and quality control, research and development, administration and other) in examine different patterns of risk. The production grouping includes workers involved in any processes that produce the rubber and who may, therefore, have had some exposure to the basic chemicals that form the raw product. Maintenance includes workers exposed to materials related to their trades and incidental exposures to the agents used in the industrial processes. Utilities represent support facilities whose workers may have exposures to specific agents. The other-job category includes warehouse, laboratory, and administration work sites. This diverse group had workers who probably had exposures in laboratories and other workers such as administrative personnel who received no exposure. A comparison with the general population has been done with the help of standardised mortality ratios (SMR), after adjustment for age, calendar time and race. Maintenance workers showed increased SMR for digestive cancers (SMR=1.06, 95% CI=0.81-1.35). SMR of arteriosclerotic heart disease (SMR =1.76; 95% CI: 1.36-2.33) as well as of the combined category of circulatory disease (SMR = 1.38; 95% CI: 1.14-1.66) were significantly elevated in blacks who worked in maintenance jobs. This study is further

referred to in Section 7.7.1.

- Shin et al. (2015) examined associations between personal exposures to volatile organic compounds (VOC) and cardiovascular parameter alterations in 63 participants engaged in the US EPA's cross-sectional Detroit Exposure and Aerosol Research Study (DEARS). Measured exposure to 1,3-BD varied between 0.13 µg/m^3 to 14.82 µg/m^3 (mean = 1.38 µg/m^3). A principal component analysis (petroleum, 1,3-BD and ambient (Freon) VOC sources) and a linear mixed model were employed. More than half of 63 participants were black people (n =35, 55.6%). Authors observed that 1,3-BD related VOC decreased diastolic blood pressure (association estimate=-25.368, p-value=0.019) but increased heart rate (association estimate=30.307, p-value=0.048) and brachial artery diameter (association estimate=1.277, p-value=0.008).
- McGraw et al. (2021) examined the vascular effects of exposure to individual VOCs and mixtures of VOCs (related to acrolein, 1,3-BD, and crotonaldehyde) in 346 black non-smokers with varying levels of CVD risk. Authors measured urinary metabolites of acrolein (CEMA and 3-HPMA), 1,3-BD (DHBMA and 3-MHBMA), and crotonaldehyde (HPMMA). The urinary levels of DHBMA were observed to exhibit strong associations with the urinary levels of norepinephrine (15.7% higher, 95%CI=5.6-27%, p-value=0.001) and normetanephrine (11.6% higher, 95%CI=3.7-20%, p-value=0.002) (two CVD risk markers). According to authors, this finding may indicate endothelial dysfunction and contribute to elevated risk of hypertension in people with increased sympathetic tone.
- Lin et al. (2020) examined associations between urinary levels of the DHBMA and CVD risk factors in 853 Taiwanese study participants. A linear regression with adjustment for sex, age, smoking, sweets in the diet, and fat in the diet was used. Linear regression coefficients (standard error) of CVD risk factors and unit increase in ln-DHBMA concentrations (µg/g creatinine) were reported. Authors reported a positive association between the urinary DHBMA levels and the CVD risk factors, including low density lipoprotein cholesterol (β=1.81, SE=0.84, p-value=0.031) and carotid intima-media thickness (β=5.76, SE=1.44, p-value=<0.001).

7.3.1.2 Neurological effects

Carpenter et al. 1944 (as reported by ATSDR (2012)) evaluated psychomotor responses of two men inhaling 2,000, 4,000, or 8,000 ppm 1,3-BD for 6–8 hours/day on different days. At the two higher concentrations, the subjects performed a steadiness test. At the highest concentration, a tapping rate test was also performed. However due to a lack of details this study is considered inadequate for evaluation of the potential effects of 1,3-BD in humans (Health Canada, 2000).

7.3.1.3 Pulmonary functions

Acute respiratory effects of 1,3-BD in humans are described in Section 7.4.1.

- Sadeghi-Yarandi et al. (2020) evaluated pulmonary function among 50 workers exposed to 1,3-BD at a petrochemical industry in Iran. The study participants consisted of 50 male workers with current respiratory exposure to 1,3-BD and 50 non-exposed workers as the control group. Workers were exposed to high 1,3-BD concentrations (mean±SD (standard deviation) = 560.82 ± 811.36 µg/m^3 (0.253 ± 0.367 ppm); mean= 560.82 µg/m^3). Workers had significantly higher prevalence rates of cough (odds ratio (OR) = 6.02, p-value=0.013), phlegm (OR = 15.58, p-value=0.01), productive cough (OR=9.41, p-value=0.003), wheezing (OR=8.82, p-value=0.01), dyspnea (OR=19.17, p-value=0.01), chest tightness (OR=6.74, p-value=0.018), and episodes of chest illness associated with cold (OR=10.41, p-value=0.01).
- Sathiakumar et al. (2021a) examined exposure-response relationships between 1,3-BD and styrene and selected diseases among styrene-butadiene rubber (SBR) workers. The cohort included 21087 workers (16579 men and 4508 women) that were followed from 1943 through 2009. The mortality analysis included cancer outcomes (summarised in Section 7.7.1.1) and non-malignant respiratory disease (NMRD), chronic obstructive pulmonary disease (COPD) and pneumonia. Authors observed no evidence of exposure-response between 1,3-BD

exposure and all NMRD or pneumonia among men or women. Results for COPD among men indicated a slightly increased rate in each quartile (quartile (Q) cut points were at the following values of ppm-years: Q2, 25.36; Q3, 81.18; Q4, 235.56, maximum = 7026.58) of exposure compared to the unexposed and a statistically significant, positive exposure-response trend in analyses using all person-time but not in analyses restricted to the exposed person-time. According to Sathiakumar et al. (2021a), "*This pattern of results suggests that chance or a difference in a confounding factor, such as smoking, between the unexposed and exposed person-time is a more likely explanation of the results rather than a causal relationship between monomer exposure and COPD in men*." Among women, COPD RR was largely below or close to the null, and there was no evidence of an exposure-response trend in any analysis.

7.3.1.4 Diabetes

Liang et al. (2023) examined associations between the urinary 1,3-BD metabolite DHBMA (quartiles (Q), µg/L : Q1 =≤198.50, Q2 =198.51–327.00, Q3= 327.01–502.00, Q4 = >502.00) and glucose homeostasis in 5092 US general residents from the National Health and Nutrition Examination Survey (NHNES). Glucose homeostasis was evaluated by fasting plasma glucose (FPG), fasting serum insulin (FINS), glycohemoglobin (HbA1c), and homeostasis model assessment of insulin resistance (HOMA-IR). DHBMA urinary level was associated with IR (OR=1.36, 95%CI=1.14-1.61), prediabetes 1.51 (OR=1.51, 95%CI=1.26-1.83) and diabetes (OR=1.20, 95%CI=0.9-1.61).

7.3.1.5 Childhood diseases

A recent epidemiological study provided some indications on potential associations between 1,3-BD and childhood diseases:

- Kuang et al. (2021) compared the differences in several biomarker levels between 252 asthmatic and 69 healthy children. Urinary DHBMA was used as the biomarker of parental 1,3-BD exposure. Levels of DHBMA were measured in asthmatic (geometric mean=108 µg/g creatinine, range=11.7-452 µg/g creatinine) and healthy (geometric mean=72.7 µg/g creatinine, range=5.31-572 µg/g creatinine) children. The results indicated that the increased urinary levels of DHBMA were significantly associated with asthma, after adjustment for gender, age and body mass index (OR = 2.76, 95% CI = 1.73-4.43).

7.3.2 Animal data

Several studies have assessed the repeated dose toxicity of 1,3-BD (Table 15).

7.3.2.1 Studies in rats

- Carpenter et al. (1944) performed a repeated dose toxicity inhalation study in rats and guinea pigs, exposed at doses of 0, 600, 2300 and 6700 ppm (equivalent to 0, 1350, 5175 and 15075 mg/m^3), for 7.5 hours/day, 6 days/week for 8 months. The highest concentration caused slight growth retardation and, in some animals, a mild reversible degeneration of the liver. ('light cloudy swelling'). There were no treatment-related effects reported in hematologic parameters, or blood or urine chemistry, nor were there pathological changes in the eye, adrenal gland, heart, kidney, skeletal muscle, pancreas, spleen, testis or ovary. A NOAEC of 600 ppm (1350 mg/m^3) in rats and 2300 ppm (5175 mg/m^3) in guinea pigs were established (both cited in SCOEL (2007); NTP (1993); EU RAR (2002)).

- Sprague-Dawley rats (males and females) were exposed to 0, 1000, 2000, 4000, and 8000 ppm 6 hours/day, 5 days/week for 13 weeks. No treatment-related gross, microscopic changes or effects were reported on growth, blood biological parameters, urinary measurements or neuromuscular functions (Crouch et al. (1979) as cited in NTP (1993)).

- Information on the repeated dose toxicity of 1,3-BD to the rat is also available from a carcinogenicity study, described in detail in section 7.7.2. Three groups of 110 rats per sex, were exposed to 0, 1,000 or 8,000 ppm 1,3-BD, 6 hours/day, 5 days/week for up to 105 weeks for females or 111 weeks for males (Owen, 1981; Owen and Glaister, 1990). An interim kill of 10 animals per sex per group was conducted at 52 weeks. Clinical chemistry and

haematological parameters were investigated at 3-6 monthly intervals and all animals were subjected to gross necropsy and comprehensive histopathological examination at sacrifice. The study was well-conducted and conformed to current regulatory guidelines.
There was a slight, statistically significant reduction in survival in animals exposed to 8,000 ppm. In the first 12 weeks of exposure, a transient, statistically significant reduction in body weight gain was seen in both sexes at 8,000 ppm and in males at 1,000 ppm. Minor, treatment-related clinical signs of toxicity – wet and ruffled fur together with slight limb weakness or incoordination following dosing on the first day of the 5-day schedule – were seen between 2 and 5 months of treatment in animals at 8,000 ppm. At the end of the study, statistically significant increases were seen in liver weight in all exposure groups, but there was no associated pathology. In addition, males at 8,000 ppm had statistically significantly increased kidney, heart, lung and spleen weights, with associated nephrosis of the kidney and focal metaplasia in the lung. There were no treatment related changes in clinical chemistry or haematological parameters, urinalysis or neuromuscular function. Overall, 1,3-BD is of low toxicity to the rat when administered at high concentrations over an extended period. A NOAEC of 1,000 ppm for systemic toxicity can be identified, with minimal toxic effects at 8,000 ppm.

7.3.2.2 Studies in mice

- NTP (1984) reported a repeated dose toxicity inhalation study in male and female B6C3F1 mice, exposed to 625 and 1250 ppm, 6 hours/day, 5 days/week for 14 weeks. Since this study was terminated after 60 weeks of exposure because of reduced survival due to fatal tumors, and because dose-response relationships for 1,3-BD-induced neoplastic and nonneoplastic lesions were not clearly established. A NOAEC of 625 ppm (1406 mg/m^3) was established.

- Exposure of male B6C3F1 mice or NIH Swiss mice to 1,250 ppm (2812 mg/m^3) of 1,3-BD for 6 weeks caused decreases in erythrocyte counts, hemoglobin concentrations, and hematocrit, and an increase in mean erythrocyte volume (Irons *et al.,* 1986a,b). Anemia was not accompanied by increases in reticulocyte counts or in the frequency of nucleated erythrocytes in peripheral blood. These changes were considered to represent a macrocytic- megaloblastic anemia, because they were accompanied by mild megaloblastic changes in bone marrow cells.

- In related studies, (Tice et al., 1987) reported that exposure of male B6C3F1 mice to 1,3-BD for 10 days caused decreases in the number and rate of dividing cells in the bone marrow. Thus, in mice exposed to 1,3-BD, hematopoiesis in the bone marrow is suppressed, and younger, larger cells are probably released into the blood from extramedullary sites. These findings established the bone marrow as a site of toxicity for 1,3-BD in mice.

- Consistently, exposure of B6C3F1 male mice to 1,250 ppm for 6 hours/day, 5 days/week, for 6 or 12 weeks produced extramedullary hematopoiesis in spleens of the mice but did not produce any persistent defects in humoral or cell-mediated immunity (Thurmond et al., 1986) The purpose of the study was to evaluate specific humoral and cell-mediated immunity and spontaneous cytotoxicity; lymphoid organ histopathology was also evaluated.

- Melnick et al. (1988) exposed B6C3F1 mice to 625 and 1250 ppm of 1,3-BD (equivalent to 1406 mg/m^3 and 2812 mg/m^3) for up to 61 weeks. The effects observed at both doses included non-neoplastic lesions such as epithelial hyperplasia of the forestomach, endothelial hyperplasia and mineralisation of the heart, alveolar epithelial hyperplasia, haemorrhage and necrosis of the liver, thymus and bone marrow atrophy, testicular atrophy, ovarian atrophy. Lesions in nasal tissues of males exposed to 1250 ppm included chronic inflammation, fibrosis, osseous and cartilaginous metaplasia, and atrophy of the olfactory epithelium (NTP, 1984, Melnick *et al.,* 1988). The proliferative lesions in the forestomach, heart, and lung may represent early preneoplastic changes in the development of neoplasms induced by 1,3-BD. This study demonstrates that butadiene causes severe toxicity in the mouse at these concentrations (as cited in NTP (1993); EU RAR (2002)).

- In an NTP (1993) study, groups of 70 male and 70 female B6C3F1 mice were administered 0 (chamber control), 6.25, 20, 62.5, or 200 ppm of 1,3-BD by inhalation for 6 hours/day, 5 days/week, for up to 103 weeks. Also 2 groups (90 male and 90 female mice) were administered 625 ppm 1,3-BD on the same schedule (Melnick et al. (1990b) as cited in (NTP, 1993)).
  Hematologic changes after 9 months of exposure included concentration-dependent decreases in erythrocyte counts, hemoglobin concentration, and packed red cell volume at exposure levels from 62.5 to 625 ppm in males and at levels of 200 or 625 ppm in females. These changes were not accompanied by significant increases in reticulocyte counts or in the frequency of polychromatic erythrocytes in peripheral blood. However, there was a statistically significant increase in the percentage of erythrocytes with Howell-Jolly body inclusions. Other hematologic changes caused by exposure to 625 ppm were an increase in mean erythrocyte volume and an increase mean in erythrocyte hemoglobin.
  Additionally, changes at other organ sites, bone marrow atrophy, and increases in splenic and hepatic extramedullary hematopoiesis were observed in mice exposed to 625ppm. These findings indicate partial or poorly regenerative, macrocytic anemia. The mechanism of the anemia cannot be determined from the data available from these studies. However, a mild megaloblastic anemia resulting from ineffective erythropoiesis in the bone marrow cannot be excluded.
  Thus, in mice exposed to 1,3-BD, hematopoiesis in the bone marrow is suppressed, and younger, larger cells are probably released into the blood from extramedullary sites. Consistent with this explanation, Thurmond et al. (1986) observed extramedullary hematopoiesis in spleens of male B6C3F1 mice exposed to 1,250 ppm for approximately 6 months.

- Himmelstein et al. (1997) reviewed the toxic effects (other than cancer) in animals. Higher inhaled concentrations oof 1,3-BD were related to biochemical alterations, including glutathione depletion in liver, lung and heart. This depletion was noted to be more complete and at lower inhaled concentrations in mice than in rats and was correlated with increased concentrations of the metabolites, butadiene monoepoxide and butadiene diepoxide.

- Studies on the ovarian toxicity of the mono- and diepoxide of 1,3-BD, as well as parallel studies on the epoxide metabolites of 4-vinyl-cyclohexene provide evidence that the diepoxide exerts specific ovarian toxicity, and that the formation of such a diepoxide metabolite may therefore be linked with ovarian toxicity and carcinogenicity of 1,3-BD (Bolt, 1996).

Table 15: Summary of studies on repeated dose toxicity after inhalation exposure to 1,3-BD

Species, strain, sex, number/ group	Doses, duration of exposure	Results	References*
Rats + guinea pigs	600, 2300 and 6700 ppm (equivalent to 0, 1350, 5175 and 15075 mg/m^3 7.5 hours/day, 6 days/week for 8 months	Top concentration caused slight growth retardation and, in some animals, a mild reversible degeneration of the liver. ('light cloudy swelling'). There were no treatment-related effects reported in hematologic parameters, or blood or urine chemistry, nor were there pathological changes in the eye, adrenal gland, heart, kidney, skeletal muscle, pancreas, spleen, testis or ovary. NOAEC = 600 ppm (1350 mg/m^3) in rats NOAEC = 2300 ppm (5175 mg/m^3) in guinea pigs	Carpenter et al. 1944 *
Sprague-Dawley rats (M+F)	0, 1000, 2000, 4000, and 8000 ppm 6 hours/day, 5 days/week for 13 weeks	No treatment-related gross, microscopic changes or effects were reported on growth, blood biological parameters, urinary measurements or neuromuscular functions NOAEC> 8000 ppm	Crouch et al. 1979 *
Rats (M+F)	0, 1,000 or 8,000 ppm, 6 hours/day, 5 days/week for up to 105 weeks for females or 111 weeks for males	Statistically reduction in body weight gain was seen in both sexes at 8,000 ppm and in males at 1,000 ppm. Statistically significant increases in liver weight in all groups, but no associated pathology Males at 8,000 ppm had statistically significantly increased kidney, heart, lung and spleen weights, with associated nephrosis of the kidney and focal metaplasia in the lung. No treatment related changes in clinical chemistry or haematological parameters, urinalysis or neuromuscular function.	Owen, 1981; Owen and Glaister, 1990
B6C3F1 mice	625 and 1250 ppm, 6 hours/day, 5 days/week for 14 weeks	A NOAEC of 625 ppm (1406 mg/m^3)	NTP, 1984
B6C3F1 mice or NIH Swiss mice	1250 ppm For 6 weeks	Decreases in erythrocyte counts, hemoglobin concentrations, and hematocrit, and an increase in mean erythrocyte volume (Anemia was not accompanied by increases in reticulocyte counts or in the frequency of nucleated erythrocytes in peripheral blood. These changes were considered to represent a macrocytic- megaloblastic anemia, because they were accompanied by mild megaloblastic changes in bone marrow cells. NOAEC = 1000 ppm	Irons et al., 1986a,b *
B6C3F1 male mice	6.25, 62.5 and 625 ppm For 10 days	Hematopoiesis in the bone marrow is suppressed, and younger, larger cells are probably released into the blood from extramedullary sites	Tice et. 1987 *
B6C3F1 male mice	1,250 ppm for 6 hours/day, 5	Extramedullary hematopoiesis in spleens but no persistent defects in humoral or cell-	Thurmond et al., 1986 *

Species, strain, sex, number/ group	Doses, duration of exposure	Results	References*
	days/week, For 6 or 12 weeks	mediated immunity	
B6C3F1 mice	625 and 1250 ppm 6 hours/day, 5 days/week, for up to 61 weeks	non-neoplastic lesions such as epithelial hyperplasia of the forestomach, endothelial hyperplasia of the heart, alveolar epithelial hyperplasia, hepatocellular necrosis, and lesions in nasal tissues including chronic inflammation, fibrosis, osseous and cartilaginous metaplasia, and atrophy of the olfactory epithelium	Melnick et al. 1988 *
B6C3F1 mice	6.25, 20, 62.5 and 200 ppm + 625 ppm 6 hours/day, 5 days/week, for up to 103 weeks	Concentration-dependent decreases in erythrocyte counts, hemoglobin concentration, and packed red cell volume at exposure levels from 62.5 to 625 ppm in males and at levels of 200 or 625 ppm in females. Partial or poorly regenerative, macrocytic anemia. Hematopoiesis in the bone marrow is suppressed, and younger, larger cells are probably released into the blood from extramedullary sites NOAEC = 20 ppm (hemato)	Melnick et al. 1990b NTP, 1993

* References are cited in NTP (1993).

7.3.3 Summary

Human data provided some evidence on the associations between 1,3-BD exposure and several adverse health effects (cardiovascular disease, respiratory diseases, diabetes, childhood diseases). The majority of epidemiological studies failed to examine dose-response relationships or examined associations with the urinary metabolites of 1,3-BD exposure.

The target organs of 1,3-BD in mice are the central nervous system, the bone marrow (and consequently blood cells) and also the reproductive organs (see also section 7.8.2).
Overall, 1,3-BD is of low toxicity to the rat when administered at high concentrations over an extended period.
Long-term exposure of animals to 1,3-BD resulted (in addition to cancer) in biochemical alterations such as glutathione depletion in liver, lungs and heart, which was more extensive in mice than in rats. In exposed mice toxicity of the haematopoietic system also was seen; semi-chronic exposure to 2,750 mg/m^3 resulted in anaemia, while leukopenia and an increase in the number of circulating erythrocyte micronuclei were reported..

7.4 Irritancy and corrosivity

7.4.1 Human data

No human data exist on skin irritation and corrosivity after exposure to 1,3-BD.
Eye and respiratory irritation effects at very high doses has been reported in these studies:

- Slight irritation and dryness of the nose and mouth were reported by human volunteers (no details on number and sex) exposed to 10000 ppm 1,3-BD for 5 minutes (Larionov et al. 1934; as cited in EU RAR (2002)).
- Two males were exposed to 2000 ppm 1,3-BD for 7 hours or 4,000 ppm for 6 hours reported eye irritation and blurred vision; however, repeated exposure resulted in less awareness of these symptoms (Carpenter et al. 1944; as cited in EU RAR (2002)).
- Irritation of mucosal surfaces has been reported by volunteers (no details on number and sex) exposed to 226 ppm 1,3-BD for 1 minute (Gostinsky 1965, as cited in EU RAR (2002)).

- Irritation of the eyes, nasal passages, throat and lungs with, on occasion, coughing and drowsiness, were noted in men exposed to 1,3-BD ((no details on number and sex; exposure concentration not stated) in American synthetic rubber plants (Wilson 1944, as cited in EU RAR (2002)).
- Irritation of the upper respiratory tract was also reported in workers (no details on number and sex) in Russian synthetic rubber plants (Bashirov 1975; Mukhametova et al. 1976; Nadirova 1967; Ripp 1967, as cited in EU RAR (2002)). According to Abdullaeva 1974 (as cited in EU RAR (2002)), it is not possible to relate these effects to 1,3-BD because there was also exposure to other chemicals.

7.4.2 Animal data

There have been no reports of skin or eye irritation for 1,3-BD (SCOEL, 2007)). An acute exposure to high concentrations of 1,3-BD (>100 000 ppm) leads to eye irritation (conjunctivitis or lacrimation) in mice or rabbits (see section 7.2.2) (EU RAR, 2002).

DECOS (2013) concluded that following acute exposure of humans and animals to high 1,3-BD concentrations in air, irritation of the eyes, nasal passage, throat and lungs were noted. Clinical signs of intoxication of animals included hyperventilation, twitching, excitation, anaesthesia and narcosis. LC_{50} values ranged from 270 000 (mice) to 550 000 mg/m^3 (rabbits).

7.4.3 Summary

No skin irritation has been mentioned in any studies in humans following single exposure to high concentrations, this suggests that 1,3-BD does not exhibit this property.

1,3-BD is an irritant to the eyes, nose and mouth as reported in humans at very high exposure concentrations (of the order of thousands of ppm).
Very limited information is available *in vivo*, showing that 1,3-BD is not an eye or skin irritant at reasonable concentrations.

7.5 Sensitisation

7.5.1 Human data

There are no human data on the skin or respiratory sensitisation potential of 1,3-BD.

7.5.2 Animal data

There are no *in vivo* or *in vitro* data on the skin or respiratory sensitisation potential of 1,3-BD.

7.5.3 Summary

There have been no reports of skin and respiratory sensitisation in both humans and laboratory animals.

7.6 Genotoxicity

1,3-BD has an entry in Annex VI of the CLP regulation as a category 1B mutagen.

7.6.1 Human data

7.6.1.1 Mutagenicity (gene level)

Several studies have examined the mutagenic potential of 1,3-BD in occupationally exposed workers, producing inconsistent results.

(Albertini et al., 2010) reported a number of studies (published between 1993-2009; cited therein) of workers exposed in either a monomer production facility or in a styrene-butadiene rubber plant in Texas (USA), assessing the frequencies of *HPRT* mutations in peripheral blood lymphocytes (PBL). Although significant elevations of the mean variant/mutation frequencies

were initially reported in exposed workers over controls - associated to the length of employment- associations between individual variant frequencies and 1,3-BD exposure levels within preassigned groups could not be established in follow-up studies. A 'memory' of *HPRT* mutations from higher past exposures or a potential confounding agent could not be excluded. The Texas studies would indicate that mean BD exposure levels of >3.0 ppm (TWA in some studies) or greater, are required to induce these mutations. Additionally, an inverse correlation between mEH activity (resulting from specific *mEH* polymorphisms) and *HPRT* mutation frequencies was reported among exposed Texas workers (Abdel-Rahman et al., 2001, 2003, as cited in (Albertini et al., 2010)).

The National Cancer Institute (NCI) study (Hayes et al., 1996, 2000, 2001; Zhang et al., 2004, as cited in Albertini et al. (2010)), a multi-institution study of polybutadiene rubber workers in Yanshan, China yielded negative results for somatic (*HPRT, GPA*) mutations increases between exposed male and female workers (n=48; median 1,3-BD level/NOAEL for the overall exposed group was 2.0 ppm with an interquartile range of 20.6 ppm) and controls (n=38).

(Liu et al., 2008) also reported no significant increases in *HPRT* mutation frequencies in workers (n=74; exposure range/mean BD levels: 0-25.8/7.2 ± 7.6 ppm) in a large petrochemical facility in Nanjing, China, compared to controls (n=157). The frequency of (multiple) exon deletions in *HPRT* mutant clones from exposed workers was however significantly higher than that in control subjects ((27.4% vs 12.5%, $p<0.05$).

(Tates et al., 1996) reported no significant increases in mutations at the *HPRT* locus between Czech workers (n=19; mean exposure 1.76 ± 4.20 ppm) and unexposed controls (n=19). Two later studies of Czech workers (see Table 16 for exposure details) with well-documented external 1,3-BD exposures and with the urinary metabolites/haemoglobin adducts revealing appreciable internal doses, also reported no evidence of *HPRT* mutation associations to either 1,3-BD-exposure or specific worker genotypes (Albertini et al., 2001, Albertini et al., 2003, Albertini et al., 2007). The NOAEL for *HPRT* mutations in Czech workers was determined at 1.794 mg/m^3 (0.812 ppm)-the mean exposure level for the highest exposed worker group in this initial study.

Collectively, no consistently positive *HPRT* mutation associations with – generally low - 1,3-BD exposure levels and no specific gene polymorphism effects have been established.

7.6.1.2 Mutagenicity/Genotoxicity (Other)

Table 16 presents the outcomes of cytogenetic studies probing chromosomal damage in the form of micronuclei induction, chromosomal aberrations and sister chromatid exchanges in exposed workers.

While sister-chromatid exchanges have consistently remained undetected, significant increases in micronuclei induction and other genotoxic biomarkers (e.g. nuclear buds, nucleoplasmic bridges) in the lymphocytes of exposed workers compared to controls have been reported ((Tan et al., 2010, Wang et al., 2010, Xiang et al., 2012, Cheng et al., 2013, Federico et al., 2019).

(Albertini et al., 2007) reported a NOAEL of 0.8 ppm for chromosomal aberrations/MN based on the respective negative studies in Czech workers (Table 16). Polymorphisms of genes involved in the bioactivation and detoxification of 1,3-BD (*GSTT1*, *GSTM1, CYP2E1*, *mEH, MTHFR*), as well as DNA-repair (BER; certain *XRCC1* diplotypes) have been shown – inconsistently - to affect the levels of chromosomal damage in exposed workers (e.g., null genotypes conferring increased MN frequencies, in proportion to the number of deleted alleles), indicating a potential role for individual susceptibility to BD (Xiang et al., 2012, Xiang et al., 2015, Xiang et al., 2021). The alkaline comet assay has also revealed positive correlations between DNA damage in the form of strand breaks and occupational exposures to 1,3-BD, among a number of genotoxicants (Cemeli et al., 2009; Ruchirawat et al., 2009, as cited in (Albertini et al., 2010)).

A systematic review of published studies on the applicability of the Lymphocyte Cytokinesis-Block Micronucleus (L-CBMN) assay as a probe for detecting 1,3-BD exposure and genotoxicity identified eight reliable ones (all included in Table 16) (Bolognesi and Kirsch-Volders, 2016). In four European studies, no increase in MN frequency was reported, at mean individual exposure levels of < 3 ppm (Sorsa et al., 1994, Sorsa et al., 1996a, Tates et al., 1996, Srám et al., 1998). In contrast, studies involving industrial facilities in China showed an increase in MN frequencies

with the oldest one reporting significant positive responses at a median annual cumulative exposure of 266 ppm (Wang et al., 2010, Xiang et al., 2012, Cheng et al., 2013).

Collectively, despite small size and heterogeneity of data, an increase in MN in human PBLs appears to be associated with the intensity of BD exposure and unfavourable metabolic and DNA repair polymorphisms.

Table 16: Cytogenetic studies on 1,3-BD occupationally exposed subjects

Exposure details/ exposure levels/ test system	Genotoxicity endpoint	Results	References
Butadiene production unit of a petrochemical plant, Portugal Plant A: workers: M+F, n=17 controls: M+F, n=10; employees in manufacturing unit 1-3 ppm Butadiene/polymer production plant, Czech Republic Plant B (sampling I): workers: M, n=23(BD-only: n=10, BD+styrene: n=13) matched controls: M, n=20 ambient exposure levels: Plant A: 1-3 ppm, 72% <1ppm Plant B: mean 1.8 ppm, 43% <1 ppm Exposed to BD-only: 5-10 ppm, 40% >10 ppm control levels: 0.01-0.3 ppm PBLs	CA, MN SCE	negative	Sorsa et al. (1994)
workers in a 1,3-BD production plant Very low exposure levels (on average < 1 ppm) PBL	CA MN SCE	negative	Ahlberg et al., 1992 as cited in Sorsa et al. (1996b)
European BD production facilities Plant A as above and Plant B as above including samplings I (styrene-butadiene polymerisation and manufacturing unit) and II (processing plant)) workers: M+F, n=56 controls: M+F, n=50 < 3 ppm (exposed to BD only: 5-10 ppm, 40% > 10 ppm) PBL and plasma	CA SCE Plasma levels of Ras oncoproteins	negative	Sorsa et al. (1996b) Sorsa et al. (1994)
Pooled analysis of Plant A, Portugal and B, Czech Republic (as above) Plant A+B: workers: M, n=53 controls: n=46	CA (gaps excluded)	**positive** among workers: • *GSTT1*(-)>*GSTT1*(+)* • *GSTM1*(-)>*GSTM1*(+)	Sorsa et al. (1996a)

Exposure details/ exposure levels/ test system	Genotoxicity endpoint	Results	References
GSTT1(-): n=17 (17.2%) *GSTM1*(-): n=57 (57.6%) Plant A: 60-70%<0.2 ppm Plant B: 50%<1 ppm, 10%>10 ppm PBL	MN SCE	among controls: • *GSTM1*(-)<*GSTM*(+)* • *GSTT1*(-)>*GSTT1*(+) negative No significant changes between *GSTT1, GSTM1* polymorphisms in workers/controls	
Petrochemical company, Czech Republic workers: M, n=19; monomer production unit controls: M, n=19; Heat production unit in same company median value of 0.53 mg/m^3 (range: 0.024-23.0); 58% <1 mg/m^3 (0.45 ppm), 21% >5 mg/m^3 (2.25 ppm) Controls: 0.009-0.27 mg/m^3 (0.004-0.12 ppm) PBL	CA SCE SCE (HFC) MN DNA strand breaks: Comet assay	**positive** workers>controls* workers/controls: • *GST1* null> positive workers: • *GSTM1* null<positive* **positive** workers>controls* no significant effects of *GSTT1/GSTM1* genotype negative	Srám et al. (1998)
Rubber tyre factory exposed to benzo[α]pyrene, benzo-fluoranthene, naphthalene, acetonaphthene, alkenes and 1,3-butadiene workers: M/F, n=29 controls: M/F, n=22 (administrative staff) 22 laboratory workers PBL	DNA strand breaks: Comet assay MN	**positive** workers>controls* (office and laboratory)	Somorovská et al. (1999)
Polymer rubber production facility, Yanshan, China workers: M/F, n=41 Controls: M/F, n=38 workers: Median air exposure = 2 ppm (6h-TWA)	CA (FISH) SCE	negative no significant difference between exposure groups No effect of *GSTM1/GSTT1* polymorphisms	(Hayes et al., 2000)
Two BD facilities, Czech Republic BD monomer production workers: M, n=24 polymerisation workers: M, n=34 controls: M, n=25 (administration unit)	CA (including FISH) SCE (+% HFC)	negative No significant difference between exposure groups No associations with *GSTM1* and *GSTT1* genotype polymorphisms	Albertini et al. (2001) Albertini et al. (2003)

Exposure details/ exposure levels/ test system	Genotoxicity endpoint	Results	References
mean BD exposures: monomer: 0.642 mg/m^3 (0.290 ppm) polymer: 1.794 mg/m^3 (0.812 ppm) mean BD exposure: 0.023 mg/m^3 (0.010 ppm)		NOAEL=1.794 mg/m^3 (0.812 ppm)	
Petrochemical plant, Italy workers: M, n=42 controls: M, n= 43 median airborne BD exposed workers: 1.5 μg/m^3 controls: 0.4 μg/m^3 PBL	CA SCE	negative	Fustinoni et al. (2004)
Petrochemical plant, Italy workers: M, n=27 controls: M, n=26 Mean individual exposure to airborne BD: Exposed workers: 0.2-69 μg/m^3 Controls: <0.1–3.8 μg/m^3 PBL	CA SCE SCE (HFC) proliferation index (PRI)	negative	Lovreglio et al. (2006)
Related to (Albertini et al., 2003), Czech Republic BD-exposed workers: F, n=23; M, n=30 controls: F, n=26; M=25 Mean 8-h TWA: controls: F: 0.008 mg/m^3 (0.0035 ppm) M: 0.007 mg/m^3 (0.0032 ppm) Exposed workers: F: 0.397 mg/m^3 (0.180 ppm) M: 0.808 mg/m^3 (0.370 ppm) 8-h TWA in exposed groups up to: F: 9.793 mg/m^3 (4.45 ppm) M: 12.583 mg/m^3 (5.72 ppm)	CA (including FISH) SCE (+% HFC)	negative No associations with BD exposures No effects of genotype polymorphisms NOAEL= 1.794 mg/m^3 (0.812 ppm)	Albertini et al. (2007)
Polybutadiene Latex production plant, China workers: M/F, n=166 controls: M/F, n=41 plant BD concentrations: 0.05-1985.99 mg/m^3 (0.02-898.6 ppm) median concentration=4.48 mg/m^3 (2 ppm) Cumulative exposure dose=587 mg/m^3 (266 ppm)/ year	MN	**positive** workers > controls* High BD exposures (>587 mg/m^3)>low (≤587 mg/m^3) BD exposures Males<females* • *GSTM1*(+)>*GSTM1*(−) • *CYP2E1* (c1c2*/c2c2)>*CYP2E1* (c1c1) • *mEH* intermediate (I)	Tan et al. (2010)

Exposure details/ exposure levels/ test system	Genotoxicity endpoint	Results	References
PBL		group* > *mEH* high (H) group No associations for *GSTT1* polymorphism	
As above; workers carrying *APE1*, *ADPRT*, *MGMT* and *XRCC1* genotypes	MN	**positive** Significant increases among workers with multiple BER polymorphisms • *XRCC1* -77 C/T*>*XRCC1* -77 T/T • *ADPRT* 762 Ala/Ala*>*ADPRT* Val/Val • *XRCC1* 194 Arg/Trp*>*XRCC1* 194 Arg/Arg • *XRCC1* 280 Arg/His* > *XRCC1* 280 Arg/Arg • *XRCC1* 399 Arg/Gln*>*XRCC* 399 Arg/Arg Gln/Gln • *XRCC1* (-77)-(194)-(280)-(399) diplotype TCGA/TCGA*>wild-type CCGG/CCGG No associations in workers carrying *MGMT* Leu84Phe and *APE1* Asp148Glu polymorphisms	Wang et al. (2010)
Petrochemical company, Nanjing, China workers: M/F, n=45 controls: M/F, n=45; matched volunteers from administrative office and circulatory water workshop mean concentration production plants: 2.27 ppm administrative office: 0.84 ppm average BD measurement: exposed group: 0.34 ± 0.61 ppm or 0.75 ± 1.35 mg/m3control group: 0.04 ± 0.01 ppm or 0.09 ± 0.02 mg/m3 PBL	MN NDI NBUDs (Nuclear buds) NPBs	**positive** workers>controls* Females>males workers<controls* workers>controls No associations between MN, NBUDs, or NDI with the *CYP2E1, mEH* and *GSTT1/ GSTM1* polymorphisms in the exposed group **positive** workers > controls* among exposed workers: • *CYP2E1* c1c2*/c2c2 • *mEH* His139Arg HR*>*mEH* His139Arg HH • *CYP2E1* c1c2/c2c2*>*CYP2E1* c1c1 • *mEH* intermediate (I)/high (H)* >mEH low(S) group	Xiang et al. (2012)

Exposure details/ exposure levels/ test system	Genotoxicity endpoint	Results	References
		no association with *GSTT1/GSTM1* polymorphism	
As above	NBUDs	**positive** among exposed workers • *XRCC1* diplotypes TCGA-CCGG and TCGG-TCGA*>TCGG-TCGG	Xiang et al. (2015)
As above	NBUDs	**positive** among exposed workers • *MTHFR* C677T CC* or *MTHFR* C677T CT*< *MTHFR* 677 TT	Xiang et al. (2021)
Polybutadiene rubber workshop of a rubber factory, Shandong, China workers: M/F, n=44 controls: M/F, n=39 TWA for each subject: 0.06-12.41 mg/m^3 (0.03-5.6 ppm), median 1.48 mg/m3 (0.66 ppm) Urinary DHBMA: controls: 57.47-443.77 ng/ml (mean 185.39 ng/ml) Workers: 18.02-9932.16 ng/ml (mean 617.82 ng/ml) Individual BD cumulative exposure levels ranged from 0.70-278.99 mg/m^3 Median, cut-off concentration for high/low exposed groups: 24.80 mg/m3 (11.22 ppm) PBL	MN SCE	**positive** workers>controls* among exposed workers: • *GSTT1*(-)>*GSTT1*(+)* • *GSTM1*(-)>*GSTM1*(+)* • *GSTP1* AG/GG>GSTP1 AA • *GSTT1/GSTM1*(-/-)* > *GSTT1/GSTM1* (+/-)*> *GSTT1/GSTM1* (-/+)*> *GSTT1/GSTM1* (+)(+) among exposed workers: • Higher cumulative levels>lower cumulative levels • Older>younger • More years of work>fewer years of work negative	Cheng et al. (2013)
Petrochemical industry pollutants (PIP), such as benzene and 1,3-butadiene in 2 industrialised areas in South/East Sicily, Italy Total subjects: M/F, n=50 n=25 (living in the PIP areas) Petroleum refinery (PR) Exposed workers: n=10 PR non-exposed workers: n=15 Non-exposed: n=25 (living in rural areas far from these industries) exfoliated buccal cells	MN Karyolitic cells Differentiated cells NPB NBD	**positive** PIP-exposed PR workers or subjects not working in PR but living in the industrialised area>controls exposed>unexposed* exposed<unexposed*no significant differences between exposed vs unexposed	Federico et al. (2019)

CA: Chromosome aberration; MN: Micronuclei; SCE: Sister chromatid exchange; HFC: cells with High Frequency of SCE; FISH: fluorescence *in situ* hybridisation; PBL: Peripheral Blood Lymphocytes; NDI: Nuclear Division Index; NDUs: Nuclear Buds; NPB: Nucleoplasmic Bridges;
* statistically significant effect.
Note: Only positive outcomes are presented in bold.

7.6.2 Animal data

7.6.2.1 DNA adducts

Specific DNA lesions following systemic exposure to 1,3-DB or its metabolites comprising: N7-Guanine (i.e., N7THBG>N7HBG) and N3 and N^6-adenine (N^6THBA, N^6HBA>N3HBA) mono-adducts, reflecting those identified *in vitro* (see section 7.6.3.1), have been detected in mouse and rat liver, lung, kidney and testes. Following inhalation of 1,3-BD, G-N7 adduct levels were overall greater in mice than in rats (e.g. mouse liver DNA adducts levels ranged from 2 to 5-fold those in rat liver DNA. EBD-associated N7THBG adducts in all tissues tested, were 6 to 27-fold greater in abundance than the EB-derived N7HBG adducts, with the tendency for adduct formation to plateau in rats, at lower concentrations) (Tretyakova et al., 1998, Oe et al, 1999, Koc et al 1999, as cited in Albertini et al. (2010)).

1,3-BD adenine adducts (e.g. N^6HBA), although formed at a fraction of the guanine ones in mouse lung, persisted for longer (Tretyakova et al., 1998, Koivisto et al., 1996, Koivisto et al, 1997, Koivisto et al, 1998a, Oile et al, 1999 as cited in (Albertini et al., 2010)).

Differences in relative adduct abundance and tissue specificities were noted between species depending on the substance tested and the route of administration (e.g. EB favoured the formation of N7HBG over N7THBG adducts, with levels in rat greater than in mouse, when administered intraperitoneally) (Boogaard et al., 1998, 2001, 2004 as cited in Albertini et al. (2010)).

Bifunctional adducts have also been detected in rodents; DEB-derived racemic guanine-guanine (bis-N7G-BD) adducts were identified in the liver, lung, kidney, brain and thymus tissues in mice and rats, and guanine-adenine (N7G-N1A-BD) adducts in the liver DNA of mice following 1,3-BD exposure by inhalation. Again, bifunctional adduct levels in rats were 4- to 10-fold lower than in mice, depending on the tissue, and greater in females compared to males. DNA-DNA and DNA-protein cross-linking has been detected in mouse liver and lung DNA following 1,3-BD inhalation, but not in the rat (Goggin et al., 2007, 2008, 2009, Jelitto et al., 1989, Vangala et al., 1993 as cited in Albertini et al. (2010)).

The strong genotoxicity and mutagenicity of DEB is attributed to its ability to produce these highly deleterious DNA-DNA crosslinks. Strain (C57BL/6J>CAST/EiJ), tissue (lung>kidney, liver) and sex-related differences (female<male in C57BL/6J, mainly confined in the lung) in the levels of 1,3-BD-induced DNA adducts have been further confirmed in mice, in more recent studies (Chappell et al., 2014a, Lewis et al., 2019).

7.6.2.2 Mutagenicity/Genotoxicity

Genomic damage in the form of DNA strand breaks, has been detected by the comet assay for the metabolites ED/DEB delivered by injection. Genotoxic effects are tissue and species-specific and have been overall greater in mice than in rats. 1.3-BD inhalation produced sister chromatid exchanges (SCE), chromosomal aberrations and induction of micronuclei in various tissues in mice but was mostly ineffective in rat somatic cells at concentrations as high as 10.000 ppm (Table 17).

Increased *Hprt* mutation frequencies were induced by 1,3-BD (inhalation) and metabolites (mostly administered by injection) in both mice and rats and mutations in the *lacZ* and *lacI* gene in mice (Table 17). Analysis of the *Hprt* mutational spectra in mice revealed point mutations in both GC and AT base pairs for 1,3-BD and metabolites (e.g. G->C and A->T transversions, G-> A transitions), but preferentially in AT base pairs for 1,3-BD. Single-base deletions and insertions, and larger deletions have also been reported (Meng at al., 2000, Meng et al., 2004, as cited in Albertini et al. (2010)). 1,3-BD's mutagenic potency was 5 to 9-fold greater in mice than in rats, depending on the exposure levels; the lowest effective dose for mutations in mice was 3.0 ppm, whereas in rats it was 62.5 ppm (Meng et al., 1999a, 2001, 2007a; Walker and Meng 2000, as cited in Albertini et al. (2010)). It has been proposed that DEB is the effective mutagenic metabolite at low to moderate 1,3-BD exposure levels (Boysen et al., 2007). The relative potencies of the 1,3-BD metabolites for inducing mutations in somatic cells have been shown by several means to be DEB >> EB >EBD, however rats are particularly sensitive in direct

DEB exposure.

Specific mutations in oncogenes and tumour suppressor genes and signalling pathways (e.g. *K-ras*, *H-ras*, *Trp53*, *Catnb*) have been documented in a variety of tumours derived from 1,3-BD-exposed mice (Table 17). Additional genetic analyses revealed a variety of large genetic changes such as deletions and/or losses of heterozygosity (LOH) in genomic regions presumably near or encompassing tumour suppressor genes, implicating these events in 1,3-BD carcinogenesis.

Table 17: Summary of *in vivo* genotoxicity studies

Study endpoint	Test system/substance (route of exposure)	Results	References*
DNA strand breaks (alkaline elution, alkaline unwinding)	Liver, lung; mice, rats/ BD (inhalation)	**positive**	Vangala et al., 1993; Walles et al., 1995
	chromosome breaks in germ cells; mice/ DEB (i.p)	**positive**	Moutschen, 1961
Comet assay	Bone marrow and testis; mice, rats/ BD (inhalation)	negative	Anderson et al., 1997; Brinkworth et al., 1998; Wickliffe et al., 2003; Vodicka et al., 2006
	Testicular DNA; mice/ BD (inhalation)	Marginally **positive**	
	Bone marrow; mice, rats/ DEB (i.p)	**positive**	
	Testis; mice, rats/ DEB (i.p)	negative	
	Testis; mice, / EB (i.p)	**positive**	
	Testis; rats/ EB (i.p)	negative	
	Bone marrow; mice/ EB (i.p)	negative	
	Bone marrow; rats/ EB (i.p)	**positive**	
	Liver; mice/ BD (inhalation)	**positive**	
	Wild type and microsomal epoxide hydrolase (mEH) null mice	**positive** (mEH null>wt)	
DNA repair; Unscheduled DNA synthesis	Hepatocytes; mice, rats/ BD (inhalation)	negative	Arce et al., 1990 ; Anderson et al., 1997 ; Brinkworth et al., 1998
	Testis; mice/ BD (inhalation)	negative	
	DEB (i.p)	**positive**	
	EB (i.p)	negative	
	Spermatozoa; mice/ BD (inhalation)	negative	
DNA repair; comet assay	Hepatocytes, mice/ BD (inhalation)	**positive** significant increase in gamma-irradiation-specific DNA-repair activity	Vodicak et al., 2006
SCE	Bone marrow cells, alveolar macrophages, hepatocytes, splenic lymphocytes; mice,	**positive**	Conner et al., 1983 ; Cunningham et al., 1986 ; Tice et al.,

Study endpoint	Test system/substance (route of exposure)	Results	References*
	Chinese hamsters/ BD (inhalation), DEB (inhalation, i.p or iv), EB (i.p) Bone marrow cells; rats/ BD (inhalation)	 negative	1987 ; Walk et al., 1987 ; Arce et al., 1990 ; Sharief et al., 1986 ; Stephanou et al., 1997 ; (NTP, 1993) ; Cunningham et al., 1986; Arce et al., 1990 ;
Chromosomal aberrations	Bone marrow cells, spermatozoa, spermatids, spermatocytes; mice, Chinese hamsters/ BD (inhalation), EB (i.p), DEB (inhalation, i.p)	**positive**	Sharief et al., 1986 ; Irons et al., 1987 ; Tice et al., 1987 ; Walk et al., 1987 ; Adler et al., 1995a ; Pacchierotti et al., 1998 (NTP, 1993)
Micronucleus assay	Peripheral blood cells, bone marrow cells, splenic lymphocytes, lung fibroblasts, spermatids/germ cells; mice/ BD (inhalation), DEB (i.p), EB (i.p)	**positive** + weak aneuploidy DEB>EB>EBD	Cuningham et al., 1986 ; Tice et al., 1987 ; Jauhar et al., 1988 ; Victorin et al., 1990 ; Autio et al., 1994 ; Adler et al., 1994 ; Adler et al., 1995a ; 1997, Xiao et al., 1996 ; Russo et al., 1997 ; Stephanou et al., 1997, 1998 ; Tommasi et al., 1998 ; Jackson et al., 2000 ; Bevan et al., 2001 ; Ranaldi et al., 2001 ; Fred et al., 2005 ; Vodicka et al., 2006 (NTP, 1993)
	Spermatids/spermatocytes; mice, rats/ BD (inhalation), EB (i/p), DEB (i.p), EBD (i.p)	**positive** (mice: BD, EB, DEB; rats: BD, EB, DEB, EBD) negative (rats: BD)	Xiao and Tates, 1995 ; Lahdetie et al., 1997
	Peripheral blood cells, bone marrow cells, splenic lymphocytes; rats / BD (inhalation), EB (i.p), EBD (i.p), DEB (i.p)	negative (BD, EB) Weakly **positive** (EBD) **positive** (DEB)	Cuningham et al., 1986 ; Autio et al., 1994 ; Xiao and Tates, 1995 ; Xiao et al., 1996 ; Lahdetie and Grawe, 1997 ; Fred et al., 2005
Dominant lethal + Heritable translocations	Germ cells; mice/ DEB (i.p) BD (inhalation), DEB (i.p),	negative **positive**	Epstein and Shafner, 1968; Epstein et al., 1972 Adler et al., 1994, 1995a, 1995b, 1998 ; Adler and Anderson, 1994 ; Anderson et al., 1993, 1996, 1998 ; Brinkworth et al., 1998 ; Morrissey et al., 1990

Study endpoint	Test system/substance (route of exposure)	Results	References*
	EB (i.p), EBD (i.p) Germ cells; rats/ BD (inhalation)	negative negative	Adler et al., 1995b ; Adler et al., 1997 ; Anderson et al., 1998
Hprt mutations	Splenic/thymic lymphocytes; mice (including wt, mEH and NER (Xpc $^{-}/^{-}$)-deficient)/ BD (inhalation), EB (i.p), DEB (i.p)	**positive** mice>rats mEH-deficient (BD, DEB, EB) NER-deficient (BD and EB) wt mice (DEB) negative (BD, EB in NER-proficient mice)	Cochrane and Skopek, 1993; Tates et al., 1994; Meng et al., 1999a, 2001, 2007a; Walker and Meng 2000; Wickliffe et al., 2003, 2006, 2007; Boysen, 2007
	Splenic lymphocytes; mice, rats/ EB (inhalation), DEB (inhalation)	Mice **positive** (DEB>EB) Rats DEB: **positive** EB: negative	Meng et al., 1999b; Walker and Meng, 2000
	Splenic lymphocytes; mice/ BD (inhalation), EB (i.p) DEB (i.p) Splenic lymphocytes; rats/ EB, DEB (drinking water or i.p)	**positive** negative negative	Tates et al., 1998 ; Boysen et al., 2007
lacZ	Lung, bone marrow, liver Bone marrow; mice/ BD (inhalation)	**positive** (lung) negative (bone marrow, liver)	Recio et al., 1993, 1996; Recio and Meyer, 1995; Sisk et al., 1994
lacI	Bone marrow and splenic lymphocytes; mice/ BD (inhalation) Bone marrow, splenic lymphocytes, lung; mice/ BD (inhalation) Bone marrow, splenic lymphocytes, lung; mice/ EB (inhalation) DEB (inhalation) Bone marrow, splenic lymphocytes; rats/ EB, DEB (inhalation)	**positive** (bone marrow) negative **positive** (lung) negative (spleen, bone marrow) negative (all tissues) **positive** (bone marrow) negative (spleen)	Recio et al., 1993, 1996 1998 Recio et al., 2000, 2001; Saranko et al., 2001 Recio et al., 2000, 2001 ; Saranko et al., 2001 Recio et al., 2000
Mutations in proto-oncogenes/tumour suppressor genes	*K-ras* mutations: codon 13 G->C transversions in lung, liver tumours, cardiac hemangiosarcomas, forestomach tumours and lymphomas *H-ras* mutations: codon 61	**positive**	Wiseman et al., 1994 ; Zhuang et al., 1996, 1998 ; Zhuang and Soderkvist, 2000 ; Kim et al., 2005 ; Ton et al., 2007

Study endpoint	Test system/substance (route of exposure)	Results	References*
	A->T transversions in lung tumours, cardiac hemangiosarcomas, forestomach tumours, neuroblastomas and harderian gland tumours; G-> C transversions in *H-ras* codon 13 in mammary adenocarcinoma *Trp53* mutations in lymphomas, brain tumours, cardiac hemangiosarcomas and mammary adenocarcinomas; GC and AT base substitutions, G -> A transitions at non-CpG sites (exons 5-8) β-catenin (*Catnb*) point mutations + Frequent inactivation of the *Cdkn2a/Cdkn2b* loci encoding p16/p15 cyclin dependent kinase inhibitors in mouse lymphomas Deletions and/or losses of heterozygosity (LOH) in genomic regions (e.g. brain tumours)/ BD or metabolites in exposed mice		(Goodrow et al., 1990) (Sills et al., 2001, Sills et al., 1999, Hong et al., 2000)

* References – unless in brackets - taken from (Albertini et al., 2010). Note: Only positive outcomes are presented in bold

The mutagenic effects of 1,3-DB, EB and DEB are dependent to different degrees upon functional mEH and NER with mEH knock-out or NER-deficient mice being more susceptible to mutagenicity than their wild-type counterparts.

Although DNA damage is sustained and accumulates in all tested tissues of exposed mice (lung, kidney, liver), a number of key 1,3-BD-related epigenetic effects have been shown to be strain-, sex- and tissue-specific in mice.

- 1,3-BD exposure is associated with loss of cytosine DNA methylation – indicative of genomic instability - in the liver of male C57BL/6J mice and in the lung of females; both being target organs for 1,3-BD carcinogenesis (see section 7.7.2).
- No exposure-related effects on cytosine methylation were observed in the kidney (non-target organ) of either sex in this strain (Lewis et al., 2019, Chappell et al., 2014b).
- In contrast, in CAST/EiJ mice, 1,3-BD induced hypermethylation (2 to 4-fold increases) in the liver and lung of female mice while only minor, sporadic DNA hypomethylation was found in the liver, lung, and kidney of male mice (Lewis et al., 2019).
- Extensive histone deacetylation - associated with impaired DNA repair, major chromosomal rearrangements, and genomic instability - has been observed in the lungs of 1,3-BD-exposed male C57BL/6J mice (Chappell et al., 2014b).

Markers of genome protective remodelling such as condensed heterochromatin and transcriptional silencing (e.g. histone-lysine trimethylation) have also been differentially

reported as:
- increases in the kidney of male and female C57BL/6J mice and decreases in CAST/EiJ mice;
- significant increase in the liver of CAST/EiJ mice (Lewis et al., 2019, Koturbash et al., 2011).

Overall minor 1-3-BD-related effects on the transcriptome in liver and kidney tissues in either strain have been reported (Israel et al., 2018).

Collectively, epigenetic alterations in addition to genotoxic effects, appear to underpin the mechanisms of strain and tissue-specific 1,3-BD chemical carcinogenesis.

7.6.3 *In vitro* data

7.6.3.1 DNA adducts

The DNA adducts produced by the three major electrophilic 1,3-BD metabolites (and pertinent stereoisomeric forms) have been described by Albertini et al. (2010):
- EB produces monoadducts *in vitro* (on nucleobases, nucleosides, salmon sperm and calf thymus DNA), the most abundant of which have been identified as N7 guanine lesions (N7G). Other guanine adducts include the more hydrolytically stable N1G and N^2G. EB also forms regioisiomeric adenine N3 (N3A), N1A and N^6A adducts, at approximately 1/10 the extent of the guanine adducts. EB also produces adducts on the thymine and cytosine bases (i.e. N3T, N3C and resulting N3 uracil (N3U) and O^2C adducts).
- DEB treatments of isolated DNA generated monofunctional adducts at the N7 position of Guanine (N7-trihydroxybutylguanine (N7THBG), along with N1G and N^2G lesions. Adenine alkylations occurred at the N^6 position (N^6A), with adducts also verified at the N1, N3, N7 and N9 positions. DEB also produces bifunctional G-G and G-A DNA adducts in the form of intra and inter-strand DNA crosslinks whose ratio depends on the stereoisomers. DEB was shown to additionally produce DNA-protein crosslinks (e.g. with rat and human glutathione S-transferase (GST) and human O^6-alkylguanine-DNA-alkyltransferase (hAGT).
- EDB produced N^6A (N^6-(2,3,4-trihydroxybutyl)-adenine) and N7G (N7THBG) monoadducts, the latter stereomerically different to the similar DEB-derived ones. Recent cell-based findings have suggested that EDB could be potentially further transformed by cytosolic ADH to a bifunctional epoxy aldehyde and produce DNA-interstrand crosslinks, analogous to the DEB-induced ones (Nakamura et al., 2021).

7.6.3.2 Mutagenicity/Genotoxicity

The three major electrophilic metabolites of 1,3-BD produce site-specific, potentially mutagenic adducts formed on all four nucleobases (as discussed above). DNA damage, including the highly deleterious DEB-induced DNA ICLs, has been detected in mammalian cells, modulated by deficiencies in Fanconi anemia and NER (*XPA*, *XPD*) genes (Table 18).

The *in vitro* mutagenic potencies of some of these lesions have been probed in site-directed mutagenicity studies (reviewed by (Albertini et al., 2010); references therein). Although the most abundant N7G monoadducts are non-promutagenic and hydrolytically unstable, other monofunctional adducts (e.g. EB-derived N^2G and N3U, EBD-derived N^6A and N^2G) or bifunctional (e.g. DEB-derived N^6-N^6A and N^2-N^2G) are mutagenic causing replication blockage, point mutations at adenines and deletion mutations. Bifunctional adducts formed only by DEB, are considerably more mutagenic that monoadducts and are capable of producing both point and deletion mutations, rendering DEB the most mutagenic of the metabolites.

EB and DEB and/or exposure have produced positive *his* reversions in relevant *Salmonella typhimurium* tester strains (TA100, TA1530, TA1535, TA1538) with or without metabolic activation (when both conditions were tested). Exposure to gaseous 1,3-BD required external activation for mutagenic activity (Table 18). Universally positive gene mutation results have been obtained for EB and DEB in other prokaryotic and eukaryotic systems (*WP2, uvrA E.coli, Klebsiella Pneumoniae, Neurospora, Aspergillus nidulans, Schizosaccharomyces pombe, S. cerevisiae, Penicillium multicolor, Vicia faba, Sulfolobus acidocaldarius* (relevant studies

described in (Albertini et al., 2010).

Gene-level mutations induced by 1,3-BD and metabolites have been observed in mammalian cells (L5178Y mouse lymphoma, V79 fibroblasts, CHO cells, TK6 human fibroblasts, and mouse and rat transgenic fibroblasts). The relative mutagenic potencies of the three major electrophilic metabolites of BD have been determined to be DEB >> EB > EBD in both endogenous gene loci and transgenes (*cII* locus; Table 18). At chromosomal level, DEB was the most widely studied and most potent metabolite for the induction of aberrations and micronuclei in different mammalian cells, producing consistently positive results. The lack of observed activity for EB was attributed to effective repair of EB-induced damage prior to DNA synthesis. Sensitivity to the DEB-induced cytogenetic effects was enhanced by mutation of the Fanconi anaemia gene or a GST-T1 null genotype in human lymphocytes (Table 18).

Most *in vitro* studies of non-mutational endpoints involved studies of sister chromatid exchange (SCE) and were mostly positive (Table 18). Among the three metabolites, DEB was again the most efficient SCE inducer in both rodent and human lymphocytes; the relative SCE potencies in peripheral blood lymphocytes of healthy individuals followed the order: DEB>> EB> EBD. Genetic factors can modulate SCE induction, with the *GST-T1* and *GST-M1* null genotypes conferring increased susceptibility to DEB and EB respectively, indicating that these pathways comprise major detoxification steps in 1,3-BD metabolism.

Table 18: Summary of *in vitro* genotoxicity studies

Study endpoint	Test system/substance	Results	References*
Mutagenicity in prokaryotes	*Salmonella Typhimurium*: TA97, TA98, TA100, TA1535 (including transfectants expressing rat GST 5-5, human GST-T1), TA1537, TA1538, TA97A, TA98, TA1530/ DB, EB, DEB	**positive** BD : (+) TA100, TA1530, TA1535 DEB: (-/+) TA98, TA100, TA1535, TA1535 transfectants containing rat GSH S-transferase (GST) and human GST-T1, TA1538 EB : (-/+) TA97A, TA98, TA100, TA1535, TA1538 EBD : (-/+) TA100 negative BD : TA97, TA98, TA100, TA1537 DEB : TA97, TA98, TA100, TA1535, TA1537 EB : TA98	McCann et al., 1975 ; Rosenkranz and Poirier, 1979 ; de Meester et al., 1978 ; de Meester et al, 1980 ; Dunkel et al., 1984 ; Gervasi et al., 1985 ; Zeiger and Pagano, 1989 ; Arce et al., 1990 ; (NTP, 1993) ; Araki et al., 1994 ; Adler et al., 1997 ; Himmelstein et al., 2001 ; Mahhusree et al., 2002
	Salmonella Typhimurium YG7108 (TA1535 derivative) and *E. coli* TRG8 +/- plasmid expressing human AGT/ DEB	**positive** Mutagenicity enhanced by hAGT in YG7108 decreased in *E.coli* single/double hAGT mutants	Valadez, et al, 1984, Kalapila et al., 2008
Mutagenicity in mammalian cells	*Tk* locus in L5178Y cells/ BD *Tk* locus in L5178Y cells/ DEB	negative (-/+) **positive** (-)	Sinha and Helgason, 1969; McGregor et al., 1988; McGregor et al.,

Study endpoint	Test system/substance	Results	References*
	Hprt locus in V79 and CHO cells/ DEB, EBD	**positive** (-)	1991; NTP 1993 Nishi et al., 1984 ; Zhu and Zeiger, 1993 ; Lee et al., 2002
	lacI in rat transgenic fibroblasts (Rat2 cells)/ EB	**positive**	Saranko et al., 1998 ; Recio et al., 2000, 2001
	DEB	negative	
	cII locus in transgenic Big Blue® mouse (BBM1) and rat (BBR1) fibroblasts/ DEB	**positive** BBM1=BBR1	Erexson and Tindall, 2000b
	EB and EBD	**positive** only in BBM1; ineffective in rat	
	HPRT, Tk in human TK6 cells/ EB, EBD, DEB	**positive** DEB 50-100x and 100-200x more potent to EB and EBD, respectively DEB >> EB > EBD DEB induces large deletions, rearrangements or single base substitution mutations EB primarily induces single base substitutions	Cochrane and Skopek, 1993, 1994 ; Recio et al., 2000, 2001 ; Steen et al., 1997a,b
DNA damage; Comet assay	Mouse and rat splenocytes/ EB, DEB	negative	Kligerman et al., 1996
	V79 cells over-expressing mGSTT1 (V79 mGSTT1) vs empty vector cells (V79 MZ)/ DEB	**positive** V79 mGSTT1<V79 MZ Protective effect of GSTT1	(Kligerman and Hu, 2007)
	V79 hamster cells (including *FANCA* and *XPD*-deficient isogenic clones/ DEB	**positive** *FANCA*$^-$ > XPD$^-$= wt	(Chesner et al., 2017)
	Human lymphocytes/ EB, DEB	**positive** EB induces oxidative damage DEB induces DNA crosslinks and DNA double-strand breaks	Cemeli et al., 2009
DNA damage (ICL) and repair; HPLC-ESI$^+$ -MS/MS assay	V79 hamster cells (including *FANCA* and *XPD*-deficient isogenic clones/ DEB	**positive** increased ICL formation and persistence in NER-deficient cells (*XPD*) and *FANCA*$^-$ vs wt	(Chesner et al., 2017)
	Human cells deficient in *FANCD2* and *XPA* and isogenic wild-type clones	**positive** no difference in ICL formation and repair kinetics between wt and deficient	
DNA damage and repair;	V79 hamster cells (including *FANCA* and *XPD*-deficient	**positive** increased levels and	(Chesner et al., 2017)

Study endpoint	Test system/substance	Results	References*
γ-H2AX	isogenic clones/ DEB	persistence of γ-H2AX foci *XPD*$^-$ > *FANCA*$^-$> wt	
	A549 cells/ BD	**positive** (-/+)	(Zhang et al., 2019)
	Human TK6 lymphoblast cells; wt and *FANCD2* k.o cells/ EDB (synthetic analogues)	**positive** *FANCD2*$^-$ > wt co-localisation of γ-H2Ax with 53BP1	(Nakamura et al., 2021)
Unscheduled DNA synthesis	Mouse and rat hepatocytes/ EB, DEB	negative (-)	Arce et al., 1990
	Syrian hamster hepatocytes/ DEB	**positive** (-)	Kornbrust and Barfknecht, 1984
	Sprague-Dawley rat hepatocytes/ DEB	negative (-)	
SCE	CHO and V79 cells/ DEB, EB, BD	**positive** BD: (+) EB, DEB: (-/+)	Perry and Evans, 1975; Nishi et al., 1984; Sasiadek et al., 1991a
	mouse and rat splenocytes/ DEB	**positive** DEB: (-)	Kligerman et al., 1996; Kligerman et al., 1999a; (Kligerman and Hu, 2007)
	EB	negative (-)	
	transgenic (Big Blue®) mouse and rat fibroblasts/ DEB	**positive** glutathione peroxidase (GSH-Px) and mammalian erythrocytes (RBCs) reduced the response	Erexson and Tindall, 2000a
	human PBLs/ DEB	**positive** responses in GST-T1-null > GST-T1 positive Combined polymorphisms: GST-T1 positive background: increased responses in CYP2E1 c2 variant allele heterozygotes (significant) GST-T1 null background: no effect of CYP2E1 polymorphism; increased responses in mEH high activity genotypes> low activity (significant) no correlation of sensitivity to DEB with GSTM1 deficiency Biomodal SCE responses among healthy subjects; positive correlation with baseline (uninduced) SCE	Wiencke et al., 1982; Porfirio et al., 1983; Wiencke et al., 1991; Kelsey et al., 1991; Sasiadek et al., 1991b; Wiencke and Kelsey, 1993; Wiencke et al., 1995; Landi et al., 1995; Norppa et al., 1995; Landi et al., 1996 a,b; Pelin et al., 1996; Kligerman et al., 1999a; Schlade-Bertusiak et al., 2000; Schlade-Bartusiak et al., 2004, (Kligerman and Hu, 2007)

Study endpoint	Test system/substance	Results	References*
		frequencies negative for FA and FA heterozygotes no difference in responses between cultured lymphocytes of normal vs cancer patients GST-M1 null	
	human PBLs/ BD	negative (-/+) weakly **positive** (-/+)	Arce et al., 1990; Sasiadek et al., 1991b
	human PBLs/ EB	negative (G0) **positive** effect of prolonged period for DNA repair before DNA synthesis; unstimulated < actively cycling cells GST-M1 null hypersensitive GST-T1 null > GST-T1+ in a GST-M1 null background	Sasiadek et al., 1991b; Wiencke and Kelsey, 1993; Uuskula et al., 1995; Bernardini et al., 1998; Sasiadek et al., 1999; Kligerman et al., 1999a; Kligerman et al., 1999b; (Kligerman and Hu, 2007)
	human PBLs/ EBD	**positive** No effect of GST-T1 or GST-M1 genotypes DEB>> EB> EBD	Bernardini et al., 1996
Chromosome aberrations	Rat embryo fibroblast, liver cells/ DEB	**positive**	Wolman and Sivak, 1975; Dean and Hodson-Walker, 1979
	Mouse and rat splenocytes (G0)/ DEB	**positive**	Kligerman et al., 1996;(Kligerman and Hu, 2007)
	EB	negative	
	Mouse, rat and human lymphocytes (G0)/ DEB	**positive**	Kligerman et al., 1999a
	EB	negative	
	human PBLs (+DNA repair inhibition)/ EB	**positive** only + ara-C	Kligerman et al., 1999b; (Kligerman and Hu, 2007)
	human PBLs/ EB	negative	Murg et al., 1999a
	DEB	**positive**	Wiencke et al., 1991 ; Murg et al., 1999a ; Murg et al., 1999b
	human skin fibroblasts	**positive**	Wolman and

Study endpoint	Test system/substance	Results	References*
	(normal and FA heterozygotes FA homozygotes/ DEB	hypersensitive to cells from FA patients negative (normal)	Auerbach, 1975; Auerbach and Wolman, 1978
	Lymphoblastoid cells (normal and FA homozygotes/ DEB	**positive**	Cohen et al., 1982
	Heterozygotes, normal / DEB	negative	
	PBL and bone marrow cells (FA homozygotes, heterozygotes)/ DEB	**positive** in FA samples negative (normal)	Marx et al., 1983 ; Porfirio et al., 1983
Micronuclei induction	rat spermatids/ DEB	**positive**	Sjoblom and Kahdetie, 1996
	EB, EBD	negative	
	transgenic (Big Blue®) mouse (BBM1) and rat (BBR1) fibroblasts/ DEB, BDE	**positive** in both BBM1 and BBR1 DEB>BDE	Erexson and Tindall, 2000b
	human PBLs (including GST-M1 +ve and GST-T1 null)/ EB, DEB	**positive** (GST-T1 null hypersensitive)	Xi et al., 1997 ; Vlachodimitropoulos et al., 1997 ; Murg et al., 1999a ; Murg et al., 1999b
	EB	negative (1cen-q12 region)	Murg et al., 1999a

* References – unless in brackets - are taken from (Albertini et al., 2010).
SCE: Sister chromatic exchange; PBL=peripheral blood lymphocytes; (-/+): absence or presence of external metabolic activation (if reported)
Note: Only positive outcomes are presented in bold

7.6.4 Summary

Available biomonitoring studies in workers indicate that 1,3-BD at levels of occupational exposure can be genotoxic to humans and can cause various chromosomal damages. Results of studies of gene mutations—primarily *HPRT* mutations— have been predominantly negative, not showing any significant associations with 1,3-BD exposures. Reports on increased frequencies of chromosomal aberrations and micronuclei induction have been inconsistent with significant findings only at higher exposure levels. Polymorphisms of genes involved in the bioactivation/detoxification of 1,3-BD (e.g. *GSTT1*, *GSTM1, CYP2E1*, *mEH)* and repair (e.g. *XRCC1*) of induced adducts have been reported to influence chromosomal damage, in a subset of studies.

1,3-BD has been tested in a wide variety of *in vitro* and *in vivo* genotoxicity assays. It is not genotoxic in the absence of metabolic activation but its epoxide metabolites readily react with DNA to form alkylation monoadducts and bifunctional lesions including – in the case of DEB - DNA interstrand cross-links, detected both in naked and in genomic DNA (cell and animal studies).

The *in vivo* formation of DNA adducts (mono and bifunctional; DNA-DNA, DNA-protein crosslinks) and pertinent genotoxic effects (DNA strand breaks, somatic mutations, chromosome aberrations, micronuclei induction) have been greater in mice than in rats, with DEB identified as the effective and most potent mutagenic metabolite; when comparison among the metabolites

was possible, the potency ranking followed the order: DEB>EB>EBD. Genetic deficiencies in Nucleotide Excision Repair (NER) and microsomal epoxide hydrolase activity increase susceptibility to 1,3-BD/metabolite-induced *Hprt* mutations. Mutations in oncogenes, tumour suppressor genes and key signalling pathways (*K-ras, H-ras, p53, Catnb*) in different target tissues of the cancer susceptible species – mice.

Mutagenic activity of the metabolites – albeit with conflicting results - has been observed in a number of *Salmonella typhimurium* tester strains (e.g. TA100, TA1535, TA1538) with 1,3-BD requiring metabolic activation to produce positive results. Each of the metabolites has been shown to induce gene mutations *in vitro*, in both endogenous genes (*Hprt*, *Tk*) and transgenes, in various mammalian cell systems, with their activity following the order: DEB >> EB> EBD. Chromosome level mutations (aberrations, micronuclei induction) have been documented, with DEB being the most widely studied and most potent metabolite.
DEB was the most efficient sister chromatid exchange-inducer in both rodent and human lymphocytes, with the ranking of relative potencies among the metabolites, mirroring the one above for gene and chromosome-level mutations.
As observed in humans and in animals, genetic factors such as deficiencies in DNA repair genes (Nucleotide excision repair, Fanconi anemia) and *GST-T1/GST-M1* genotypes can modulate the *in vitro* genotoxic effects of butadiene and metabolites.

Apart from genotoxicity, 1,3-BD also elicits epigenetic effects which are strain-, sex- and tissue specific. Epigenetic alterations (e.g. cytosine methylation) were evident in target tissues (lung and liver), but were insignificant in non-target tissues (kidneys), where the formation of genome protecting chromatin condensation was observed instead.

7.7 Carcinogenicity

1,3-BD may cause cancer and has an entry in Annex VI of the CLP regulation as a category 1A carcinogen.

IARC (2012) concluded that 1,3-BD is "*carcinogenic to humans*" (Group 1). This conclusion was based on "*sufficient in humans*" (cancer of the haematolymphatic organs) and "*sufficient evidence*" in experimental animals for the carcinogenicity of 1,3-BD.

In their evaluation IARC (2012), noted that "*There is strong evidence that the carcinogenicity of 1,3-butadiene in humans operates by a genotoxic mechanism that involves formation of reactive epoxides, interaction of these direct acting mutagenic epoxides with DNA, and resultant mutagenicity. The metabolic pathways for 1,3-butadiene in experimental animals have also been demonstrated in humans.*"

SCOEL (2007) reviewed large North-American epidemiological studies of styrene-butadiene workers and agreed on a significant dose-response relationship between leukaemia mortality and cumulative exposure to 1,3-BD. Moreover, 1,3-BD was adequately tested for carcinogenicity in mice and rats by inhalation of both sexes, and induced various tumours, including heart angiosarcoma, malignant lymphomas, lung alveolar/bronchiolar adenomas and carcinomas, and forestomach papillomas and carcinomas. In their recommendation, SCOEL (2007) concluded that 1,3-BD should be treated as a possible human carcinogen, operating via a genotoxic mechanism (Group A).

7.7.1 Human data

Cancer follow-up studies have been conducted both on workers employed in 1,3-BD manufacturing facilities (exposure to butadiene monomer alone), and on workers exposed to 1,3-BD during styrene-butadiene rubber (SBR) production. In general population, the relationship between environmental exposure to 1,3-BD and cancer survival in children has been examined by several authors.

Statistical analysis included within-cohort (internal) and external comparisons. In external

comparisons, a standardised mortality ratio (SMR) has been often calculated. An SMR describes whether a specific population (workers) are more (excess in deaths), less (death deficit) or equally as likely to die than a standard or reference population (general population).

Occupational epidemiologic studies of cancer and exposure to 1,3-BD as considered by IARC (2008), IARC (2012), DECOS (2013) and ANSES (2022) are summarised in Table 19 and Table 20, and updated with more recent publications.

7.7.1.1 Occupational populations

7.7.1.1.1 1,3-butadiene monomer production

Three independent cohorts of monomer production workers in the USA examined mortality due to lymphohematopoietic cancer:

- two Union Carbide plants in West Virginia (Ward et al., 1995, Ward et al., 1996),
- a Texaco plant in Texas (Divine, 1990, Divine and Hartman, 2001, Downs et al., 1987), and
- a Shell plant in Texas (Cowles et al., 1994, Tsai et al., 2001).

Statistical analyses of these cohorts showed increased mortality from leukaemia and non-Hodgkin lymphoma after exposure to 1,3-BD. The risk did not increase with duration of exposure and was elevated among workers who had been exposed during the Second World War, when exposures to butadiene had probably been higher. However, the interpretation of these studies is difficult because no quantitative exposure information was available, exposure group analyses were based on small numbers, and comparison was with the general population only.

These studies have been reviewed and adapted from IARC (2012) and DECOS (2013), no more recent 1,3-BD monomer worker studies were identified since then (Table 19).

Table 19: Summary of epidemiological studies of 1,3-BD monomer workers and the risk of for lymphohematopoietic cancer (adapted from IARC (2008) and DECOS (2013))

Reference	Description	Exposure assessment	Organ site	Exposure categories	SMR (95% CI)	Adjustment for confounders	Comments
(Ward et al., 1995, Ward et al., 1996)	364 male workers in three units between 1941-1971, followed-up until 1990 (USA)	Employment in butadiene departments, no benzene or ethylene oxide present	Lymphohematopoietic Lympho-/reticulosarcoma Leukaemia		1.8 (0.7-3.6), n=7 1.8 (0.7-3.6), n=4 1.2 (0.2-4.4), n=2	Age, time period, country, reference rates	Lympho-/reticulosarcoma cases had been employed >2 years, 3 worked in the rubber reserve plant
(Divine and Hartman, 2001)	2800 male workers employed >= 6 months in 1943-1996, followed up until 1999 (USA)	Industrial hygiene sampling data	Lymphohematopoietic	Employed <5 yrs 5-19 yrs ≥20 yrs High exposure <5 yrs ≥5 yrs First employed 1942-1949 ≥1950	1.4 (1.1-1.9), n=50 1.6 (1.0-2.3), n=25 1.2 (0.5-2.4), n=8 1.3 (0.8-2.2), n=16 1.8 (1.1-2.8), n=20	Age, time period, age at hire	Update of Downs et al. (1987). No increasing trend by duration of employment or exposure group. Lymphohematopoietic tumours and lymphosarcoma significantly increased in the highest exposure category, elevations were found in workers employed <1950 and short-term workers.
			NHL	Employed <5 yrs 5-19 yrs ≥20 yrs High exposure <5 yrs ≥5 yrs First employed 1942-1949 ≥1950	1.5 (0.8-2.5), n=14 1.5 (1.1-2.1), n=46 0.7 (0.2-1.8), n=4 1.5 (0.9-2.3), n=19		
			Leukaemia	Employed <5 yrs 5-19 yrs ≥20 yrs High exposure <5 yrs ≥5 yrs First employed 1942-1949 ≥1950	1.3 (0.3-3.7), n=12 0.9 (0.3-2.3), n=3 2.0 (0.9-3.9), n=4 1.1 (0.3-2.9), n=8 1.6 (0.9-2.6),		

Reference	Description	Exposure assessment	Organ site	Exposure categories	SMR (95% CI)	Adjustment for confounders	Comments
					n=4 1.6 (0.9-2.6), n=17 0.9 (0.1-3.2), n=2 1.3 (0.8-2.0), n=18 1.4 (0.6-2.6), n=9 0.7 (0.1-2.6), n=2 1.5 (0.6-3.1), n=7 1.9 (0.8-3.7), n=8 1.4 (0.4-3.2), n=5 1.5 (0.9-2.4), n=18 0 (0-178), n=0		
(Tsai et al., 2001)	614 male workers, employed ≥5 yrs between 1948-1989 in butadiene production, followed-up until 1998 (USA)	Most 8-h TWA for butadiene <2.2 mg/m^3 (geometric mean <6.6 mg/m^3)	Lymphohematopoietic		1.1 (0.3-1.5), n=3	Age, race, calendar year, reference country-specific rates	Update of Cowles et al. (1994). A concurrent morbidity study failed to show differences in haematological values between butadiene-exposed and unexposed workers within the complex.

1,3-BD: 1,3-butadiene; NHL, Non-Hodgkin lymphoma; SMR, standardised mortality ratio; CI, confidence interval; TWA, time-weighted average.

7.7.1.1.2 Styrene-butadiene rubber production

In the US and Canada, a cohort of SBR manufacturing workers has been set up. These studies include the following epidemiological cohorts:

- Two plants in the state of Ohio (USA) (McMichael et al., 1976, McMichael et al., 1974, Meinhardt et al., 1982, Melnick et al., 1990, Santos-Burgoa et al., 1992)
- Eight plants in the US and Canada (Matanoski et al., 1993, Matanoski et al., 1990, Matanoski and Schwartz, 1987). More recently, these cohorts were later followed up by a group of researchers at the University of Alabama in Birmingham (USA) (Cheng et al., 2007, Delzell et al., 2001, Delzell et al., 2006, Delzell et al., 1996, Graff et al., 2005, Macaluso et al., 1996, Sathiakumar et al., 2021a, Sathiakumar et al., 2021b, Sathiakumar et al., 2009, Sathiakumar et al., 2015, Sathiakumar and Delzell, 2007, Sathiakumar et al., 2005, Sathiakumar et al., 2019)

Compared to the butadiene monomer production plants, SBR employees moved between plants, and were co-exposed to styrene and dimethyldithiocarbamate (DMDTC). Cohorts of SBR workers attempted to estimate individual exposure to 1,3-BD and styrene and focused mainly on lymphohematopoietic cancer (Table 20).

The most recent epidemiological analysis of SBR workforce confirmed a positive exposure–response relationship between 13-BD and all leukaemia among SBR workers, most of whom had co-exposed to styrene. More specific analyses supported an association between butadiene and lymphoid leukaemia, but not myeloid leukaemia, and provided little evidence of any association of butadiene or styrene exposures with major subtypes of B-cell malignancies other than lymphoid leukaemia, including NHL and multiple myeloma (Sathiakumar et al., 2021b).

Among all other examined cancer outcomes (cancers of the bladder, lung, kidney, oesophagus, and pancreas), only the bladder cancer was found to be associated with the highest quartile of cumulative 1,3-BD exposure (≥328.79 ppm-years); the relative risk (RR) was 2.13 (95% CI = 1.03-4.41) (Sathiakumar et al., 2021a).

The studies have been reviewed and summarised by (IARC (2012); (ANSES, 2011, ANSES, 2022, DECOS, 2013).

Only the most recent publications of SBR workers with dose-response analyses for lymphohematopoietic cancer (also referred to in Section 9.1.1) are summarised in Table 20.

Table 20: Summary of epidemiological studies of styrene-butadiene workers and the risk of for lymphohematopoietic cancer (adapted from IARC (2008) and DECOS (2013))

Reference	Description	Exposure assessment	Organ site	Exposure categories	Risk estimate (RR/HR (95% CI)	Adjustment for confounders	Comments
Delzell et al. (1996)	15649 workers employed for at least 1 year in 8 production plants in 1943-1991 (USA/Canada)	8281 unique combinations of work area/job title, grouped in 308 work areas with similar exposure (5 main process groups and 7 sub-groups)	Lymphosarcoma Other lymphopoietic cancer Leukaemia	 Polymerisation Maintenance Labour Laboratories Ever hourly workers Employed >10 yrs Hired >20 yrs ago	0.8 (0.4-1.4), n=11 1.0 (0.7-1.3), n=42 1.3 (1.0-1.7), n=48 2.5 (1.4-4.1), n=15 1.1 (0.6-1.9), n=12 2.2 (1.3-3.6), n=16 4.3 (2.1-7.9), n=10 1.4 (1.0-1.9), n=45 2.2 (1.5-3.2), n=28	Age, race, calendar year	Overlap with (Delzell et al., 1996, Lemen et al., 1990, Matanoski et al., 1993, Matanoski et al., 1990, Matanoski and Schwartz, 1987, Meinhardt et al., 1982, Santos-Burgoa et al., 1992).
Graff et al. (2005)	16579 men working at 6 plants >1 yr by 1991 and followed through 1998 (USA/Canada)	Cumulative exposure estimates for 1,3-BD (ppm-years), styrene and DMDTC	Leukaemia*	0 0-<75 75-<408 408-<939 ≥939 p-trend= <0.001	Ref., n=10 1.4 (0.7-3.1), n=17 1.2 (0.6-2.7), n=18 2.9 (1.4-6.4), n=18 3.7 (1.7-8.0), n=18	Age, years since hire	SMR analyses for leukaemia consistent with those of internal analysis using Poisson regression models
			Leukaemia*	0 0-<75 75-<408 408-<939 ≥939 p-trend= 0.03	Ref., n=10 1.4 (0.5-3.9), n=17 0.9 (0.3-2.6), n=18 2.1 (0.7-6.2), n=18 3.0 (1.0-9.2), n=18	Age, years since hire, other chemicals	
			Chronic lymphocytic leukaemia	<75 75-<939 ≥939 p-trend= 0.01	Ref., n=7 1.5 (0.6-4.0), n=11 3.9 (1.3-11.0), n=7		
			Chronic myelogenous leukaemia	<75 75-<939 ≥939 p-trend= 0.01	Ref., n=3 2.7 (0.7-10.4), n=8 7.2 (1.7-30.5), n=5		
			Other leukaemia	<75 75-<939 ≥939	Ref., n=5 1.1 (0.3-3.9), n=5 4.0 (0.3-15.0), n=4		

Reference	Description	Exposure assessment	Organ site	Exposure categories	Risk estimate (RR/HR (95% CI)	Adjustment for confounders	Comments
				p-trend= 0.06			
Cheng et al. (2007)	16091 male workers employed >1 year before 1992 followed through 1998 (USA/Canada)	Same as Graff et al. (2005)	Leukaemia	Continuous 0 0-<12.1 12.1-<22.9 22.9-<38.8 38.8-<78.1 78.1-<184.6 184.6-<251.1 251.1-<318.5 318.5-<450.9 450.9-<829.6 ≥829.6 Continuous, mean scored deciles Continuous peaks 0 0-<22.8 22.8-<241.9 241.9-<295.1 295.1-<434.9 434.9-<985.4 985.4-<1878.9 1878.9-<2901.2 2901.2-<3837.8 3837.8-<5715.5 ≥5715.5 Continuous peaks, mean scored deciles Average intensity, continuous	 Ref., n=10 1.0 (0.4-2.6), n=7 1.7 (0.6-4.5), n=7 1.4 (0.5-4.0), n=7 0.8 (0.3-2.3), n=7 0.6 (0.2-1.7), n=7 1.8 (0.6-5.2), n=7 2.5 (0.8-7.4), n=7 2.0 (0.6-5.9), n=7 1.9 (0.6-5.6), n=7 2.6 (0.8-7.7), n=8 $\beta=3.0*10^{-4}$, $SE=1.4*10^{-4}$, p=0.04 $\beta=5.8*10^{-4}$, $SE=2.7*10^{-4}$, p=0.03 Ref., n=10 3.6 (1.4-9.2), n=8 1.2 (0.5-3.3), n=7 8.9 (3.4-23.4), n=7 4.0 (1.5-10.5), n=7 1.6 (0.6-4.2), n=7 2.3 (0.9-6.1), n=7 3.7 (1.4-9.8), n=7 6.9 (2.6-18.2), n=7 5.8 (2.2-15.2), n=7 4.3 (1.6-11.2), n=8 $\beta=5.6*10^{-5}$, $SE=2.4*10^{-5}$, p=0.02 $\beta=7.5*10^{-5}$, $SE=3.7*10^{-5}$, p=0.04 $\beta=3.6*10^{-3}$, $SE=2.1*10^{-3}$, p=0.09	Age, year of birth, race, plant, years since hire, DMDTC	Lymphoid Neoplasms associated with 1,3-BD mg/m^3-years and myeloid neoplasms with 1,3-BD peaks, neither trend significant after adjusting for covariates; DMDTC as a continuous variable not associated with leukaemia, risk estimates for quartiles of exposure to DMDTC significantly increased without monotonic trend.

Reference	Description	Exposure assessment	Organ site	Exposure categories	Risk estimate (RR/HR (95% CI)	Adjustment for confounders	Comments
				Average intensity, mean scored deciles	$\beta=3.8*10^{-3}$, $SE=3.7*10^{-3}$, p=0.4		
Sathiakumar et al. (2015)	16579 men employed >1 year before 1992, followed through 2009 (USA/Canada)	Same as Graff et al. (2005). Quartile cutpoints (ppm-years) for each outcome	Leukaemia	0 0-<12.47 12.47-<25.84 25.84-<45.51 45.51-<70.05 70.05-<124.38 124.38-<213.43 213.43-<289.91 289.91-<448.17 448.17-<908.35 >=908.35	Ref., n=15 0.98 (0.44-2.18), n=10 1.72 (0.77-3.85), n=10 1.83 (0.82-4.09), n=10 1.96 (0.88-4.38), n=10 1.47 (0.66-3.27), n=10 1.57 (0.7-3.51), n=10 3.2 (1.42-7.18), n=10 2.64 (1.18-5.91), n=10 2.78 (1.24-6.24), n=10 3.76 (1.59-8.89), n=9		
			NHL	0 0-<4.06 4.06-<11.23 11.23-<24.88 24.88-<56.13 56.13-<114.98 114.98-<167.41 167.41-<301.48 301.48-<355.58 355.58-<490.66 >=490.66	Ref., n=17 1.32 (0.54-3.22), n=7 1.29 (0.53-3.13), n=7 1.04 (0.43-2.5), n=7 0.75 (0.31-1.81), n=7 0.68 (0.28-1.64), n=7 1.36 (0.56-3.29), n=7 0.94 (0.39-2.27), n=7 3.55 (1.46-8.6), n=7 2.96 (1.27-6.91), n=8 1.3 (0.55-3.06), n=8		
			MM	0 0-<2.92 2.92-<26.09 26.09-<44.65 44.65-<67.89 67.89-<107.78 107.78-<124.94 124.94-<364.52 364.52-<660.98	Ref., n=14 0.89 (0.25-3.14), n=3 0.29 (0.08-1.0), n=3 0.62 (0.18-2.19), n=3 0.63 (0.18-2.23), n=3 0.52 (0.15-1.84), n=3 1.71 (0.49-6.04), n=3 0.31 (0.1-0.95), n=4 2.48 (0.8-7.7), n=4		

Reference	Description	Exposure assessment	Organ site	Exposure categories	Risk estimate (RR/HR (95% CI)	Adjustment for confounders	Comments
				435.19-<660.98 >=660.98	1.33 (0.43-4.11), n=4 0.63 (0.2-2.04), n=4		
Sathiakumar et al. (2021b)	21087 (16579 men and 4508 women) workers employed >1 year before 1992 followed through 2009 (USA/Canada)	Same as Graff et al. (2005). Quartile cutpoints (ppm-years) for each outcome	Leukaemia	0 0-<34 34-<121.28 121.28-<363.64 >=363.64	Ref., n=29 1.04 (0.6-1.83), n=26 1.37 (0.76-2.46), n=26 1.6 (0.87-2.94), n=25 2.53 (1.37-4.67), n=26 $\beta=2.5*10^{-4}$, $CI_{up}=4.73*10^{-4}$, p=0.03	Age at hire, year of hire, race, sex, plant, ever hourly status	Styrene exposure–response trends for all leukaemia and lymphoid leukaemia were less consistent than those for 1,3-BD. 1,3-BD and styrene not associated consistently with myeloid leukaemia, BCM, NHL and MM.
			Lymphoid leukaemia	0 0-<44.73 44.73-<213.43 213.43-<376.31 >=376.31	Ref., n=13 0.72 (0.29-1.78), n=9 0.85 (0.34-2.14), n=10 2.61 (1.02-6.67), n=10 1.95 (0.76-5.03), n=10 $\beta=4.78*10^{-4}$, $CI_{up}=8.23*10^{-4}$, p=0.01		
			Myeloid leukaemia	0 0-<25.2 25.2-<70.05 70.05-<230.08 >=230.08	Ref., n=14 1.16 (0.53-2.56), n=13 1.96 (0.88-4.38), n=14 1.47 (0.64-3.4), n=13 1.72 (0.73-4.07), n=13 $\beta=0.63*10^{-4}$, $CI_{up}=5.15*10^{-4}$, p=0.8		
			AML	0 0-<19.14 19.14-<59.19 59.19-<185.63 >=185.63	Ref., n=9 1.28 (0.47-3.48), n=8 2.04 (0.73-5.7), n=8 1.85 (0.64-5.36), n=8 1.9 (0.63-5.73), n=8 $\beta=-2.27*10^{-4}$, $CI_{up}=7.7*10^{-4}$, p=0.6		
			NHL	0 0-<17.94 17.94-<117.22 117.22-<334.83 >=334.83	Ref., n=34 0.9 (0.5-1.61), n=19 0.57 (0.31-1.04), n=19 0.94 (0.5-1.75), n=19 1.33 (0.71-2.49), n=19 $\beta=1.55*10^{-4}$,		
			MM	0 0-<31.42 31.42-<107.78 107.78-<386.04			

Reference	Description	Exposure assessment	Organ site	Exposure categories	Risk estimate (RR/HR (95% CI)	Adjustment for confounders	Comments
			BCM	>=386.04 0 0-<27.04 27.04-<124.38 124.38-<370.89 >=370.89	$CI_{up}=5.58*10^{-4}$, p=0.4 Ref., n=20 0.61 (0.27-1.35), n=10 0.74 (0.32-1.71), n=10 0.69 (0.29-1.6), n=10 1.01 (0.43-2.42), n=10 $\beta=-0.36*10^{-4}$, $CI_{up}=4.14*10^{-4}$, p=0.9 Ref., n=65 0.75 (0.49-1.15), n=37 0.7 (0.45-1.08), n=37 0.94 (0.6-1.49), n=37 1.39 (0.88-2. 2), n=37 $\beta=1.95*10^{-4}$, $CI_{up}=4.02*10^{-4}$, p=0.06		

1,3-BD, 1,3-butadiene; DMDTC, dimethyldithiocarbamate; AML, acute myeloid leukaemia; NHL, Non-Hodgkin lymphoma; MM, multiple myeloma; BCM, B-cell malignancy; CI_{up}, upper confidence interval; CI, confidence interval; RR, risk ratio; HR, hazard ratio; TWA, time-weighted average.
* Different adjustment variables.

7.7.1.1.3 Population-based studies

- Loughlin et al. (1999)examined the risk of lymphohematopoietic cancer among students of a high school in eastern Texas, USA, that situated near facilities that produced synthetic SBR since 1943. A cohort of 15043 students who had attended the school for at least 3 consecutive months during a school year between 1963 and 1993 was constructed. In total, 338 graduates (241 men and 97 women) had died during the follow-up period of 1963–95, which were fewer than expected. The SMR for all lymphohaematopoietic cancer was 1.64 (95% CI= 0.85–2.87) for men (12 observed deaths) and 0.47 (95% CI= 0.06–1.70) for women (two observed deaths). The SMR was higher for 1530 men who had attended the school for 2years than for the 6352 who had attended for 2 years. For the former group, the SMR for all lymphohematopoietic cancer was 3.20 (95% CI, 0.87–8.20).
- Parent et al. (2000) set up a population-based case–control study in the Montréal area (Canada). The study assessed the association between renal-cell carcinoma and a large number of occupational exposures (including SBR) among men aged 35–70 years between 1979 and 1985. Cases were identified at all large hospitals in the area and were histologically confirmed. Questionnaires on cancer risk factors that included lifetime occupational history were administered. In the analysis, 142 cases and 533 population controls and 1900 other cancer controls were available. The odds ratio (OR) for exposure to 'styrene–butadiene rubber' was 2.1 (10 exposed cases; 95% CI= 1.1–4.2) after controlling for age, family income, tobacco smoke and body mass index and 1.8 (95% CI= 0.9–3.7) after controlling for other occupational exposures. The Working Group of IARC (IARC, 2008) noted that *"it was unclear what was meant by exposure to styrene–butadiene rubber."*

7.7.1.2 General population

Epidemiological studies investigated the associations between environmental 1,3-BD exposure and diseases in children: leukaemia, brain tumours and other childhood tumours.

7.7.1.2.1 Children leukaemia

- Whitworth et al. (2008) performed analysis included 977 cases of childhood lymphohematopoietic cancer diagnosed from 1995–2004 in the USA. Modelled estimates of benzene and 1,3-BD for 886 census tracts surrounding Houston, Texas were obtained. Statistical models were adjusted for age, sex, race/ethnicity, and community-level socioeconomic status (cSES). 1,3-BD levels were associated with all leukaemia (RRs = 1.4, 95% CI= 1.07–1.81), acute myeloid leukaemia (RR=1.68, 95% CI= 0.84–3.35), and acute lymphoblastic leukaemia (RR=1.32, 95% CI=0.98–1.77). No association was found between 1,3-BD and lymphoma and lymphoma incidence.
- Heck et al. (2014) set up a case–control study, where ascertained 69 cases of acute lymphoblastic leukaemia (ALL) and 46 cases of acute myeloid leukaemia (AML) from California Cancer Registry records of children <age 6, and 19209 controls from California birth records within 2 km (ALL) and 6 km (AML) of an air toxics monitoring station between 1990 and 2007. Information on air toxics (including 1,3-BD) exposures was taken from community air monitors. 1,3-BD was only associated ALL during 3rd trimester (OR=1.54, 95%CI=1.19-1.99) or the entire pregnancy (OR=1.94, 95%CI=1.09-2.86), and AML during the child's first year (OR=2.35, 95%CI=1.02-5.39).
- Symanski et al. (2016) conducted a population-based case-control study (1248 cases and 12172 controls) to examine individual effects of benzene, 1,3-BD and polycyclic organic matter (POM) in ambient residential air on acute lymphocytic leukaemia (ALL) diagnosed in children under age 5 years in Texas from 1995–2011. Maternal and infant characteristics from birth certificates were abstracted to obtain information about potential confounders. Modelled estimates of benzene, 1,3-BD and POM exposures at the census tract level were assigned by linking geocoded maternal addresses from birth certificates to U.S. EPA National-Scale Air Toxics Assessment data for single and co-pollutant statistical analyses. In adjusted single pollutant models, odds of childhood leukemia among mothers with the highest with the highest ambient air exposures compared to those in the lowest quartile were 1.29 (95 % CI: 1.08–1.52) for 1,3-BD. In co-pollutant models, odds ratios for childhood leukaemia remained

elevated for 1,3-BD.

- Filippini et al. (2019) performed a review of 29 original studies and cohort studies that had investigated the risk of childhood leukaemia in relation to exposure either to motorized traffic and related contaminants, based on various traffic-related metrics (number of vehicles in the closest roads, road density, and distance from major roads),or to measured or modelled levels of air contaminants such as benzene, nitrogen dioxide, 1,3-BD,and particulate matter. An association between 1,3-BD as an air pollutant and the risk of death from acute leukaemia [lymphoid and/or myeloid] leukaemia in childhood was also reported (RR = 1.45; 95% CI = 1.08 - 1.95 - based on two studies including 1,3-BD).

7.7.1.2.2 Children brain tumours

- Danysh et al. (2015) conducted a population-based study evaluating the association between traffic-related hazardous air pollutants (1,3-BD, benzene, diesel particulate matter [DPM]) and the incidence of childhood central nervous system (CNS) tumours among 1949 children diagnosed with a CNS tumor at <15 years of age, in Texas (USA), for the period of 2001–2009. Census tracts with medium (RR= 1.46, 95%CI=1.05-2.01) and medium-high (RR=1.69, 95%CI=1.22-2.33) 1,3-BD concentrations had higher astrocytoma incidence rates, compared with low concentrations.
- von Ehrenstein et al. (2016) examined risks for brain tumors (medulloblastoma, central nervous system primitive neuroectodermal tumor (PNET), and astrocytoma) in children < 6 years diagnosed in 1990-2007, after prenatal and infant exposure to monitored ambient air toxics. Cases of primary neuroectodermal central nervous system tumours (≤38) were positively associated with various pollutants, including 1,3-BD (OR = 2.23; 95% CI = 1.28-3.88).

7.7.1.2.3 Other childhood tumours

- Hall et al. (2019) set up a case-control study, including 243 cases of germ cell tumours in children under 6 years of age, and reported an increased risk of cancer in children under 6 years old, reported an increased risk of germ cell tumours (particularly tumours of the yolk sac) with exposure to air pollutants, including 1,3-BD (OR = 1.51; 95% CI = 1.01-2.26), during the second trimester of pregnancy.

7.7.2 Animal data

1,3-BD has been evaluated for carcinogenicity by inhalation exposure in one study in rats and four studies in mice, described below and in
Table 21.

In Sprague-Dawley rats (n=110/sex/group), exposed to 1000 and 8000 ppm 1,3-BD the following significant/noteworthy findings were reported by (Owen et al., 1987, Owen and Glaister, 1990):

- in males: significantly increased incidences of exocrine adenomas in the pancreas at the highest dose (8000 ppm) and testicular Leydig cell tumours in all dosed animals (≥ 1000 ppm). The pancreatic tumours were considered by the authors as *"doubtful biological evidence of oncogenicity"* as they were confined to one sex and were not easily distinguished from hyperplastic foci or nodules, while the testicular tumours were considered *"treatment-related".* The occurrence of nine glial cell neoplasms of the brain in exposed male rats *"may have been related"* to 1,3-BD due to the rarity of this malignancy in untreated rats.
- in females: significant trends were reported for Zymbal gland carcinomas, sarcomas of the uterus, adenomas and carcinomas (combined) of the mammary gland, and follicular cell adenomas of the thyroid gland. Of those, the incidences of uterine sarcomas and Zymbal gland tumours were similar to those occurring in untreated rats and were deemed *"not treatment-related"*, in contrast to the mammary and thyroid tumours which were considered

"treatment-related".

Collectively, the two-year toxicity/carcinogenicity study in rats concluded that under the experimental conditions, 1,3-BD is a weak carcinogen to the rat (Owen et al., 1987, Owen and Glaister, 1990).

The NTP initially conducted a long-term inhalation carcinogenicity studies in B6C3F1 mice only (n=50/sex/group), exposed to 625 and 1000 ppm for 60-61 weeks (NTP, 1984). The significant/noteworthy findings, largely distinct from the rat study, are summarised in Table 21. Neoplasms occurred in exposed mice at significantly increased incidences:

- in males and females (at ≥ 625 ppm): in the lung (alveolar/bronchiolar adenomas and carcinomas), hematopoietic system (malignant lymphomas), heart (hemangiosarcomas) and in the forestomach (papillomas or carcinomas)
- in females: in the ovary (granulosa cell tumour; at ≥ 625 ppm) and in the liver (hepatocellular adenomas or carcinomas) and mammary gland (acinar cell carcinoma), at 1000 ppm

The increased incidences of the above lesions, along with their early detection comprised *"clear evidence of carcinogenicity"* for 1,3-BD (NTP, 1984).

Marginal numerical increases in incidences were noted in neoplasms in the preputial gland (squamous cell carcinomas) and the brain (gliomas) in males, mammary gland (adenosquamous carcinomas) in females and in Zymbal gland (carcinomas) in both sexes.
Collectively, the results suggested that B6C3F1 mice are more susceptible to the carcinogenic effects of 1,3-BD than rats (NTP, 1984).

Another 2-year inhalation study was later conducted by the NTP, employing an expanded range of exposure concentrations for better characterisation of the concentration-dependent responses for 1,3-BD induced neoplasms (NTP, 1993). B6C3F1 mice (n=70-90/sex/group) were exposed to 0, 6.25, 20, 62.5 and 200 ppm 1,3-BD. Statistically significant increases in the incidences of neoplasms, exceeding historical controls, were observed in the following organs:

- in males and females in: hematopoietic system (malignant lymphoma: at 625 ppm in M; ≥ 200 ppm in F), heart (hemangiosarcoma: ≥ 62.5 ppm in M; ≥ 200 ppm in F), lung (alveolar/bronchiolar adenocarcinoma or carcinoma: ≥ 20-200 ppm in M; ≥ 6.25 ppm in F), forestomach (squamous cell papilloma or carcinoma: at 200 ppm in M; at 625 ppm in F), liver (hepatocellular adenomas and carcinomas: at 200 ppm in M; 62.5-200 ppm in F), harderian gland (adenoma or carcinoma: ≥ 62.5 ppm in M; 62.5-200 ppm in F),
- in females: ovary (malignant granulosa cell tumour: at 62.5 and 200 ppm) and mammary gland (carcinoma: ≥ 200 ppm)
- in males only: preputial gland (carcinomas: at 200 ppm)

There was no exposure level (≥ 6.25 ppm), at which a significant carcinogenic response was not observed. The above neoplastic lesions comprised *"clear evidence of carcinogenicity"* of 1,3-BD in mice (NTP, 1993).

Low incidences of uncommon neoplasms including intestinal carcinomas and brain gliomas and neuroblastomas (in the "stop-study"; see below) in males, renal tubule adenomas in males and females, skin sarcomas and Zymbal's gland adenomas and carcinomas in females, were also reported and *"may have been related"* to chemical administration (NTP, 1993).

A "stop-exposure" study comprising shorter exposures of male mice (n=50/group), for 13, 26, 40 and 52 weeks to 625, 625, 200 and 312 ppm 1,3-BD, respectively, was conducted as part of the NTP (1993) assessment. These exposures induced neoplasms at the same sites as those identified in the 2-year study.

Collectively, the (NTP, 1993) studies further describe *"clear evidence of carcinogenicity"* of 1.3-BD, at multiple sites in mice.

No carcinogenic activity was evident in a study by Bucher et al. (1993) in B6C3F1 mice (n=60/sex/group) subjected to a single, short (2 h) exposure to high doses (up to 10000 ppm) 1-3-BD, designed to reflect an occupational high-exposure situation.

Table 21: Summary of inhalation carcinogenicity studies in rodents

Species/strain, sex, No/group	Route, dose levels, duration of exposure	Results/Remarks	Reference
Sprague-Dawley CD rats; M/F (n=110/sex/group)	0, 1000, 8000 ppm (0, 2200 or 17 600 mg/m^3) 6 h/d, 5d/wk for 111 wks (M) or 105 wks (F) (study terminated at 20-25% survival; n=10/sex/group sacrificed at wk 52)	Dose-dependent mortality in the 2nd year; Survival was significantly reduced in low- and high-dose F and in high-dose M; increased mortality resulted in F: primarily from mammary tumours (80% cases) and in M: renal lesions; no significant differences in body weight post wk 12 **Pancreas: exocrine adenoma** (in the 0, 1000, 8000 ppm groups) M: 3/100, 1/100, 10/1000** **Testis: Leydig cell tumours** M: 0/100, 3/100**, 8/100** **Uterine Sarcoma** F: 1/100, 4/100, 5/100† **Zymbal gland carcinoma** F: 0/100, 0/100, 4/100† **Mammary adeno/carcinoma** F: 50/100, 79/100, 81/100† **Thyroid follicular cell adenoma** F: 0/100, 2/100, 10/100† **Brain: gliomas** M: 1/100, 4/100, 5/100†	(Owen and Glaister, 1990, Owen et al., 1987)
B6C3F1 mice; M/F (n=50/sex/group)	0, 625, 1250 ppm (1380 or 2760 mg/m^3) 6 h/d, 5d/wk for 60 wks (M) or 61 wks (F)	Studies planned for 103-wks but abbreviated because of rapidly declining survival, primarily due to malignant lymphomas; no effect on mean body weights **Lung (alveolar/bronchiolar) carcinoma** (in the 0, 625, 1250 ppm groups) M: 0/50^{a}, 2/49, 5/49* F: 0/49, 6/48*, 8/49* **Lung (alveolar/bronchiolar) adenoma or carcinoma** M: 2/50, 14/49**, 15/49** F: 3/49, 12/48*, 23/49** **Haematopoietic: Lymphoma (all malignant)** M: 0/50, 23/50**, 29/50** F: 1/50, 10/49*, 10/49* **Heart: hemangiosarcoma** M: 0/50, 16/49**, 7/49** F: 0/50, 11/48**, 18/49** **Forestomach: Squamous cell carcinoma**	(NTP, 1984); (Huff et al., 1985)

Species/strain, sex, No/group	Route, dose levels, duration of exposure	Results/Remarks	Reference
		M: 0/49, 2/40, 1/44 F: 0/49, 1/42, 1/49 **Forestomach: all papillomas or carcinoma** M: 0/49, 7/40*, 1/44 F: 0/49, 5/42*, 10/49** **Liver: hepatocellular carcinoma** F: 0/50, 1/47, 1/49 **Liver: hepatocellular adenoma or carcinoma** F: 0/50, 2/47, 5/49* **Mammary gland: acinar cell carcinoma** F: 0/50, 2/49, 6/49* **Mammary gland: Adenosquamous carcinoma** F: 0/50, 4/49, 0/49 **Ovary: Granulosa cell tumour** F: 0/49, 6/45*, 12/48** **Preputial gland: carcinoma** M: 0/50, 3/50, 2/50 **Brain: Gliomas** M: 0/50, 2/50, 1/50 **Zymbal gland carcinoma** M: 0/50, 0/50, 2/50 F: 0/50, 0/49, 1/49	
B6C3F1 mice; M/F (n=70-90/sex/group)	0, 6.25, 20, 62.5, 200, 625 ppm (0, 14, 44, 138, 440 or 1380 mg/m^3) 6 h/d, 5d/wk for up to 103 wks	Significant decrease in survival in M/F at ≥20 ppm due to chemical-related malignancies. No F exposed to ≥ 200 ppm or M exposed to 625 ppm survived to the end of the studies (M: 35/50, 39/50, 24/50, 22/50, 4/50, 0/70; F: 37/50, 33/50, 24/50, 11/50, 0/50, 0/70). No effects in mean body weights. Lymphocytic lymphomas, seen as early as wk 23, were the principal cause of death in M/F at 625 ppm **Lymphocytic malignant lymphoma** (in the 0, 6.25, 20, 62.5, 200 and 625 ppm group) M: 2/50, 0/50, 2/50, 4/50, 2/50, 49/73** F: 1/50, 3/50, 6/50*, 3/50, 8/50**, 31/80** **Histiocytic sarcoma** M: 0/50, 0/50, 4/50, 5/50, 7/50, 4/73* F: 3/50, 2/50, 7/50, 4/50, 7/50*, 4/80* **Malignant lymphomas (all)** M: 4/50, 2/50, 4/50, 6/50, 2/50, 51/73** F: 6/50, 12/50, 11/50, 7/50, 9/50**, 32/80** **Heart: hemangiosarcoma** M: 0/50, 0/49, 1/50, 5/48*, 20/48**, 4/73** F: 0/50, 0/50, 0/50, 1/49, 21/50**, 23/80** **Lung: Alveolar/bronchiolar**	(NTP, 1993)

Species/strain, sex, No/group	Route, dose levels, duration of exposure	Results/Remarks	Reference
		adenocarcinoma, or carcinoma M: 5/50, 6/50, 11/50*, 12/49*, 22/50**, 3/73 F: 0/50, 5/50*, 11/50**, 9/50*, 19/50**, 8/78* **Forestomach: squamous cell carcinoma** M: 0/50, 0/50, 0/50, 1/50, 2/73* F: 0/50, 0/50, 1/50, 1/50, 1/50, 6/80** **Forestomach: squamous cell papilloma or carcinoma** M: 1/50, 0/50, 0/50, 1/50, 8/50**, 4/73**,[b] F: 0/50, 0/50, 3/50, 2/50, 4/50, 22/80** **Liver: Hepatocellular carcinoma** M: 11/50, 16/50, 16/50, 17/48, 26/48**, 1/72 F: 4/49, 6/49, 8/50, 9/50, 8/50*, 1/80 **Liver: hepatocellular adenoma or carcinoma** M: 21/50, 23/50, 30/50, 25/48, 33/48*, 5/72[b] F: 15/49, 14/49, 15/50, 19/50*, 16/50*, 2/80[b] **Harderian gland: carcinoma** M: 0/50, 1/50, 1/50, 3/50, 2/50, 0/73 F: 0/50, 1/50, 1/50, 0/50, 1/50, 0/80 **Harderian gland: adenoma or carcinoma** M: 6/50, 7/50, 9/50, 20/50**, 31/50**, 6/73* F: 8/50, 10/50, 7/50, 15/50*, 20/50**, 9/80 **Kidney: renal tubule adenoma** M: 0/50, 1/50, 0/50, 3/48, 1/49, 0/73 F: 0/49, 0/49, 0/48, 0/50, 2/50, 0/80 **Preputial gland: carcinoma** M: 0/50, 0/50, 0/50, 0/50, 5/50*, 0/73 **Ovarian: Malignant granulosa cell tumour** F: 0/49, 0/49, 0/48, 3/50*, 2/50*, 0/79 **Ovarian: Benign or malignant granulosa cell tumour** F: 1/49, 0/49, 1/48, 9/50**, 8/50**, 6/79 **Mammary gland: carcinoma** F: 0/50, 2/50, 2/50, 6/50, 11/50*, 12/80* **Mammary gland: adenoacanthoma, carcinoma, or malignant tumour** F: 0/50, 2/50, 4/50, 12/50**, 15/50*, 16/80**	
B6C3F1 mice; M (n=50/group)	0, 200 pm for 40 wk, 312 ppm for 52 wks, 625 ppm for 13 or 26 wk The product of the exposure	Survival significantly lower that than of controls in all exposed groups, due to malignant neoplasms, particularly malignant lymphomas and hemangiosarcomas of the heart. No effect on mean body weights **Hematopoietic system: Lymphomatic**	(NTP, 1993)

Species/strain, sex, No/group	Route, dose levels, duration of exposure	Results/Remarks	Reference
	concentration x the duration of exposure (ppm–weeks) was approximately 8000 ppm–weeks or 16 000 ppm–weeks for the four exposure groups 6 h/d, 5 d/wk Upon termination of exposure, animals were evaluated at wk 103	**malignant lymphoma** ((in 0, 200 ppm (40 wk), 625 ppm (13 wks), 312 (52 wks), 625 (26 wks) group)) M: 2/50, 6/50, 17/50**, 4/50*, 30/50** **Histiocytic sarcoma** M: 0/50, 5/50*, 2/50*, 7/50**, 2/50* **Heart: hemangiosarcoma** M: 0/50, 15/50**, 7/50**, 33/50**, 13/50** **Lung: Alveolar/bronchiolar adenocarcinoma, or carcinoma** M: 5/50, 22/50**, 18/50**, 16/50**, 11/50** **Forestomach: squamous cell papilloma or carcinoma** M: 1/50, 3/50, 7/50*, 9/50*, 10/50 **Liver: Hepatocellular adenoma or carcinoma** M: 21/50, 33/49*, 24/49, 24/50, 13/50 **Forestomach: squamous cell carcinoma** M: 0/50, 0/50, 4/50**, 5/50**, 6/50** **Harderian gland: adenoma or carcinoma** M: 6/50, 27/50**, 23/50**, 30/50**, 13/50** **Kidney: renal tubule adenoma** M: 0/50, 4/48, 1/50, 1/49, 2/50 **Brain: malignant glioma** M: 0/50, 0/50, 2/50, 1/50 **Brain: malignant neuroblastoma** M: 0/50, 0/50, 2/50, 0/50	
B6C3F1 mice; M/F (n=60/sex/group)	0, 1000, 5000, 10000 ppm (0, 2200, 11000 or 22000 mg/m^3) Single 2-h exposure, mice evaluated at 2 years	No effect on survival or body weights. No significant differences in tumour incidences were observed between control and exposure groups.	(Bucher et al., 1993)

[a] overall rates: animals bearing lesions at the specific anatomic site/number of animals examined at that site/necropsied; [b] low incidences reflecting the occurrence of early deaths/lower survival from malignancies; p≤ 0.05, ** p≤ 0.001 (Fisher exact or life table tests or logistic regression analysis); † significant positive trend

The induction of thymic lymphomas was investigated in B6C3F1 and NIH Swiss mice (n=60) exposed to 1,250 ppm 1,3-BD for 52 weeks, to probe whether the high incidence of this tumour type in the mouse strain in the original NTP (1984) study was at least partially dependent on the activation of an endogenous retrovirus. The NIH Swiss mouse strain does not express the ecotropic murine leukemia viruses expressed in B6C3F1 mice and it has a background rate of

nearly zero for thymic lymphoma. The finding that exposure to 1,3-BD caused a 14% (8/57) incidence of thymic lymphomas in NIH Swiss mice, as opposed to an incidence of 57% (34/60) in B6C3F1 mice suggested that the lack of retroviruses provides resistance to the induction of the lymphomas by 1,3-BD (Irons et al, 1987, 1989, Irons, 1990 as cited in (IARC, 2008)).

The 1,3-BD metabolite, 1,2:3,4-diepoxybutane (DEB) has been evaluated in long-term (18 months) inhalation studies in female B6C3F1 mice and Sprague-Dawley rats (n=56/group), exposed to 0, 2.5 and 5 ppm (Henderson et al., 1999, Henderson et al., 2000). Significantly increased incidences of Harderian gland adenomas in female mice at 5 ppm (0/40, 2/42, 5/36 in controls, 2.5 and 5 ppm groups, respectively), and of squamous cell carcinoma of the nose in all exposed female rats (0/47, 12/48, 21/48) were reported. These findings indicate that DEB is carcinogenic in the respiratory tract of rats.

In earlier studies, D,L- and L-DEB administration via subcutaneous or intraperitoneal injection in rats and mice resulted in increased incidences of fibrosarcomas in female rats and female mice at the injection site and lung tumours in mice of both sexes. A gavage study in mice did not produce any tumours, while life-long dermal applications of D,L-DEB and *meso*-DEB (3- 100 mg) in Swiss male and female mice produced skin papillomas and squamous-cell carcinomas (Van Duuren et al, 1963, Van Duuren et al, 1965, Van Duuren et al, 1996, and Shimkin et al, 1996, as cited in IARC (2008)).

7.7.3 Summary

Extensive human data on 1,3-BD cancer effects have been published. The data mainly concern significantly increased mortality due to leukaemia and other types lymphohematopoietic cancer. The reported associations remained statistically significant after adjusting the risk estimates by age and other confounding factors.

1,3-BD has been evaluated for its carcinogenic potency in chronic inhalation studies in rats and mice, revealing species-related differences in the multiple sites of neoplasm induction and the magnitude of dose-dependent responses:

- in rats: neoplasms present in the pancreas, testis, mammary gland and thyroid gland
- in mice: neoplasms present in the hematopoietic system, heart, lung, forestomach, liver, Harderian gland, preputial gland, brain, ovary, mammary gland and kidney.

Significantly increased incidences of multiple tumour types in either sex, in the relevant NTP bioassay in mice, provided *'clear evidence of carcinogenicity'* of 1,3-BD.

Overall, mice were markedly more sensitive to the carcinogenic potential of 1,3-BD compared to rats; the lung was the most sensitive site, presenting significantly increased incidences of alveolar/bronchiolar adenocarcinoma or carcinoma in females exposed to concentrations as low as 6.25 ppm (14 mg/m^3).
In rats, a statistically significant increase in exposure-related tumour incidences was only seen in the Leydig cells of the testes in males, and positive, dose-related trends in mammary and thyroid tumours in females (at ≥ 1000 ppm; 2200 mg/m^3), rendering 1,3-BD an overall weak carcinogen to the rat.

7.8 Reproductive toxicity

7.8.1 Human data

The studies of the reproductive effects of 1,3-BD in humans have been extremely scarce.

- von Ehrenstein et al. (2014) examined the association between *in utero* exposure to toxic air pollutants (including 1,3-BD, meta/para-xylene, other aromatic solvents, lead, perchloroethylene and formaldehyde) and risk of childhood autism among a cohort of 148722 children born in Los Angeles County (US) between 1995-2006 and whose mothers resided during pregnancy in a 5-km buffer around air toxic monitoring stations. Exposure to 1,3-BD

(ppbV) was: mean±SD=0.31±0,17, interquartile range (IQR)=0.28. In the cohort, 768 cases of autism have been identified. Statistical analyses were adjusted for maternal age, race/ethnicity, nativity, education, insurance type, parity, child sex, and birth year. Authors found that the childhood autism risk was elevated per interquartile range increase in 1,3-BD exposure (OR =1.59, 95% CI = 1.18–2.15). In 3-toxics models (1,3-BD, formaldehyde and trichloroethylene), the significant association with 1,3-BD disappeared (OR=1.15, 95%CI=0.79-1.68).

- Willis and Hystad (2019) assessed an impact of multiple hazardous air pollutant (HAP) exposures (including 1,3-BD) during pregnancy on adverse birth outcomes among 289651 births of Portland (USA). Associations between cumulative and individual multiple hazardous air pollutant exposures were examined. Prenatal exposure to 19 multiple hazardous air pollutants was assessed using a dispersion model applied to maternal residential address at delivery. Linear and logistic multivariate regression models to assess associations between individual and cumulative multiple hazardous air pollutant exposures and preterm term, term birth weight, and small for gestational age, adjusting for several potential individual and neighbourhood confounding factors (birth year, birth month, infant sex, maternal and paternal race, maternal and paternal ethnicity, maternal and paternal education, payment mechanism, maternal alcohol and tobacco use during pregnancy, gestational or chronic diabetes, gestational or chronic hypertension, maternal weight gain, census tract median household income, census tract percent population below poverty line, census tract percent racial minority, and gestational age). 99th percentile of 1,3-BD exposure yielded 0.62 µg/m^3. Small size for gestational age was significantly associated with 1,3-BD (β=1.18 (95%CI=1.07-1.3)). No associations were observed between term birth weight and preterm birth.
- Poli et al. (2020) conducted a cross sectional study in Italy that involved 86 males with diagnosis of idiopathic male infertility, and 46 controls with no alteration in sperm characteristics. Seminal plasma and urine samples were analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS) to quantify biomarkers of exposure. 1,3-BD biomarkers included DHBMA, mandelic, phenylglyoxylic , and phenylhydroxyethylmercapturic acids. The authors concluded that urinary DHBMA levels were negatively correlated with sperm count and sperm abnormal forms.

7.8.2 Animal data

As shown in Figure 4 of PEROSH (2021), 12 studies with LOELs are available, and all studies were of high quality (codes 1-2). The median of the LOELs was 200 ppm (equivalent to 450 mg/m^3), with LOAELs ranging from 6 to 1250 ppm ((equivalent to 13.5-2812.5 mg/m^3). Results from both toxicological and epidemiological studies were for the most part heterogeneous. However, the scientific evidence shows a dose or concentration-dependent effect of 1,3-BD on human health and a hazard characterization based on animal studies (PEROSH (2021)). The main limitations of the review lie in the fact that the authors did not consider exposure duration and animal species, nor the relevance of these studies for humans.

Below ECHA has reviewed the relevant information.

7.8.2.1 Reproductive toxicity/ Fertility

Several studies are reported regarding the potential reproductive toxicity of 1,3-BD (Table 22).

- In Anderson et al. (1996), groups of 25 male rats were exposed to 0, 65, 400 or 1,250 ppm butadiene for 6 hours/day, 5 days/week for 10 weeks prior to mating. An additional group of 50 males was kept as an untreated control group. Three days after the last exposure, each male was allowed to mate with two untreated females, over a 10-day mating period. Females were sacrificed on day 20 of pregnancy and numbers of corpora lutea, live implantations, early deaths, late deaths and dead fetuses were counted. All malformed fetuses were stored for further examination, along with one normal litter mate and one concurrent control fetus. Exposure to 1,3-BD had no effect on mating success nor on fertility. There was no effect of

treatment on pre- or post-implantation losses, early or late deaths nor on the numbers of dead or abnormal fetuses. Overall, this study showed no evidence for a dominant lethal effect of 1,3-BD in the rat (as cited in (EU RAR, 2002)).

- In Anderson et al. (1996) CD-1 male mice were exposed either singly or repeatedly to 1,3-BD, in addition to a control group (n=25).
  In the single exposure protocol, males were exposed to 1,250 ppm (n=25) and 6,250 ppm (n=50) for 6 hours.
  In the repeated exposure regime, males were exposed to 12.5 ppm (n=25) and 1,250 ppm (n=50), 6 hours/day, 5 days/week for 10 weeks. Each male was mated with 2 unexposed females.
  Following mating, one female per pair was sacrificed on day 17 of gestation while the other was allowed to deliver and rear the litter. Females sacrificed on day 17 of gestation were examined for the number of implantations and post implantation deaths (early and late deaths, including dead fetuses). No effect of treatment on bodyweight in surviving animals was reported and no clinical observations of systemic toxicity were seen.
  Following single exposure there was no treatment-related effect on the pregnancy rate, the number of implantations nor on post-implantation deaths, and therefore no evidence of a dominant lethal effect.
  Following repeated exposure, the pregnancy rate was unaffected. At 12.5 ppm, there was no change in the total number of implantations per pregnancy. At 1,250 ppm there was a statistically significant reduction in the total number of implantations per pregnancy and also a statistically significant increase in post-implantation losses (mainly due to an increase in early deaths). Overall, these results indicate that repeated exposure of male mice to 12.5 or 1,250 ppm of 1,3-BD can induce dominant lethal mutations in the germ cells which lead to an increase in post-implantation losses. (as cited in (EU RAR, 2002)).

- CD-1 male mice were investigated for the effect of shorter duration exposure to 1,3-BD (Anderson, 1998). Groups were exposed to 0, 12.5, 65 and 130 ppm 6 hours/day, 5 days/week for 4 weeks. Each male was then mated with 2 females for up to one week. Females were necropsied on day 17 of gestation and examined for the presence of live implantations, early and late deaths and dead fetuses.
  There were no treatment-related mortalities, and no clinical signs nor any treatment-related effect on bodyweight. Fertility was unaffected by exposure. There was no statistically significant difference in the total number of implantations per pregnancy in treated groups compared with controls. However, a statistically significant increase in the number of early deaths per implantation per pregnancy was observed at 65 and 130 ppm (not in the 12.5 ppm group). The numbers of late deaths, excluding or including dead fetuses, were not increased in any exposure group. One possible explanation may be that several females were necropsied prior to gestation day 17, and therefore effects arising late in gestation were not detected. Overall, this study confirms other results, namely that exposure to 1,3-BD causes dominant lethal mutations in mice, as indicated by an increase in early deaths. This effect was evident following repeated exposure to 65 or 130 ppm, but not to 12.5 ppm (as cited in (EU RAR, 2002)).

- As described in section 7.3.2.2, in an NTP (1993) study, groups of 70 male and 70 female B6C3F1 mice were administered 0 (chamber control), 6.25, 20, 62.5, or 200 ppm of 1,3-BD by inhalation for 6 hours/day, 5 days/week, for up to 103 weeks. Also 2 groups (90 male and 90 female mice) were administered 625 ppm 1,3-BD on the same schedule.

  At 625 ppm,
  - testicular atrophy was induced in male B6C3F1 mice exposed (also in higher doses, as per above previous studies (NTP, 1984));
  - ovarian atrophy of moderate severity was observed in female mice exposed for 9 months, (and also in the 200-ppm animals), while the ovaries of mice exposed to 62.5 ppm appeared normal. The atrophic ovaries had no identifiable oocytes, follicles, or corpora lutea.

After 15 months of exposure, ovarian atrophy was observed at exposure levels of 20 ppm and above.
Up to 2 years of exposure, the incidence of ovarian atrophy was increased at all exposure concentrations (6.25 to 625 ppm) compared with controls.

Even though ovarian atrophy in the 6.25 ppm group was not observed until late in the study, when reproductive senescence was likely occurring, the dose-response data clearly establish the ovary as a target organ of 1,3-BD toxicity in female mice (but also testicular atrophy). Therefore, the LOAEC, the lowest concentration studied, was defined as 6.25 ppm (equivalent to 14.06 mg/m^3).

Fertility studies revealed no adverse effects in guinea pigs, rabbits and rats at exposure concentrations up to 6700 ppm (15075 mg/m^3) for 8 months (Carpenter et al., 1944) (as cited in SCOEL (2007)).

Table 22: Summary of inhalation studies on reproductive toxicity of 1,3-BD

Species, strain, sex, number/ group	Doses, duration of exposure	Results	References*
Rats, mice	Rats: 65, 400, 1250 ppm For 6h/day, 5 days/ week for 10 weeks Mice: 1250-6250 ppm/ single and 12.5-1250 ppm/ repeated For 6h/day, 5 days/ week for 10 weeks (unless single expo)	In rats: Exposure to 1,3-BD had no effect on mating success nor on fertility. There was no effect of treatment on pre- or post-implantation losses, early or late deaths nor on the numbers of dead or abnormal fetuses. Overall, this study showed no evidence for a dominant lethal effect of 1,3-BD. In mice: After single expo, no treatment-related effects; After repeated expo, at 1250 ppm, statistically significant reduction in the total number of implantations per pregnancy and also a statistically significant increase in post-implantation losses (mainly due to an increase in early deaths). Overall, these results indicate that repeated exposure can induce dominant lethal mutations in the germ cells which lead to an increase in post-implantation losses. In addition there was a statistically significant increase in the incidence of abnormal fetuses at both 12.5 and 1,250 ppm. The incidence was higher at 12.5 ppm. The toxicity observed in the fetuses of the 12.5 ppm group was late fetal death, exencephaly and skull abnormalities. However these findings are not clearly reproducible and the significance of these observations for human health is unclear.	Anderson, 1996
Mice and rats	12.5, 65, 130 ppm; 6h/day, 5 days/wk, 4 weeks 65, 400, 1250 ppm; 6h/day, 5 days/wk, 10 weeks	Mice: LOAEC = 65 ppm Reduction in the number of round and elongated sperm heads was seen in mice exposed to 130 ppm 4 weeks. This was not associated with changes in fertility or significant effect for implantations and abnormal foetuses, but to was associated in an increase in early fetal deaths. Rats: No significant effects that was ascribed to exposure	Anderson, 1998
B6C3F1 Mice	0, 625, 1250 ppm;	LOEC = 625 ppm	Melnick, 1988 (NTP

Species, strain, sex, number/ group	Doses, duration of exposure	Results	References*
(male+ female)	6h/day, 5 days/wk For up to 61 weeks	Significant testicular atrophy, ovarian atrophy	study) (see Table 15)
B6C3F1 mice	6.25, 20, 62.5 and 200 ppm + 625 ppm 6 hours/day, 5 days/week, for up to 103 weeks	Testicular atrophy, of minimal to mild severity, was present in 6 of 10 males exposed to 625 ppm 1,3-BD. Ovarian atrophy was marked in female mice exposed to 200 or 625 ppm 1,3-BD, while ovaries of mice exposed to 62.5 ppm appeared normal. Until the end of the study, the dose-response data clearly establish the ovary as a target organ of 1,3-BD toxicity at concentrations as low as 6.25 ppm, the lowest concentration studied. LOAEC = 6.25 ppm (equivalent to 14 mg/m^3).	Melnick et al. 1990b NTP, 1993 (see Table 15)

* References are taken from (EU RAR, 2002).

7.8.2.2 Other study type

A sperm-head morphology assay was conducted in mice (Hackett et al., 1988a). Details of the study are provided by Morissey et al. (1990) (as cited in (EU RAR, 2002)). Groups of 20 male mice were exposed to 0, 200, 1,000 or 5,000 ppm 1,3-BD 6 hours/day for 5 days and sacrificed in the fifth week post-exposure. Mice were examined for lesions of the reproductive tract and for gross tissue abnormalities.
At least 500 sperm heads per mouse were examined for morphological abnormalities. Transient signs of toxicity, piloerection and dyspnea, were observed immediately following exposure to 5,000 ppm, but there were no mortalities and no effect on bodyweight in any groups compared with control. There was a concentration-related increase in the percentage of abnormal sperm in exposed mice, with an increase of 21% in abnormal sperm at 200 ppm, 73% at 1,000 ppm and 129% at 5,000 ppm, relative to controls. The increases at 1,000 and 5,000 ppm were statistically significant.

The reproductive effects of 1,3-BD by inhalation have been evaluated in male mice by reduction of post-meiotic germ cells, alteration of sperm chromatin structure and transmission of chromosome aberrations to one-cell embryos (Pacchierotti, 1988). Mice were exposed 130, 500 or 1300 ppm for 6 hr/ day and for 5 consecutive days. The testicular fraction of post-meiotic germ cells was measured by flow cytometric analysis based on their DNA content. Round spermatids were discriminated from mature, elongated spermatids by their different degree of chromatin condensation. 1,3-BD-induced cytotoxic effects on differentiating spermatogonia were shown by a concentration-dependent decrease of round spermatids occurring 21 days after chemical exposure, confirmed by a similar decrease of elongated spermatids measured in testes sampled 7 days later. Statistically significant effects were seen already at 130 ppm. An incomplete repopulation of the elongated spermatid compartment observed 35 days after exposure to 1300 ppm suggested that the toxicity extended to stem cells.
Chromosome-type structural aberrations were significantly elevated in first-cleavage embryos conceived by males mated during the first and second week after the end of exposure. The lowest effective tested concentration was 500 ppm, the same reported for dominant lethal induction under identical exposure conditions. As in the dominant lethal assay, the effect of this dose was confined to exposed sperm, while both sperm and late spermatids were affected by the inhalation of 1300 ppm. A quantitative comparison between the effects induced by intraperitoneal injections of DEB or by 1,3-BD inhalations suggested that other reactive intermediates, in addition to DEB, might contribute to mediate 1,3-BD-induced reproductive toxicity.

Table 23: Summary of inhalation studies on other toxicity effects of 1,3-BD

Species, strain, sex, number/ group	Doses, duration of exposure	Results	References
mice	200, 1000, 5000 ppm; 6 hrs/day 5dys/ wk for 5 weeks	Significant concentration-related increase in the percentage of sperm with abnormal heads and decrease of normal sperm	Hackett, 1988a
CD1 mice (M – n=20)	200, 1000, 5000 ppm; 6 hrs/day 5dys/ wk for 5 weeks	There was an indication from effects seen in the first 2 weeks post-exposure that 1,3-BD may result in damage to the more mature sperm cells, spermatozoa and spermatids. However, the results were not conclusive.	Hackett, 1988b (summarised by Morissey et al., 1990 and cited in EU RAR 2002)
mice	130, 500, 1300 ppm; 6 hrs/day for 5dys	LOAEC=130 ppm (decreased testis weight and reduction in round spermatids) Chromosome-type structural aberrations were significantly elevated in first-cleavage embryos conceived by males mated during the first and second week after the end of exposure.	Pacchierotti, 1988

7.8.2.3 Developmental toxicity

Developmental toxicity studies have shown no effects at exposures below those causing maternal toxicity (Hackett et al., 1987a,b; Irvine, 1981) (as cited in SCOEL (2007); (EU RAR, 2002); ATSDR (2012)).

- (Irvine, 1981) exposed female rats to 0, 200, 1,000 or 8,000 ppm of 1,3-BD for 6 hours/day on days 6-15 of gestation and sacrificed on day 20. There were 40 negative controls, 24 females in each test group and 26 females in a positive control group dosed with aspirin. There was a statistically significant concentration-related reduction (14-45%) in maternal bodyweight gain at all exposure levels. There was a marginal concentration-related lowering of fetal weight and size (crown/rump length) which reached statistical significance at 8,000 ppm (mean fetal weight 6% less than control; crown/rump length 5% less than control). The values of these parameters were low in all groups, compared with historical controls. Statistically significantly increased incidences of marked and severe forms of wavy ribs, irregular rib ossification and incomplete ossification were noted at 8,000 ppm. These effects are considered to be indicative of delayed development. There was a statistically significantly increased incidence of bipartite thoracic centra in all exposure groups. This study demonstrates that 1,3-BD has a minor effect on fetal development at concentrations which are toxic to the dam. These effects can be attributed to delayed development, secondary to maternal toxicity and are therefore of low concern for human health (as cited in (EU RAR, 2002) and in ATSDR (2012)).

- Groups of 31-33 pregnant Swiss CD-1 mice and 30 pregnant Sprague-Dawley rats were exposed to 0, 40, 200 or 1,000 ppm butadiene for 6 hours/day on days 6-15 of gestation (Hackett et al., 1987a,b; as cited in (EU RAR, 2002)). Mice were sacrificed on day 18 of gestation and rats on day 20. Implantation sites were recorded. Live fetuses were weighed and gross, visceral and skeletal examination made.
  In rats, the top dose 1000 ppm induced maternal toxicity, observed as a statistically significant 31% reduction in bodyweight gain during gestation. Exposure to 1,3-BD had no effect on developmental parameters at any exposure concentration.
  In mice, a statistically significant reduction in maternal bodyweight gain during gestation was seen at 200 ppm (14% reduction) and 1,000 ppm (20% reduction). Fetal weight was statistically significantly lower at 200 ppm (16% less than control) and 1,000 ppm (22% less than control). There were no statistically significant increases in percentage resorptions or malformations per litter although there was a slight, statistically significant increase in minor

skeletal abnormalities at 200 ppm and/or 1,000 ppm, indicative of growth retardation (supernumerary ribs, reduced sternebral ossification and misaligned, scrambled or cleft sternebrae).
These studies demonstrate that 1,3-BD is not a developmental toxicant to the rat following inhalation exposure. However, in the mouse, 1,3-BD appears to have a minor effect on development, with retardation in fetal bodyweight and skeletal development seen at 200 and 1,000 ppm, concentrations which also produced evidence of maternal toxicity.

- In Anderson et al. (1996), as described in section 7.8.2.1 / Table 22 above, the results suggest that repeated exposure of males CD1 mice to 12.5 or 1,250 ppm 1,3-BD may be associated with the presence of abnormalities in the offspring. However these findings are not clearly reproducible and the significance of these observations for human health is unclear (as cited in (EU RAR, 2002)).

Table 24: Summary of inhalation studies on developmental toxicity of 1,3-BD

Species, strain, sex, number/group	Doses, duration of exposure	Results	References*
Rats	200, 1000, 8000 ppm 6hrs/day on Gestation Days (GD) 6-15	LOEC= 200 ppm From 200 ppm: depressed maternal body weight gain At 200 ppm: increased number of litters with minor skeletal defects, but not at higher dose levels; At 1,000 or 8,000 ppm: significant increases in litters with major skeletal defects At 8000 ppm: decreased fetal growth (lower body weights and crown-rump length)	Irvine, 1981 (cited in EU RAR, 2002, ATSDR, 2012)
SD rats (F)	0, 40, 200 and 1000 ppm 6 hours/day on days 6-15 of gestation	NOAEC = 1000ppm for fetus tox. 1000 ppm dose induced maternal toxicity, observed as reduction in bodyweight gain during gestation No evidence for teratogenic response in rats at any exposure concentration.	Hackett, 1987a (cited in EU RAR 2002)
B6C3F1 mice (F)	0, 40, 200 and 1000 ppm 6 hours/day on days 6-15 of gestation	NOAEC=40 ppm (decreased body weight of male fetuses), or LOAEC=200 ppm (decreased fetal body and placental weights, increased skeletal variations) Both maternal BW gain and fetus weight were reduced at 200 ppm Also increase in minor skeletal abnormalities at 200 ppm and/or 1,000 ppm, indicative of growth retardation (supernumerary ribs, reduced sternebral ossification and misaligned, scrambled or cleft sternebrae). alternative	Hackett, 1987b (cited in EU RAR 2002)

* References are taken from EU RAR (2002) and ATSDR (2012)

7.8.3 Summary

The studies of the reproductive effects of 1,3-BD in humans have been extremely scarce and no information on developmental toxicity in humans was located. This precludes establishing strong associations with 1,3-BD exposure.

In animals, several studies have led to the following conclusions:

Fertility studies with rats exposed to 2,200 mg/m^3 (980 ppm) showed decreased weight gain during pregnancy.
Fertility studies revealed no adverse effects in guinea pigs, rabbits and rats at exposure concentrations up to 6700 ppm (15075 mg/m^3) for 8 months (Carpenter *et* al., 1944) (as cited

in SCOEL (2007).
Finally in mice, testicular and ovarian atrophy were observed following long-term exposure to 1,375 and 13.8 mg/m^3, respectively. Male mice exposed to 2,200-11,000 mg/m^3 showed abnormal sperm head morphology.
The overall LOAEC for reproduction is 14 mg/m^3 (based on ovarian atrophy in mice, NTP (1993)).

In addition, developmental toxicity studies have shown no effect at exposures below those causing maternal toxicity. Therefore 1,3-BD is considered to not induce malformations in rats or mice. However, developmental toxicity in mice was indicated because of the decrease in fetal weight of male mice where the dams were exposed to 40 ppm or higher (Melnick et al. (1990a).

A developmental inhalation study indicated growth retardation in rat fetuses and an increase in major skeletal abnormalities as of a concentration of 1,000 ppm of 1,3-BD. The testes are also a target organ in the rat (Leydig cell tumour formation was observed in a 2-year study at 1000 and 8000 ppm).

The developmental effects following other routes of exposure have not been studied.

In mice, a decrease in fetal body weight gain in males was observed following exposure of dams to 88-2,210 mg/m^3 (40-1000 ppm) during GD 6-15. Increased incidences of extra ribs and reduced ossification of sternebrae were found in groups exposed to 442 and 2,210 mg/m^3 (200-1000 ppm), respectively. Observed effects included increased late fetal death, exencephaly and skull abnormalities. The LOAEC for developmental toxicity in mice is 27.5 mg/m^3 (12.2 ppm).

The results of developmental studies in the rat and mouse suggest development effects are secondary to maternal toxicity and are of low concern for human health (OECD SIDS, 1996).

8. Other considerations

8.1 Mode of action (MoA) considerations

Based on the available evidence, SCOEL (2007) agreed that 1,3-BD should be treated as a possible human carcinogen, operating via a genotoxic mechanism.

All three of 1,3-BD major electrophilic metabolites (namely EB, EBD, and DEB) react with DNA, to form a number of mono- and bifunctional adducts, including DNA-DNA crosslinks (Figure 1).

The resulting genotoxic effects including DNA strand breaks, unscheduled DNA synthesis and DNA repair, induction of sister chromatid exchange and ultimately mutations at the gene and chromosome levels (chromosomal aberrations, micronuclei induction) are mediated by the reactive metabolites – rather than by 1,3-BD itself - and have been documented in several test systems *in vitro* and *in vivo*. In humans, no consistent results were reported for gene mutations in exposed workers, but chromosomal damage affected by polymorphisms of genes involved in the bioactivation and detoxification of 1,3-BD (*GSTT1, GSTM1, CYP2E1, mEH, MTHFR*), as well as DNA-repair deficiencies (BER; certain *XRCC1* diplotypes; Fanconi anemia) has been shown, albeit inconsistently, to increase susceptibility.

The order of potency for mutagenicity *in vitro* or the induction of micronuclei in bone marrow cells of exposed animals is: DEB >> EB > EBD.

Mice are overall more susceptible than rats: when internal concentrations of DEB were measured using hemoglobin adducts, the relative mutagenicity between these two species correlated with the amounts of DEB produced, rendering DEB the key metabolic mediator of 1,3-BD-related mutagenicity.

Animal studies provide "clear evidence" for the multiple-site carcinogenicity of 1,3-BD with mice

exhibiting a higher sensitivity than rats, attributed to metabolic differences between the two species. Mutations in oncogenes/tumour-suppressor genes with human homologues frequently mutated in cancer, and the disruption of multiple genetic pathways in 1,3-BD-induced tumours from chronically exposed mice (e.g., *K-* and *H-ras*, *TP53*, *Catnb,* p16/p15 and Wnt pathway), further establish the mutagenic activity as the key mediator of the carcinogenic mode of action of 1,3-BD and 1,3-BD-derived epoxides.

Although DNA damage accumulates in all tissues investigated in animal studies, not all of them are equally susceptible to carcinogenesis. Recent studies indicate that the variation in epigenetic alterations may be a key driver in the susceptibility of target organs (and 'resistance' of non-target organs or strains). Sex, strain, and tissue-dependent effects such as loss of cytosine DNA methylation or hypermethylation, and genome protective chromatic condensation, transcriptional silencing have been reported in mice.

Overall, sufficient information is available to conclude on a non-threshold MoA for carcinogenic action, which is considered the critical effect for exposure to 1,3-BD.

8.2 Lack of specific scientific information

Not identified.

8.3 Groups at extra risk

Not identified.

9. Evaluation and recommendations

9.1 Cancer risk assessment

9.1.1 Published approaches for cancer risk assessment

A non-threshold MoA is assumed for 1,3-BD (see Section 8). This chapter describes the cancer risk assessment of 1,3-BD performed by numerous EU and international bodies, and individual authors. The summary of the assessments by the regulatory bodies is presented in Table 27.

9.1.1.1 Occupational population

9.1.1.1.1 SCOEL

SCOEL (2007) considered 1,3-BD as a possible human carcinogen, operating via a genotoxic mechanism, and calculated the excess risk entailed to exposure during a working life to various concentrations of 1,3-BD, according to the established approach for such carcinogenic substances.

As a key study, SCOEL chose the data from the "Delzell" cohort of SBR workers (Delzell et al., 2001) to assess the risk of leukaemia. The authors used two different statistical models, to estimate a risk coefficient (β) per unit of exposure:

- *Linear non-threshold where the excess risks are of the form "β x cumulative exposure"*. To obtain the risk coefficient per unit of exposure, each observed excess risk (RR or SMR-1) was divided by the associated cumulative exposure. When a set of median cumulative exposures and associated relative risks were available, the risk coefficient per unit exposure was obtained by applying a linear interpolation to the data via Poisson regression techniques.
- *Step model*. The risk coefficient per exposure unit remains constant in a certain range of exposure and then changes abruptly (step) moving to the next range. Ranges of cumulative exposure above 0.0 ppm and associated relative risk estimates were combined with a dummy variable indicating a specific range.

Also, the number of expected deaths from leukaemia in the absence of the exposure of interest

was estimated in a reference male population (England and Wales) with a lifetable approach. Assuming that exposure lasted for a working life (40 years, between the ages of 20 and 65), the number of predicted leukaemia deaths associated with different cumulative exposure to 1,3-BD were calculated, using the estimated coefficients indicating the excess relative risk for each ppm of cumulative exposure, for a population of 1000 exposed male workers between the ages of 20 and 85.

Predicted and expected deaths were compared, and results expressed as either additional deaths (predicted deaths - expected deaths) or excess SMR (Standardized Mortality Ratios; predicted deaths/expected deaths).

Table 25 presents the assessment of the number of excess deaths and excess SMR associated with lifetime exposure to 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10 ppm of 1,3-BD, obtained with the "step" model.

Table 25: Number of excess deaths and SMR based on risk assessment (SCOEL, 2007)

	Number of EXCESS DEATHS and SMR based on risk assessment													
	TWA (ppm)													
	0.1		0.2		0.5		1		2		5		10	
	Excess Deaths	SMR	Excess Deaths	SMR	Excess Deaths	SMR	Excess Deaths	SMR	Excess Deaths	SMR	Excess Deaths	SMR	Excess Deaths	SMR
Min	-1.02	0.80	-1.02	0.80	-1.02	0.80	-0.09	0.98	-0.47	0.91	-0.05	0.99	1.73	1.34
Max	7.64	2.50	7.64	2.50	7.64	2.50	10.78	3.12	9.88	2.94	11.67	3.30	21.45	5.26

SCOEL (2007) gives an example of how this table reads: *"In a population of 1000 adult males experiencing a mortality rate similar to that of the male population of England and Wales, occupational exposure to 1 ppm of 1,3-butadiene for a working life (40 years between the ages of 25 and 65), will cause from 0.0 to 10.78 extra leukaemia deaths between the ages 25-85 years, in addition to the 5 leukaemia deaths expected to occur in the absence of exposure to 1,3-butadiene."*

Using the central estimate (10.78/2), an excess risk of 4 x 10^{-3} leukaemia deaths corresponds to an exposure level of 0.74 ppm (NEG, 2022).

SCOEL (2007) did not consider necessary to set a STEL or propose a "skin" notation.

9.1.1.1.2 AGS

AGS (2010) identified 1,3-BD as a genotoxic carcinogen and derived an ERR for leukaemia risk, based on epidemiological evidence from a cohort of US/Canadian SBR workers (Graff et al., 2005). As described in Section 7.7.1, the estimated ERR considered cumulative exposures to 1,3-BD below 100 ppm and used adjustments for age and date of hire.

For the calculations of excess risk, AGS (2010) used a rounded slope value of two (i.e., excess risk of 2 x 1000 for exposure to 1 ppm for 35 years).

The calculations took into account the residual risk of leukaemia in the population (reported at 1% in the US male population and other industrialised countries). The duration of occupational exposure was 35 years, considered equivalent to 40 years. The exposure-risk relationship (ERR) is presented in Table 26.

Table 26: Exposure-risk relationship for 1,3-butadiene (AGS, 2010)

1,3-BD concentration, long-term mean, 35 to 40 years of occupational exposure		Exposure-related lifetime leukaemia risk
ppm	µg/m³	
15	33660	3%
5	11220	1%
2	4488	4 to 1000
1	2244	2 to 1000
0.5	1122	1 to 1000
0.05	112	1 to 10000
0.005	11	1 to 100000

9.1.1.1.3 ANSES

ANSES (2011) prepared a scientific recommendation for an atmospheric occupational exposure limit (OEL) of 1,3-BD. It was not possible to determine a threshold-based health effect, given the known non-threshold MoA for 1,3-BD.
Studies among SBR workers were considered relevant for risk assessment and leukaemia was chosen as the critical endpoint, referring to an evaluation of Health Canada (2000) (see Section 9.1.1.2.1).

An initial assessment concluded that using the model best suited to the data, the concentration of 1,3-BD corresponding to 1% excess risk of mortality from leukaemia was estimated at 8 mg/m^3. With low-dose linear extrapolation and an occupational exposure scenario for 1,3-BD of 8h/day, 240 days/year, over 45 years of professional life (probability calculated up to 70 years), the additional risk of death due to leukaemia was estimated to be:

- 1 x 10^{-4} for 45 years of exposure at a concentration of 0.08 mg/m^3
- 1 x 10^{-5} for 45 years of exposure at a concentration of 0.008 mg/m^3
- 1 x 10^{-6} for 45 years of exposure at a concentration of 0.0008 mg/m^3.

9.1.1.1.4 DECOS

DECOS (2013) concluded that 1,3-BD is a genotoxic carcinogen, expressing its genotoxicity through its reactive metabolites (EB, DEB and EBD), which are alkylating agents. DECOS (2013) noted that *"1,3-butadiene expresses its carcinogenicity at lower exposures compared to the lowest exposure at which toxic effects other than carcinogenicity become manifest."*

DECOS (2013) selected Cheng et al. (2007) as the key study because this study provided the *"most extensive set of quantitative data between leukaemia death risk and 1,3-butadiene exposure, was most transparent in the methods used regarding exposure data and exposure-response modelling, including corrections for co-exposure to styrene and DMDTC."*

DECOS (2013) employed the life-table analysis to estimate cancer risk values, by assuming that:

- the leukaemia mortality of the general population was calculated on the basis of national data on leukaemia mortality in five-year age bands (mortality data for the years 2000-2010, separated by age and sex; rates for women and men were averaged to describe the average risk for the population; to soften the transitions between age categories, the mortality data were 'smoothed');
- the occupational exposure of the cohort started at the age of 20 and continued until the age of 60. The cohort was followed until it reached the age of 100;
- the leukaemia included in the analysis consisted of categories C81-C96 according to 10th International Code of Diseases (ICD-10). DECOS (2013) noted that *"The diagnosis and classification of lymphatic and haematopoietic malignancies is very complex, and has*

undergone several changes in the course of time. Thus, limiting the risk evaluation to leukaemia only would certainly result in an underestimation of the risk of developing cancer following butadiene exposure";

- the linear additive model (RR = 1 + 0.001159 x (cumulative exposure)) was chosen for life-table analysis, as it had a slightly better fit compared to an exponential model (RR = 1 + 0.005934 x (cumulative exposure)$^{0.7626}$).

The excess risk of leukaemia mortality due to the 1,3-BD exposure yielded the following values:
- Extra risk of 4 x 10^{-3} deaths, at 40 years of occupational exposure, equals to 10 mg/m^3 (5 ppm)
- Extra risk of 4 x 10^{-5}, at 40 years of occupational exposure, equals to 0.1 mg/m^3 (0.05 ppm)

9.1.1.1.5 BAuA

In 2015, the Federal Institute for Occupational Safety and Health in Germany (BAuA, 2015) prepared a substance evaluation report (under REACH). They noted that "*Some uses (PROCs) mentioned in the registration dossier indicated that potential worker exposure might occur. Exposure scenarios to these uses needed to be evaluated for the quality of data and plausibility. The levels of exposure should be compared with available DMEL[6]/DNEL[7] and exposure risk relationships. Present data indicate that the DMELs calculated may give rise to exposures well above a risk ratio of 4: 1,000.*"

The eMSCA had two conclusions:

- Workers. After carrying out an evaluation of registrants and [AGS 2010] approaches, they noted that "*The risk calculation of the registrants is not supported. Nevertheless, the proposed DMEL of 1 ppm (2.21 mg/m^3) has been taken for risk assessment. Based on the registrants' DMEL of 1 ppm, the reported exposure values do not exceed this DMEL in general. Within the AGS concept the reported exposure values are between the tolerance level of 2 ppm and the acceptance level of 0.2 ppm. Due to the fact that the exposure values are closer to the acceptance level both approaches lead to the conclusion that there is no need for further activities like the initiation of a restriction or an authorisation procedure*".
- Consumers. "*Based on epidemiological studies in workers exposed to butadiene an inhalative DMEL for consumers was derived with 1.50 µg/m^3 (0.0007 ppm)*."

9.1.1.1.6 NFA

In 2022, the National Research Centre for the Working Environment (NFA, 2022), under the request of the Danish Working Environment Authority, set a working group to review data relevant to assess the hazard of 1,3-BD and to calculate health-based OELs relying on data from both human and animal studies.

NFA (2022) regarded 1,3-BD-induced cancer (leukaemia) and ovarian atrophy as critical effects. Leukaemia was considered a non-threshold effect due to the formation of DNA adducts, whereas ovarian atrophy was considered a threshold effect.
NFA (2022) calculated 'health-based OELs' equivalent to ECHA's ERR, based on cancer (leukaemia) data from both human and animal studies.

Human studies

NFA (2022) used two recent updates of a cohort of SBR workers (Cheng et al., 2007, Sathiakumar et al., 2015) as their key studies, and a life-table analysis. using Danish mortality data from all lymphohematopoietic malignancies (ICD-10 C81-C96):

- The average risk of dying from all lymphohematopoietic malignancies in the Danish general population between 1997-2016 (background mortality) resulted in 1.015 % for

[6] Derived Minimal Effect Level
[7] Derived No Effect Level

both sexes (men = 1.26 %, women = 0.77 %).

- The RR of mortality of from all lymphohematopoietic malignancies caused by 1,3-BD was calculated by adding 1 extra case to the background mortality at the different risk levels (RR=1.099 for 1 x 1 000, RR=1.0099 for 1 x 10 000, RR=1.00099 for 1 x 100 000).
- Assuming 1 x 1 000 excess deaths among men and women and *RR = 1 + 0.0007 x cumulative exposure* (ERR for all leukaemia mortality obtained from Sathiakumar et al. (2015)), the corresponding cumulative exposure in mg/m^3-years resulted in 140.7 mg/m^3-years (3.1 mg/m^3 for a 45-year work life).

The excess risk of all lymphohematopoietic mortality due to the 1,3-BD exposure yielded the following values:
- 1 x 10^{-3} for 45 years of exposure at a concentration of 3.1 mg/m^3
- 1 x 10^{-4} for 45 years of exposure at a concentration of 0.31 mg/m^3
- 1 x 10^{-5} for 45 years of exposure at a concentration of 0.031 mg/m^3

Animal studies

As comparison, NFA (2022) also performed risk assessment based on lymphoma incidence data, based on a 2-year inhalation study in mice (NTP, 1993). Correction to an 8-hour working day (the mice were exposed 6 h/day) and for a higher breathing rate in workers at light work (10 m^3/day) compared to rest (6.7 m^3/day) resulted in an exposure of 222.36 µg/m^3. After emphasizing the fact that the comparison of the excess risk between mice and humans was not straightforward, NFA noted that *"the risk estimates were remarkably similar."*

The excess risk of lymphoma incidence due to the 1,3-BD exposure yielded the following values (based on mice data):
- 1 x 10^{-3}: concentration of 1.566 mg/m^3
- 1 x 10^{-4}: concentration of 0.1566 mg/m^3
- 1 x 10^{-5}: concentration of 0.01566 mg/m^3

9.1.1.2 General population

9.1.1.2.1 Health Canada

In 2000, Health Canada performed its cancer risk assessment for the general population using human data of leukaemia from a cohort of SBR workers (Health Canada, 2000, Delzell et al., 1996). A life-table analysis was undertaken, for which the following assumptions were used:

- Use of Canadian all-cause and mortality rates, follow-up until 70 years.
- Four risk functions tested but the linear risk function had the better fit.
- ERL of 1%
- Correction for environmental exposure (240/345 days and 8/24 hours)

The levels of leukaemia mortality risk reached:
- 1 x 10^{-6}: 0.17 µg/m^3
- 1 x 10^{-5}: 1.7 µg/m^3
- 1 x 10^{-4}: 17 µg/m^3

9.1.1.2.2 US EPA

In 2002, US EPA performed its cancer risk assessment for the general population using the human data on leukaemia from a cohort of SBR workers (Delzell et al., 1996, US EPA, 2002).

A life-table analysis with the following assumptions was used:
- Use of US all-cause and leukaemia mortality and incidence rates 1994-1998, both sexes combined; follow-up until 85 years
- Several dose-response relationships (DRR) functions tested (linear, log-linear, power)
- ERL of 1%
- Correction for environmental exposure (240/345 days and 10/20 m^3 for the quantity of

inhaled air)

Depending on the model used, a PoD corresponded to ERL of 1% varied between 0.037 and 0.87 ppm. The linear model reached 0.027 ppm and was chosen for extrapolation.

The levels of leukaemia mortality risk reached:
- 1 x 10^{-6}: 0.03 µg/m^3
- 1 x 10^{-5}: 0.3 µg/m^3
- 1 x 10^{-4}: 3 µg/m^3

9.1.1.2.3 OEHHA

In 2011, the Californian Office of Environmental Health Hazard Assessment performed its cancer risk assessment for the general population (OEHHA, 2011), taking into account the genotoxic MoA of 1,3-BD.
Tumour occurrence in mice was the toxic effect taken into consideration (Melnick et al., 1990), because human data from epidemiologic studies were considered inadequate. However, OEHHA noted that differences exist in kinetics between rats and mice, with a higher metabolic rate for 1,3-BD in mice, limited detoxification and hence accumulation of 1,3-BD in rats.
Moreover, the formation of the primary reactive genotoxic metabolite (EB) may be a significant factor in the increased susceptibility of mice to 1,3-BD. Therefore, OEHHA chose the increase in alveolar and bronchiolar lung tumours as the most critical effect.

The levels of cancer risk (alveolar and bronchiolar lung tumours) reached:
- 1 x 10^{-6}: 0.006 µg/m^3
- 1 x 10^{-5}: 0.06 µg/m^3
- 1 x 10^{-4}: 0.6 µg/m^3

9.1.1.2.4 TCEQ

In 2015, the Texas Commission on Environmental Quality performed its cancer risk assessment for the general population (TCEQ, 2015), assuming genotoxic MoA of 1,3-BD and using the human data on leukaemia from a cohort of SBR workers (Cheng et al., 2007). A life-table analysis for leukaemia with the following assumptions was done:
- Use of the US all-cause and leukaemia mortality rates for the years 2000-2003.
- Follow-up until 70 years
- Log-linear risk function
- ELR of 0.1%

After correction for environmental exposure (5 days/week versus 7 days/week and 10/20 m^3 for the quantity of inhaled air), the ERL yielded 5.07 x10^{-7} µg/m^3.

The levels of leukaemia mortality risk reached:
- 1 x 10^{-6}: 2 µg/m^3
- 1 x 10^{-5}: 20 µg/m^3
- 1 x 10^{-4}: 200 µg/m^3

9.1.1.2.5 ANSES

In 2022, ANSES published a report focusing on the derivation of reference values for the general population (ANSES, 2022). The latest follow-up study of SBR workers (Sathiakumar et al., 2021b) was used and chronic lymphoid leukaemia was chosen as the most critical endpoint.

The following assumptions for life-table analysis were used:
- Use of French all-cause mortality and incidence of lymphoid leukaemia (International Classification of Diseases 10th revision (ICD-10) C91) for years 2015-2017, both sexes combined.
- Lifelong exposure to 1,3-BD, follow-up until 84 years.
- RR of lymphoid leukaemia obtained from the recent study of SBR workers:

RR=2.7 (95%CI=1.08-6.76), $\beta=4.91x10^{-4}$ (95% $CI_{up}=8.31 \times 10^{-4}$)
- Log-linear risk function: RR=exp(βx)
- Excess lifetime risk (ELR) of 1%.

A point of departure (PoD) of 6 ppm was calculated (equivalent to 13.5 mg/mg[3]) using linear extrapolation.

After a correction for workers-to-general population (days of exposure per year (240/365) and quantity of inhaled air (20/10 m^3)), the PoD resulted as 41 062µg/m^3.

The levels of lymphoid leukaemia mortality risk were estimated to be:
- 1×10^{-4} for lifelong exposure at a concentration of 410 µg/m^3
- 1×10^{-5} for lifelong exposure at a concentration of 41 µg/m^3
- 1×10^{-6} for lifelong exposure at a concentration of 4 µg/m^3.

9.1.1.3 Other reports and scientific publications

9.1.1.3.1 PEROSH (2021)[8]

The joint research program of the Partnership for European Research in Occupational Safety and Health (PEROSH) reviewed toxicological (animal) and epidemiological data on 1,3-BD (PEROSH, 2021).

Human studies of SBR were used to derive DRR for the following lymphohematopoietic cancers:
- Leukaemia (y= 0.0025x + 1.4792; R^2 = 0.5474)
- Non-Hodgkin's lymphoma (NHL) (y=-7E-06x2+ 0.0073x + 0.8012 ; R^2 = 0.5373)
- Multiple myeloma (y =-6E-08x3+ 4E-05x2-0.0059x + 0.8434 ; R^2 = 0.4426)

The authors noted that it was common in epidemiological studies that a DRR does not to have a continuous monotonic course. A positive trend line was observed for risk of leukaemia, non-Hodgkin lymphoma (NHL) and multiple myeloma (MM).
The 1,3-BD doses in ppm-years for the doubling of the relative risk, RR=2 (i.e., for an attributable fraction > 50%) were as follows:
- leukaemia and NHL: approximately 200 ppm-years,
- multiple myeloma: approximately 400 ppm-years.

9.1.1.3.2 Sielken & Valdez-Flores (2015)

Sielken and Valdez-Flores (2015) quantitatively assessed cancer risks of environmental and occupational exposure to 1,3-BD, using human data on lymphohematopoietic cancer of SBR workers. Risk assessment was performed for all leukaemia and leukaemia subtypes (acute myelogenous leukaemia (AML), chronic lymphocytic leukaemia (CLL), chronic myelogenous leukaemia (CML), and the groups of lymphoid and myeloid neoplasms). Cox proportional hazards log-linear models were used to fit exposure–response models. Follow-up was until 70 years.

The excess risk for the general population was the largest for lymphoid neoplasms.

The best estimates of the environmental concentrations of 1,3-BD associated with an excess risk of 10^{-5} were: lymphoid leukaemia (0.06 ppm), all leukaemia (0.16 ppm) and chronic lymphoid leukaemia (0.38 ppm).

The best estimates of the occupational 1,3-BD exposure from 20 to 65 years of age associated with an excess risk of 1×10^{-4}:
- lymphoid leukaemia (2.7 ppm)
- all leukaemia (7.3 ppm)
- CLL (15.1 ppm).

[8] Only review of toxicological data and dose-response relationship derivation was performed.

9.1.1.3.3 Valdez-Flores et al. (2022)

Valdez-Flores et al. (2022) explored the relationship between six endpoints (all leukaemia, lymphoid leukaemia, myeloid leukaemia, multiple myeloma, non-Hodgkin's lymphoma, and bladder cancer) and exposures to 1,3-BD using the most recent exposure metrics and the most recent update of the SBR study by Sathiakumar et al. (2021b).

The statistical analysis resulted in an upper 95% confidence level on the cancer potency based on leukaemia of 0.000086 per ppm.

9.1.1.3.4 Khoshakhlagh et al. (2022)

Khoshakhlagh et al. (2022) performed a probabilistic human health risk assessment of 1,3-BD and styrene in the carpet production industry. The sensitivity and uncertainty analysis were performed by the Monte Carlo simulation (MCS) technique.

The average air concentration measured of 1,3-BD during work shifts of employees were 0.039 mg/m^3 (0.017 ppm).

The mean ± standard deviation (SD) value of estimated cancerogenic risk in inhalation exposure to 1,3-BD were $5.13 \times 10^{-3} \pm 3.85 \times 10^{-4}$ and, respectively, exceeding the acceptable risk level of 10^{-6} defined by US EPA.

The average non-carcinogenic risk (HQ) values of 1,3-BD 8.50×10^{0}, exceeding the acceptable risk level of 1.
The authors concluded that *"As the results of our studies exceeded both cancerogenic and non-carcinogenic risk values it indicates that adverse health effects due to inhalational exposure to 1,3-BD and styrene for workers in the machine carpet industry are very likely."*

9.1.1.3.5 Kirman & Hays (2022)

Kirman and Hays (2022) used biomarker data and relative potencies of mutagenic metabolites to support derivation of cancer unit risk (UR) values for 1,3-BD from rodent tumour data.

UR were derived for 1,3-BD based upon its ability to cause tumours in laboratory mice and rats. Metabolism has been established as the significant molecular initiating event of 1,3-BD's carcinogenicity. This approach used biomarker data (haemoglobin adducts) to quantify species differences in the internal doses of 1,3-BD metabolites experienced in mice, rats, and humans.

Using these methods, the dose–response relationships in mice and rats resulted in upper bound UR values ranging from 2.1×10^{-5} to 1.2×10^{-3} per ppm of 1,3-BD.

Table 27: Summary of existing cancer risk assessments based on human data and 1,3-BD concentrations corresponding to 4 x 10^{-5} risk

Reference	Key study	Cancer type	DRR to derive PoD	Unit of cancer risk	1,3-BD concentration for 4 x 10^{-5} risk	Comments
Occupational population						
SCOEL (2007)	Delzell et al. (2001)	All leukaemia	Linear and "step"	Not available	0.007 ppm (0.016 mg/m^3)	Human data. 40 years of occupational exposure.
AGS (2010)	Graff et al. (2005)	All leukaemia	Linear	2 x 10^{-3} per ppm	0.02 ppm (0.045 mg/m^3)	Human data. 35 years of occupational exposure.
ANSES (2011)	Delzell et al. (1996)	All leukaemia	Linear	Not available	0.03 mg/m^3	Human data. Relied on Health Canada (2000) evaluation.
DECOS (2013)	Cheng et al. (2007)	All leukaemia	Linear	Not available	0.1 mg/m^3	Human data. 40 years of occupational exposure.
NFA (2022)	Sathiakumar et al. (2015)	All leukaemia	Linear	Not available	0.1 mg/m^3	Human data. 45 years of occupational exposure.
General population						
Health Canada (2000)	Delzell et al. (1996)	All leukaemia	Linear	5.9 x 10^{-6} per µg/m^3	0.007 mg/m^3	Human data. 70 years of exposure.
US EPA (2002)	Delzell et al. (1996)	All leukaemia	Linear	3 x 10^{-5} per µg/m^3	0.001 mg/m^3	Human data. 85 years of exposure.
OEHHA (2011)	Melnick et al. (1990)	Lung tumours	Linearised multistage model (LMS)	1.7 x 10^{-4} per µg/m^3	0.0002 mg/m^3	Animal (mice) data. Exposure duration not available.
TCEQ (2015)	Cheng et al. (2007)	All leukaemia	Linear	5 x 10^{-7} per µg/m^3	0.08 mg/m^3	Human data. 70 years of exposure.
BAuA (2015)	Not available	All leukaemia	Not available	6.7 x 10^{-6} per µg/m^3	0.06 mg/m^3	Human data. 70 years of exposure.
ANSES (2022)	Sathiakumar et al. (2021b)	Lymphoid leukaemia	Log-linear	2.43 x 10^{-7} per µg/m^3	0.16 mg/m^3	Human data. 85 years of exposure.

Notes: 1,3-BD, 1,3-butadiene; NHL, Non-Hodgkin lymphoma; SMR, standardised mortality ratio; CI, confidence interval; DRR, dose-response relationship.

9.1.2 Cancer risk assessment

There is clear evidence of carcinogenic effects of 1,3-BD in humans.

A statistically significant increase in all leukaemia deaths after exposure to 1,3-BD was observed in a cohort of US and Canadian SBR workers: RR in the range of 2.63 (95% CI=1.18-5.86) to 3.63 (95% CI=1.59-8.32), for exposure > 213.43 ppm-years (Sathiakumar et al., 2015). This study is described in detail in Section 7.7.1.

The present risk assessment was performed using these good quality epidemiological human data and Sathiakumar et al. (2015) was selected as a key study (because of a higher number of 1,3-BD dose categories in comparison to Sathiakumar et al. (2021b))[9].

All leukaemia deaths were chosen as the most sensitive endpoint. Assessing the risk for specific leukaemia sub-types or using incidence data was not possible because of the lack of statistical data at EU level.

A life-table analysis (National Research Council, 1988) was conducted to estimate lifetime risks of dying from all leukaemia for the EU population.

- The life-table analysis calculates the excess risk using a life-table by age category, taking into account the fraction of the (hypothetical) original population cohort which would still be available to experience the excess risk in each age category and then sums up these to a life-time risk.
- The life-table analysis is preferred because it takes into account the shrinking of the population at risk due to other causes of death.

The input for the life-table analysis (leukaemia deaths (ICD-10 C91-C95) and all-cause deaths were mortality rates, averaged for both sexes across 27 EU countries for the years 2012-2017 from the Eurostat database. The occupational exposure was 40 years (between 20 and 60 yrs) and the excess risk was calculated until 85 years of age[10].

The lifetime risk of dying from all leukaemia (background mortality, 0-85 yrs) in the EU population (2012-2017) was 0.57% (5.7 per 1 000, 57 per 10 000 or 570 per 100 000).

As done in previous recent risk assessments (see Table 27Table 27), we fitted a linear dose-response curve to the human data from Sathiakumar et al. (2015), because the data from Sathiakumar et al. (2015) seemed to follow linear dose-response relationship below the highest two deciles, being consistent with the current low 1,3-BD exposure levels. Therefore, the two highest dose deciles (448.17-<908.35 ppm-years and ≥908.35 ppm-years) have been omitted to allow a better fit to lower dose range and more conservative risk estimation.

The dose-response relationship (1,3-BD exposure in ppm-years/all leukaemia deaths) can be written as:

$$RR = 1 + 0.00553 \times cumulative\ exposure.$$[11]

The extra-risk of 4 x 100 000 resulted in 0.07 mg/m^3 (0.03 ppm) of 1,3-BD concentration.

The resulting excess risk of leukaemia mortality by the level of exposure is described in Table 28 and expressed per 100 000 exposed individuals.

[9] Sathiakumar et al. (2021b) is the most recent update of the same cohort as Sathiakumar et al. (2015). A request was sent to their research team to request data on RRs (hazard ratios) and mean and range of 1,3-BD exposure in ppm-years within each decile (similar to Sathiakumar et al. (2015)). In the absence of this information, the decision was made to use the data from Sathiakumar et al. (2015).

[10] ECHA 2019. Guidance on information requirements and chemical safety assessment Appendix to Chapter R.8: Guidance for preparing a scientific report for health-based exposure limits at the workplace.

[11] The slopes with all dose range and with only the highest decile omitted, yielded 0.00155 and 0.00354, respectively.

Table 28: Cancer exposure-risk relationship (all leukaemia deaths) after working life exposure to a given 8-hour air concentration for five working days a week over a 40-year working life period

1,3-BD concentration in air (mg/m³)	1,3-BD concentration in air (ppm)	Excess life-time leukaemia risk (Cases per 100 000 exposed)
0.07	0.03	4
0.68	0.3	40
6.8	3	400
68	30	4000

9.2 Derived Occupational Exposure Limit (OEL) values

9.2.1 Published approaches to establish OELs

9.2.1.1 DECOS

DECOS (2013) noted that toxic effects (other than cancer) have been reported in experimental animals: ovarian atrophy in female mice (NTP, 1993).

To establish a PoD, DECOS (2013) chose the lowest overall LOAEC of 6.25 ppm based on ovarian atrophy observed in mice in the two-years carcinogenicity/toxicity inhalation study (NTP, 1993).

A benchmark dose (BMD) analysis (10% response level) yielded a BMDL of 1.0 mg/m³.
To derive a health-based OEL for humans, DECOS (2013) applied two uncertainty factors of 3: one to correct for interspecies differences, and one to correct for intraspecies differences.
Because the exposure of the experimental animals was for 6h/day, 5 days/week for 103 weeks, additional uncertainty or uncertainty factors were not applied.

This resulted in an OEL of 0.11 mg/m³. DECOS (2013) considered that this OEL is practically equal to the 4 x 10^{-5} risk (0.1 mg/m³ or 0.047 ppm) derived for humans using mortality data on leukaemia in SBR workers.

9.2.1.2 NFA

NFA (2022) considered that 1,3-BD-induced ovarian atrophy is of relevance to humans, and that it is the critical effect, based on a 2-year inhalation study in female mice (NTP, 1993). NFA also noted that ovarian atrophy is probably caused by 1,3-BD and that mice produce more DEB than humans during metabolism of 1,3-BD.

The histopathological changes in NTP (1993) were clearly dose-dependent and the incidence of ovarian atrophy was increased at all exposure concentrations (6.25 to 625 ppm) compared with controls. Therefore NFA (2022) used the LOAEC of 6.25 ppm to derive a hypothetical OEL based on reproductive effects *("Derived No-Effect Level (DNEL) for toxicological effects having thresholds"*).

The calculation performed by NFA (2022) consisted of several steps:
- The LOAEC corrected for an 8-hour working day and for a higher breathing rate in workers at light work (10 m³/day) compared to at rest (6.7 m³/day): LOAECcorr = 6.9 mg/m³
- The overall assessment factor of 15 (3 as very severe adverse outcome, 1 for interspecies extrapolation, 5 for intraspecies extrapolation) or 50 (10 as very severe adverse outcome, 1 for interspecies extrapolation, 5 for intraspecies extrapolation) was used.
  This resulted in a value between from 0.46 mg/m³ (460 µg/m³) to 0.138 mg/m³ (138 µg/m³).

NFA (2022) developed an alternative approach that included the following assessment factors:
- Use of LOAEC instead of a NOAEC: 10 (the highest factor due to the complete depletion of follicles). This resulted in 6.9 mg/m³.

- Human inherent variation in number of follicles at birth: 8.5
- Late age at birth of first child: 3 (there is a 5-fold decrease in the follicle reserve from the age of 15 until the age of 29 in Denmark.
- Inherent susceptibilities e.g. genetic polymorphisms in humans: 3
- Smokers are more exposed, because 1,3-BD is an abundant constituent in cigarette smoke: 2
- Humans are less fertile than rodents. Furthermore, humans differ substantially from mice in life span and in the time available for chronic exposure to induce ovotoxicity which is far longer in humans, and the generally greater robustness of the mouse reproductive system relative to the human: 10
- Lack of multigenerational studies and of dose-response data for partial follicle depletion which are the precursor step to ovarian atrophy: 3
- This resulted in 0.00015 mg/m^3 (15 µg/m^3).

NFA (2022) noted that there is strong evidence that ovarian atrophy is mediated by the formation of DEB because the interspecies variation between humans and mice is a 720-fold difference in DEB blood levels following metabolism of 1,3-BD, the corrected value should be multiplied by 720 resulting in 0.108 mg/m^3 (108 µg/m^3).

9.2.2 Published approaches to establish reference values for the general population

Several European and international regulatory bodies (OEHHA, 2013, TCEQ, 2015, US EPA, 2002) published approaches to establishing reference values for the general population for 1,3-BD based on toxic effects other than cancer. As the most sensitive effect, all organisations chose ovarian atrophy in female mice, based on NTP (1993).

These PoD were used:
- $BMCL_{10\ corrected}$ (lowest confidence interval corresponding to 10% response): 0.88 ppm (US EPA, 2002).
- $BMCL_5$ (lowest confidence interval corresponding to 5% response): 1.01 ppm (OEHHA, 2013). This value derived by OEHHA (2013) was also used by ANSES (2022).
- $BMCL_{5\ corrected}$: 0.462 ppm (TCEQ, 2015).
- LEC_5 (lowest confidence interval corresponding to 5% effect response): 1.01 ppm. Used by ANSES (2022) based on OEHHA (2013).

9.2.3 8h TWA for non-cancer effects

1,3-BD has been shown to cause carcinogenicity in humans and it is assumed to be related to a non-threshold MoA. For that reason, it is not possible to derive a health-based OEL, and exposure-risk relationships (ERR) were calculated from human data (see Section 9.1).

If an 8h-TWA level for non-cancer effects was to be derived from data on threshold effects, ovarian atrophy observed in repeated dose studies could be used as a starting point.

Ovarian atrophy was observed in a lifetime bioassay of female mice (NTP, 1993). The animals were exposed to 0, 6.25, 20, 62.5, 200, or 625 ppm for 6 h/day, 5 days/week for up to 65 weeks. While there was significant mortality, a concentration-related increase in ovarian atrophy was also observed (see Section 7.3.2).

Based on these results, NTP investigators identified a chronic LOAEC of 6.25 ppm (14.06 mg/m^3) for target organ effects on ovaries (atrophy) (NTP, 1993).

To derive a 8 h TWA value, the calculations would include the following steps:

1) Correct the point of departure (PoD) to correspond to worker exposure conditions:
$$14.06\ mg/m^3 \times 6h/8h \times 6.7\ m^3/10\ m^3 = 7.06\ mg/m^3.$$

2) Apply assessment factors: factor of 3 for LOAEC to NOAEC extrapolation, factor of 2.5 to cover interspecies differences, and 5 for worker intraspecies differences.
Therefore the 8h TWA= 7.06 mg/m^3 / (3 x 2.5 x 5) ≈ 0.19 mg/m^3 (equivalent to 0.08 ppm)

Assuming linearity, at this exposure level the excess life-time cancer risk would be approximately 1 x 10^{-4}.

9.2.4 Short-Term Exposure Limits (STELs)

A non-threshold MoA is assumed for the carcinogenic effects of 1,3-BD and an ERR is derived. There is no data of short-term effects necessitating additionally a STEL.

9.2.5 Biological Limit Value (BLV)

A non-threshold mode of action is assumed for the carcinogenic effects of 1,3-BD and it is not possible to identify a BLV under which no excess risk would occur.

There are some methods available to measure urinary levels of 1,3-BD and its (unspecific) metabolites, but biomonitoring has not been commonly used at workplaces. No BLV is proposed.

9.2.6 Biological Guidance Value (BGV)

No sufficient or robust information is available that would support the identification of background levels of 1,3-BD or specific metabolites in the general population. No BGV is proposed.

9.3 Notations

There are no data indicating skin irritation or respiratory sensitisation effects after 1,3-BD exposure.

Furthermore, 1,3-BD is highly volatile and transported as a liquid under pressure. Contact with rapidly vaporizing liquid can cause frostbite, so routine dermal exposure of compressed 1,3-BD is not expected to occur (due to concerns of frostbite).

Thus, no 'skin' notation or notation for skin or respiratory sensitisation are proposed.

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