National Research Centre
for the Working Environment

1,3-butadiene:

Scientific basis for setting a health-based occupational exposure limit

(1,3-butadien:

Videnskabelig dokumentation for helbredsbaserede risikoestimater)

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Report

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Foreword

In 2007, SCOEL produced a document with recommendations of an occupational exposure limit (OEL) for 1,3-butadiene (SCOEL, 2007). In 2008, the International Agency for Research on Cancer (IARC) classified 1,3-butadiene as carcinogenic to humans (IARC, 2008). IARC later updated their monograph with the latest scientific data (IARC, 2012). In 2013, the Dutch Expert Committee on Occupational Safety (DECOS) produced a criteria document on 1,3-butadiene (DECOS, 2013). In their report, DECOS criticized the risk assessment done by SCOEL. The risk assessment by DECOS resulted in less conservative risk estimates based on the exposure-response relationships from the most recent cohort study available at that time. In 2016, the European Commission proposed to revise or to introduce occupational exposure limit values for 13 carcinogenic chemical agents, among them 1,3-butadiene, out of 25 priority chemicals. ACSH (Advisory Committee on Safety and Health) performed the evaluation, taking economic, social and health impact into account, and suggested an EU-OEL of 1 ppm (2.2 mg/m^3) 1,3-butadiene, which scientifically was based on the preceding SCOEL recommendation from 2007. The suggested OEL was adopted by the European Committee by the European Parliament and the Council of the European Union in 2017 and was introduced in 'Directive 2004/37/EC of the European Parliament and of the Council on the protection of workers from the risks related to exposure to carcinogens or mutagens at work'. The current Danish OEL (TWA 8h) for 1,3-butadiene was accordingly updated in 2020 to 1 ppm (2.2 mg/m^3) based on the EU-OEL value.

At the request of the Danish Working Environment Authority, a working group at the National Research Centre for the Working Environment (NFA) reviewed data relevant to assess the hazard of 1,3-butadiene and calculate health-based OELs founded on data from both human and animal studies.

The working group wishes to thank Chief Toxicologist Poul Bo Larsen, DHI, Denmark for reviewing the report.

Copenhagen, 2022

Contents

Executive summary

In this rapport, a working group at the National Research Centre for the Working Environment (NFA) reviewed data relevant to assess the hazard of 1,3-butadiene and calculate health-based occupational exposure limits (OELs) based on data from both human and animal studies. The current Danish OEL for 1,3-butadiene is 1 ppm (2.2 mg/m^3).

1,3-butadiene is a colorless gas used as monomer and polymers in the production of a variety of synthetic rubber/plastic products. Workers in 1,3-butadiene monomer production plants and styrene-1,3-butadiene-based rubber/polymer plants are most likely to receive the largest occupational exposure by inhalation. Furthermore, workers can be exposed to 1,3-butadiene from petroleum refinery product streams or smoke produced during electrosurgery.

In 2008, IARC classified 1,3-butadiene as carcinogenic to humans (Group 1), as IARC evaluated that there was sufficient evidence in humans that 1,3-butadiene is causally related to leukemia. IARC found strong evidence that the carcinogenicity of 1, 3-butadiene operates by a genotoxic mechanism that involves formation of reactive epoxides and subsequent interaction with DNA. The main documentation on the carcinogenic potential of 1,3-butadiene in this report is based on the two most recent IARC monographs (IARC, 2008) and the update (IARC, 2012), and on the latest DECOS evaluation (DECOS, 2013).

Studies in experimental animals also provide evidence of significant effects on the reproductive system, such as ovarian and testicular atrophy and developmental toxicity. Reproductive toxicity is therefore included in the present report. The documentation is primarily based on a report by the California Office of Environmental Health Hazard Assessment (OEHHA, 2013) and (DECOS, 2013).

The current working group regards 1,3-butadiene-induced cancer (leukemia) and ovarian atrophy as critical effects. The current working group considers 1,3-butadiene-induced cancer (leukemia) to be a non-threshold effect due to the formation of DNA adducts, whereas ovarian atrophy is considered a threshold effect.

The current working group calculates health-based OELs based on cancer (leukemia) data from both human and animal studies, and in addition, Derived No-Effect Level (DNEL) for toxicological effects having thresholds based on reproductive toxicity data from animal studies (ovarian atrophy).

OEL based on leukemia mortality data from human studies
Different publications using the same cohort on synthetic rubber production workers in Nothern America have been used for risk assessment and calculations of health-based occupational cancer risk values (DECOS, 2013; SCOEL, 2007). DECOS based their assessment on modelled exposure data and leukemia mortality data from a cohort with 16,000 subjects (men) working at styrene–butadiene rubber production plants in Northern America during 1944-1998 (Cheng et al., 2007). The current working group identified a

new publication on the same cohort with an 11-years' follow-up (1944-2009) (Sathiakumar et al., 2015). We used both publications in our derivation of an OEL based on leukemia under the assumption of a non-threshold mechanism of action. The exposure-response relationship from the most recent Sathiakumar et al. study was similar to the Cheng et al. study. Overall, our calculations showed that the estimate of an excess risk of mortality is practically equal to the value calculated by DECOS. Thus, the current working group calculates the excess mortality risk to be 1:1,000 at 3.1 mg/m^3 1,3-butadiene based on the Sathiakumar et al. study.

OEL based on leukemia incidence in mice

In a 2-year inhalation study, mice exposed to 1,3-butadiene showed increased incidences of benign and malignant neoplasms at multiple sites (NTP, 1993). Significant carcinogennic response was observed at all assessed dose levels. The calculation of risk estimates resulted in 1:1,000 excess risk of cancer incidence at 1.566 mg/m^3. In the risk assessment, the current working group notes that although the calculations are based on incidence in mice and mortality in humans, respectively, the risk estimates were remarkably similar, despite observations showing that mice are more effective in metabolizing 1,3-butadiene to reactive epoxides.

OEL based on reproductive toxicity in mice

The current working group considers both cancer and ovarian atrophy as critical effects, as both adverse effects were observed in chronic long-term studies in female mice (NTP, 1993). The calculation of a DNEL for ovarian atrophy resulted in DNELs of 138 or 460 µg/m^3 depending on the choice of assessment factor for LOAEL (3 or 10, as suggested by ECHA). The current working group notes that compared to controls, the lowest air concentration of 1,3-butadiene tested induced an almost 5-fold increase in the incidence of ovarian atrophy in female mice. Based on this observation, the current working group regards the highest assessment factor of 10 as most appropriate.

Derived excess mortality risks and DNELs

In the table below, exess mortality risk at 1 in 1,000, 1 in 10,000 and 1 in 100,000 derived based on a human epidemiological cohort (Sathiakumar et al., 2015) and DNELs for reproductive toxicity derived based on the 2-year inhalation study of mice (NTP, 1993) is presented:

		Suggestion of an OEL for 1,3-butadiene	
Type of effect		**Leukemia mortality**	**Reproductive toxicity**
Non-threshold based	Extra mortality risk		
	1:1,000	3.1 mg/m^3	
	1:10,000	0.31 mg/m^3	
	1:100,000	0.031 mg/m^3	
Threshold- based	$DNEL_{max}$		0.138 mg/m^3
	$DNEL_{NFA}$		0.108 mg/m^3

The current working group considers both cancer and reproductive toxicity as critical effects. Therefore, the current working group recommends that both endpoints are taken into consideration.

Dansk sammenfatning

Ved fastsættelse af grænseværdier i arbejdsmiljøet indgår en række hensyn. Det drejer sig om helbredsrisikoen, men også tekniske og samfundsmæssige hensyn.

I NFA's arbejde med grænseværdidokumentation anvendes risikoestimater, som er et teoretisk mål for hvor mange, der ved dagligt udsættelse for stoffet ved grænseværdien efter et helt arbejdsliv (typisk efter 40-45 år) vil blive syge. I disse beregninger, er der *ikke* taget hensyn til personlige værnemidler eller andre kendte foranstaltninger til beskyttelse mod eksponering.

NFA udarbejder dokumentation for helbredsbaserede grænseværdier. Der tages udgangspunkt i publiceret systematisk litteraturgennemgang af epidemiologiske studier, dyrestudier og cellestudier af sammenhængen mellem udsættelse og risiko for forskellige helbredsudfald og de biologiske virkningsmekanismer. På baggrund af dette videnskabelige arbejde beregnes risikoestimaterne.

Dokumentation for helbredsbaserede grænseværdier vil sammen med de tekniske og samfundsmæssige betragtninger ligge til grund for forhandlinger mellem arbejdsmarkedets parter om endelig fastsættelse af grænseværdierne.

I denne rapport vurderer en arbejdsgruppe ved NFA data, der er relevante for at evaluere faren ved udsættelse for 1,3-butadien og beregne helbredsbaserede græseværdier for 1,3-butadien i arbejdsmiljøet. Beregningerne baseres på data fra både humane studier og dyreforsøg. Den nuværende danske grænseværdi for 1,3-butadien i arbejdsmiljøet er 1 ppm (2,2 mg /m^3).

1,3-butadien er en farveløs gas, der anvendes som monomer og polymerer i fremstillingen af forskellige syntetiske gummi- og plastprodukter. Arbejdstagere i produktionsanlæg af disse produkter er sandsynligvis dem, der modtager den største erhvervsmæssige eksponering ved indånding. Desuden kan arbejdstagere udsættes for 1,3-butadien fra råolieraffinaderiproduktion eller røg produceret ved anvendelsen af elektrokirurgi.

I 2008 klassificerede WHO's kræftagentur (IARC) 1,3-butadien som kræftfremkaldende for mennesker (Gruppe 1), da de vurderede, at der var tilstrækkelig dokumentation fra humane studier for, at 1,3-butadien kan forårsage leukæmi. IARC fandt betydeligt evidens for, at de kræftfremkaldende effekter af 1,3-butadien opererer ved en genotoksisk mekanisme, der involverer dannelse af reaktive epoxider og efterfølgende interaktion med DNA. Dokumentationen af de kræftfremkaldende effekter for 1,3-butadien, er i denne rapport især baseret på den seneste IARC-monografi (IARC, 2008) og dennes opdatering (IARC, 2012) og på den seneste evaluering foretaget af Den Hollandske Komité for Arbejdsmiljøsikkerhed (The Dutch Expert Committee on Occupational Safety) (DECOS, 2013).

Undersøgelser med forsøgsdyr har vist signifikante effekter på reproduktionssystemet, såsom ovarie- og testikelatrofi. Reproduktionstoksicitet er derfor inkluderet i denne rapport. Dokumentationen er primært baseret på en rapport fra California Office of Environmental Health Hazard Assessment (OEHHA, 2013) og DECOS, 2013.

Arbejdsgruppen betragter 1,3-butadien-induceret kræft (leukæmi) og ovarieatrofi som kritiske effekter. Arbejdsgruppen anser 1,3-butadien-induceret kræft (leukæmi) for at være en ikke-tærskeleffekt på grund af dannelsen af DNA-addukter, mens ovarieatrofi anses som værende en tærskeleffekt.

Arbejdsgruppen beregner helbredsbaserede grænseværdier baseret på kræft (leukæmi) data fra både humane studier og dyreforsøg, og derudover Derived No-Effect Level (DNEL) for toksikologiske virkninger med tærskeleffekt baseret på reproduktionstoksicitetsdata (ovarieatrofi).

Beregning af grænseværdi baseret på leukæmidødelighedsdata fra humane studier
Flere videnskabelige publikationer har anvendt samme kohorte med arbejdstagere fra produktionsanlæg af syntetiske gummiprodukter i Nord Amerika til risikovurdering af 1,3-butadien og beregninger af helbredsbaserede grænseværdier i arbejdsmiljøet (DECOS, 2013; SCOEL, 2007). DECOS baserede deres vurdering på modellerede eksponeringsdata og leukæmidødelighedsdata fra kohorten med 16.000 forsøgspersoner (mænd), der arbejdede på produktionsanlæg af styren-butadiengummi i løbet af 1944-1998 (Cheng et al., 2007). Arbejdsgruppen identificerede en ny publikation fra samme kohorte med 11 års opfølgning (1944-2009) (Sathiakumar et al., 2015). Vi anvendte begge publikationer i beregninger af helbredsbaserede risikoestimater baseret på leukæmidødelighed under antagelse af en ikke-tærskelmekanisme. Dosis-responsforholdet fra den seneste publikation af Sathiakumar et al. svarede til Cheng et al. Samlet set viste vores beregninger, at estimatet for en overskydende dødsrisiko praktisk talt er lig med værdien beregnet af DECOS. Arbejdsgruppen beregner således risikoen for overdødelighed til 1:1.000 ved 3,1 mg / m^3 1,3-butadien baseret på publikationen af Sathiakumar et al.

Beregning af grænseværdi baseret på forekomst af lymfomer hos mus
Et 2-årigt inhalationsstudie hos mus viste en øget forekomst af godartede og ondartede neoplasmer mange forskellige steder (NTP, 1993). De signifikante effekter blev observeret ved alle anvendte dosisniveauer. Beregningen resulterede i 1:1.000 overskydende risiko for kræftincidens ved 1,566 mg / m^3. I risikovurderingen bemærker arbejdsgruppen, at skønt beregningerne er baseret på henholdsvis lymfomforekomst hos mus og leukæmidødelighed hos mennesker, var resultaterne bemærkelsesværdigt ens på trods af, at mus er mere effektive til at metabolisere 1,3-butadien til reaktive epoxider.

Beregning af grænseværdi baseret på reproduktionstoksicitet hos mus
Arbejdsgruppen betragter både kræft og ovarieatrofi som kritiske effekter, da begge blev observeret i kroniske langtidsstudier på hunmus (NTP, 1993). Beregningen af en DNEL for ovarieatrofi resulterede i værdier på 138 eller 460 µg / m^3 afhængigt af valget af sikkerhedsfaktor for brugen af en LOAEL-værdi (3 eller 10, som foreslået af ECHA).

Arbejdsgruppen bemærker, at den lavest anvendte koncentration af 1,3-butadien inducerede en næsten 5 gange forøget forekomst af ovarieatrofi hos hunmus sammenlignet med kontroller. Grundet denne observation anser arbejdsgruppen den højeste sikkerhedsfaktor på 10 som den mest passende. Derudover beregnes en $DNEL_{NFA}$ hvor der tages højde for yderligere usikkerhedsfaktorer.

I nedenstående tabel præsenteres dødsrisiko ved 1 ud af 1.000, 1 ud af 10.000 og 1 ud af 100.000 baseret på humane studier (Sathiakumar et al., 2015) og DNELs for reproduktionstoksicitet baseret på et 2-årigt inhalationsstudie med mus (NTP, 1993):

		Forslag til grænseværdi for 1,3-butadien	
Effekt		**Leukæmidødelighed**	**Reproduktionstoksicitet**
Ikke-tærskelbaseret	Overskydende dødsrisiko		
	1:1.000	3,1 mg/m^3	
	1:10.000	0,31 mg/m^3	
	1:100.000	0,031 mg/m^3	
Tærskelbaseret	$DNEL_{max}$		0,138 mg/m^3
	$DNEL_{NFA}$		0,108 mg/m^3

Arbejdsgruppen betragter både kræft og reproduktionstoksicitet som kritiske effekter. Derfor anbefaler arbejdsgruppen, at begge endepunkter tages i betragtning.

Abbreviations

ACSH	Advisory Committee on Safety and Health
AF	Assessment Factor
BD	1,3-butadiene
BMD	Benchmark Dose
BMDL	Benchmark Dose Level
BMCL	Benchmark Concentration Level
CI	Confidence Interval
DEB	diepoxybutane
DECOS	The Dutch Expert Committee on Occupational Safety
DMDTC	dimethyldithiocarbamate
DNEL	Derived No-Effect Level
EB	epoxybutene
EBD	epoxybutane diol
ECHA	European Chemicals Agency
EU	European Union
IARC	The International Agency for Research on Cancer
ICD	International Code of Diseases
LOAEL	No Observed Adverse Effect Level
LOAEL	Lowest Observed Adverse Effect Level
NFA	National Research Centre for the Working Environment
NIOSH	The National Institute of Occupational Safety and Health
NTP	National Toxicology Program
OEHHA	California Office of Environmental Health Hazard Assessment
OEL	Occupational Exposure Limit
OSHA	Occupational Safety and Health Administration
REL	Reference Exposure Level
RR	Relative Risk
SCOEL	Scientific Committee on Occupational Exposure Limit Values
TWA	Time Weighted Average

Introduction

The chemical formula of 1,3-butadiene is C_4H_6 (CAS No: 106-99-0). 1,3-butadiene is a colorless gas with a mild aromatic or gasoline odor, and is highly volatile (boiling point -4.4°C) (IARC, 2008). 1,3-butadiene can be manufactured in different ways, but 95 % of the global production arises during co-production with ethylene production (IARC, 2008).

1,3-butadiene is used as a monomer in the production of a variety of synthetic rubber products and polymers, which are used as components in primarily tire products and different rubber/plastic materials. The synthetic rubbers that are produced from butadiene include styrene-butadiene rubber, polybutadiene rubber, styrene-butadiene latex, chloroprene rubber and nitrile rubber. Plastics that contain butadiene as a monomeric component are: shock-resistant polystyrene (a two-phase system of polystyrene and polybutadiene), polymers that consist of acrylonitrile, butadiene and styrene; and a copolymer of methylmethacrylate, butadiene and styrene (DECOS, 2013).

Workers in 1,3-butadiene monomer production plants and styrene-1,3-butadiene-based rubber/polymer plants are most likely to receive the largest exposure by inhalation. Petroleum refinery workers can be exposed, as 1,3-butadiene are present in petroleum refinery product streams due to its presence in crude oil, but exposure are generally low (Akerstrom et al., 2016). A study has furthermore reported that smoke produced during electrosurgery contained a considerable amount of 1,3-butadiene; however, the estimated continuous exposure per day seems to be low (Oganesyan et al., 2014).

In 2008, IARC classified 1,3-butadiene as carcinogenic to humans (Group 1), as IARC evaluated that there was sufficient evidence in humans that 1,3-butadiene is causally related to leukemia.

The current Danish OEL (TWA 8h) for 1,3-butadiene is 1 ppm (2.2 mg/m^3), and is regulated by the Danish Working Environment (updated in 2020).

The main documentation on the carcinogenic potential of 1,3-butadiene in this report is based on the two most recent IARC monographs (IARC, 2008) and the update (IARC, 2012), and on the latest DECOS evaluation (DECOS, 2013).

Studies in experimental animals also provide evidence of significant effects on the reproductive system, such as ovarian and testicular atrophy and developmental toxicity. Reproductive toxicity is therefore included in the present report. The documentation is primarily based on a report by the California Office of Environmental Health Hazard Assessment (OEHHA, 2013) and (DECOS, 2013).

Our literature search strategy matches the procedure suggested by DECOS in a new guidance document (DECOS, 2021). DECOS states: "The search starts with the search for reports that were published by other scientific organizations", such as e.g. SCOEL, IARC, and ECHA. "If such reports are available, the literature search starts at the last date of the search mentioned in the relevant assessment report". In line with this, we performed

a literature search (2010-2020) in the PubMed database overlapping in time with the 1,3-butadiene report from DECOS published in 2013. Thus, we put weight on other scientific organizations reports and their conclusions, although not uncritically. Furthermore, our critical appraisal was limited to original literature published after 2010. The search resulted in 831 publications using the keyword 1,3-butadiene. Of these, publications were manually excluded if they related only to synthesis and/or chemistry (e.g. polymerization), ambient emissions, smoking, or chlorinated butadiene. Publications that remained were those related to *in vivo* experiments (n=16), *in vitro* experiments (n=30), human studies (occupational and biomarker/exposure) (n=24), reproductive toxicity (n=8) and reviews (n=18). Of these, only some were relevant for inclusion in this report. 16 of the N=92 publications are cited directly in the present report. Due to the overlap in search periods, other five publications out of the 92 were included in the report by DECOS, but are not cited directly in the present report.

The OEL derivation and risk assessment methodology of this report will follow the guidelines outlined by REACH guidance documents (ECHA-RAC/SCOEL, 2017b; ECHA, 2012, 2019; ECHA/RAC-SCOEL, 2017a).

Human data

Human exposure

IARC and DECOS reviewed the occupational 1,3-butadiene exposure data:

"The highest exposure to butadiene occurs in occupational settings. No measurements of exposure in butadiene monomer production before the 1970s are available, but levels of exposure have decreased from the late 1970s to the early 2000s from < 20 mg/m³ to < 2 mg/m³."

"In styrene–butadiene polymer production, the estimated median levels of exposure to butadiene in earlier decades varied from 8 mg/m³ to 20 mg/m³, while current measurements of exposure in modern facilities in North America and Western Europe are generally below 2 mg/m³. Levels reported in China are somewhat higher (~4 mg/m³). Regardless of the type of factory, production process or country, some tasks are still characterized by very high exposures (~200 mg/m³) that are typically short in duration" (IARC, 2008).

"The average occupational exposure to butadiene in the European Union in 1995 as reviewed by IARC in 2008 was 3.1-7.5 mg/m³ for exposed production workers at 15 monomer production facilities, and 0.06-2.2 mg/m³ for controls (supposedly non-exposed) laboratory workers" (DECOS, 2013).

Electrosurgery is a commonly used technique in dermatology. A study showed that the smoke produced during electrosurgery contained a considerable amount of 1,3-butadiene. While monitoring of exposure during active electrosurgery, the probe collecting the smoke was placed at approximately the same point as where the surgeons' head would be located under normal operating position. Analysis of the smoke revealed air concentrations of 1,3-butadiene of 707 μg/m³. Surgeons were estimated to actively perform electrosurgery for 18 minutes of continuous exposure per day, corresponding to approximately 50 hours of continuous smoke exposure per year. However, these estimates will vary based on the particular practice and the number of surgeries done by each surgeon (Oganesyan et al., 2014).

The estimated number of workers exposed to 1,3-butadiene in Denmark are 422 (Directive, 2004//37/EC).

Cohort studies

1,3-butadiene is classified by IARC as *carcinogenic to humans* (group 1) because there is *sufficient evidence* in humans for the carcinogenicity of 1,3-butadiene as well as sufficient evidence from animal studies.

IARC reviewed studies of three cohorts of workers in the butadiene monomer industry and two cohorts of workers in the styrene-butadiene rubber industry (IARC, 2008):
"Three independent cohorts of monomer production workers in the USA have been studied: at two Union Carbide plants in West Virginia (Ward et al., 1995), at a Texaco plant in Texas (Divine & Hartman, 2001) and at a Shell plant in Texas (Tsai et al., 2001)".

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

Subsequently, researchers from the University of Alabama at Birmingham (Delzell et al., 1996) studied the two-plant complex originally investigated by NIOSH plus seven of the eight plants studied by the Johns Hopkins' University. The Johns Hopkins' researchers also conducted nested case–control studies within this working population (Matanoski et al., 1997; Santos-Burgoa et al., 1992). The University of Alabama at Birmingham group recently updated the follow-up of the cohort and revised and refined their assessment of exposures both to butadiene and to possible confounding co-exposures (Macaluso et al., 2004). A number of largely overlapping publications from these groups have been reviewed. The most recent results were publications by (Cheng et al., 2007; Graff et al., 2005; Sathiakumar et al., 2005)" (IARC, 2008).

IARC concluded that:
"Overall, the epidemiological studies provide evidence that exposure to butadiene causes cancer in humans. This excess risk cannot be reasonably explained by confounding, bias or chance. This conclusion is primarily based on the evidence for a significant exposure–response relationship between exposure to butadiene and mortality from leukaemia in the University of Alabama in Birmingham study, which appears to be independent of other potentially confounding exposures. It is also supported by elevated relative risks for non-Hodgkin lymphoma in other studies, particularly in the butadiene monomer production industry. The Working Group was unable to determine the strength of the evidence for particular histological subtypes of lymphatic and haematopoietic neoplasms because of the changes in coding and diagnostic practices for these neoplasms that have occurred during the course of the epidemiological investigations. However, the Working Group considered that there was compelling evidence that exposure to butadiene is associated with an increased risk for leukaemias" (IARC, 2008).

DECOS reviewed the same cohort studies mentioned above (no new cohort studies was published between the IARC and DECOS evaluations) and concluded:
"In two of the three butadiene monomer industry studies a slight overall excess of mortality from leukaemia was observed, whereas the third study reported a small deficit in mortality from leukaemia. The excess of mortality from leukaemia in one of the monomer industry cohorts was more pronounced among workers who had been exposed at high levels during the first years of production (Second World War). In this cohort, no increase in excess of leukaemia was observed with duration of exposure or cumulative exposure.

A review of the studies of styrene-butadiene rubber production workers by researchers at the University of Alabama in Birmingham (Cheng et al., 2007) was considered to be the most informative. In this review the mortality rates of approximately 17,000 workers from eight facilities in the USA and Canada were examined, and the authors included earlier studies of some of these facilities. A limiting factor in the evaluation was that the diagnosis and classification of lymphatic and haematopoietic malignancies are very complex and have undergone several changes over the course of time. The study used Cox regression procedures to examine further the exposure-

response relationships between several continuous time-dependent butadiene exposure indices: butadiene mg/m³-years, the total number of exposures to butadiene peaks > 221 mg/m³, and average intensity of butadiene. All three ways of expressing butadiene exposures were associated positively with leukaemia, supporting the presence of a causal relationship between high cumulative exposure and high intensity of exposure to butadiene and leukaemia. The analyses indicated that the exposure-response relationship for butadiene and leukaemia was independent of exposure to dimethyldithiocarbamate (DMDTC)" (DECOS, 2013).

The current working group notes that both IARC and DECOS conclude that the dose-related induction of leukemia appears to be independent of concurrent exposure to other possible carcinogens. Especially the concurrent exposure to styrene and DMDTC has been a common concern in the cohort studies of styrene-butadiene rubber production workers (Cheng et al., 2007). In 2019, IARC classified styrene in Group 2A, "probably carcinogenic to humans" based on limited evidence in humans and sufficient evidence in experimental animals for carcinogenicity (IARC, 2019). A carcinogenic mechanism of DMDTC has not been established in animals or humans; however, the immunesuppressive activity of DMDTC suggests it could play a role in the formation of lymphoid tumors (Kirman et al., 2010).

The current working group has identified a more recent publication on the styrene–butadiene rubber production workers cohort with a follow-up of 11 years (Sathiakumar et al., 2015). The current working group will use this new publication as well as the Cheng et al. 2007 study in the risk assessment.

Here follows brief descriptions of the two publications:

<u>Cheng et al. 2007:</u>

Follow-up period: 1944-1998

The cohort included 16,579 men classified as having worked at any of the six synthetic rubber plants, located in Texas (two plants), Louisiana (two plants), Kentucky (one plant) and Canada (one plant) for at least one year before 1992.

Several publications describe the methods used to identify subjects, develop work histories and how exposure estimates of the synthetic rubber worker cohort were modelled (Delzell et al., 1996; Graff et al., 2005; Sathiakumar et al., 2005). The development of quantitative estimates has been described in detail in (Macaluso et al., 1996; Macaluso et al., 2004).

In brief, job-exposure matrices with quantitative estimates of exposure to 1,3-butadiene, styrene, and DMDTC were developed for specific job titles and work areas of the different plants. The estimates were linked with individual workers' jobs as determined from personnel records to indicate individuals assigned to carry out operations with potential for exposure. Job- and year-specific estimates were linked with subjects' work histories to obtain cumulative exposure estimates. Exposure estimates varied among tasks, jobs, plants, and time periods (Sathiakumar et al., 2015).

These estimation procedures have several limitations. Although the estimates were quantitative, they were not actual measurements. Consequently, the validity of the estimates depends on the assumptions used in the models, and misclassification of exposure levels is likely (Sathiakumar et al., 2015). However, data from a validation study at one of the plants found that the correlation between estimated and measured 1,3-butadiene was moderate overall (Spearman's $r = 0.45$, $p < 0.0001$) and was high for jobs that pertained to typical styrene-butadiene rubber operations ($r = 0.81$, $p < 0.0001$) (Sathiakumar et al., 2007).

Information on cause of death was obtained from death certificates, the US National Death Index and the Canadian Mortality Data Base, mentioning leukemia or any other cancer of the lymphatic and hematopoietic tissues. A total of 81 decedents with leukemia were identified.

Potential confounders included in the analyses were air concentrations of DMDTC, race (nonwhite, other), plant, years since hire (<20, 20-29, 30+) and year of birth (<1909, 1909-1915, 1916-1922, 1923-1932, 1933+). Data were not adjusted for styrene exposure.

The analyses of 1,3-butadiene exposure relative to leukemia (cox regression) were based on 16,091 subjects and 485,732 person-years of observation for leukemia (488 men dropped out of follow-up at ages younger than the youngest leukemia decedent (age 33 years), as they were considered too young to develop leukemia). The relationship between 1,3-butadiene and leukemia is shown in Table 1.

Table 1. Mean values of 1,3-butadiene exposure, estimated rate ratio (RR) and 95% confidence interval (CI) for data included in Cox regression (Cheng et al., 2007).

Mean exposure ppm-years	Mean exposure mg/m^3	RR[a]	95% CI
0,00	0.00	1.00	1.0
4.82	10.66	1.13	(0.43, 2.98)
17.20	38.05	2.12	(0.81, 5.56)
30.52	67.52	2.03	(0.77, 5.34)
56.88	125.83	1.22	(0.47, 3.22)
124.02	274.37	0.94	(0.36, 2.46)
215.34	476.39	2.96	(1.13, 7.79)
282.31	624.55	4.00	(1.52, 10.51)
374.93	829.45	3.37	(1.28, 8.86)
606.37	1,341.46	2.94	(1.12, 7.73)
1,852.59	4,098.45	3.84	(1.51, 9.76)

[a] Rate ratio, controlling for age.

Conclusion of Cheng et al. 2007: *"The present analyses support the presence of a positive exposure-response relationship between several indices of 1,3-butadiene exposure and leukemia".*

Sathiakumar et al. 2015:

Follow-up period: 1944-2009

This study used the same cohort and historical exposure estimates as the Cheng et al. 2007 study, but had a longer follow-up.

Potential confounders included were race (nonwhite, other), plant, years since hire (<20, 20-29, 30-39, 40+ years), year of birth (leukemia: <1913, 1913-1919, 1920-1926, 1927-1934, 1935+), payroll status (ever hourly, never hourly) and year of hire (<1950, 1950-1959, 1960). Analyses of 1,3-butadiene and styrene exposure were conducted separately, and were not adjusted for each other.

A total of 114 decedents with leukemia were identified (i.e. 33 additional cases compared to Cheng et al. 2007). Decedents with non-Hodgkin lymphoma (NHL) (n=89) or multiple myeloma (MM) (n=48) were included in separate analyses. However, there was no evidence of an association of 1,3-butadiene with NHL or MM.

The leukemia analyses (cox regression) were based on 16,411 subjects and 611,880 person-years of observation for leukemia (168 men dropped out of follow-up at ages younger than the youngest leukemia decedent (age 32 years), as they were considered too young to develop leukemia). The relationship between 1,3-butadiene and leukemia is shown in Table 2.

Table 2. Mean values of 1,3-butadiene exposure, estimated rate ratio (RR) and 95% confidence interval (CI) for data included in Cox regression (Sathiakumar et al., 2015).

Mean exposure ppm-years	Mean exposure mg/m^3	RR[a]	95% CI
0	0	1.00	1.0
8.45	18.69	0.98	(0.44, 2.18)
19.73	43.65	1.70	(0.76, 3.78)
37.56	83.09	1.79	(0.80, 3.98)
56.23	124.40	1.91	(0.86, 4.24)
90.50	200.21	1.43	(0.64, 3.19)
185.16	409.63	1.54	(0.69, 3.43)
258.20	571.21	3.23	(1.45, 7.19)
378.94	838.32	2.63	(1.18, 5.86)
675.63	1,494.68	2.68	(1.20, 5.97)
2,273.85	5,030.40	3.63	(1.59, 8.32)

[a] Rate ratio, controlling for age.

Conclusion of Sathiakumar et al. 2015: "*The present analyses support the presence of a positive exposure-response relationship between cumulative exposure to BD and leukemia. These results along with other research and biological information support an interpretation that BD causes leukemia in humans*".

Toxicokinetics

Toxicokinetics of 1,3-butadiene has been reviewed by (Kirman et al., 2010). Here follows a brief summary:

"The metabolism of BD to reactive intermediates has been well studied (Figure 1) (reviewed in, e.g., (Albertini et al., 2003; Himmelstein et al., 1997)). The parent compound is initially oxidized to the 1,2-epoxy-3-butene (EB), a reaction mediated primarily by cytochrome P450 (CYP) isozyme CYP2E1, although other isozymes such as CYP2A6 have also been shown to be involved (Duescher & Elfarra, 1994). Further oxidation of EB produces the 1,2:3,4-diepoxybutane (DEB). Detoxification of EB proceeds by conjugation with glutathione (GSH) (mediated by glutathione S-transferase) or by hydrolysis (mediated by epoxide hydrolases), the latter producing the 1,2-dihydroxy-3-butene (butenediol or B-diol) metabolite. Both DEB and B-diol undergo further conversions in vivo, the former by EH hydrolysis and the latter by CYP2E1 oxidation, to produce the 1,2-dihydroxy-3,4-epoxybutane (epoxybutane diol or EBD) metabolite. B-diol can also be metabolized by alcohol dehydrogenase (ADH) and CYP2E1 to form hydroxymethylvinylketone (HMVK). The epoxide metabolites of BD can be detoxified via conjugation with glutathione via glutathione S-transferase".

Genetic variation might lead to variation in toxicokinetics in humans, as human genetic polymorphisms may underlie differences in metabolism between individuals. It follows, that this may lead to differences in susceptibility to 1,3-butadiene exposure. The specific impact of these polymorphisms is not completely known, but it likely involves complex interactions. *In vitro* studies and *in vivo* molecular epidemiological studies indicate the range of increased sensitivity that may be attributed to some human genetic polymorphisms is approximately 2- to 3.5-fold in humans as a worst-case scenario (Kirman & Grant, 2012).

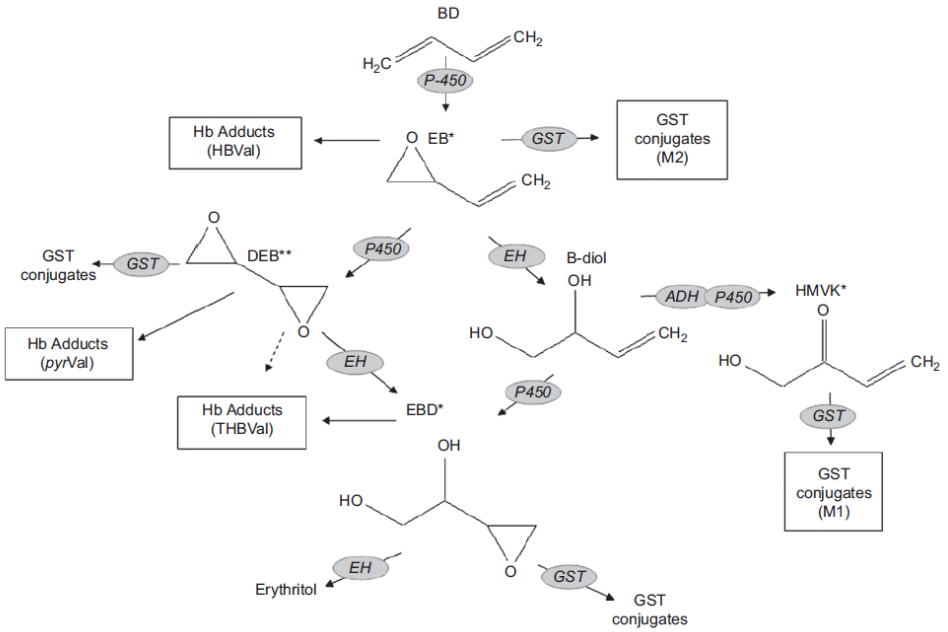

Figure 1. Metabolism of 1,3-butadiene. BD = 1,3-butadiene; EB = epoxybutene; DEB = diepoxybutane; B-diol = butenediol; HMVK = hydroxymethylvinyl ketone; EBD = epoxybutane diol; * = monofunctional alkylating agent; ** = bifunctional alkylating agent; P450 = cytochrome P450; GST = glutathione *S*-transferase; EH = epoxide hydrolase; ADH = alcohol

dehydrogenase; HBVal = *N*-(2-hydroxy-3-butenyl)-valine; M1 = 1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane; M2 = 1-(*N*-acetylcysteinyl)-2-hydroxy-3-butene; *pyr*Val = *N,N*-(2,3-dihydroxy-1,4-butadiyl)-valine; THBVal = *N*-(2,3,4-trihydroxybutyl)-valine. Boxes indicate where in the metabolic pathway biomarkers of exposure have been measured in exposed workers (reprint from Kirman et al., 2010).

The differences in metabolism between mice, rats and humans are also reviewed in Kirman et al. 2010:

"*Data collected from in vitro studies, measurements of metabolites in tissues, measurements of hemoglobin adduct biomarkers, and measurements of metabolites in urine describe a consistent pattern of species differences between mice, rats, and humans. Mice are more efficient in the production of epoxide metabolites of BD (especially DEB), whereas rats and humans are more efficient in hydrolytic detoxification of these metabolites. Blood and tissue concentrations and accumulations of all three electrophilic metabolites are greater in mice than in rats—in some cases (depending on test concentration and metabolite) much greater. Of importance are the higher concentrations of the reactive metabolites EB, EBD, and DEB, especially DEB. BD total metabolite concentrations determined by exposures to radiolabeled BD are even lower in subhuman primates than in either mice or rats, whereas the hydrolytic detoxification of these metabolites in primates is greater*".

"Hemoglobin adduct data indicate that the levels of DEB in humans are lower than levels observed in rats, and much lower than levels observed in mice. First, the species differences in the metabolism of BD suggest that use of rodent tumor data expressed in terms of external concentration (i.e., ignoring species differences in metabolism) will overestimate the potential risks to human populations. Assuming that humans and rodents are similarly susceptible to the effects of BD metabolites (e.g., DEB), the degree of overestimation would likely be approximately 2 orders of magnitude if based on mice, and 1 order of magnitude if based on rats".

The current working group notes the species differences where mice seem to be more efficient in the production of epoxide metabolites of BD (especially DEB) than rats and humans.

Animal data

Carcinogenicity

1,3-butadiene are classified by IARC as carcinogenic to humans because there is sufficeent evidence in humans for the carcinogenicity of 1,3-butadiene as well as *sufficient evidence* in experimental animals for the carcinogenicity of 1,3-butadiene and DEB (metabolite of butadiene).

DECOS (2013) and the IARC (2008) monograph both evaluated two US National Toxicology Program (NTP) studies exposing mice to 1,3-butadiene by inhalation. Both animal studies showed increased incidences of lymphoma and neoplasms with increasing exposure. These studies are briefly described:

In the first study by (Huff et al., 1985; NTP, 1984), groups of 50 male and 50 female mice were exposed 6 hours per day, 5 days per week, for 60 to 61 weeks to air containing 0, 625, or 1,250 ppm 1,3-butadiene (0, 1,380 or 2,760 mg/m^3). The study was designed for 104 weeks of exposures but had to be terminated after 61 weeks because of cancer-related mortality in both sexes at both exposure concentrations. Early in the study, there was induction and significantly increased incidences of especially malignant lymphomas, but also hemangiosarcomas of the heart, alveolar-bronchiolar neoplasms, squamous cell neoplasms of the forestomach in males and females. In females also acinar cell carcinomas of the mammary gland, granulosa cell neoplasms of the ovary, and hepatocellular neoplasms were observed.

In the second study by (Melnick et al., 1990; NTP, 1993) lower exposure concentrations were applied. Groups of 70 male and 70 female mice were exposed to air containing 0, 6.25, 20, 62.5, or 200 ppm (0, 14, 44, 138 or 440 mg/m^3) 1,3-butadiene for 6 hours per day, 5 days per week for up to 2 years; groups of 90 male and 90 female mice were exposed to 625 ppm (1,380 mg/m^3) 1,3-butadiene on the same schedule. Up to 10 animals from each group were examined after 9 and 15 months of exposure. The two-year survival was decreased for males and females exposed to concentrations of 20 ppm or above, primarily due to the development of chemical-related malignant neoplasms. No female mice exposed to 200 or 625 ppm or males exposed to 625 ppm survived to the end of the studies.

Exposure of mice to 1,3-butadiene induced benign and malignant neoplasms at multiple sites. Statistically significant increases in the incidences of neoplasms at one or more sites were seen at concentrations of 20 ppm (44 mg/m^3) and above in males and at 6.25 ppm (14 mg/m^3) and above in females. There was no exposure level in this study at which a significant carcinogenic response was not observed. Statistically significant increases occurred in the incidences of malignant lymphoma; histiocytic sarcoma; cardiac hemangiosarcoma; harderian gland adenoma; hepatocellular adenoma and carcinoma; alveolar-bronchiolar adenoma and carcinoma; mammary gland carcinoma, adenoacanthoma, and malignant mixed tumor (females only); benign and malignant ovarian granulosa cell tumor; and forestomach squamous cell papilloma and carcinoma. Lymphocytic lymphomas appeared as early as week 23 and were the principal cause of death of male and

female mice exposed to 625 ppm (1,380 mg/m^3) 1,3-butadiene. The early and extensive development of lethal lymphocytic lymphomas in mice exposed to 625 ppm resulted in a reduced number of mice at risk for neoplasms developing later at other sites.

Long-term studies in rats have been conducted by (Owen & Glaister, 1990) (also evaluated by (DECOS, 2013) and (IARC, 2008)). Rats were exposed by inhalation to 0, 2,200 or 17,600 mg/m^3 1,3-butadiene for 6 hours per day, for 5 days per week for 105 weeks (females) or 111 weeks (males). Survival was reduced in low- and high-dose females and in high-dose males. Females died as result of mammary tumors and fibrous tumors of the skin, whereas renal lesions were likely the major cause of death in males.

A clear species difference with respect to sites of tumor development and potency of response was demonstrated in the carcinogenic studies of mice and rats, and it has been concluded that mice are more sensitive to 1,3-butadiene than rats (DECOS, 2013).

Carcinogenicity studies with mice and rats exposed to metabolites of 1,3-butadiene have been reviewed by DECOS (2013) and IARC (2008, 2012). IARC concluded that there is sufficient evidence in experimental animals for the carcinogenicity of diepoxybutane (IARC 2008, 2012). These carcinogenicity studies of metabolites will not be discussed further in the present report.

The current working group notes that, to our knowledge, no long-term studies on butadiene has been conducted at exposure concentrations that have not shown a carcinogenic response (down to the lowest exposure level of 14 mg/m^3 in mice). Thus, a NOAEL cannot be established based on animal studies. In addition, no newer data from long-term studies are available.

Reproductive toxicity

The current working group notes that reproductive toxicity, in terms of ovarian atrophy, was a significant non-neoplastic effect in the 2-year study at low levels of exposure (NTP, 1993). In female mice exposed to 1,3-butadiene for up to 2 years, 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 probably occurring, the dose-response relationship observed clearly establishes the ovary as a target organ of 1,3 butadiene toxicity at concentrations as low as 6.25 ppm, the lowest concentration studied. Characteristically, affected females had no evidence of oocytes, follicles, or corpora lutea in the ovary (NTP, 1993).

Female rats did not develop ovarian atrophy after exposure to 1,3-butadiene at comparatively high concentrations (1,000, 8,000 ppm) in a 2-year inhalation study (Owen et al., 1987).

This clear interspecies variation in the sensitivity to 1,3-butadiene between mice and rats are partly due to the metabolic differences between species. Differences in the formation rate and detoxification of epoxide metabolites have been observed, in terms of higher

tissue levels of epoxide metabolites in rodents, predominantly in mice, than in humans. For example, in vitro and perfusion data show that mice are more efficient than rats at oxidizing 1,3-butadiene to form EB, and that the conversion of EB to DEB in mice is 3.3-fold greater than in rats and 2.4–61-fold greater than in humans (Kirman et al., 2010). In addition, in vitro studies designed to assess interspecies differences in the activation of 1,3-butadiene and inactivation of epoxides revealed that the overall activation/detoxication ratio for metabolism was approximately 10 times higher in mice compared to that of rats or humans (Bond et al., 1993). Biomarkers of exposure have been identified for the epoxide metabolites including pyr-Val hemoglobin adducts, which have shown a good surrogate biomarker for DEB (Georgieva et al., 2010). DEB is the metabolite with the highest genotoxic potency and the metabolite suggested being the causative agent for ovarian atrophy (Kirman & Grant, 2012; NTP, 1993). The DEB dose-equivalent in human blood (measured as pyr-Val hemoglobin adducts) was shown to be approximately 16 times lower than in rats, which in turn was approximately 45 times lower than the DEB blood levels in mice (Swenberg et al., 2011), thus there is a 720-fold difference between mice and humans. Furthermore, follicle cell depletion has been observed in mice following short-term exposures (30-day) to EB and DEB, and in rats following short-term exposures to DEB (Doerr et al., 1996).

In male mice, testicular atrophy has been observed, but primarily after high exposures (NTP, 1984, 1993). Other reproductive endpoints related to developmental effects, as well as dominant lethal effects, have been observed in animal studies following 1,3-butadiene exposure (OEHHA, 2013), i.e. male mice were exposed to 1,3-butadiene and effects on litters were measured after mating to unexposed females. The dominant lethal responses are believed to represent a genotoxic effect, i.e. the exposure damages DNA in sperm cells.

In relation to dominant lethal effects, OEHHA concludes:

"Accumulated data appear to suggest that inhalation exposure of butadiene is associated with an increase in dominant lethal effects even at concentrations below the threshold for acute toxicity. There is evidence of species and strain differences in susceptibility, with mice being more susceptible than rats, and outbred CD-1 mice appearing to show dominant lethality at lower butadiene concentrations than other strains of mice. Regardless of the length of pre-mating dosing (i.e., a single 6 hr, 5 day, or 4-10 week exposures), dominant lethal effects were associated with butadiene effects in the more mature male germ cells, specifically mature sperm and late spermatids" (OEHHA, 2013).

Developmental toxicity has been observed following maternal 1,3-butadiene exposure during gestation (Hackett et al., 1987); 78 pregnant female mice were exposed whole-body to 0, 40, 200, or 1,000 ppm butadiene for 6 hours per day on gestation days (gd) 6-15, with necropsy on gd 18.

The incidences of fetal variations (supernumerary ribs and reduced ossification of the sternebrae) were significantly elevated in litters from mice exposed to 200 and 1,000 ppm. At these exposure levels, there was also evidence of maternal toxicity as shown by significantly lower maternal weight gain. There was, however, also significant dose-

dependent reduction of fetal body and placental weights at the two higher dose levels for female fetuses, and at all dose levels in males.

Mechanisms of toxicity

Mutagenicity and genotoxicity

IARC extensively reviewed the available literature on mutagenicity and genotoxicity (IARC, 2008). At that time, the literature consisted of 54 different scientific publications on 1,3-butadiene exposure to different experimental test systems. 18 publications were about the metabolite epoxybutene (EB), 4 publications were about the metabolite epoxybutanediol (EBD) and 22 publications were about the metabolite diepoxybutane (DEB) exposure to different test systems.

The most common test systems were:
-Salmonella typhimurium or E.coli reverse mutation
-DNA cross-links in vivo
-DNA single-strand breaks in vivo
-Sister chromatid exchange in vitro (rodent or human whole blood, rodent fibroblasts)
-Gene mutations (LacI/ Hprt locus in vivo or Hprt locus in human lymphoblastoid TK6 cells)
-Micronucleus formation in vivo (liver/ lung) or in vitro (rodent fibroblasts)
-Chromosomal aberrations in vivo
-Dominant lethal test
-Binding to DNA at N7 of guanine in vivo (various tissues).
Less common test systems are cell cycle arrest, hyperdiploidy, chromosomal breakage, inhibition of clonogenic activity, aneuploidy and comet tail moment.

IARC summarizes the mutagenic potential of 1,3-butadiene (IARC, 2012):

"The mechanistic link between animal and human neoplasia induced by butadiene is supported by the identification in mice of genetic alterations in butadiene-induced tumours that are frequently involved in the development of a variety of human cancers as well. The K-Ras, H-Ras, p53, p16/p15 and β-catenin mutations detected in tumours in mice probably occurred as a result of the DNA-reactive properties and the genotoxic effects of butadiene-derived epoxides. A consistent pattern of K-Ras mutations (G→C transversion at codon 13) was observed in butadiene-induced cardiac haemangiosarcomas, neoplasms of the lung and fore-stomach, and lymphomas (Hong et al., 2000; Sills et al., 2001; Ton et al., 2007). Alterations in the p53 gene in mouse-brain tumours were mostly G→A transition mutations (Kim et al., 2005). Inactivation of the tumour-suppressor genes p16 and p15 may also be important in the development of butadiene-induced lymphomas (Zhuang et al., 2000). Mammary gland adenocarcinomas induced by butadiene in mice frequently had mutations in the p53, H-Ras and β-catenin genes (Zhuang et al., 2002).

IARC concluded:

"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" (IARC, 2012).

Several studies in humans have demonstrated DNA-binding properties and clastogenicity in lymphocytes of workers occupationally exposed to 1,3-butadiene (IARC 2008).

1,3-butadine is clastogenic in mice and induces chromosomal aberrations, micronucleus formation and sister chromatid exchange, but it has not been found to be clastogenic in rats (IARC 2008).

Related to the genotoxicity of individual epoxide metabolites of 1,3-butadiene, Kirman et al. 2010 summarizes:

"EB, DEB, and EBD, as reactive electrophilic compounds, are capable of reacting with DNA, resulting in one or more genotoxicity events likely relevant to the carcinogenic mode of action for BD; however, their genotoxic potencies are remarkably different (DEB >> EB > EBD) (reviewed in (Albertini et al., 2010). The potency of DEB is likely attributed to its ability to serve as a bifunctional alkylating agent, capable of binding to two cellular macromolecules (e.g., DNA-protein cross-links; (Loeber et al., 2006) or to the same molecule twice (e.g., DNA cross-links; (Goggin et al., 2007; Goggin et al., 2009), whereas the other three metabolites are all monofunctional agents. DNA cross-links are relatively poorly repaired when compared to DNA damage produced by monofunctional agents" (Kligerman & Hu, 2007; Vock et al., 1999).

Generally, the metabolites have been investigated to a minor extent in vivo compared to in vitro test systems.
A few publications have assessed the same end point simultaneously for both 1,3-butadiene and metabolites. The effects can be difficult to compare across studies due to differences in exposure concentrations, exposure duration or administration method. Three studies were described in the IARC 2008 report:

1) (Recio et al., 2001). Male lacI mice were exposed to BD by whole-body inhalation (62.5 ppm, 625 ppm or 1250 ppm for 6 h/day) and female lacI mice were exposed to EB and DEB (29.9 ppm for 2 weeks and 3.8 ppm for 2 weeks, respectively). Conclusion: The data presented clearly indicate that BD exposure induces specific point mutations in tissues of lacI mice and that EB and DEB differ in the mechanisms by which they induce mutation in mammalian cells. In the experimental systems examined, EB primarily acts via the induction of point mutations, while DEB induces point mutations, deletions, and chromosomal alterations. In mice exposed to BD, both metabolites and EBD may act in concert to induce the range of genotoxicity observed.

2) (Walker & Meng, 2000) only abstract available. The relative contribution of BDO (EB) versus BDO2 (DEB) to overall BD mutagenicity was evaluated by exposing mice and rats to carefully chosen concentrations of BD and racemic mixtures of BDO and BDO2 (that is, 62.5, 2.5, and 4.0 ppm, respectively) and comparing the mutagenic potency of each compound when comparable blood levels of metabolites were achieved. The resulting MF (mutant frequency) data indicate that (+/-)-BDO2 is a major contributor to the mutagenicity of BD in mice at lower BD exposure levels (< or = 62.5 ppm).

3) (Wickliffe et al., 2007). Knockout mice (Ephx1-null and Xpc-null) were exposed either to BD by inhalation or to the reactive epoxide metabolites, EB or DEB by intraperitoneal

injection. The doses were 20 ppm (7h/day, 5 days/week for 4 weeks) of BD by inhalation or ED (Ephx1-null: 240 mg/kg (three separate injections of 80 mg/kg every 48 h); Xpc-null: 300 mg/kg (three separate injections of 100 mg/kg every 48 h)) or DEB (Ephx1-null: 30 mg/kg (two separate injections of 15 mg/kg every 24 h). The EPHX gene codes for the detoxification enzyme epoxide hydrolase and the XPC gene is involved in nucleotide excision repair mechanisms. Genetic susceptibility was measured using the Hprt cloning assay measuring mutant frequencies. Conclusion: Both deficient strains of mouse were significantly more sensitive to the mutagenic effects of BD and the injected epoxides, which indicate that individuals deficient in both hydrolytic detoxification and repair of premutagenic DNA adducts may be at a particularly high risk following exposure to BD.

The evidence for mutagenicity and genotoxicity in humans has been investigated in workers in styrene-butadiene or butadiene monomer facilities. Effects have been assessed in workers exposed to 1,3-butadiene and control groups as e.g. HPRT variant (mutant) frequency in lymphocytes, concentration of urinary metabolite of butadiene, chromosomal aberrations and sister chromatid exchange in blood cells, and DNA adducts in lymphocytes. However, there are also studies showing conflicting results in the literature (reviewed by IARC).

The current working group notes that there is evidence of primary gentoxicity of 1,3-butadiene, i.e. a non-threshold mode of action. Also, there is strong evidence of primary genotoxicity of the individual epoxide metabolites of 1,3-butadiene.

Epigenetic changes

Even though 1,3-butadiene primarily acts by a genotoxic mechanism, epigenetic effects of 1,3-butadiene have been of increasing interest during the last decade. This subject has not been covered by IARC or DECOS; however, here we will add the most recent *in vivo* studies in the area.

In a study by (Koturbash et al., 2011b), mice (n=5) were exposed to 6.25 ppm or 625 ppm 1,3-butadiene by inhalation for 6 hours per day, 5 days per week for 2 weeks whereafter epigenetic alterations were examined in liver tissue. The results showed loss of global DNA and LINE1 methylation, decreases in histone methylation, and altered expression of proteins responsible for the accurate maintenance of the epigenetic marks. The epi-genetic effects were most pronounced in the 625 ppm exposure group, although some effects were also observed in mice exposed to 6.25 ppm 1,3-butadiene.

(Chappell et al., 2014) exposed mice (n=3) to 425 ppm of 1,3-butadiene by inhalation (6 hours per day, 5 days per week) for 2 weeks. Epigenetic alterations indicative of genomic instability, including demethylation of repetitive DNA sequences and alterations in histone-lysine acetylation, were evident in liver and lung tissues.

The same group of scientists behind these two studies have further investigated mouse inter-strain- and tissue-specific variability in the epigenetic responses after 1,3-butadiene exposure in several publications (Chappell et al., 2017; Israel et al., 2018; Koturbash et al.,

2011a; Lewis et al., 2019), as well as in a systematic literature review (Chappell et al., 2016).

Overall these results suggest that 1,3-butadiene might act as both a genotoxic and an epigenotoxic chemical.

Non-neoplastic toxicity

The current working group notes that reproductive toxicity, in terms of ovarian and testicular atrophy, appears as significant non-neoplastic effects in studies of the toxicity of 1,3-butadiene (NTP, 1984, 1993). The mechanism underlying ovarian atrophy probably operates by a threshold effect, and is primarily caused by the 1,3-butadiene metabolite DEB. The molecular mechanism is not clearly understood, but the diepoxides appear to selectively destroy the primordial and primary follicles via programmed cell death or apoptosis. This accelerates depletion of oocytes in the ovary. Follicle cell depletion has been observed in mice following short-term exposure to EB and DEB, and in rats following exposures to DEB. Toxicity of DEB to human ovarian follicles is assumed, supported by studies in nonhuman primates for the structurally similar diepoxide (vinylcyclohexane diepoxide), summarized in (Iorio et al., 2014; Kirman & Grant, 2012).

Previous evaluations

SCOEL (2007)

In 2007, SCOEL produced a recommendation report on 1,3-butadiene (SCOEL, 2007). The report, which was quite short, used a previous report by (ECETOC, 1997) as the main source of documentation for the evaluation. SCOEL agreed with the IARC evaluation at that time that 1,3-butadiene was *probably carcinogenic to humans (gr. 2A)* (IARC, 1999). SCOEL did not include animal experiments in their risk assessment.

SCOEL used exposure-response relations published in the cohort study by (Delzell et al., 2001) to calculate the risk estimates (risk coefficient (β) per unit of exposure) for leukaemia, using two different methods:

"One was based on excess relative risk "linear model" without a threshold. 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."

"The second method was a "step model" in which the risk coefficient per exposure unit remains constant in a certain range of exposure and then changes abruptly (step) moving to the next range. Here, ranges of cumulative exposure above 0.0 ppm and associated relative risk estimates were combined with a dummy variable indicating a specific range. 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, taking into account the mortality decline that naturally occurs in an ageing population. Assuming that exposure lasts 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-butadiene were calculated, using the estimated coefficients indicating the excess relative risk for each ppm of cumulative exposure, for a population of 1,000 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 (predicted deaths/expected deaths)."

"The "step model" was considered the most appropriate (Zocchetti 2002)."

The Zocchetti 2002 paper was an internal SCOEL document, but has later been published in Italian (Zocchetti et al., 2004).

The recommendation section of the SCOEL document includes the following final conclusion:

"In a population of 1,000 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."

In the later report by DECOS (2013), the SCOEL risk assessment data are not used. DECOS states that the motives for this are: "(*1) the availability of more recently published data, (2) lack of clarity on the criteria used by SCOEL to model the data (SCOEL used various models to calculate the upper and lower risk levels at the different exposure levels, without explanation), and (3) SCOEL's use of out-of date mortality data of a local population, whereas national or European and up-to-date date are preferred".*

DECOS (2013)

In 2013, DECOS produced a report on 1,3-Butadiene: "Health–based calculated occupational cancer risk values" (DECOS, 2013).

After reviewing all cohort studies on 1,3-butadiene, DECOS choose the study by (Cheng et al., 2007) for their risk assessment, because of the extensive set of quantitative data, including corrections for co-exposure to styrene and DMDTC.

The current working group notes that DECOS in their risk assessment used the data on relative risk and cumulative exposure adjusted only for age instead of the data adjusted for year of birth, race, DMDTC exposure, years since hire and industrial plant. DECOS does not explain this choice in their report. However, in the Cheng et al. study (2007) it is stated that "adjustment of the association between DMDTC and leukemia is appropriate only if either 1) DMDTC causes leukemia, or 2) it correlates with some unknown causal factor". At present, the evidence of a causal association between DMDTC and leukemia is limited and other correlating causal factors have not been identified. Therefore, use of the adjusted data could lead to underestimation of the risk of developing leukemia following 1,3-butadiene exposure.

As reference data, DECOS used leukemia mortality data of the general population in the Netherlands from 2000 to 2010, where rates for women and men were averaged. Leukemias included in the analysis are the malignancies listed in WHO's 10th International Code of Diseases with the codes C81-C96 (Malignant neoplasms of lymphoid, hematopoietic and related tissue). DECOS states that: *"Limiting the risk assessment to leukemia only would certainly result in an underestimation of the risk of developing cancer following 1,3-butadiene exposure"*. However, *"The committee prefers to use leukemia data of Cheng et al. 2007 and to extrapolate these to the malignancies listed in WHO's ICD codes 81-96. The committee is aware of the resulting possible slight overestimation by limiting the risk to leukemia only"*.

DECOS used the software program SAS for the analyses and obtained the relationship with the best fit:

- RR= 1 + 0.001159 x (cumulative exposure in mg/m^3-years)

Thereafter a survival analysis was performed using the software R. DECOS subsequently calculated the concentration of 1,3-butadiene in the air which corresponds to an excess risk of cancer mortality of:

- 4 per 1,000 ($4x10^{-3}$) deaths in the general population, at 40 years of occupational exposure, equals to 10 mg 1,3-butadiene per m^3 (4.7 ppm)
- 4 per 100,000 ($4x10^{-5}$) deaths in the general population, at 40 years of occupational exposure, equals to 0.1 mg 1,3-butadiene per m^3 (0.047 ppm)

DECOS also included a benchmark dose (BMD) analysis based on non-neoplastic effects reported in a two-years inhalation study in mice. Here the lowest overall LOAEL was 13.8 mg/m (based on ovarian atrophy) (NTP, 1993). DECOS used the software of the US-EPA. DECOS concludes:

"Taking into account the seriousness of the effect, the 10% extra risk level was taken as the point of departure. This analysis resulted in a BMDL (BMD at lower risk level with 95% confidence interval) of 1.0 mg/m^3. To derive a health-based occupational limit value for humans, two uncertainty factors of 3 were applied, one to correct for interspecies differences, and one to correct for intraspecies differences. Since the exposure of the experimental animals in the cited study was for 6 h/day, 5 days/week during 103 weeks, additional uncertainty or uncertainty factors were not needed. This resulted in a human occupational limit value of 1.0/9 = 0.11 mg/m^3. This value is practically equal to the 4 x 10-5 risk of 0.1 mg/m^3 that the Committee derived above. Hence, this health-based calculated occupational cancer risk value is not expected to result in effects other than carcinogenicity" (DECOS, 2013).

OEHHA (2013)

OEHHA describes the development of acute and 8-hour reference exposure levels (RELs) based on the critical effects; lowered male fetal weight (acute) following exposure during gestation, and ovarian atrophy in female mice, following exposure during adulthood (8-hour) (OEHHA, 2013). Here we summarize the calculations:

Acute REL

As described above, maternal 1,3-butadiene exposure during gestation has been associated with maternal and fetal toxicity (Hackett et al., 1987). In mice, there was a significant dose-dependent reduction of fetal body and placental weights at the two higher exposure levels for female fetuses (200 and 1,000 ppm, 6 hours per day on gd 6-15), and at all dose levels in males (40, 200 and 1,000 ppm, 6 hours per day). The observation that male fetuses appeared to be susceptible to butadiene at levels that were not maternally toxic forms the basis of the acute REL.

OEHHA's acute RELs are levels at which intermittent one-hour exposures are not expected to result in non-cancer adverse health effects. The study by Hackett et al. 1987 was later reanalyzed by (Green, 2003). OEHHA performed a continuous BMD analysis of the values from the Green re-analysis of the Hackett data. OEHHA choses the lowest BMCL value giving the best model fit (male pups, Green), i.e. 17.7 ppm for the mouse and 29.7 ppm for the human equivalent concentration. After applying uncertainty factors of 100, the **acute REL** was calculated to **297 ppb (660 μg/m³)** (OEHHA, 2013).

8-hour REL

Ovarian atrophy was a significant non-neoplastic effect in the 2-year mouse study by (Melnick et al., 1990; NTP, 1993). The animals were exposed to 0, 6.25, 20, 62.5, 200, or 625 ppm for 6 hours per day, 5 days per week for up to 65 weeks. At 40 weeks, ovarian atrophy was present in females exposed to 200 and 625 ppm butadiene. At 65 weeks, ovarian atrophy was present in all groups exposed to ≥ 20 ppm butadiene, and female mice exposed to the lowest concentrations of butadiene (6.25 ppm) exhibited atrophy at the end of the study at 105 weeks, compared to controls. Based on these results, NTP investigators identified a chronic LOAEL of 6.25 ppm for reproductive toxicity. OEHHA performed a BMD analysis on the 9-, 15- and 24-month ovarian atrophy data. If the 9 and 24 months data were included in a time-adjusted model, all of the data could be fit (N = 435). Using the log probit model, a $BMCL_{05}$ of 1.01 ppm butadiene was obtained. Adjustment of exposure time and the human equivalent concentration and after applying uncertainty factors of 300, the **8-hour REL** was calculated to be **4 pbb (9 μg/m³)** (OEHHA, 2013).

Other regulatory values

The European Commission proposed in 2016 to revise or to introduce occupational exposure limit values for 13 carcinogenic chemical agents, among them 1,3-butadiene, out of 25 priority chemicals. **ACSH** (Advisory Committee on Safety and Health) performed the evaluation (taking economic, social and health impact into account) and suggested an EU-OEL of 1 ppm (2.2 mg/m³) 1,3-butadiene, which scientifically were based on the preceding SCOEL recommendation (2007). The suggested OEL was adopted by the European Committee, by the European Parliament and by the Council of the European Union in 2017 and introduced in 'Directive 2004/37/EC of the European Parliament and of the Council on the protection of workers from the risks related to exposure to carcinogens or mutagens at work' (ACSH, 2016).

Danish Working Environment. The current Danish OEL (TWA 8h) for 1,3-butadiene (updated in 2020) is 1 ppm (2.2 mg/m^3). This is in line with the EU-OEL value of 1 ppm.

Occupational Safety and Health Administration (OSHA) set a permissible exposure limit (PEL) for 1,3-butadiene to an 8-hour TWA of 1 ppm and a short-term exposure limit (STEL) of 5 ppm for 15 minutes. The limits have not been revised since 1996. A detailed report including risk assessment is available (OSHA, 1996).

The National Institute of Occupational Safety and Health (NIOSH) considers 1,3-butadiene to be a potential occupational carcinogen as defined by the OSHA carcinogen policy (current OSHA PEL: 1 ppm (2.2 mg/m^3) TWA; 5 ppm (11.0 mg/m^3) ST). The Immediately Dangerous to Life or Health Concentration (IDLH) value was revised by NIOSH in 1994 and based primarily on older acute inhalation toxicity data (LC50) from the 1940s. The revised IDLH value was set to 2,000 ppm; however, NIOSH recommends as part of its carcinogen policy that the "most protective" respirators should be worn for 1,3-butadiene at any detectable concentration (NIOSH, 1994).

United States Environmental Protection Agency (US EPA) established in 2002 a Reference Concentration (RfC) and a chronic reference level of 0.002 mg/m^3 for 1,3-butadiene for the general population (i.e. for 24 h continuous exposure/ day) based on reproductive effects in mice. US EPA is currently evaluating the risk of 1,3-butadiene under the Toxic Substances Control Act (TSCA), initiated in 2019 (USEPA, 2019).

Scientific basis for setting an occupational exposure limit

The working groups of SCOEL and DECOS considered cancer as the critical health effect following 1,3-butadiene exposure. 1,3-butadiene induces mutations by a genotoxic mechanism that involves the formation of reactive epoxides and their interaction with DNA.

Based on the presented evidence and evaluations by other working groups, the current working group is of the opinion that 1,3-butadiene operates by non-threshold mechanisms relative to induction of cancer. The current working group calculates health-based OELs based on cancer (leukemia) data from both human and animal studies. Additionally, the current working group notes that significant reproductive toxicity was observed in female mice (NTP) and furthermore consider this as a critical effect. Therefore a calculation of the Derived No-Effect Level (DNEL) for toxicological effects having thresholds is also performed.

Health-based exposure limit based on epidemiological data

In addition to the Cheng et al. 2007 study used by DECOS in their calculations of excess risk of cancer (leukemia) mortality, the current working group will use the most recent publication on the styrene–butadiene rubber production workers' cohort (Sathiakumar et al., 2015). Both studies have already been described above.

The Cheng study included ~16,000 subjects and examined mortality due to leukemia from 1944 to 1998. During the period, 81 decedents with leukemia or cancer of the lymphatic and hematopoietic tissues were identified based on information from death certificates. Methods were developed for quantitative estimates of each subject's exposure to 1,3-butadiene (Macaluso et al., 2004).

The figure below shows the exposure-response relationship from Cheng at al. 2007, which was also used by DECOS (2013):

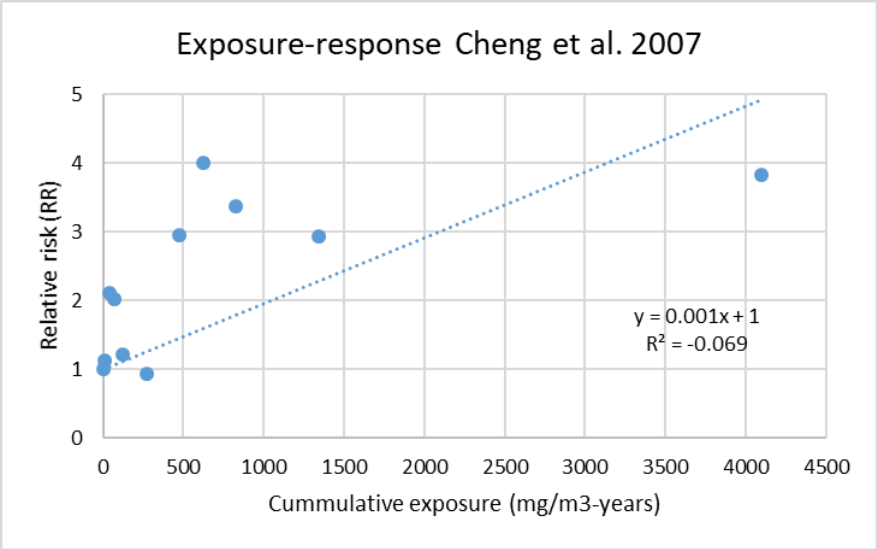

Figure 2. Relative risk of leukaemia mortality following occupational exposure to 1,3-butadiene according to Cheng et al. (2007).

RR for leukemia mortality = intercept + slope x cum exposure (mg/m^3-years)

RR = 1 + 0.001 x cum exposure

In our analysis, we used Danish mortality data from all hematologic malignancies covering ICD codes 81-96, i.e. malignant neoplasms of lymphoid, hematopoietic and related tissue (Table 3). The Danish cancer mortality data were obtained from the NORDCAN database. Data are available from 1943 until 2016.

Table 3. Hematologic malignancies covering ICD codes 81-96.

	ICD codes
Akut lymfatisk leukæmi	*C91.0*
Kronisk lymfatisk leukæmi	*C91.1*
Anden og uspecificeret lymfatisk leukæmi	*C91.2-9*
Akut myeloid leukæmi	*C92.0+C93.0+C94.0+C94.2+C94.4-5*
Kronisk myeloid leukæmi	*C92.1+C93.1+C94.1*
Anden og uspecificeret myeloid leukæmi	*C92.2-9+C93.2-9+C94.3+C94.7*
Leukæmi, uspecificerede celler	*C95*
Leukæmi	*C91-95*
Myelomatose	*C90*
Myeloproliferative sygdomme	*D45+D47.1,3-5*
Myelodysplastiske syndromer	*D46*
Hodgkins lymfom	*C81*
Non– Hodgkin lymfom	*C82-86*
Andre maligne hæmatologiske sygdomme	*C88,C96,D47.0,7-9*
Maligne hæmatologiske sygdomme	*C81-86,C88,C90-96,D45-47.0-1,3-9*

We obtained the Danish lifetime risks of dying from hematologic malignancies (0-74 years) for men and women for the period covering the last 20 years (1997-2016). Rates for

women and men were averaged to describe the average risk for the general population. These are given in Table 4.

Table 4. Risk of dying from hematologic malignancies in the Danish population 1997-2016.

	Men	Women	Average
Risk of dying (0-74 years)	1.26 %	0.77 %	1.015 %

The relative risk of mortality of hematologic malignancies caused by 1,3-butadiene at the different risk levels (0.1%, 0.01% and 0.01%) are given in Table 5.

Relevant Danish relative risk levels for mortality from hemalologic malignancies were calculated for the Danish population by adding 1 extra case to the background incidence of hemalologic malignancies (1.015 % or 10.15 per 1000 or 1015 per 100 000), as shown in table 5.

Table 5. Relative risk of mortality caused by 1,3-butadiene.

Excess risk of mortality	
1: 1,000	RR = (10.15+1)/10.15 = 1.099
1: 10,000	RR = (101.5+1)/101.5 = 1.0099
1: 100,000	RR = (1,015+1)/1,015 = 1.00099

We hereafter use RR = 1 + 0.001 x cum exp to calculate the corresponding cumulative exposure in mg/m^3-years.

We include the corresponding exposure for a work life of 40 years, which is the number of work years DECOS used in their risk assessment, and of 45 years, corresponding to the expected worklife in Denmark.

Assuming 1:1,000 excess deaths among men and women (average), the calculation would be:

Corresponding cum exp = (RR - 1) / 0.001 = (1.099 – 1) / 0.001 = 99 mg/m^3-years.

For a 45-year work life this would correspond to 99 mg/m^3-years/ 45 years = 2.2 mg/m^3

The calculations are given in Table 6.

Table 6. Excess mortality risk (based on the Cheng et al. 2007 study).

	Corresponding cum exp (mg/m^3-years)	Exposure using 40-year work life	Exposure using 45-year work life
1:1,000	(1.099-1)/0.001 = 99	99/40 = 2.475	99/45 = 2.2
1:10,000	(1.0099-1)/0.001 = 9.9	9.9/40 = 0.2475	9.9/45 = 0.22
1:100,000	(1.00099-1)/0.001 = 0.99	0.99/40 = 0.02475	0.99/45 = 0.022

The current working group performed similar calculations based on the exposure-response relationship from the Sathiakumar et al. 2015 study, which is a follow-up of the same cohort used in the Cheng study. The Sathiakumar study examined mortality due to leukemia from 1944 to 2009. During the period, 114 decedents with leukemia were

identified based on information from death certificates. The figure below shows the exposure-response relationship from Sathiakumar at al. 2015:

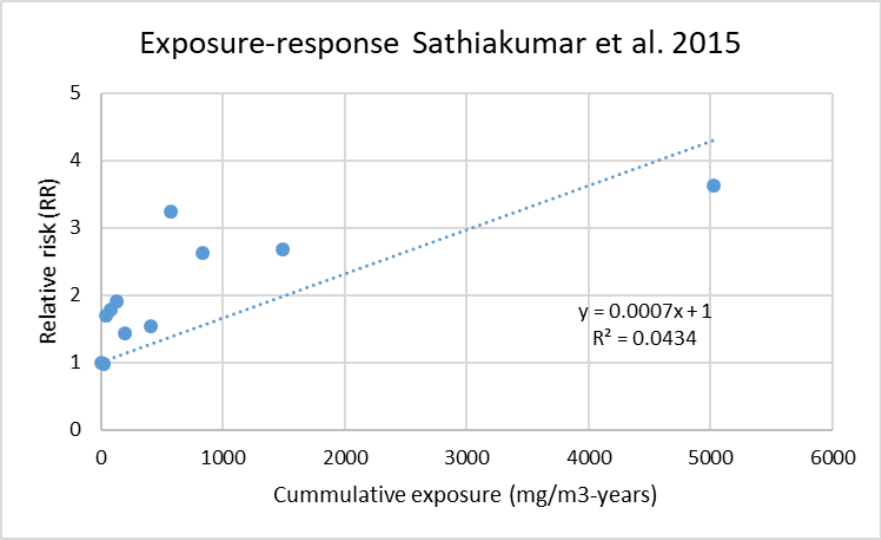

Figure 3. Relative risk of leukaemia mortality following occupational exposure to 1,3-butadiene according to Sathiakumar et al. (2015).

We used the lifetime risks from Table 3 and RR = 1 + 0.0007 x cum exp to calculate the corresponding cumulative exposure in mg/m^3-years. The relative risks and excess mortality risks are shown in Table 7.

Table 7. Excess mortality risk (based on the Sathiakumar et al. study).

Excess mortality risk	RR (men/women average)	Cum exp (mg/m^3-years)	Exp for 45-year work life ((mg/m^3)
1:1,000	(10.15+1)/10.15 = 1.099	(1.099-1)/0.0007 = 140.7	140.7/45 = 3.1
1:10,000	(101.5+1)/101.5 = 1.0099	(1.0099-1)/0.0007 = 14.07	14.07/45 = 0.31
1:100,000	(1015+1)/1015 = 1.00099	(1.00099-1)/0.0007 = 1.407	1.407/45 = 0.031

Summary

The health-based risk estimates of an excess risk of mortality using the Cheng et al. study are very similar to the health-based risk estimates calculated by DECOS in 2013. The exposure-response relationship from the most recent study (Sathiakumar et al. 2015) was fairly similar to the Cheng et al. 2017 study resulting in risk estimates allowing 1:1,000 excess deaths at 45 years of occupational exposure at 1,3-butadiene concentrations of 3.1 and 2.2 mg/m^3, respectively.

Health-based exposure limit based on inhalation studies in mice

The current working group calculated health-based exposure limits based on a 2-year inhalation study in mice for non-threshold mechanisms (leukemia) and threshold mechanisms (ovarian atrophy), respectively (NTP, 1993).

Endpoint: Cancer (leukemia)

The derivation of an OEL based on leukemia incidence was made under the assumption of a non-threshold driven mechanism. Risk estimates were calculated as recommended by (ECHA, 2012). The lowest exposure levels at which lymphocytic malignant lymphoma was statistically significantly elevated was 442.5 mg/m^3 (200 ppm) in female mice and 1,382.7 mg/m^3 (625 ppm) in male mice exposed to 1,3-butadiene, 6 hours per day, 5 days per week for up to 2 years (Table 8) (NTP, 1993). We chose to use the lowest concentration in the calculation (442.5 mg/m^3 (200 ppm) in female mice).

Table 8: Lymphoma incidence in a 2-year inhalation study in mice (NTP, 1993).

		Male mice	Female mice
ppm	mg/m^3	Lymphoma	Lymphoma
0	0	2/50	1/50
6.25	13.8	0/50	3/50
20	44.2	2/50	6/50
62.5	138.3	4/50	3/50
200	442.5	2/50	8/50*
625	1,382.7	49/73*	31/80*

* Indicates statistically increased cancer incidence

Excess cancer risk was calculated according to ECHA (ECHA, 2012):

Observed excess cancer incidence at 442.5 mg/m^3 (200 ppm) for female mice:

$$(\text{Incidence}_{200\ \text{ppm (female)}} - \text{Incidence}_{\text{control (female)}}) / (1-(\text{Incidence}_{\text{control (female)}})) =$$

$$(8/50 - 1/50) / (1-(1/50)) = 0.143 = 14.3\%$$

Correction to an 8-hour working day (the mice were exposed 6 hours a day) and for a higher breathing rate in workers at light work (10 m^3/day) compared to at rest (6.7 m^3/day):

442.5 mg/m^3 x (6h/day)/ (8h/day) x (6.7 m^3/10 m^3) = 222.36 mg/m^3 = 222,360 μg/m^3

Calculation of unit risk factor for cancer:

Risk level = exposure level x unit risk
0.143 = 222,360 μg/m^3 x unit risk
Unit risk = 6.4 x 10^{-7} per μg/m^3

At an exposure level of 1 μg/m^3, 6.4 x 10^{-7} excess cancers are expected.

Calculation of exposure level corresponding to risk level of 10^{-5} (and other risk levels)

10^{-5} risk level = exposure level x unit risk (6.4 x 10^{-7} per μg/m^3)
Exposure level (10^{-5}) = 15.66 μg/m^3

Thus, at 15.66 μg/m^3 (0.01566 mg/m^3), 1:100,000 excess risk of cancer incidence can be expected (Table 9).

Table 9. Excess risk of cancer incidence in mice and cancer mortality in humans (based on NTP, 1993 and Sathiakumar et al. 2015, respectively).

	1,3-butadiene (mg/m^3)	
Excess risk of cancer	Mice (incidence)	Humans (mortality)
1:1,000	1.566	3.1
1:10,000	0.1566	0.31
1:100,0000	0.01566	0.031

Summary

The current working group notes that although we cannot compare the excess risk between mice and humans directly because the calculations are based on incidence in mice and mortality in humans, respectively, the risk estimates were remarkably similar.

Endpoint: Reproductive toxicity (ovarian atrophy)

In the current report, we first calculate the DNEL as recommended by ECHA for toxicological effects having thresholds (ECHA 2012).

The current working group is of the opinion that 1,3-butadiene-induced ovarian atrophy is of relevance to humans. The current working group therefore considers ovarian atrophy as a critical effect. The current working group notes that ovarian atrophy is probably caused by the 1,3-butadiene metabolite DEB. The current working group notes that mice produce more DEB than humans during metabolism of 1,3-butadiene.

Ovarian atrophy is observed in several studies in mice and there is evidence to support that there is a threshold for this effect (Kirman & Grant, 2012). The LOAEL of 13.8 mg/m^3 (6.25 ppm) is identified for ovarian atrophy in female mice exposed to 1,3-butadiene, 6 hour/day, 5 days/week for up to 2 years (NTP, 1993). The histopathological changes were clearly dose dependent (Table 10). The incidence of ovarian atrophy was increased at all exposure concentrations (6.25 to 625 ppm) compared with controls.

Table 10. Incidence of ovarian atrophy in female mice in a 2-year inhalation study (NTP, 1993).

		Female mice
ppm	mg/m^3	Ovarian atrophy
0	0	4/49
6.25	13.8	19/49*
20	44.2	32/48*
62.5	138.3	42/50*
200	442.5	43/50*
625	1,382.7	69/79*

* Indicates statistically increased incidence

First, the LOAEL is modified to correct for an 8-hour working day and to correct for a higher breathing rate in workers at light work (10 m^3/day) compared to at rest (6.7 m^3/day):

$LOAEL_{corr}$ = 13.8 mg/m^3 * 6 hour/8 hour * 6.7 m^3/10 m^3 = 6.9 mg/m^3

Secondly, the corrected LOAEL is adjusted by a number of assessment factors (most of these are default values suggested by ECHA).

The following default assessment factors (AF) are used:
Use of LOAEL: ovarian atrophy causes infertility and is consequently considered a very severe adverse outcome. When a LOAEL is the starting point for the DNEL calculation, it is suggested to use an assessment factor between 3 (as minimum/majority of cases) and 10 (as maximum/exceptional cases) (ECHA). The current working group therefore performs calculations for both assessment factors 3 and 10.

Interspecies extrapolation: 1, since mice produce more DEB than humans during metabolism of 1,3 butadiene, the current working group therefore considers the effects in mice to represent a precautious estimate in humans.

Intraspecies interpolation (default factor for workers): 5

The overall assessment factor, $AF_{overall\ min}$ = 3 * 1 * 5 = 15
The overall assessment factor, $AF_{overall\ max}$ = 10 * 1 * 5 = 50

This results in a DNEL for chronic inhalation for reproductive toxicity:
$DNEL_{min} = LOAEL_{corr}/AF_{overall\ min}$ = 6.9 mg/m^3 / 15 = 0.460 mg/m^3 = 460 μg/m^3
$DNEL_{max} = LOAEL_{corr}/AF_{overall\ max}$ = 6.9 mg/m^3 / 50 = 0.138 mg/m^3 = 138 μg/m^3

The current working group notes that there are several intra- and interspecies uncertainties, that is not taken into consideration in the DNEL calculation approach suggested by ECHA, such as on one hand, the species-dependent differences in 1,3-butadiene metabolism and on the other hand, inherent genetic susceptibilities affecting the metabolic pathway, the individual variation in follicle reserves, that the size of the follicle reserve decreases with age, and that smokers would be exposed to 1,3-butadiene from cigarette smoke in addition to the occupational exposure. In the above calculation of DNEL, we used the approach by ECHA, where the intra-species factor is set to 5 as a default value for workers. Also the appropriate setting of the interspecies extrapolation factor to 1 can of be discussed. Therefore we will use an additional approach to calculate a DNEL taking these uncertainties into account alongside the ECHA approach.

The interspecies variation between humans and mice is a 720-fold difference in DEP blood levels following metabolism of 1,3-butadiene (Swenberg et al., 2011).

For ovarian atrophy in mice, the point of departure is a LOAEL. All mice with ovarian atrophy had complete depletion of follicles which represent the final state of ovary degeneration. Hence, much less histologically apparent adverse effects (i.e. gradual

decrease in follicle number) may have occurred at lower exposure levels and could have resulted in reduced fertility. Follicle cell depletion has been observed both in mice and rats following short-term exposures (30-day) to DEB (Doerr et al., 1996). Rats did not develop ovarian atrophy after exposure to 1,3-butadiene (Owen et al., 1987), but they could potentially have undergone a gradual decrease in numbers of follicles (to the best of our knowledge this has not been assessed).

The current working group notes that there may be important inter-species differences in fertility between mice and women, and that Danish women on average give birth to their first child at 29 years of age, an age where they are already subject to age-related decline in ovarian follicles.

Infertility is a severe adverse effect, which will affect ca 2/3 of the average lifespan (when the women live without having achieved desired children due to infertility). Hence, the uncertainty related to establishment of a threshold effect should be carefully considered.

We assume in the following calculation that ovarian atrophy is mediated by the formation of DEP based on evidence from the existing literature (Kirman et al. 2012).

The following assessment factors (AF) are used:

Use of LOAEL instead of a NOAEL: 10 (the highest factor due to the complete depletion of follicles).

The following assessment factors (intraspecies) are included:

- Human inherent variation in number of follicles at birth (Kirman & Grant, 2012; Wallace & Kelsey, 2010): 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 (Wallace & Kelsey, 2010).
- Inherent susceptibilities e.g. genetic polymorphisms in humans (Kirman & Grant, 2012): 3
- Smokers are more exposed, because 1,3-butadiene is an abundant constituent in cigarette smoke (Soeteman-Hernandez et al., 2013): 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 (OEHHA, 2013): 10
- Lack of multigenerational studies and of dose-response data for partial follicle depletion which are the precursor step to ovarian atrophy: 3

LOAELcorr = 6.9 mg/m^3

$DNEL_{NFA}$ = ((6.9 mg/m^3 / (10 * 8. 5 * 3 * 3 * 2 * 10 * 3)) = 0.00015 mg/m^3

We then multiply with 720 reflecting the difference in DEP levels between mice and humans:

$DNEL_{NFA}$: 0.00015 mg/m³ x 720 = 0.108 mg/m³ = 108 μg/m³

Summary

Our calculation of a DNEL, based onthe approach recommended by ECHA, results in 138 or 460 μg/m³ depending on the choice of assessment factor for LOAEL. The calculation of a DNEL, taking on one hand, species-dependent differences in metabolism and on the other hand, more assessment factors into account instead of ECHAs default values, resulted in a DNEL of 108 μg/m³. DECOS used the same data on ovarian atrophy to calculate a human occupational limit value of 0.11 mg/m³ (110 μg/m³). OEHHA also used the data on ovarian atrophy and calculated an 8-hour REL of 9 μg/m³ which is fairly lower that our and DECOS' results. This is mainly because of the high total assessment factor in the latter case.

Table 11. Overview of assessment factors used by DECOS, OEHHA and NFA.

	DECOS	OEHHA	$DNEL_{min}$	$DNEL_{max}$	$DNEL_{NFA}$
Dose descriptor (mg/m^3)	$BMDL_{10}$ = 1[g]	$BMCL_{05}$ = 2.2[g]	LOAEL = 13.8	LOAEL = 13.8	LOAEL = 13.8
Corrected for exp time	-	6 h/ 8 h	6 h/ 8 h	6 h/ 8 h	6 h/ 8 h
Corrected for respiration	-	-	6.7 mg/m^3 /10 mg/m^3	6.7 mg/m^3 /10 mg/m^3	6.7 mg/m^3 /10 mg/m^3
Human equivalent conc.	-	1.68 (DAF) [b]	-	-	-
AF Use of LOAEL	-	-	3_{low}[a]	10_{high}[a]	10
AF Interspecies differences	3	1[c] * 10[d]	1[c]	1[c]	720[h]
AF Intraspecies differences	3	10[e] * 3[f]	5[a]	5[a]	10 * 8. 5* 3 * 3 * 2 * 10 * 3
AF overall	9	300	15	50	45 900
Results ($\mu g/m^3$)	OEL = 110	8 h REL = 9	$DNEL_{low}$ = 460	$DNEL_{high}$ = 138	$DNEL_{NFA}$ = 108

[a] ECHA default values (ECHA, 2012).

[b] Dosimetric adjustment factor (DAF) = predicted human blood concentration/predicted animal blood concentration and; Human equivalent concentration = Animal experimental concentration x DAF (OEHHA, 2013).

[c] Set to 1, because the mouse is more active in metabolizing butadiene to reactive epoxide metabolites than either the rat or human (toxicokinetic) (OEHHA, 2013).

[d] Set to 10, based on the uncertainty arising from potentially greater human response to the ovotoxic effects of butadiene epoxide metabolites, particularly diepoxybutane (DEB), as compared to the mouse (toxicodynamic) (OEHHA, 2013).

[e] Set to 10, this is specifically justified to account for observed human variability due to polymorphisms in the microsomal epoxide hydrolase gene (toxicokinetic) (OEHHA, 2013).

[f] Set to √10 = 3 (toxicodynamic) (Grant et al., 2010; OEHHA, 2013).

[g] BMDL10 and BMCL05 derived by DECOS and OEHHA, respectively, are different because they use different approaches, albeit the same Benchmark Dose Software (version 2.3.0 and 2.1.2, respectively). The approaches are different since DECOS used the ovarian atrophy data from 103 weeks of exposure as point of departure, whereas OEHHA used the full data sets of 9, 15, and 24 months of exposure as the best basis for the deviation. Other differences are possible, but it is difficult to compare 1:1 because the level of detail in reporting differs considerably between the two – OEHHA are very detailed about their approach compared to DECOS that supply far less detail.

[h] The interspecies variation reflects a 720-fold higher level of DEP in blood of mice compared to humans (Swenberg et al., 2011).

Sensitive groups

Women are born with a finite number of ovarian follicles that gradually diminishes until menopause. It is therefore assumed that the number of follicles at birth reflects human susceptibility to depletion of follicle reserves (premature menopause) by diepoxides such as DEB. Hence, individuals born with low follicle counts, or acquiring low follicle counts due to surgery (ovariectomy) or medication (chemotherapy) would constitute potential sensitive subpopulations (Kirman & Grant, 2012). The average age for women's birth of the first child is approximately 29 years in Denmark, a time where fertility is already reduced (Baird et al., 2005). Assuming an entry to the labor market at 18 years of age, this leaves several years for exposure before pregnancy is pursued. These considerations have not been taken into consideration in the default intraspecies assessment factor by ECHA, but is included in the $DNEL_{NFA}$ calculation.

1,3-butadiene is generated from the combustion of natural precursors found in tobacco leafs itself and precursors from additives including cellulose, paraffin, and sugars. 1,3-butadiene is therefore found in both tobacco smoke and in second hand tobacco smoke. Hence, smokers might be a sensitive group relative to exposure to 1,3-butadiene (Soeteman-Hernandez et al., 2013).

Conclusion

IARC has classified 1,3-butadiene as a group 1 carcinogen based on sufficient evidence in humans and experimental animals for carcinogenicity (IARC, 2008). The working groups of SCOEL and DECOS considered cancer as the critical health effect following 1,3-butadiene exposure.

The current working group notes that there is evidence of primary gentoxicity, i.e., there is no threshold for effect, and strong evidence of primary genotoxicity of the individual epoxide metabolites of 1,3-butadiene.

Based on the presented evidence and evaluations by other working groups, the current working group is of the opinion that 1,3-butadiene operates by non-threshold mechanisms relative to induction of cancer.

The current working group notes the species differences where mice seem to be more efficient in the production of epoxide metabolites of BD than rats and humans, and takes this into account in the risk assessment.

Additionally, the current working group notes that significant reproductive toxicity, in terms of ovarian atrophy, was observed in female mice (NTP, 1993).

The current working group calculates health-based OELs based on cancer (leukemia) data from both human and animal studies, and in addition, DNEL for toxicological effects having thresholds based on reproductive toxicity data.

Cancer, based on epidemiological studies:
Different publications using the same cohort on synthetic rubber production workers have been used for risk assessment and calculations of health-based occupational cancer risk values based on leukemia mortality (DECOS, 2013; SCOEL, 2007). DECOS based their assessment on the Cheng et al. study with 16,000 subjects (men) examining leukemia mortality during 1944-1998, where 81 decedents with leukemia were identified based on information from death certificates (Cheng et al., 2007). The current working group identified a new publication on the same cohort with an 11-year follow-up (1944-2009), where 114 decedents with leukemia were identified (Sathiakumar et al., 2015). We used both publications in our derivation of an OEL based on leukemia under the assumption of a non-threshold driven mechanism. The exposure-response relationship from the most recent Sathiakumar et al. study was similar to the Cheng et al. study. Overall, our calculations showed that the estimate of an excess risk of mortality is practically equal to the value calculated by DECOS:

Table 12. Excess mortality risks based on the exposure-response relationship from Cheng et al. 2007 and Sathiakumar et al. 2015.

	Cheng et al. 2007	Sathiakumar et al. 2015
Excess mortality risk	Exp for 45-year work life	Exp for 45-year work life
1:1,000	2.2 mg/m^3	3.1 mg/m^3
1:10,000	0.22 mg/m^3	0.31 mg/m^3
1:100,000	0.022 mg/m^3	0.031 mg/m^3

The current working group notes that the Danish OEL (TWA 8h) for 1,3-butadiene at 1 ppm (2.2 mg/m^3) corresponds to ~1:1,000 excess deaths at 45 years of occupational exposure at 1,3-butadiene concentrations.

Cancer, based on studies in mice:
In a 2-year inhalation study, mice exposed to 1,3-butadiene showed increased incidences of benign and malignant neoplasms at multiple sites (NTP, 1993). There was no exposure levels where a significant carcinogenic response was not observed. Lymphocytic lymphomas appeared early and were the principal cause of death of male and female mice exposed to 1,3-butadiene. The current working group notes that, to our knowledge, no other long-term studies on butadiene have been conducted at exposure concentrations that have not shown a carcinogenic response. In the risk assessment, the current working group notes that although we cannot compare the excess risk between mice and humans directly because the calculations are based on incidence in mice and mortality in humans, respectively, the risk estimates were remarkably similar, despite observations showing that mice are more effective in metabolizing 1,3-butadiene to DEP which is regarded as the most gentoxic metabolite of 1,3-butadiene.

Reproductive toxicity, based on studies in mice:
The current working group considers both cancer and ovarian atrophy as critical effects, as both adverse effects were observed in chronic longterm studies in female mice (NTP, 1993). Ovarian atrophy is observed in several studies in mice and there is evidence to support that there is a threshold for this effect (Kirman & Grant, 2012).

The calculation of a DNEL using the approach recommended by ECHA resulted in DNELs of 138 or 460 μg/m^3 depending on the choice of assessment factor for LOAEL (3 or 10). The current working group notes that compared to controls, the lowest air concentration of 1,3-butadiene tested induced an almost 5-fold increase in the incidence of ovarian atrophy in female mice (from 4/49 to 19/49, repectively). Based on this observation, the current working group regards a LOAEL to NOAEL assessment factor of 10 as most appropriate.
The calculation of a DNEL, taking inter-species differences in metabolism and more assessment factors into account instead of ECHAs default values, resulted in a DNEL of 108 μg/m^3.
The current working group furthermore notes that DECOS used the same data on ovarian atrophy to calculate a human occupational limit value of 0.11 mg/m^3 (110 μg/m^3). OEHHA also used the data on ovarian atrophy and calculated an 8-hour REL of 9 μg/m^3 which is fairly lower that our and DECOS' results.

Table 13 shows exess mortality risk at 1 in 1,000, 1 in 10,000 and 1 in 100,000 derived based on a human epidemiological cohort (Sathiakumar et al., 2015) and DNELs for reproductive toxicity derived based on the 2-year inhalation study of mice (NTP, 1993):

Table 13. Overview of exposure levels resulting in extra mortality risk levels at 1:1,000, 1:10,000 and 1: 100,000 based on a non-threshold based mechanism and DNELs based on a threshold-based mechanism.

		Suggestion of an OEL for 1,3-butadiene	
Type of effect		**Leukemia mortality**	**Reproductive toxicity**
Non-threshold based	Extra mortality risk		
	1:1,000	3.1 mg/m^3	
	1:10,000	0.31 mg/m^3	
	1:100,000	0.031 mg/m^3	
Threshold- based	$DNEL_{max}$		0.138 mg/m^3
	$DNEL_{NFA}$		0.108 mg/m^3

The current working group considers both cancer and reproductive toxicity as critical effects. Therefore, the current working group recommends that both endpoints are taken into consideration.

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