Health Council of the Netherlands

1,3-Butadiene

GSW 1399
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Health-based calculated occupational cancer risk values

Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *1,3-Butadiene*
Uw kenmerk : DGV/MBO/U-932342
Ons kenmerk : U-7739/BJB/fs/459-K68
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Geachte minister,

Graag bied ik u hierbij aan het advies over de gevolgen van beroepsmatige blootstelling aan 1,3-butadieen.

Dit advies maakt deel uit van een uitgebreide reeks waarin concentratieniveaus in de lucht worden afgeleid die samenhangen met een extra kans op overlijden aan kanker door beroepsmatige blootstelling van 4 per 1.000 en 4 per 100.000 sterfgevallen in de algemene bevolking. De conclusies van het genoemde advies zijn opgesteld door de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. W.A. van Gool,
voorzitter

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1,3-Butadiene

Health-based calculated occupational cancer risk values

Dutch Expert Committee on Occupational Safety
a Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2013/08, The Hague, May 31, 2013

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Samenvatting

Vraagstelling

Op verzoek van de minister van Sociale zaken en Werkgelegenheid schat de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS) van de Gezondheidsraad het extra kankerrisico als gevolg van beroepsmatige blootstelling aan stoffen die door de Europese Unie of door de Commissie GBBS als stochastisch genotoxisch kankerverwekkend zijn aangemerkt. In dit rapport presenteert de commissie een dergelijke schatting voor blootstelling aan 1,3-butadieen. Zij heeft daarbij gebruik gemaakt van de methode die beschreven is in het rapport 'Leidraad berekening risicogetallen voor carcinogene stoffen' van de Gezondheidsraad (2012).

Fysische en chemische eigenschappen

1,3-Butadieen (CAS nummer 106-99-0; hierna "butadieen") is een kleurloos gas met een moleculair gewicht van 54,1 dalton en wordt gebruikt in de bereiding van verschillende synthetische rubberproducten en -polymeren en als intermediair in de productie van basale petrochemicaliën. Producten gebaseerd op butadieen zijn belangrijke componenten van motorvoertuigen, constructiematerialen, apparaatonderdelen, computers en telecommunicatie-apparatuur, (beschermende) kleding, verpakkingen en huishoudelijke artikelen.

Grenswaarden

De huidige grenswaarde voor 1,3-butadieen in Nederland is 46,2 mg/m^3 (8-uurs tijdgewogen gemiddelde, TGG). Groot-Brittannië heeft een TGG van 22 mg/m^3, Finland en Noorwegen hebben een TGG van 2,2 mg/m^3, Zweden heeft een TGG van 1 mg/m^3, en de USA hebben TGG's van 4,4 ('threshold limit value' van de American Conference of Governmental Industrial Hygienists) en 2,2 mg/m^3 ('permissible exposure limit' van de Occupational Safety and Health Administration). De genoemde landen hebben een notificatie dat 1,3-butadieen kankerverwekkend is voor mensen, dan wel verdacht wordt kankerverwekkend te zijn voor mensen.

Kankerverwekkendheid

Veel epidemiologische onderzoeken tonen een verhoogd risico voor leukemie of andere vormen van lymfo-haematopoietische kankers na blootstelling aan 1,3-butadieen. Er zijn echter maar drie onderzoeken met werknemers die uitsluitend aan de stof zijn blootgesteld. De meeste onderzoeken zijn uitgevoerd met werknemers blootgesteld aan 1,3-butadieen gedurende de productie van styreen-butadieen rubber (SBR) en deze mensen waren behalve aan 1,3-butadieen ook blootgesteld aan andere potentieel gevaarlijke stoffen. Hoewel er een groot aantal onderzoeken is gepubliceerd, betreft het vaak actualisaties van eerder uitgevoerde onderzoeken; er is dus sprake van dezelfde of overlappende cohorten.

In twee van de onderzoeken in 1,3-butadieen monomeer-fabrieken vond men een iets hogere sterfte door leukemie, in het derde onderzoek werd een kleine afname van die mortaliteit gevonden. Het in verhouding hoogste aantal sterfgevallen werd vastgesteld in het onderzoek onder werknemers die waren blootgesteld aan hoge concentraties gedurende de eerste productiejaren (Tweede Wereldoorlog). In dit cohort werd geen verband gevonden tussen de toename van leukemie en de cumulatieve blootstelling of de duur van de blootstelling.

Met hun langlopend onderzoek onder werknemers in de SBR-productie verschaften epidemiologen van de Universiteit van Alabama in Birmingham (USA) de meeste informatie. Zij onderzochten de mortaliteit van ongeveer 17.000 werknemers in acht fabrieken (USA en Canada). Een beperking van deze evaluaties was dat de diagnose en classificatie van leukemie en andere kwaadaardige nieuwvormingen van het lymfatische en haematopoietische systeem zeer complex zijn en in de loop der jaren diverse veranderingen hebben ondergaan. Hoewel in de meest recente actualisatie van dit cohort de totale mortaliteit ten

gevolge van leukemie slechts licht verhoogd was, werden grotere verhogingen van die mortaliteit gevonden bij mensen werkzaam in delen van de fabrieken met hoge blootstellingen, alsmede bij werknemers die per uur werden betaald, vooral bij hen die werkzaam waren in de vroege productiejaren en die daar tien jaar of langer gewerkt hadden. Voorts werd er een significante associatie vastgesteld tussen mortaliteit ten gevolge van leukemie en de cumulatieve blootstelling aan 1,3-butadieen. Uit de recente analyses is tevens gebleken dat deze blootstellings-respons relatie onafhankelijk was van blootstelling aan styreen en dimethyl-dithiocarbamaat.

Onderzoek met muizen heeft aangetoond dat zowel mannetjes als vrouwtjes meer tumoren ontwikkelden nadat ze aan respectievelijk ongeveer 14 mg/m^3 (vrouwtjes) en 44 mg/m^3 1,3-butadieen (mannetjes) waren blootgesteld. In ratten blootgesteld aan concentraties tot 2.200 mg/m^3 is dit niet waargenomen. Waarschijnlijk moet dit worden toegeschreven aan de cruciale rol van het oxidatieve metabolisme. De carcinogene werking van 1,3-butadieen vereist namelijk activering tot electrofiele epoxiden, en daarin bestaan belangrijke soortverschillen: muizen zijn efficiënter in de productie van epoxidemetabolieten van 1,3-butadieen, terwijl ratten en mensen efficiënter zijn in de hydrolytische detoxificatie van deze metabolieten.

Uit vele mutageniteits- en genotoxiciteitstesten, evenals uit onderzoek naar het carcinogene werkingsmechanisme, is gebleken dat 1,3-butadieen in mensen en proefdieren het genetische materiaal kan beschadigen.

Algemene toxiciteit

Korte blootstelling aan hoge concentraties 1,3-butadieen leidde bij mensen en proefdieren tot irritatie van de ogen, neusholte, keel en longen. Tot de klinische vergiftigingsverschijnselen behoorden hyperventilatie, krampen, opwinding, anesthesie en narcose. LC_{50} waarden* varieerden van 270 (muizen) tot 550 (konijnen) g/m^3.

Langdurige blootstelling van proefdieren aan 1,3-butadieen resulteerde (behalve in kanker) in biochemische veranderingen, zoals depletie van glutathion in lever, longen en hart; bij muizen was dit ernstiger dan bij ratten. In blootgestelde muizen werd ook toxiciteit van het haematopoietische systeem waargenomen: subchronische blootstelling aan 2.750 mg/m^3 leidde onder meer tot bloedarmoede en een verminderde hoeveelheid circulerende witte bloedcellen.

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* De LC_{50} is de concentratie waarbij 50% van de blootgestelde dieren binnen 24 uur overlijdt.

Onderzoek naar de voortplantingseffecten met ratten blootgesteld aan 2.200 mg/m^3 lieten een afname van het lichaamsgewicht gedurende de zwangerschap zien. In muizen werden testiculaire en ovariële atrofie waargenomen na langdurige blootstelling aan respectievelijk 1.375 en 13,8 mg/m^3. Mannelijke muizen blootgesteld aan 2.200 tot 11.000 mg/m^3 hadden afwijkende spermamorfologie.

In ontwikkelingsonderzoek met ratten blootgesteld aan 2.230 of 17.680 mg/m^3 gedurende dagen 6-15 van de dracht vertoonden de foetussen kleine respectievelijk grote skeletafwijkingen. Bij muizen blootgesteld aan 88 - 2.210 mg/m^3 gedurende dagen 6-15 van de dracht lieten de mannelijke foetussen een afname van het foetale lichaamsgewicht zien. In de groepen blootgesteld aan 442 en 2.210 mg/m^3 nam respectievelijk het aantal gevallen van extra ribben toe en het aantal gevallen van verbening van het borstbeen af. Foetale toxiciteit (late dood, ontbreken van schedeldak en schedelafwijkingen) werd gevonden wanneer onbehandelde vrouwtjesmuizen werden gepaard met mannetjes die blootstonden aan 27,5 mg/m^3 (6 uur per dag, 5 dagen per week gedurende 10 weken).

De commissie concludeert dat de LOAEL* (algemene toxiciteit en in het bijzonder de voortplantingseffecten) voor blootstelling aan 1,3-butadieen 13,8 mg/m^3 bedraagt (gebaseerd op ovariële atrofie bij muizen). De LOAEL voor ontwikkelingstoxiciteit bedraagt 27,5 mg/m^3 (muizen). Niveaus waarbij geen toxisch effect optrad (NOAEL's**) konden uit deze onderzoeken niet worden afgeleid.

Evaluatie

Op basis van voorgaande informatie beschouwt de commissie het optreden van kanker na langdurige blootstelling aan 1,3-butadieen als het kritische effect. Op advies van de Subcommissie Classificatie van kankerverwekkende stoffen van de Commissie GBBS, concludeert de commissie dat 1,3-butadieen kankerverwekkend is voor de mens (categorie 1A). De stof veroorzaakt kanker via een zogenaamd stochastisch genotoxisch mechanisme. De commissie leidt daarom voor 1,3-butadieen concentratieniveaus in de lucht af (risicogetallen, HBC-OCRV***) die samenhangen met een kans op 4 extra sterfgevallen door kanker door beroepsmatige blootstelling per 1.000 en per 100.000 sterfgevallen in de algemene bevolking.

* Lowest observed adverse effect level: het laagste niveau waarbij nog juist toxische effecten optreden.
** No observed adverse effect level: het hoogste niveau waarbij nog juist geen toxische effecten optreden.
*** HBC-OCRV: health based calculated occupational cancer risk value.

Voor het afleiden van de risicogetallen gaat de commissie uit van het onderzoek van Cheng en medewerkers (2007) die een verhoogd aantal gevallen van leukemie beschreven onder een grote groep werknemers in de SBR-productie.

Advies

De Commissie GBBS schat dat de 1,3-butadieenconcentratie in de lucht die samenhangt met een kans op 4 extra sterfgevallen door leukemie:

- per 100.000 sterfgevallen in de algemene bevolking ($4x10^{-5}$), bij een beroepsmatige blootstelling gedurende 40 jaar, 0,1 mg/m^3 (0,05 ppm) bedraagt
- per 1.000 sterfgevallen in de algemene bevolking ($4x10^{-3}$), bij een beroepsmatige blootstelling gedurende 40 jaar, 10 mg/m^3 (5 ppm) bedraagt.

De geadviseerde risicogetallen zijn uitgedrukt als 8-uurs tijdgewogen gemiddelde concentraties.

Daarnaast beveelt de commissie aan om 1,3-butadieen te classificeren als 'kankerverwekkend voor de mens' (categorie 1A).

Executive summary

Scope

At the request of the Minister of Social Affairs and Employment, the Dutch Expert Committee on Occupational Exposure Safety (DECOS; hereafter called 'the Committee'), a committee of the Health Council of the Netherlands, estimates the additional cancer risk associated with occupational exposure to substances that have been classified by the European Union or the Committee as a stochastic genotoxic carcinogen. In this report the Committee presents such an estimate for 1,3-butadiene, using the method described in the report 'Leidraad berekening risicogetallen voor carcinogene stoffen (in Dutch)' of the Health Council of the Netherlands (2012).

Physical and chemical properties

1,3-Butadiene (CAS number 106-99-0; hereafter "butadiene") is a colourless gas with a molecular weight of 54.1 dalton and is used for the preparation of a variety of synthetic rubber products and polymers, and as an intermediate in the production of basic petrochemicals. Butadiene-based products are important components of automobiles, construction materials, appliance parts, computers and telecommunications equipment, (protective) clothing, packaging and household articles.

Guidelines

Currently, the limit value for occupational exposure to 1,3-butadiene in The Netherlands is 46.2 mg/m^3 (8-hour time-weighted average: TWA). The UK has a TWA of 22 mg/m^3, Finland and Norway have a TWA of 2.2 mg/m^3. Sweden has a TWA of 1 mg/m^3, and the USA has TWAs of 4.4 (threshold limit value of the American Conference of Governmental Industrial Hygienists) and 2.2 mg/m^3 (permissible exposure limit of the Occupational Safety and Health Administration). All mentioned countries either have a notification that 1,3-butadiene is carcinogenic to humans or that it is suspected to be carcinogenic to humans.

Carcinogenicity

Many epidemiological studies show an elevated risk of leukaemia or other cancers of the lymphohaematopoietic system following exposure to 1,3-butadiene. Only three studies have been conducted on workers employed in 1,3-butadiene manufacturing facilities, where exposure is to 1,3-butadiene monomer alone. Most studies have been done on workers exposed to 1,3-butadiene during styrene-butadiene rubber (SBR) production. Although a relative large number of studies has been reported, many of these studies update previously reported findings and thus relate to the same or overlapping cohort populations.

In two of the butadiene monomer industry studies a slight overall excess of mortality from leukaemia was observed, whereas in the third study a small decrease in mortality from leukaemia was observed. The increased 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 leukaemia was observed with duration of exposure or cumulative exposure.

Studies on SBR workers by researchers of the University of Alabama at Birmingham (USA) were considered to be the most informative. In these studies the mortality rates of approximately 17,000 workers from eight facilities in the USA and Canada were examined. A limiting factor in the evaluations was that the diagnosis and classification of lymphatic and haematopoietic malignancies are very complex and have undergone several changes over the course of time. Although overall mortality from leukaemia was only slightly elevated in the most recent update of this cohort, larger increases of mortality from leukaemia were seen in workers in the most highly exposed areas of the plants and among

hourly paid workers, especially those who had been hired in the early years and had ten years or longer employment. Furthermore, a significant exposure-response relationship between cumulative 1,3-butadiene exposure and mortality from leukaemia was observed in this study. Recent analyses indicate that the exposure-response relationship for 1,3-butadiene and leukaemia was independent of exposure to styrene and dimethyldithiocarbamate.

Studies with mice showed increased tumour formation in various organs in both sexes at 1,3-butadiene exposures to approximately 14 mg/m^3 (females) and 44 mg/m^3 (males). This was not observed in rats at exposures up to 2,200 mg/m^3, likely due to the crucial role of oxidative metabolism: 1,3-butadiene requires metabolic activation to generate electrophilic epoxides in which important species differences exist (mice are more efficient in the production of epoxide metabolites of butadiene, while rats and humans are more efficient in the hydrolytic detoxification of these metabolites).

Many tests on mutagenicity, genotoxicity and mechanism of action clearly indicate that 1,3-butadiene is a genotoxic compound in humans and in experimental animals, requiring metabolic activation to generate electrophilic and DNA-reactive epoxides (epoxybutene, epoxybutanediol and diepoxybutane), one or more of which are considered to be the ultimate carcinogens.

General toxicity

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

Long-term exposure of animals to 1,3-butadiene resulted (in addition to cancer) in biochemical alterations such as glutathione depletion in liver, lungs and heart, which was more extensive in mice than in rats. In exposed mice toxicity of the haematopoietic system also was seen: semi-chronic exposure to 2,750 mg/m^3 resulted in anaemia, while also leukopenia and an increase in the number of circulating erythrocyte micronuclei were seen.

Reproductive studies with rats exposed to 2,200 mg/m^3 showed decreased weight gain during pregnancy. In mice testicular and ovarian atrophy were observed following long-term exposure to 1,375 and 13.8 mg/m^3, respectively.

* The concentration at which 50% of the exposed animals dies within 24 h.

Male mice exposed to 2,200-11,000 mg/m^3 showed abnormal sperm head morphology.

In developmental studies with pregnant rats which were exposed to concentrations of 2,230 or 17,680 mg/m^3 during gestational days (GD) 6-15, the fetuses showed minor or major abnormalities, respectively. In mice, a decrease in fetal body weight gain in males was observed following exposure of dams to to 88 - 2,210 mg/m^3 during GD 6-15. Increased incidences of of extra ribs and reduced ossification of sternebrae were found in groups exposed to 442 and 2,210 mg/m^3, respectively. Fetal toxicity was seen following mating untreated female mice with males exposed to 27.5 mg/m^3 (6 h/day, 5 days/week, 10 weeks). Observed effects included increased late fetal death, exencephaly and skull abnormalities.

In conclusion, the overall LOAEL* for reproduction is 14 mg/m^3 (based on ovarian atrophy in mice). The LOAEL for developmental toxicity is 27.5 mg/m^3 (mice). It was not possible to derive NOAELs** from these studies.

Evaluation and advice

Based on the information summarized above the Committee considers the induction of cancer following long-term exposure to 1,3-butadiene to be the critical effect. Following the advice of the DECOS Subcommittee on the Classification of carcinogenic substances the Committee concludes that 1,3-butadiene is "carcinogenic to humans" (category 1A). The substance induces cancer by a stochastic genotoxic mechanism. Hence the Committee derives risk values for concentrations of 1,3-butadiene in ambient air (HBC-OCRV: health based calculated occupational cancer risk value) that are related to the risk for 4 extra cancer deaths due to occupational expusure per 1,000 and 100,000 deaths in the general population.

The Committee uses the epidemiological study of Cheng and coworkers (2007), in which the mortality of approximately 17,000 workers in the SBR production was examined. From this study the Committee calculates that 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 (5 ppm)

* Lowest observed adverse effect level.
** No observed adverse effect level.

- 4 per 100,000 ($4x10^{-3}$) deaths in the general population, at 40 years of occupational exposure, equals to 0,1 mg 1,3-butadiene per m^3 (5 ppm).

The recommended values are expressed as 8-hour time-weighted average concentrations.

The Committee also recommends to classify 1,3-butadiene as "carcinogenic to man" (category 1A).

Chapter 1

Scope

1.1 Background

In the Netherlands, occupational exposure limits for chemical substances are set using a three-step procedure. In the first step, a scientific evaluation of the data on the toxicity of the substance is made by the Dutch Expert Committee on Occupational Exposure Safety (DECOS), a committee of the Health Council of the Netherlands, at request of the Minister of Social Affairs and Employment (Annex A). This evaluation should lead to a health-based recommended exposure limit for the concentration of the substance in air. Such an exposure limit cannot be derived if the toxic action cannot be evaluated using a threshold model, as is the case for carcinogens acting by stochastic genotoxic mechanism. In that case, an exposure-response relationship is recommended for use in regulatory standard setting, i.e., the calculation of so-called health-based calculated occupational cancer risk values (HBC-OCRVs). The Committee calculates HBC-OCRVs for compounds, which are classified by the European Union or by the Committee in category 1A of 1B.

For the establishment of the HBC-OCRVs, the Committee generally uses a linear extrapolation method, as described in the Committee's report 'Calculating cancer risk due to occupational exposure to genotoxic carcinogens'. The linear model to calculate occupational cancer risk is used as a default method, unless scientific data would indicate that using this model is not appropriate.

In the next phase of the three-step procedure, the Social and Economic Council advises the Minister of Social Affairs and Employment on the feasibility of using the HBC-OCRVs as regulatory occupational exposure limits. In the final step of the procedure, the Minister sets the official occupational exposure limits.

1.2 Committee and procedure

The present report is an update of the health-based recommended occupational exposure limits for 1,3-butadiene of the Health Council from 1990[1] and contains the derivation of HBC-OCRVs by the DECOS, hereafter called the Committee. The members of the Committee are listed in Annex B. The submission letter (in English) to the Minister can be found in Annex C.

In 2012, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex D. The Committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

In order to calculate the HBC-OCRV and to evaluate other toxic effects of 1,3-butadiene, the safety evaluation of butadiene by the International Agency for Research on Cancer (IARC 2008[2]) has been used as a basis for the update of the health-based recommended occupational exposure limit (DECOS 1990[1]). Where relevant, the original publications were reviewed and evaluated as indicated in the text. In addition, literature was retrieved from the on-line databases Medline, Toxline and Chemical Abstracts until September 2012. For the present evaluation, only the literature from 2006-2012 was used to update the information reviewed in the cited IARC monograph[2].

Chapter 2

General information

2.1 Identity, and physical and chemical properties

1,3-Butadiene (hereafter "butadiene"), a colourless gas, is manufactured primarily as a co-product of the steam cracking of hydrocarbon streams to produce ethylene. This process accounts for over 95% of global butadiene production[2]. The purity of the technical product is approximately 99.5%. Butadiene is used for the preparation of a variety of synthetic rubber products and polymers. Butadiene-based products are important components of automobiles, construction materials, appliance parts, computers and telecommunications equipment, clothing, protective clothing, packaging and household articles.

The synthetic rubbers that are produced from butadiene include styrene-butadiene rubber (SBR), polybutadiene rubber, styrene-butadiene latex, chloroprene rubber and nitrile rubber. Important plastics that contain butadiene as a monomeric component are shock-resistant polystyrene (a two-phase system of polystyrene and polybutadiene), polymers that consists of acrylonitrile, butadiene and styrene (STYR); and a copolymer of methylmethacrylate, butadiene and STYR (which is used as a modifier for polyvinylchloride). Butadiene is also used as an intermediate in the production of chloroprene, adiponitrile and other basic petrochemicals. It is not known to occur as a natural product[2].

Butadiene is very reactive: it may form acrolein and peroxides upon exposure to air, it can react with oxidizing materials, and it polymerizes readily, particularly if oxygen is present. Butadiene is stabilized with hydroquinone, catechol, t-butyl catechol or aliphatic mercaptans[1,2].

The identity and most important physicochemical properties of butadiene are presented in Table 1.

The following measured ambient concentrations have been reported[3]:

- Air concentrations in urban/suburban areas: 0.02-2 µg/m^3
- Air in heavy traffic area: 2-13 µg/m^3
- Air in homes and restaurants where smoking is allowed: 1.7-4.3 µg/m^3
- Air in homes and restaurants where smoking is not allowed: 0.1-0.9 µg/m^3.

Lovreglio et al. (2006[4]) reported that environmental mean levels of butadiene in Italy ranged between 0.2 and 7.9 µg/m^3, with lower concentrations measured in indoor non-smoking environments and higher concentrations measured in indoor smoking environments and in cars. Saborit et al. (2009[5]) reported environmental levels of 0.4 ± 0.7 µg/m^3 in both urban and suburban, but also in rural areas in the UK.

The highest exposure to butadiene occurs in occupational settings. In general, actual workplace exposure levels were not determined until 1970-1980, but it is assumed that during early years of butadiene and SBR production (1940 to ~1970) workplace butadiene levels were higher (approximately 8-20 mg/m^3) compared to more recent workplace levels[7] (< 2 mg/m^3). However, peak exposures to high concentrations still occur for some activities in the production of butadiene[2].

The average occupational exposure to butadiene in the European Union in 1995 as reviewed by IARC in 2008[2] was 3.1-7.5 mg/m^3 for exposed production workers at 15 monomer production facilities, and 0.06-2.2 mg/m^3 for controls (supposedly non-exposed) laboratory workers.

It must be noted that also smoking contributes to butadiene exposure. Hurst reported in 2007[6] that the tobacco smoke of one cigarette contained 0.4 mg butadiene. The average breath concentration of butadiene was 0.014 and 0.353 mg/m^3 in nonsmokers and smokers, respectively[2].

2.2 IARC conclusion

In 2008 IARC[2] concluded that butadiene is carcinogenic to humans (Group 1), because:

- There is sufficient evidence in humans for the carcinogenicity of butadiene

- There is sufficient evidence in experimental animals for the carcinogenicity of butadiene
- There is sufficient evidence in experimental animals for the carcinogenicity of D,L-diepoxybutane (diepoxybutane is a metabolite of butadiene, see Section 2.6).

In 2009 a working group of IARC (IARC 2009[7]) updated the evaluation of 2008[2] and confirmed the earlier conclusion, pointing to strong evidence of genotoxicity as the mechanism of carcinogenic effects in workers in the rubber industry.

Table 1 Physical and chemical properties of 1,3-butadiene.

Chemical name	1,3-butadiene
CAS registry number	106-99-0
EINECS number	203-450-8
RTECS number	EI9275000
IUPAC name	1,3-butadiene
Synonyms	butadiene; biethylene; bivinyl; buta-1,3-diene; divinyl; erythrene; pyrrolylene; vinylethylene
Molecular weight	54.1 g/mol
Molecular formula	C_4H_6
Molecular structure	$H_2C{=}CH{-}CH{=}CH_2$
Physical appearance	colourless gas (at 100 kPa and 15.5 °C)
Boiling point	-4.4 °C (100 kPa)
Freezing point	-108.9 °C (100 kPa); -113 °C [20]
Log P_{ow}	1.99 (estimation)
Vapour pressure (21 °C)	240 kPa
Relative density of saturated vapour in air (air = 1), 21 °C, 100 kPa	3.07
Percentage [vapour in saturated] air (21 °C, 100 kPa)	237%
Odour threshold	detection: 1.0-2.1 mg/m³ air recognition: 2.4-169 mg/m³ air
Flash point	below -76 °C
Explosion limits in air	1.1-12.3% (vol/vol)
Solubility	Water: 2.3 g/L (0 °C); 1.9 g/L (50 °C); 735 mg/L [20]; soluble in ethanol, diethyl ether, benzene and organic solvents; very soluble in acetone.
Conversion factors (20 °C, 100 kPa)	1 ppm = 2.21 mg/m³ 1 mg/m³ = 0.442 ppm
EU classification and labelling (GHS)	Category 1A May cause cancer (danger) H350

Data from DECOS (1990[1]) and IARC (2008[2]) unless stated otherwise.

2.3 Other conclusions

The Scientific Committee on Occupational Exposure Limits of the European Union (SCOEL) evaluated butadiene in 2007 (SCOEL 2007[8]), and concluded that 'butadiene should be treated as a possible human carcinogen, operating via a genotoxic mechanism'. No short term exposure limit (STEL) or skin notation was considered necessary. Risk values were derived for a number of exposure scenarios, see Section 3.4.

The European Risk Assessment Report of butadiene (EU-RAR 2002[9]) classified the substance as a category 1 carcinogen ('substances known to be carcinogenic to humans'), and as a category 2 mutagen ('substances which should be regarded as if they are mutagenic to man').

In the extensive reviews of Kirman et al. (2010a[10], 2010b[11]) and Albertini et al. (2010[12]) the authors concluded that butadiene has a mutagenic mode of action in producing cancer in experimental animals (rodents) and humans. They attributed the mutagenicity of butadiene to the formation of the reactive metabolites epoxybutene, diepoxybutane and epoxybutane diol.

2.4 Carcinogenicity studies in humans

In the previous DECOS report (1990[1]) it was concluded that the available human studies were inconclusive to determine if butadiene is carcinogenic to humans. Many studies showed an elevated risk of leukaemia or other lymphopoietic cancers, but either the exposure was not exclusively to butadiene, or the cohort was too small to have enough power to detect a two-fold leukaemia excess. The Committee concluded that butadiene had to be considered a carcinogen in experimental animals but drew no conclusion about any specific mode of action. The Committee estimated an additional lifetime cancer risk of $1x10^{-4}$ for 40 years of exposure to 1.18 mg butadiene per m^3, and $1x10^{-6}$ for 40 years of exposure to 0.012 mg butadiene per m^3 (DECOS 1990[1]).

Mortality studies have been conducted both on workers employed in butadiene manufacturing facilities where exposure is to butadiene monomer alone, and on workers exposed to butadiene during SBR production. Although a relative large number of studies has been reported, many of these studies updated previously reported findings and thus relate to the same or overlapping cohort populations[2,9]. Epidemiological studies of cancer and exposure to butadiene as considered by IARC (2008[2]) are summarized in Annex F, and updated with more recent publications.

In IARC's evaluation of butadiene[2], three independent cohorts of monomer production workers in the USA were evaluated: the first at three Union Carbide plants in West Virginia (Ward et al. 1995[13]), the second at a Texaco plant in Texas (Divine & Hartman 2001[14]), and the third at a Shell plant in Texas (Tsai et al. 2001[15]). Also two independent groups of SBR production workers have been studied; one included a two-plant complex in Texas, USA (McMichael et al. 1974, 1976; Meinhardt et al. 1982; in IARC[2]) studied by the University of Pittsburgh, and the other included workers from eight facilities in the USA and Canada studied by researchers from the Johns Hopkins University (JHU; Matanoski & Schwartz 1987 in IARC[2]; Matanoski et al. 1990[16], 1993[17]). Subsequently, researchers from the University of Alabama at Birmingham (UAB) studied the two-plant complex originally investigated by the National Institute for Occupational Safety and Health (NIOSH) plus seven of the eight plants studied by the JHU (Delzell et al. 1996[18]). The JHU researchers also conducted nested case-control studies with this working population (Matanoski et al. 1997[19], Santos-Burgoa et al. 1992[20]). The UAB group recently updated the follow-up of the cohort, revising and refining their assessment of exposures to butadiene, and taking possible confounding co-exposures into account (Macaluso et al. 2004[21]). A number of largely overlapping publications from these groups have been reviewed. The most recent results that were evaluated by IARC[2] were published by Graff et al. 2005[22], Sathiakumar et al. 2005[23] and Cheng et al. 2007[24].

Compared to exposure to butadiene alone in the monomer production sites, multiple chemical exposures of SBR workers make interpretation of the results more difficult. In addition, many employees moved between plants, and have worked in both the butadiene manufacturing industry and in the SBR industry, which makes the interpretation of these studies even more complicated.

Industry-based studies - monomer production

Ward and co-workers (1995[13]) identified, among 29,139 workers at three Union Carbide Corporation facilities in West Virginia (USA), a study population of 364 men who worked in butadiene monomer production from 1940-1979. The mortality experience of the cohort was compared to USA and to Kanawha County mortality rates using a modified life-table analysis system, developed by NIOSH. SMR (standardized mortality ratio), CI (95% confidence interval) and two-sided p-values were calculated. Mortality from all cancers was not increased. SMR for lymphohaematopoietic cancers was 1.8 (CI 0.7-3.6), with an SMR for lymphosarcoma and reticulosarcoma of 5.8 (CI 1.6-14.8). The study

has several limitations, the most important of which is that no exposure monitoring data are available, so it was not possible to associate mortality from lymphosarcoma and reticulosarcoma with exposure data.

A cohort mortality study of 2,800 male workers employed > 6 months between 1943 and 1996 at a butadiene monomer production facility in Texas (USA) was described by Divine and Hartman (2001[14]) and also evaluated by IARC[2]. Earlier analyses[2] of this cohort showed a significant elevation of deaths from cancers of the lymphohaematopoietic system that was mainly due to an increase in the deaths from lymphosarcoma. In the most recent update[14], a follow-up of the patterns of mortality to the end of 1999 was included. The overall and cause-specific mortality of the study population was compared to that of the US population. The SMR for all lymphohaematopoietic cancers was 1.4 (CI 1.1-1.9) and was significantly increased. The SMRs for leukaemia and non-Hodgkin's lymphoma were 1.3 and 1.5, respectively (not significantly increased). As for the previous evaluation of this cohort, the lymphohaematopoietic cancer elevations were found only in workers first employed before 1950. Survival analyses for all lymphohaematopoietic cancers, non-Hodgkin's lymphoma and leukaemia were performed using an estimate of cumulative butadiene exposure as a time-dependent explanatory variable defined as a combination of job exposure class, calendar time, and length of time in job. The job/unit exposure classification scheme used in previous reports was used again, with a background exposure group (office, utilities, warehouse and transportation employees), a low-exposure group (workers partly working on the operating units and partly in the office or maintenance shops), and a varied exposure group (employees with the potential for exposure to butadiene on a routine basis such as laboratory workers, pumpers, pipefitters, and instrument men). The relative risks (RRs) for the above causes of death were essentially 1.0, suggesting that there was no increase in risk with increasing butadiene exposure. In addition to the above mentioned cancers of the lymphohaematopoietic system, non-significant positive associations in cancer mortality were observed for larynx-, skin- and kidney cancer, lymphosarcoma, and Hodgkin's disease. Limitations of the study were that no industrial hygiene sampling data exist for the plant for the years prior to 1981, and no quantitative exposure information was available. In addition, the cohort size was small, and the numbers became even smaller for the exposure group analyses.

The report of Cowles (1994, in IARC[2]) on employees from a petrochemical facility in Texas related the cause-specific mortality (1948-1989) of 614 employees (who had worked in the plant for 5 years or more) with potential exposure to butadiene monomer. Butadiene monomer production took place

between 1941 and 1948 and from 1970 onwards. Tsai and coworkers (2001[15], also evaluated by IARC[2]) extended this study to 1998 and found the SMR for all causes of death to be 0.6 (CI 0.4-0.7). None of the cause-specific mortality was in excess compared with coworkers without butadieen exposure. The findings in this study suggest that the butadiene exposure at this facility in the last 20 years does not pose a cancer hazard to employees. However, the mortality and morbidity numbers of the study were based on small numbers of employees and based on a 8-h time weighted average (TWA) butadiene level with a geometric mean of less than 6.6 mg/m^3 (exposure to butadiene was mostly below 2.2 mg/m^3, with few exposures exceeding 2.2 mg/m^3 as an 8-h TWA).

Industry-based studies – styrene-butadiene rubber production

Matanoski et al. (1990[16], also evaluated by IARC[2]) followed a cohort of 12,110 male workers employed >1 year in eight SBR polymer manufacturing plants in the USA and Canada for mortality over a 40-year period (1943-1982). Compared to the general population, the all-cause mortality of these workers was low (SMR 0.8), whereas the SMRs for some cancers of the digestive tract were higher than expected, especially oesophageal cancer and stomach cancer in white men. In this study, individuals were assigned to four work areas (production, maintenance, utilities, and others), based on longest job held. There were no measured exposure data for the different work areas. Production workers showed a significantly increased SMR for haematologic neoplasms. Deaths from cancers of the haematopoietic and lymphopoietic system were higher than expected in production workers, with significant excesses for leukaemia and kidney cancer in black workers and other lymphomas in all workers.

SMR production workers, cancers, white men: kidney 1.66 (95% CI 0.54-3.88), Hodgkin's 1.31 (0.16-4.75), lymphatic 2.30 (0.92-4.73); black men: lung 1.23 (0.45-2.67), liver 1.98 (no 95% CI), kidney 5.07 (1.87-11.07), lymphosarcoma 5.32 (no 95% CI), leukaemia 6.56 (1.35-19.06), lymphatic 4.82 (0.59-17.62); total: kidney 1.53 (0.50-3.57), lymphopoietic 1.46 (0.88-2.27), Hodgkin's 1.20 (0.15-4.35), leukaemia 1.34 (0.53-2.76), lymphatic 2.60 (1.19-4.94). SMR maintenance workers, cancers, white men: oesophagus 1.44 (0.53-3.14), stomach 1.66 (0.93-2.75), Hodgkin's 1.70 (0.35-4.95); black men: rectum 1.37 (no 95% CI); total: stomach 1.51 (0.90-2.39), Hodgkin's 1.51 (0.31-4.41). SMR utility workers, total: large intestine 1.61 (0.44-4.13), respiratory cancers 1.49 (0.79-2.55) with larynx 5.13 (0.62-8.52) and lung 1.22 (0.58-2.24); lymphopoietic

cancers 2.03 (0.66-4.74), with leukaemia 1.92 (0.23-6.96) and other lymphatic 3.13 (0.62-6.95).

In a later analysis of Matanoski and coworkers (1993[17], also evaluated by IARC[2]) the results of the above cohort study[16] were discussed. SMRs for some lymphohaematopoietic cancers sometimes were high in early cohort analysis. A total of 3,952 samples for butadiene were reported (personal and area monitoring). Values indicated upper ranges as high as 1,485 mg/m^3 in measurements taken in the 1980s, suggesting that previous exposures of some workers may have been even higher. In all plants there was a marked variation in measurements. The 1993 cohort study[17] included workers of eight SBR manufacturing plants in North America. All but one (1955) of the plants had begun operation in 1943. Four plants had complete personal records from the beginning; for three plants that had incomplete records, follow up was begun several years after they opened. For the largest plant only workers with >10 years of employment were included in the cohort, since follow-up was possible only through records of death-benefit claims with the employees group life insurance program. The missing records for three of the plants and limitations in follow-up in one could lead to underestimation of the workers' risk. Workers were classified in work areas to prevent masking effect of exposure because of dilution of the risk in a few people as the result of large numbers of unexposed people. New limitations were that workers had to be assigned to a single work group, and work areas were assumed to be homogeneous. Despite the continued problem of dilution of possible risks due to exposure by the presence of both exposed and unexposed workers, workers in production areas showed an increased risk for lymphohaematopoietic cancers (SMR 1.6, CI 1.1-2.3), and oesophagus cancer (SMR 1.8, CI 0.9-3.4).

Nested case-control studies within the SBR cohort in the USA and Canada were conducted by Matanoski et al. (1993[17],1997[19]), and Santos-Burgoa et al. (1992[20]). Since the presence of large numbers of unexposed workers could conceal risks within a cohort, case-control studies were designed to examine the relationship between estimated exposures and the mortality due to lymphohematopoietic cancers.

Santos-Burgoa et al. (1992[20], also evaluated by IARC[2]) conducted a case-control study of 59 cases of lymphohaematopoietic cancers within a cohort of male workers employed between 1943 and 1982 in eight North American SBR polymer-producing plants (Matanoski et al. 1990[16]). A total of 193 controls were matched by plant, age, year of hire, duration worked, and survival to case of death. Each job was assigned an estimated exposure rank, and each worker's cumulated rank score was calculated on the basis of time spent in each job during

his employment. In the mortality analysis, three subdivisions (process, utilities and maintenance) were analyzed separately according to the longest job held by workers in these areas. Matched-pair analysis identified a strong association between leukaemia and butadiene, with an odds ratio (OR) of 9.4 (CI 2.1-22.9) and an association between STYR and leukaemia (OR 3.1, CI 0.8-11.2) that did not achieve statistical significance. When exposure to both STYR and butadiene was included in a conditional logistic regression model, the OR for butadiene remained high (OR 7.4), but the estimated association of leukaemia with STYR was small. The results of this study support the hypothesis that exposure to butadiene is associated with the risk of leukaemia. The study lacked measured individual exposures over time; however, there appeared to be an additional risk from work in specific subdivisions of the industry.

Matanoski and coworkers (1993[17], also evaluated by IARC[2]) studied the same 59 cases of lymphohaematopoietic cancer and 193 controls from the study of Santos-Burgoa et al. (1992[20]). Cases and controls were divided into four cancer groups (leukaemia, other lymphatic cancers, lymphosarcoma and Hodgkin's disease), and an average exposure was calculated on the basis of the log scores (rank assigned to a job multiplied by the number of months worked in that job, summed over the total work period, and transformed into its logarithm (log score); method described by Santos-Burgoa et al. 1992[20]). The analysis of leukaemia cases and controls, using the log mean as a categorical exposure variable, showed an OR of 7.6 (CI 1.6-35.6) for butadiene alone and 2.9 (CI 0.8-10.3) for STYR alone. When both variables (butadiene and STYR) were used in the model, only butadiene was associated with a significantly increased OR (OR butadiene = 7.4 and OR STYR = 1.1). Three areas seemed to be overrepresented among the cases: operation services, laboratory, and utility. Limitation of the study is the use of controls only matching for duration of work. All but one case had been hired before 1960, 81% had been employed for 10 or more years in the industry and 73% had worked in only three of the eight plants. The absence of a risk for workers hired after 1960 may have been due to the long latency period for this cancer. An average of 24 years between the start of employment and death was observed. New sets of controls were selected that matched variables except duration of work. Addition to the model of a variable to account for duration of work improved the model and demonstrated a risk for leukaemia associated with exposure to butadiene. The importance of the duration of work variable suggested that dose per unit time may be the most important exposure variable to investigate. The same dose given over different time periods may not carry the same risk. The results suggested that the risk for leukaemia was associated with exposure to butadiene and with work in specific areas. The SMR

for leukaemia among long-term workers hired before 1960 who had worked in plants with assumed high butadiene levels was 1.8 times higher than that of the US population.

In the study of Matanoski and coworkers of 1997[19] (also evaluated by IARC[2]) the population from the Santos-Burgoa et al. (1992[20]) and Matanoski et al. (1993[17]) cohort was used for a nested case-control study of lympho-haematopoietic cancers occurring in a cohort of synthetic rubber production workers to determine the associations of these cancers with exposure to butadiene and STYR. Exposures were based on measured values of the two chemicals from personal monitoring data in seven of the eight plants under study. Plant- and work area-specific exposure estimates were linked to work histories to obtain indices of cumulative exposure (mg/m^3-months) and average intensity of exposure (mg/m^3) based on total cumulated exposure in mg/m^3-months divided by the total time employed for both STYR- and butadiene-related processes for each individual. The 59 cases studied previously by Matanoski and coworkers[17] and by Santos-Burgoa and coworkers[20] were re-evaluated, resulting in 58 lymphatic and haematopoietic cancers in this study, together with 1,242 controls from the plants. The risk of leukaemia increased with exposure to a TWA butadiene measure, with an OR of 1.5 (CI 1.1-2.1) at 2.2 mg/m^3 average butadiene exposure. Work in specific areas also contributed to the risk, possibly because these areas had not been completely characterized for differences in butadiene exposure. Hodgkin's disease was also associated with butadiene exposure (OR 1.7, CI 1.0-3.0). Multiple myeloma, lymphosarcoma and all lymphomas were associated with exposure to STYR. According to the authors, workers in this industry were apparently exposed to two carcinogenic agents, and thus they stated that more information is needed on the exposures to each chemical over time.

A retrospective follow-up study of men employed during 1943-1991 in the SBR industry in the USA and Canada evaluated the mortality outcome of 15,649 men employed for more than one year at any of eight North American SBR plants (Delzell et al. 1996[18]). The investigation included workers from seven of the eight plants previously studied by the JHU[16,17,20] and workers from the two plants previously investigated by the NIOSH (Meinhardt et al. 1982, in IARC[2]). Due to lack of information to identify individual subjects, it was not possible to determine the number of subjects in this study who were included in the earlier investigations. The overlap was estimated to be large. Complete work histories were available for about 97% of the subjects. Work area groups were combined into 5 'process main groups' (rubber production, maintenance, labour, laboratories, and other operations) and seven 'process subgroups' (polymerization,

coagulation, finishing, shop maintenance, field maintenance, production labour, and maintenance labour). The subgroup analysis excluded subjects from two plants (n=1,354) due to lack of information on specific work areas. About 75% of the men were exposed to butadiene, 83% of the men were exposed to STYR. The cohort consisted mainly of hourly-paid workers (86%). About 50% of the workers were hired before 1960 (median year of hire was 1960), 44% had worked for at least 10 years (median, 7.8 years) and 59% started work before the age of 30 years. During 1943 to 1992 the cohort had a total of 386,172 person-years and an average of 25 person-years of follow-up. The overall SMR was 0.87 (CI 0.85-0.90, 3,976 deaths observed, 4,553 deaths expected). More leukaemia deaths than expected occurred in the overall cohort (48 observed/37 expected, SMR 1.3, CI 1.0-1.7) and among hourly workers (45 observed/32 expected, SMR 1.4, CI 1.0-1.9). This increase was observed in hourly workers who worked more than ten years and who died after more than twenty years since hire (28 observed/13 expected, SMR 2.2, CI 1.5-3.2), and among workers in three areas with potential for relative high exposure to butadiene or STYR: polymerization (15 observed/6 expected, SMR 2.5, CI 1.4-4.1), maintenance labour (13 observed/4.9 expected, SMR 2.6, CI 1.4-4.5) and laboratories (10 observed/2.3 expected, SMR 4.3, CI 2.1-7.9). The most likely causal agent was butadiene or a combination of butadiene and STYR. According to the authors, some cohort subgroups had slight increases in deaths from lymphopoietic cancers other than leukaemia, but there was no indication of a causal association with occupational exposures.

In an update (an additional 7 years of follow-up and re-examination) of the study from Delzell et al. (2001[25]), a possible association between exposure to butadiene, STYR and dimethyldithiocarbamate (DMDTC), and mortality from lymphohaematopoietic cancer among 16,579 synthetic rubber industry workers followed up from 1943 to 1998 was evaluated (Graff et al. 2005[22]). All subjects were men who had worked at any of six study plants (USA and Canada) for at least one year by the end of 1991. Each of the 7,802 unique work area/job title combinations was classified into one of 296 work area/job groups. Macaluso et al. (2004[21]) described in detail the exposure estimation procedures. In short, the exposure metrics included 8-h TWA intensity, the annual number of peak exposures (butadiene > 221 mg/m^3) and TWA intensity below and above the peak threshold. butadiene TWAs were approximately 22 mg/m^3 during the 1940s-1960s and declined during the 1970s and 1980s. Butadiene peak exposure accounted for a large proportion of cumulative butadiene exposure. Multiple correlations among DMDTC, butadiene and STYR exposure estimates made it difficult to estimate agent-specific effects. Nevertheless, the new exposure

estimates were highly correlated with the old estimates, yielding equivalent exposure ranking of workers, and were comparable to limited industrial hygiene data published by NIOSH. The cumulative exposure indices used in the study of Graff and co-workers (2005[22]) were butadiene mg/m^3-years, butadiene mg/m^3-years due to exposures to intensities $\leq$ 221 mg/m^3, butadiene mg/m^3-years due to exposures to intensities > 221 mg/m^3, and the total number of butadiene peaks (> 221 mg/m^3 (100 ppm)). Poisson regression analyses were used for lympho-haematopoietic cancer rates in relation to butadiene (and STYR and DMDTC) exposure. Models provided maximum likelihood estimates of the relative rate for the contrast between categories of one agent, adjusting for other agents and for additional potential confounders. The results were consistent with the previous investigation of Delzell and coworkers[25], and indicated that leukaemia was positively associated with cumulative exposure to butadiene (relative risks (RRs) of 1.0, 1.4 (CI 0.7-3.1), 1.2 (CI 0.6-2.7), 2.9 (CI 1.4-6.4) and 3.7 (CI 1.7-8.0), respectively, for exposures of 0, > 0 to < 74, 74 to < 408, 408 to < 939 and $\geq$ 939 mg/m^3-years), whereas the association of leukaemia with cumulative exposure to butadiene together with STYR and DMDTC was observed at higher butadiene exposure levels (RRs of 1.0, 1.4 (CI 0.5-3.9), 0.9 (CI 0.3-2.6), 2.1 (CI 0.7-6.2) and 3.0 (CI 1.0-9.2), respectively, for exposures of 0, > 0 to < 74, 74 to < 408, 408 to < 939 and $\geq$ 939 mg/m^3-years). According to Graff et al. (2005[22]) the relation between butadiene and leukaemia appeared to be somewhat stronger for exposure to butadiene concentrations greater than 221 mg/m^3 (in mg/m^3-years) than for exposure to concentrations of $\leq$ 221 mg/m^3 (in mg/m^3-years), but the results did not rule out an effect of lower concentrations. Data on specific forms of leukaemia were difficult to interpret because of diagnostic uncertainty that persisted despite efforts to review medical records. The data do not preclude a role of STYR or DMDTC. Recently Graff et al. (2009[26]) reported an uncertainty analysis of their earlier findings[21,22,27], which overall confirmed the reported results.

Sathiakumar et al. (2005[23]) updated the mortality study of workers in the eight SBR plants in North America and Canada previously described by Delzell et al.[18,25], Macaluso et al.[27] and Sathiakumar et al.[2] with an additional 7 years of data (1943-1998). They observed that the 16% leukaemia increase was concentrated in hourly paid workers with 20-29 years since hire and 10 or more years of employment in the industry (SMR 2.6, CI 1.6-4.0) and in workers employed in polymerisation (SMR 2.0, CI 1.2-3.2), maintenance labour (SMR 3.3, CI 1.8-4.6), and laboratory operations (SMR 3.3, CI 1.8-5.5). Uncertainty in this study remained about the role of unidentified confounding factors.

Sathiakumar and Delzell (2009[28]) and Sathiakumar et al. (2009[29]) investigated cancer mortality among female workers in the eight SBR plants in North America and Canada previously described by Delzell et al. (1996[18], 2001[25]), Macaluso et al. (1996[27]) and Sathiakumar et al. (1998, cited in IARC[2]) for the period 1943-2002. Generally, the number of deaths observed among exposed females were approximately equal to the expected numbers of deaths, with the exception of hourly paid women who had more deaths than expected from lung (SMR 1.6, CI 1.2-2.1) and bladder cancers (SMR 3.3, CI 1.2-7.2). However, exposure-response analysis (done only for lung cancer) indicated no trend for butadiene or STYR. The authors concluded that the observed excess of lung and bladder cancers might be attributable to non-occupational factors rather than to workplace exposure.

Cheng et al. (2007[24]) used the Cox regression procedure on the data from Sathiakumar et al. (2005[23]) and Graff et al. (2005[22]) from the SBR plants in North America and Canada (1944-1998) to examine further the exposure-response relationship between several butadiene exposure indices (butadiene mg/m^3-years, the total number of exposures to butadiene peaks > 221 mg/m^3, and average intensity of butadiene), and leukaemia, lymphoid neoplasms and myeloid neoplasms. By using Poisson regression analysis in which butadiene and covariates were categorical variables, Sathiakumar et al. (2005[23]) and Graff et al. (2005[24]) had concluded that cumulative exposure to butadiene was associated positively with leukaemia, but controlling for STYR and DMDTC attenuated this association. Subjects included in the study of Cheng et al.[12] were 16,579 men, and exposure to butadiene, STYR and DMDTC was estimated quantitatively by identifying work/area groups as described by Macaluso et al. (1996[27], 2004[21]). All three ways of expressing butadiene exposures (butadiene mg/m^3-years, the total number of exposures to butadiene peaks > 221 mg/m^3, and average intensity of butadiene) were associated positively with leukaemia. Using continuous, untransformed butadiene mg/m^3-years the regression coefficient (β) from analysis that controlled only for age was 2.9×10^{-4} ($p < 0.01$), and was similar in magnitude ($\beta = 3.0 \times 10^{-4}$ ($p = 0.04$)) when adjusted for all covariates (age, year of birth, race, plant, years since hire, and DMDTC), though with reduced statistical significance (more details are shown in Annex F). The analysis of exposure to 11 mg/m^3 butadiene over a 20-year period (cumulative exposure, 221 mg/m^3) yielded an RR for leukaemia of 1.0 for the untransformed continuous butadiene mg/m^3-years variable. The analyses indicated that the exposure-response relationship for butadiene and leukaemia was independent of exposure to DMDTC. The relevant results are summarized in Table 2.

Sielken et al. (2007[30]) studied the dose-response assessment of the association between butadiene and leukaemia mortality among workers in the North American synthetic rubber industry, based on the recent UAB study and exposure estimation described by Macaluso et al. (2004[21]), by giving consideration to peak exposures to butadiene (a butadiene peak was defined as any exposure, regardless of duration, to a butadiene concentration above 221 mg/m^3). Exposure to butadiene levels above 221 mg/m^3 was rather common, in large part intermittent, frequently of short duration (several seconds to several minutes) and not uncommonly to levels of a few hundreds mg/m^3. If cumulative butadiene mg/m^3-years was used as the predictor of the leukaemia rate ratio, the performance of this predictor was indeed statistically significantly improved if the slope in the predictor was estimated with age and the cumulative number of butadiene peaks added as categorical covariates. The cumulative number of butadiene peaks counted the number of exposures above 221 mg/m^3 and not the magnitude above 221 mg/m^3 nor the duration of time above 221 mg/m^3. The inclusion of the cumulative number of butadiene peaks as covariate made a statistically significant improvement in the model for leukaemia and myeloid neoplasms, but not for lymphoid neoplasms.

Sielken et al. (2007[30]) used a Poisson regression analysis to assess the leukaemia mortality data. The UAB human epidemiological data suggested that there was no increasing risk for leukaemia at low cumulative butadiene mg/m^3-years. Even though the primary focus for regulatory environmental risk assessment is the best estimate of the slope associated with cumulative butadiene mg/m^3-years, statistical analysis suggested that cumulative butadiene mg/m^3-years by itself was not a sufficient explanation of the leukaemia mortality observed in the workers. Among the exposure variables reported in the UAB study, the observed leukaemia rate ratios were most strongly correlated with the number of butadiene peaks (slope of linear rate ratio model 5.77 x 10^{-4}, maximum log likelihood -68.75 (peaks included) or -80.50 (peaks excluded), p-value of 0.00027). There was no correlation with the cumulative butadiene exposure (in mg/m^3-years).

Sielken et al. (2007[30]) mentioned three reasons for the inclusion of butadiene peaks in the risk assessment of butadiene: (1) there were large numbers of butadiene peaks not only during the early years near the end of World War II but also during the entire period up to the 1990s, and a large number of butadiene peaks was a prominent part of the work environment throughout the study period; (2) all of the statistical analyses of leukaemia herein indicated that cumulative number of butadiene peaks was the most important exposure covariate which explained a substantial portion of the increase of leukaemia rate

ratios with cumulative exposure to butadiene; and (3) the inclusion of butadiene peaks was consistent with the current biological understanding of the mode of action of butadiene.

Table 2 Cumulative 1,3-butadiene exposure and rate ratios for leukaemia as reported by Cheng et al. 2007[24]

Butadiene mg/m³-years, decile ranges of exposure values[a]	Mean exposure (mg/m³)	Number of leukaemias	RR (95% CI)[b]	RR (95% CI)[c]
0	0	10	1.0	1.0
> 0 - < 26.7	10.7	7	1.13 (0.43-2.98)	0.98 (0.37-2.61)
26.7 - < 50.6	38.0	7	2.12 (0.81-5.56	1.67 (0.62-4.50)
50.6 - < 85.7	67.4	7	2.03 (0.77-5.34)	1.45 (0.53-3.97)
85.7 - < 173	126	7	1.22 (0.47-3.32)	0.83 (0.30-2.32)
173 - < 408	274	7	0.94 (0.36-2.46)	0.61 (0.21-1.73)
408 - < 555	476	7	2.96 (1.13-7.79)	1.77 (0.60-5.24)
555 - < 704	624	7	4.00 (1.52-10.51)	2.47 (0.82-7.44)
704 - < 996	829	7	3.37 (1.28-8.86)	1.96 (0.65-5.87)
996 - < 1,833	1,340	7	2.94 (1.12-7.73)	1.86 (0.62-5.55)
≥ 1.833	4,094	8	3.84 (1.51-9.76)	2.56 (0.85-7.66)

[a] Exposure data are split up into 10 equally large subsections ('deciles').
[b] Estimated rate ratio (RR) and 95% confidence interval (CI) controlling only for age.
[c] Estimated rate ratio (RR) and 95% confidence interval (CI) controlling for age, year of birth, race, dimethyldithiocarbamate, years since hire and plant.

In 2011 Sielken and Valdez-Flores[31] re-analyzed the earlier mortality data of the UAB cohort, using Cox regression analyses to estimate exposure-response models with cumulative butadiene mg/m³-years as the exposure metric. The authors reported a statistically significant positive correlation between occupational leukaemia and cumulative exposure in butadiene mg/m³-years, i.e. a significantly positive slope of the cumulative butadiene mg/m³-years in the log-linear rate ratio model; this slope became less steep if other butadiene exposure metrics were used. These results are difficult to interpret because the coefficients for the other exposure metrics are not reported, and confounders were not taken into account. The correction for plant, e.g., had an increasing effect on the slope of the model, but could not be interpreted without inclusion of confounders in the models. The authors argued that there was virtually no risk at low exposures. However, truncating the ranges of exposure decreases the the number of cases and hence strongly decreases the statistical power. Due to the complex structure of the correlation the estimation of the effects of the variables as presented by the authors is intricate, and it remains possible that the incidence of occupational leukaemia is fully attributable to butadiene.

Population-based studies

In addition to industry-based studies, a population-based case-control study in Canada (Parent et al. 2000[32]) and a cohort study of students at a high school adjacent to a SBR production plant in the USA (Loughlin et al. 1999[33]) were reviewed by IARC[2]. More recent information from Higashino et al. (2007[34]) and Mita et al. (2006[35]) is included.

The risk of mortality from lymphatic and haematopoietic cancers and other causes was evaluated among students of a high school adjacent to synthetic butadiene-STYR facilities in Texas (producing since 1943). In this study, school records, year books and health records for the school years 1963-64 to 1992-93 were used to construct a cohort of 15,403 students, who attended the school for at least 3 consecutive months. No data existed on environmental exposure. The SMR for all cause mortality was 0.8 (CI 0.7-1.0) for men and 0.9 (CI 0.7-1.1) for women. The SMR for all lymphatic and haematopoietic cancers was 1.6 (CI 0.8-2.9) for men and 0.5 (CI 0.1-1.7) for women. The slightly positive association for males and lymphatic and haematopoietic cancers was stronger among men who attended school for two years or less (Loughlin et al. 1999[33]).

Grant et al. (2007[36]) provided information on butadiene monitoring data in Texas (USA), where several large industrial sources of atmospheric butadiene are located. In 2003, annual average concentrations at monitoring sites ranged from 0.02 to 7.1 µg/m^3 with an overall average of 0.4 µg/m^3. Cancer incidence data from 1995 to 2002 and cancer mortality data from 1993 to 2003 from several areas were investigated by the Texas Cancer Epidemiology and Surveillance Branch (TCES). It was concluded that the cancer incidence and mortality data from all examined types of cancer in the Houston region were within normal ranges. In the Port Neches region, however, a SMR of 5.0 (99% CI 1.1-14.2) for subleukaemia and leukaemia not otherwise specified mortality was observed, but the TCES could not identify a cause for this elevated mortality. Different leukaemia subtypes have varying risk factors, and the elevation of various types of leukaemia rather than one predominant subtype is generally not indicative of an environmental exposure. However, different leukaemia subtypes could not be evaluated independently because non-specific descriptions of leukaemia on death certificates are coded to the aleukaemic category.

A population-based case-control study in Montreal, Canada, investigated the association between renal-cell carcinoma and a large number of occupational exposures among 35-70 years old men diagnosed between 1979 and 1985. A total of 142 male patients with renal cell carcinoma were compared with 1,900

controls with cancer at other sites and 533 population-based controls. Detailed job histories and relevant data on potential confounders were obtained (by interview), and each job was translated into a history of occupational exposures using a checklist of 294 substances. RRs were estimated by ORs from unconditional logistic regression models. The OR for exposure to butadiene-STYR was 2.1 (CI 1.1-4.2) if adjusted for age, family income, smoking and body mass index, and 1.8 (CI 0.9-3.7) if adjusted for former confounders including other occupational exposures (Parent et al. 2000[32]).

Higashino et al. (2007[34]) and Mita et al. (2006[35]) assessed the risk and consequences of exposure to BTD on human health in Japan. Butadiene in the general environment originates primarily from automobile emissions. Industrial emission of butadiene in Japan has decreased in recent years, primarily due to a voluntary industrial emissions reduction program. The annual mean concentration of butadiene in residential areas generally amounted to less than 0.5 $\mu g/m^3$, but exceeded 1.7 $\mu g/m^3$ at certain sites near industrial sources (data from 1997-2003). The results indicated that in 2002 the majority of the population in Japan had an excess lifetime cancer risk of less than 10^{-5} due to exposure to butadiene, whereas a small number of people living close to industrial sources had a cancer risk greater than 10^{-5}.

Summary of human data and conclusion

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[7].

A review of the studies of SBR workers by researchers at the UAB (Cheng et al. (2007[24]) 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/m^3-years, the total number of exposures to butadiene peaks > 221

mg/m^3, 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 DMDTC.

2.5 Carcinogenic activity in experimental animals, lifetime low-dose exposure

In the previous DECOS report[1] it was concluded that butadiene has a weak carcinogenic potential in the rat, but is a carcinogen in the mouse and should be regarded as a carcinogen in experimental animals.

All animal studies with butadiene and its metabolites are presented in Annex G.

Mouse

In the IARC monograph (2008[2]) two butadiene inhalation studies with mice were evaluated which showed increased incidences of lymphoma and neoplasms of the heart, lung, forestomach, liver, Harderian gland, preputual gland, and kidney in males, and increased incidences of lymphomas and neoplasms of the heart, lung, forestomach, liver, Harderian gland, ovary, and mammary gland in females. As the first study was terminated due to the high mortality mainly caused by malignant lymphomas, the second study was performed at much lower exposure levels than the first, comparable to or even lower than historical levels of occupational exposure in humans. Tumours developed at the same organ sites in both studies. The second study is described below (NTP 1993[37], Melnick et al. 1990[38]).

Groups of 70 (all dose groups except highest) to 90 (highest dose group only) B6C3F1 mice were exposed to 0, 14, 44, 138, 440 or 1,380 mg/m^3 butadiene (purity > 99%), 6 h/day, 5 days/week for 2 years[2,37,38]. After two years, survival was significantly reduced ($p < 0.05$) in all groups of mice at 44 mg/m^3 and higher; terminal survivors were: 35/70, 39/70, 24/70, 22/70, 3/70 and 0/90 for males and 37/70, 33/70, 24/70, 11/70, 0/70 and 0/90 for females at 0, 14, 44, 138, 440 or 1,380 mg/m^3, respectively. Early occurrence and development of lethal lymphocytic lymphomas of thymic origin at 44 mg/m^3 and higher reduced the number of animals at risk for the expression of later developing neoplasms at other sites. Notwithstanding the reduced survival, increased incidence of

neoplasms of the lung were found at all exposed levels, neoplasms of the liver were found at 44 mg/m^3 and higher, haemangiosarcomas of the heart, Harderian gland, mammary gland and ovarian gland at 138 mg/m^3 and higher, and neoplasms of the forestomach and preputual gland at 440 mg/m^3 and higher. Additional studies in which exposure to butadiene was terminated after limited exposure periods were included to assess the relationship between exposure level and duration of exposure on the outcome of butadiene-induced carcinogenicity. In the stop-exposure studies, groups of 50 male mice were exposed to one of the following regimens: 1,380 mg/m^3 for 13 weeks, 440 mg/m^3 for 40 weeks, 1,380 mg/m^3 for 26 weeks, or 686 mg/m^3 for 52 weeks. After exposure, the animals were held in control chambers for the remainder of the 104 weeks study. The total exposure (concentration x duration) was approximately equivalent for the first two groups and provided about half the total exposure given to the last two groups. Survival was 35/70 for controls (same group as above), 9/50 at 440 mg/m^3, 1/50 at 686 mg/m^3, and 5/50 and 0/50 at 1,380 mg/m^3 exposed for 13 and 26 weeks, respectively. Again increased incidences of lymphomas, heart haemangiosarcomas, lung alveolar/bronchiolar adenomas and carcinomas, forestomach papillomas and carcinomas, Harderian gland adenomas and adenocarcinomas, and kidney tubular adenomas were found at 440 mg/m^3, and preputial gland carcinomas at 686 mg/m^3. This exposure protocol revealed additional tumour sites in males (preputial gland and renal cortex).

The incidence of thymic lymphomas in mice exposed to higher concentrations of butadiene for a short time was greater than exposure to lower concentrations for an extended period (9/70 at 440 mg/m^3 for 2 years compared to 24/50 at 1,380 mg/m^3 for 13 weeks). Butadiene-induced neoplastic responses (other than thymic lymphomas) at multiple organ sites were also observed after only 13 weeks of exposure (Melnick et al. 1990[38]).

A benchmark dose (BMD) analysis of the main (2-years) study[37,38], performed by the Committee using US-EPA's BMD software, revealed that the log-logistic model showed the best fit and resulted in the lowest BMD* and BMDL at the 10% extra risk level of all models tested, with a BMD of 262 and a BMDL of 147 mg butadiene per m^3. The other models showing equally good fits resulted in BMDs and BMDLs varying from 330 - 593 and 211 - 401 mg/m^3, respectively.

* BMD: benchmark dose; BMDL: benchmark dose at the lower 95% confidence level.

Rat

Owen and Glaister (1990[39], also evaluated in DECOS[1] and IARC[2]) reported a study with Sprague-Dawley rats (4-5 weeks old, 100/sex/dose) exposed by whole-body inhalation to 0, 2,200 or 17,600 mg/m³ butadiene (purity ≥ 92.2%) for 6 h/day, 5 days/week for 105 weeks (females) or 111 weeks (males). Survival was reduced in low- and high-dose females and in high-dose males. During the second year of the study, increased mortality was observed. In males, renal lesions were likely the major cause of the increased death rate. Females died as result of mammary tumours (80% of subcutaneous masses) and fibrous tumours of the skin. Statistically significantly increased incidences in tumours in high dose males were observed in the exocrine pancreas (10/100, with 3/100 in controls) and the testis (interstitial cells, 8/100, with 0/100 in controls). In high dose females increases in the incidence of thyroid follicular-cell tumours (10/100, with 0/100 in controls), uterine sarcomas (5/100, with 1/100 in controls), Zymbal gland carcinomas (4/100, with 0/100 in controls) and mammary adenocarcinomas (26/100, with 18/100 in controls) were observed.

For the metabolites of butadiene, only inhalation studies with D,L-diepoxybutane were available. These studies confirmed the conclusion from the carcinogenic studies on butadiene that mice are far more sensitive than rats (see Annex G).

Conclusion

In rodents butadiene induced lymphoma, and neoplasms of the heart, lung, forestomach, liver, Harderian gland, preputual gland, kidney, ovary and mammary gland, starting in mice at exposure to 44 mg/m³, and in rats at exposure to 17,600 mg/m³ (exposure duration was 2 years, 6 h/day, 5 days/week).

2.6 Kinetics and kinetic models

Kinetics

In human volunteers exposed to 4.4 mg/m³ butadiene for 20 minutes, the absorbed fraction varied from 18 to 74%; this variation was not influenced by sex or age. Blood levels approached equilibrium by 5 minutes (ATSDR 2009[40]).

The uptake of inhaled butadiene by mice and rats was linear up to 4,400 and 2,200 mg/m³, respectively, above which metabolism appeared to be saturated. In

mice and rats inhaling up to 1,380 mg/m^3 butadiene, equilibrium in blood levels was reached by 2 h; butadiene blood levels in mice were 3- to 4-fold higher than in rats at all times (ATSDR 2009[40]).

Butadiene distributed to a variety of tissues and organs, as was shown in in vitro measurements of tissue : blood equilibrium partition coefficients: in humans these coefficients were highest in fat (18.4) and similar in both well- and poorly-perfused tissues (0.69 and 0.72, respectively). In rats, partition coefficients were highest for fat (21.9), similar for liver, kidney, muscle and spleen (0.87-0.94) and lowest in brain (0.43; ATSDR 2009[40]).

Following exposure of mice and rats to ^{14}C-butadiene, the elimination of radioactivity was rapid: 77-99% of the initial tissue concentration was eliminated with half-lives of between 2 and 10 h. At exposure concentrations of $\leq$ 2,200 mg/m^3 the elimination followed first-order kinetics in both species. The maximal metabolic elimination rate of butadiene was 400 and 200 µmol/h.kg in mice and rats, respectively. Urine and exhaled air were the major routes of elimination (75-85% of total eliminated ^{14}C-butadiene; ATSDR 2009[40]).

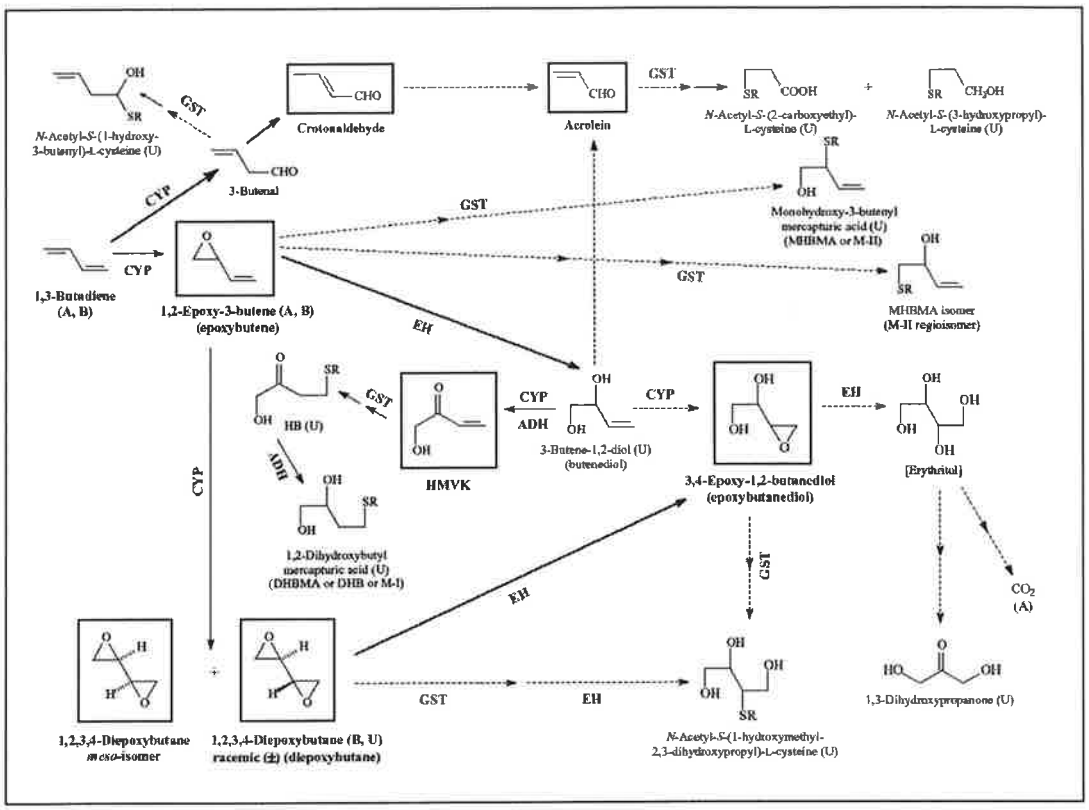

Figure 1 Metabolic pathways of 1,3-butadiene deduced from findings in mammals in vitro and in vivo (copied from IARC 2008[2]).
Solid frames: electrophilic metabolites that can form DNA or haemoglobin adducts.
Dashed lines: assumed pathways.
A, B, U: metabolites in exhaled air, blood, urine, respectively.
ADH: alcohol dehydrogenase.
DHB: 4-(N-acetyl-L-cystein-S-yl)-1,2-dihydroxybutane.
HB: 4-(N-acetyl-L-cystein-S-yl)-1-hydroxy-2-butanone.
HMVK: hydroxymethylvinyl ketone.

Butadiene is oxidized by cytochrome P450 (CYP) to electrophilic epoxides. CYP2E1 is one of the enzymes involved in butadiene metabolism. In addition, in mouse kidney and liver CYP4B1 plays a major role in butadiene epoxidation. The human CYP enzymes forms involved in butadiene conversion to epoxybutene appear to be CYP2E1 at low and CYP2A6 at high concentrations of butadiene (ATSDR 2009[40]). The primary epoxide metabolite formed is

1,2-epoxy-3-butene (also known as epoxybutene, butadiene monoepoxide, monoepoxybutene or 2-ethyloxirane). The second step in metabolism of butadiene may be conjugation of the epoxide with glutathione (GSH), hydration by microsomal epoxide hydrolase (mEH), or further oxidation to 1,2:3,4-diepoxybutane diastereoisomers (also known as diepoxybutane, diepoxide of butadiene, butane diepoxide or 2,2'-bioxirane; ATSDR 2009[40], Filser et al. 2010[41]). All epoxides may be detoxified by GSH conjugation or hydration by mEH. Additional epoxide forms, including 3,4-epoxy-1,2-butanediol (epoxybutanediol) may be involved in butadiene-related carcinogenic processes. This latter epoxide is of particular concern as it is the most abundant genotoxic butadiene metabolite in humans (IARC 2008[2], Hurst 2007[6]), and was suggested by Jackson et al. (2000, cited in Hurst 2007[6]) to be the most significant metabolite in humans. The metabolic pathways of butadiene, as summarized in IARC (2008[2]) are shown in Figure 1.

Comparative in vitro studies conducted with tissues from mice, rats and humans indicate that the relative rates of oxidation of butadiene to epoxybutene and of epoxybutene to diepoxybutane are mice > rats ≈ humans. The relative extent of mEH-catalyzed hydration of epoxybutene or diepoxybutane is humans > rats > mice, whilst glutathione S-transferase (GST) mediated conjugation of epoxybutene is mice ≈ rats > humans. Another comparison (activation by initial oxidation rates versus detoxification as the sum of initial rates for mEH-mediated hydration and GSH conjugation) among species indicated highest activation/detoxification ratios for epoxybutene and diepoxybutane in mice, intermediate in rats and lowest for humans. These observations indicate that the relative carcinogenicity of butadiene in these species may depend on the balance of activation versus detoxification (ATSDR 2009[40]).

Oral or dermal studies regarding absorption, distribution and excretion – in humans or in experimental animals – were not located.

Physiologically based pharmacokinetic models

Johanson and Filser (1993, cited in ATSDR 2009[40]) simulated absorption and disposition of butadiene and its metabolite epoxybutene in PBPK models for the mouse and the rat, including the hepatic conjugation of epoxybutene with GSH. Tissue compartments included blood, liver, fat, and a lumped compartment for muscle and richly-perfused tissues. Some, but not all, parameters were optimized against experimental data. The model predicted epoxybutene levels that were similar to experimental observations following exposure to butadiene. The model has not been evaluated for inhalation exposures below 1,100 mg/m^3, it does not

simulate other metabolites (such as the diepoxybutane, the diols and the GSH conjugation products), nor does it simulate butadiene disposition in humans.

Also in the model of Kohn and Melnick (1993, 1996, 2000, cited in ATSDR 2009[40]) the absorption and disposition of butadiene and its metabolite epoxybutene in the mouse and rat were simulated.The body was represented by compartments for venous and arterial blood, lung, liver, kidney, fat, GI tract, and lumped compartments for richly and poorly perfused tissues. Oxidative metabolism is represented by the formation of epoxybutene and subsequent oxidation to diepoxybutane, further metabolism is represented by hydration and GSH conjugation of epoxybutene. Profiles of butadiene and epoxybutene uptake data (220- 8,800 mg/m^3) in mice and rats, and single time point concentrations of butadiene following exposures of mice and rats to 15.5-2,750 mg/m^3 were predicted correctly, as was the GSH depletion in mice and rats following butadiene exposures of 100-4,400 mg/m^3. The model has not been evaluated against data for inhalation exposures of humans, and it does not simulate other metabolites (such as diepoxybutane, the diols and the GH conjugation products.

The model reported by Brochot et al. (2007, cited in ATSDR 2009[40]) simulated absorption and disposition of butadiene and the disposition of epoxybutene and diepoxybutane in the blood, fat, and lumped compartments for richly- and poorly-perfused tissues in humans. Also the disposition and clearance of 3-butene-1,2-diol (butenediol) and epoxybutanediol was modeled for the blood and the richly and poorly perfused tissues. All of the metabolic steps were described as first-order processes; the metabolic rate constants and physiological parameters were optimized against 133 datasets from individual subjects inhaling 4.4 mg/m^3 butadiene for 20 minutes. The model was intended and thus calibrated for low exposures of humans. Extrapolation to higher doses would require modification of the metabolic expressions to account for saturation of the various metabolic pathways.

Péry and Bois (2009[42]) developed a (male human) model with 23 compartments, including arterial and venous blood, lungs, liver, kidney, fat, heart, brain, bone marrow, breast, adrenals, thyroid, gonads, pancreas, spleen, stomach, and gut. The model simulated absorption and disposition of butadiene and the disposition of epoxybutene, and was optimized against human inhalation data of butadiene in Japan, with an average environmental concentration of 0,25 µg/m^3 (background in unpolluted areas was 0.06 µg/m^3, 0.8 µg/m^3 and higher was only found in the vicinity of industrial activities).

Beaudouin et al. (2010[43]) reported a human model to address tissue dosimetry over the human lifespan. It had a compartmentalization similar to the model of Péry and Bois (2009[42]) described above, extended with compartments

evolving during pregnancy (i.e., placenta and a foetal submodel). The model was only evaluated by comparing the predicted butadiene concentrations in exhaled air with human experimental data on brief and low level laboratory inhalation exposures (4.4 mg/m^3 for 20 minutes) of volunteers to butadiene and found to predict these concentrations quite well. The authors modelled occupational exposure by simulating an exposure to 22 mg/m^3 for 9 h per day, 5 days per week, which resulted in venous blood levels of 0.13-0.28 mM at the beginning (slowly increasing during the week) and 6 mM at the end of the simulated working day (equalling 7-15 and 325 mg/L, respectively).

2.7 Mechanistic and other relevant data

The carcinogenicity of butadiene is mediated by its metabolic intermediates, since butadiene-induced mutagenicity requires metabolic activation: the DNA-reactive epoxides formed during biotransformation of butadiene are direct-acting mutagens.

Biomarkers

Biomarkers of exposure to butadiene (measurable internal indicators of change at the molecular or cellular level that can signal key events between exposure and adverse health effects) include water-soluble metabolites of butadiene in urine (Hurst 2007[6], Swenberg et al. 2007[44]). Studies have quantified the presence of butadiene-derived metabolites in butadiene-exposed humans. Two urinary metabolites have been identified, both mercapturic acids derived from the GSH conjugates of electrophilic butadiene metabolites: DHBMA (1,2-dihydroxybutyl mercapturic acid; also referred to as DHB, M1, and MI) and MHBMA (monohydroxy-3-butenyl mercapturic acid; also referred to as M2 and MII) – see Figure 1. In urine of rats and mice two isomeric forms of MHBMA have been quantified[7]. The relative proportions of the metabolites DHBMA and MHBMA depend on the species. Since DHBMA shows relatively high background levels, this metabolite appears to be a less specific biomarker for butadiene exposure than MHBMA, which has relatively low background levels. However, both metabolites appeared to be elevated in butadiene exposed humans compared with unexposed controls[2].

Besides the urinary biomarkers, there is interest in developing biomarkers that are (more) correlated with carcinogenic effects of butadiene, such as butadiene-metabolite-DNA adducts. Even though not directly related to mutagenic action, covalent adducts of butadiene metabolites with haemoglobin

(Hb) protein may serve as surrogates for DNA adducts and can integrate exposure to reactive nucleophilic metabolites over periods up to the lifetime of the red cell (120 days in humans)[2,6]. The metabolite epoxybutene reacts with Hb to form N-(2-hydroxy-3-butenyl)valine (MHbVal) adducts, and epoxybutanediol and diepoxybutane form N-(2,3,4-trihydroxybutyl)valine (THbVal) adducts[2]. Also the metabolite 3-butene-1,2-diol forms Hb adducts in rats in vivo (Barshteyn & Elfarra 2009[45]). At equivalent exposures to butadiene, blood levels of the Hb adducts MHbVal and the cyclic adduct N,N-(2,3-dihydroxy-1,4-butadiyl)valine (PyrVal) were higher in mice than in rats whereas the level of the major adduct, THbVal, was similar in these species. All of these adducts have been measured in butadiene-exposed rats and mice at concentrations as low as 6 mg/m^3 (IARC 2008[2], Swenberg et al. 2007[44]); PyrVal has been demonstrated in mice and rats exposed to butadiene at levels of 0.2 and 1.1 mg/m^3, respectively (Georgieva et al. 2010[46]). Both MHbVal and THbVal have been found in exposed workers at occupational exposure levels as low as 0.09 mg/m^3. When sampling was performed on a limited number of days, correlations between concentrations of adducts in blood and butadiene air concentrations were poor, whereas good correlations were observed (1) when very frequent air monitoring was conducted, and (2) in case of continuous monitoring of an increase in adduct concentration over a short period of time and the cumulative exposure during this time. The biomarker PyrVal, specific for diepoxybutane, is not available yet in humans[2,6]. Urine metabolite excretion patterns in both sexes revealed GSH conjugation to be a minor detoxicification pathway in humans.

Filser et al. (2007[47]) have measured directly the metabolites epoxybutene, diepoxybutane and epoxybutanediol (partial hydrolysis of diepoxybutane), and butenediol (hydrolysis product of epoxybutene) in blood from mice and rats. All metabolites increased with increasing exposure concentrations; diepoxybutane was only found in blood of mice. Butenediol and epoxybutanediol were quantitatively predominant in both species. At higher butadiene concentrations epoxybutanediol blood concentrations decreased again, which, according to the authors, can be explained by a competitive inhibition of the epoxybutanediol-producing CYP by butadiene in both species. In mice, epoxybutene blood concentrations increased almost linearly with the butadiene exposure concentrations up to 2,210 mg/m^3 butadiene. In blood of rats, the increase of epoxybutene deviates from linearity at much lower butadiene concentrations. The authors stated that a species-specific saturability of CYP-mediated butadiene metabolism may contribute to this observed flattening of the epoxybutene curve.

In 2011 Swenberg et al.[48] reviewed the biomarkers of butadiene exposure. They state that the three butadiene epoxides (1,2-epoxy-3-butene, 1,2:3,4-

diepoxybutane, and 1,2-epoxy-3,4-butanediol) vary up to 200-fold in their mutagenic potency, with 1,2:3,4-diepoxybutane being the most mutagenic metabolite. Mice appeared to form approximately 200 times and 50 times more 1,2:3,4-diepoxybutane than humans and rats, respectively, at exposures of 0.2 – 3.3 mg butadiene per m^3.

DNA adducts

Many adducts with epoxybutene, epoxybutanediol and diepoxybutane have been identified in reactions of these epoxides with nucleosides and DNA in vitro (see Annex H). Many of these adducts can also block replication by many polymerases or can cause misincorporation of proper nucleotides. DNA adducts have been identified in humans exposed to butadiene and in animals exposed to butadiene and its metabolites. The most abundant DNA adduct measured in butadiene-exposed rats and mice is N7-trihydroxybutylguanine, which is derived from either epoxybutanediol or diepoxybutane. N7-guanine adducts can lead to apurinic sites. Epoxide metabolites of butadiene can also react at base-pairing sites to form adducts at N3-cytosine, N1-adenine, N6-adenine, N1-guanine and N2-guanine. The level of DNA adduct N1-(2,3,4-trihydroxybutyl)adenine was determined in workers from a butadiene monomer production plant in the Czech Republic. The level of this adduct was significantly increased in exposed workers compared to control workers. Exposure was not significantly correlated with DNA single-strand breaks or micronucleus formation (IARC 2008[2], Hurst 2007[6], Goggin et al. 2009[49]).

In a study by Fernandes et al. (2006[50]) the phosphoramidites and subsequent oligodeoxynucleotides containing N3-2'-deoxyuridine adducts (formed from diepoxybutane reacted cytosine followed by spontaneous deamination) have been constructed and characterized. The results indicate that the N3-2'-deoxyuridine adducts are highly mutagenic lesions that may contribute to butadiene-mediated carcinogenesis. Thus, the authors have established the mutagenic effect of the butadiene N3-dU adducts. These are stable adducts that are blocking to replicative and repair polymerases and mutagenic in mammalian cells. These data thus suggest the importance of the butadiene N3-dU adducts as crucial lesions contributing to butadiene carcinogenesis.

Several authors have reported the formation of DNA-protein adducts by cross-linking through 1,2:3,4-diepoxybutane (Jelitto et al. 1989[51]; Costa et al. 1997[52]; Loeber et al. 2006[53]; Michaelson-Richie et al. 2010[54]). Potentially such helix-distorting DNA-protein cross-links may interfere with critical cellular

processes like replication and transcription, ultimately triggering apoptosis or genotoxicity.

The study of Antsypovich et al. (2007[55]) indicates that diepoxybutane-induced alkylation of N6-adenine in DNA is unlikely to lead to DNA-DNA cross-linking but instead can result in the formation of exocyclic deoxyadenosine adducts.

Loecken and Guengerich (2008[56]) and Loecken et al. (2009[57]) reported diepoxybutane-mediated cross-linking between DNA and the enzyme glyceraldehyde 3-phosphate dehydrogenase and histones H2b and H3 in homogenates of human liver nuclear proteins. These cross-links did, however, not induce enhanced mutagenesis in recombinant E. coli systems.

According to Swenberg et al. (2011[58]) no gender differences have been reported for globin adducts or N7 guanine adducts, but female rats and mice had 2-3 fold higher hprt* mutations and DNA-DNA crosslinks, suggesting a gender difference in DNA repair.

Other data

According to Swenberg et al. (2007[44]), the findings of the research group at UAB suggest that lymphoid neoplasms are more strongly associated with cumulative butadiene exposure, whereas myeloid neoplasms show a stronger association with peak exposures.

The International Life Sciences Institute has developed a human relevance framework that can be used to assess the plausibility that a mode of action which is described for animal models is also valid for humans. The mode of action is described as a sequence of key events and processes that result in an adverse outcome. A key event is a measurable precursor step that is in itself a necessary element of the mode of action or is a bioindicator for such an element. A number of key events have been identified whereby DNA-reactive chemicals can produce tumours. These include DNA adducts in target tissues, gene mutations and/or chromosomal alterations in target tissues and enhanced cell proliferation in target tissues. This type of data integration approach to quantitative cancer risk assessment can be applied to butadiene, for example, using data on biomarkers in exposed Czech workers (Albertini et al. 2003[59]). Using this study, Preston (2007[60]) assessed an extensive range of biomarkers of exposure and response, including polymorphisms in metabolizing enzymes, urinary concentrations of several metabolites of butadiene, Hb adducts, mutations at the hprt locus in T-

* hprt: hypoxanthine guanine phosphoribosyltransferase.

lymphocytes, chromosome aberrations (CAs) by fluorescent in situ hybridization and conventional staining procedures, and sister chromatid exchanges (SCEs). For the human relevance framework it is necessary to establish key events for a mode of action in rodents for the induction of tumours by butadiene. There is clearly a species difference in sensitivity to tumour induction (mice being much more sensitive than rats); requirement for the identification of a key event is that it can account for this difference. For butadiene, the weight of evidence from rodents supports a mode of action of DNA-reactivity and subsequent genotoxicity, To evaluate the plausibility of this mode of action in humans, Preston (2007[60]) considered some key events in human. In general the metabolism in human liver samples was more similar to that observed in rats than in mice. Secondly, it was shown that DNA adducts can be measured in human lymphocytes following butadiene exposure in an occupational setting. Based on these observations, the author concluded that the key events in the mode of action are plausible in humans. There is a large variation in humans for the metabolism of butadiene but overall the kinetics are more similar to those of rats than to those of mice. There appears to be no unequivocal evidence for the induction of gene mutations or CAs in butadiene-exposed humans. However, this lack of response does not signify a threshold response for tumours, but rather indicates some lack of sensitivity of such bioindicator assays at relatively low levels of exposure.

Genotoxicity - in vitro and animal data

In the previous DECOS report (1990[1]) it was concluded that the mutagenicity of butadiene depends mainly on the mutagenic potential of its reactive metabolites. The mutagenic potential of epoxybutene and diepoxybutane, its most reactive metabolites, has been proven in several test organisms in vitro. The primary metabolite epoxybutene was mutagenic in Salmonella typhimurium and Klebsiella pneumoniae. The secondary epoxide metabolite diepoxybutane was mutagenic in Klebsiella pneumoniae, Saccharomyces cerevisiae, Drosophila melanogaster, and induced SCEs in CHO cells.

In the IARC monograph on butadiene (2008[2]) a comprehensive review of the genotoxicity of butadiene was reported.

Butadiene is indirectly toxic to genetic material, as a result of action of its oxidative metabolites, resulting in a variety of genotoxic effects following butadiene exposure. Genotoxic effects beyond DNA alkylation involved cytogenetic effects including induction of micronuclei in developing erythrocytes and SCEs in cytogenetic studies of bone marrow cells from mice (not rats) exposed to butadiene, together with lengthening of average generation time and a

significant depression in the mitotic index. In peripheral blood the proportion of polychromatic erythrocytes and micronucleated normochromatic erythrocytes had increased. Butadiene was mutagenic in vivo at the hprt locus of splenic T cells from mice and weakly mutagenic in rats[2,6]. A greater hprt mutation efficiency was found in rats exposed to 137 mg/m^3 or mice exposed to 6.6 mg/m^3 (LOAELs*) compared with exposure of either species to 1,380 or 2,762 mg/m^3. This may be explained by competition between butadiene and butenediol or epoxybutene for CYP oxidation, limiting the secondary oxidation reaction. Thus, high-dose studies of butadiene in animals ($\geq$ 1,380 mg/m^3) may not adequately reveal the full carcinogenic potential of this compound at lower levels of exposure[2,61].

The relative genotoxic potency of the butadiene epoxide metabolites decreases in the order diepoxybutane > epoxybutene > epoxybutanediol, based on effects observed in mice, rats and in human cells. Diepoxybutane has been found by means of in vitro studies to be formed at higher levels in mice than in rats, which may contribute to the greater susceptibility to tumour formation of mouse over rat during chronic butadiene exposure[2,6]. An overview of results of animal genotoxicity tests for in vivo exposure to butadiene or its metabolites is shown in Table 3. No in vivo data were available for epoxybutanediol.

Walker et al. (2007[62]) tested the hypothesis that the hydrolysis (detoxification) pathway of butadiene through butenediol is a major contributor to mutagenicity at high-level butadiene exposures in the mouse and the rat. To determine the relative contribution of butenediol to butadiene-induced mutagenicity in rodents, mice and rats were exposed by inhalation directly to butenediol at exposure concentrations that produce plasma levels of butenediol equivalent to those found after exposure of mice to selected levels of the parent compound. Measurements of plasma levels of butenediol showed that exposures of mice and rats to 40 mg/m^3 butenediol were equivalent to those produced by 440 mg/m^3 butadiene exposures. Measurements of hprt mutant frequencies (via T-cell cloning assay) showed that repeated exposures to 40 and 80 mg/m^3 butenediol were significantly mutagenic in mice and rats; mutagenic potency was similar between these two concentrations. The resulting data indicated that butenediol-derived metabolites (especially 1,2-dihydroxy-3,4-epoxybutane) are responsible for nearly all of the mutagenic response in the rat and for a substantial portion of the mutagenic response in the mouse following high-level ($\geq$ 440 mg/m^3) butadiene exposures.

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* LOAEL: lowest observed adverse effect level.

Correlations between the efficiency for formation of adducts with the induction of *hprt* mutants in butadiene-exposed mice showed poor correlations between epoxybutene-induced adducts (hydroxybutenyl adducts at N7 of guanine, epoxybutene-GUA) and mutagenic effects, suggesting that epoxybutene is not the primary source of butadiene-induced mutations in vivo in the mouse. In contrast, there were highly positive correlations between the formation of trihydroxybutyl adducts at N7 of guanine (THB-GUA) as a biomarker of butadiene exposure, and hprt mutation induction, as a biomarker of butadiene-induced effect. THB-GUA adducts presumably arise largely from butenediol, and to a lesser degree from diepoxybutane, and point to the relative importance of these metabolites in butadiene-induced mutagenesis in the mouse[62].

DNA sequencing revealed that about half of the mutations induced in mice in vivo by butadiene, epoxybutene and diepoxybutane were frameshift mutations, while the remaining butadiene-, epoxybutene- and diepoxybutane-induced mutations were transition and transversion mutations at both AT and GC base pairs. At the hprt locus in human cells, epoxybutene was genotoxic mainly through point mutations, while diepoxybutane caused point mutations and partial deletions[2,6].

Swenberg et al. (2007[44]) reported that rats exposed to diepoxybutane developed CAs and micronuclei, although butadiene is not clastogenic in rats. Diepoxybutane is a bifunctional alkylating agent that exhibits both mutagenic and cytotoxic activity, presumably as a result of its ability to form bifunctional DNA adducts.

Kligerman and Hu (2007[63]) investigated SCEs and CAs in vitro in lymphocytes from humans, rats and mice after exposure to epoxybutene or diepoxybutane at the G0 stage of the cell cycle. Epoxybutene induced no increases in SCEs or CAs in the cells from the three species. Diepoxybutane was a potent SCE- and CA-inducer, with the results being similar in each rodent species. The response for SCEs seen in the human cells was more complex, with genetic polymorphism for GST possibly modulating the response.

Genotoxicity - human data

In the IARC (2008[2]) monograph a number of genotoxicity tests in humans was reported, using workers from several butadiene monomer or SBR production facilities. CAs were significantly increased in 1 out of 6 studies, SCEs in 1 out of 5 studies, and hprt mutations in 4 out of 6 studies. Several studies had separated the smokers and non-smokers. Only the study of Lovreglio and coworkers (2006[4]) found a statistically significant increase in the mean SCEs in smokers

Table 3 Results of animal genotoxicity tests for in vivo exposure to 1,3-butadiene or a metabolite (derived from IARC 2008[2]).

Test type	1,3-Butadiene		Epoxybutene		Diepoxybutane	
	Result	No of studies	Result	No of studies	Result	No of studies
DNA damage, single strand / double strand breaks, cross-links	+	7	+	1	(+)	1
	–	5				
Gene mutation	+	15	+	5	+	3
	(+)	5	(+)	2	(+)	1
			–	6	–	1
Comet			+	1	+	1
					(+)	1
					–	1
Sister chromatid exchange	+	2				
	–	1				
Micronucleus	+	12	+	1		
	–	2 (rat)	–	1		
Chromosome aberration	+	3				
Aneuploidy	–	1				
Dominant lethal	+	6				
	–	2				
Heritable translocation	+	2				
DNA-binding (N7-guanine, N6-adenine)	+	7				
	–	2				
Sperm morphology	+	1				

+ = positive; – = negative; (+) = weakly positive.

(6.6 ± 1.2) compared with non-smokers (5.5 ± 0.8; p = 0.001). Exposure to butadiene was higher in smokers (mean 7.7) than in non-smokers (mean 2.0; p = 0.3). The genotoxic biomarkers (SCEs, CAs, and cells with high frequency of SCEs) could not distinguish between the exposed (6.4 ± 14.0 µg/m^3) and non-exposed group (0.8 ± 1.1 µg/m^3) in this study.

Albertini et al. (2001[64]) found no evidence that low-intensity exposure to butadiene was associated with structural changes in chromosomes or gene mutations in lymphocytes (as possible indicators for butadiene-induced lymphohaematopoietic cancer) among butadiene monomer and synthetic rubber workers in the Czech Republic. The relatively small cohort consisted of 83 male workers: 24 butadiene monomer production workers with mean butadiene exposure of 0.64 mg/m^3, 34 polymerization workers with mean butadiene exposure of 1.76 mg/m^3 and 25 controls with mean butadiene exposure of 0.3 mg/m^3. Their duration of employment was on average 15-18 years.

The mutation frequency (MF) at the hprt locus as an intermediate biomarker of butadiene carcinogenicity was investigated for its relation to mortality and cancer incidence by Liu et al. (2008[65]). A population of butadiene-exposed workers and non-exposed control subjects working at the alkenes plant at a petrochemical products company in Nanjing, China, was analyzed to determine the MF of the hprt gene in lymphocytes. Exposure of the workers was to an average butadiene concentration of 21 ± 34 mg/m^3. All control group locations were below 0.44 mg/m^3 (detection limit). There was a, not statistically significant, increase of 43% in hprt gene MFs in the exposed workers (18 ± 9x10^{-6}) as compared to controls (13 ± 7x10^{-6}) by using the T-cell cloning assay. The observed increase in the total number of hprt clones with deletions in exposed workers (27.4%) was statistically significantly increased compared to control workers (12.5%). The increase is primarily the result of an increase in multiple exon deletions (2-8) with 56% and 23% in exposed and controls, respectively, including both continuous deletions (37% and 17% in exposed and controls, respectively) and discontinuous deletions (with 19% and 6% in exposed and controls, respectively).

Wickliffe et al. (2009[66]) investigated the frequencies of hprt mutant lymphocytes in workers at a butadiene polymer plant in Texas (USA). Hprt MFs were not significantly associated with current exposures nor with age. They were, however, significantly associated with the number of years working in the butadiene industry at this plant. According to the authors this mutagenic effect might be the result of chronic and/or past high-level exposures.

DNA repair capacity

To investigate the role of DNA repair in modulating butadiene-induced genotoxicity/carcinogenicity, Vodicka et al. (2006[67]) investigated single strand breaks and endonuclease III-sensitive sites in DNA along with γ-irradiation-specific DNA-repair activity in hepatocytes and frequencies of micronuclei in polychromatic bone marrow erythrocytes of male NMRI mice (6/experimental point) during sub-acute inhalation exposure to butadiene (28 days, 500 mg/m^3) and up to 28 days after exposure. Concentrations of butadiene in blood (indicator of internal exposure) were 0.08-0.10 mg/L during the exposure period. The γ-irradiation-specific DNA repair activity gradually increased during exposure, reaching a maximum on day 1 after the termination of exposure and then returning to control levels. A significant correlation between γ-irradiation-specific DNA repair activity and the concentration of butadiene in blood supports a possible induction of DNA-repair activity by the exposure to

butadiene and formation of its metabolites. The initial increase in micronucleus frequency in the exposed mice continuously decreased from 20.4 (day 3) to 15 (day 28) within the exposure period and subsequently from 12 to 4.6 in the period following termination of the butadiene exposure, while micronucleus frequencies in control animals were significantly lower (1.7-4.2 micronuclei per 1,000 cells).

Alterations in oncogenes and suppressor genes in tumours

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. The K-ras, H-ras, p53, p16/p15 and b-catenin mutations detected in tumours in mice probably occurred as a result of the DNA reactivity and genotoxic effects of butadiene-derived epoxides. A consistent pattern of K-ras mutations (G→C transversions at codon 13) was observed in butadiene-induced cardiac haemangiosarcomas, neoplasms of the lung and forestomach and lymphomas[2,68]. Alterations in the p53 gene in mouse brain tumours were mostly G→A transition mutations. Inactivation of the tumour-suppressor genes p16 and p15 may also be important in the development of butadiene-induced lymphomas. Mammary gland adenocarcinomas induced by butadiene in mice had frequent mutations in the p53, H-ras and b-catenin genes.

Together these observations point to a genotoxic mechanism that underlies the development of butadiene-induced cancers. Although genotoxicity data indicate that diepoxybutane is the most genotoxic of the butadiene epoxides, the relative contribution of these metabolic intermediates to the mutagenicity and carcino-genicity of butadiene is not known[2,6].

Polymorphism

Metabolic activation and inactivation rates of butadiene in humans exhibit a high degree of variability and appear to span the range of activation rates between mice and rats when evaluated with in vitro systems measuring enzyme kinetics (greater than ten-fold). Other in vitro studies and in vivo molecular epidemiological studies indicate the range of increased sensitivity due to human genetic polymorphisms is approximately two- to four-fold (IARC 2008[2], ATSDR 2009[40]).

The basis of species differences between rats and mice may be related to the greater production of toxic intermediates, specifically diepoxybutane, and a lower capacity for detoxification of these intermediates in mice[3].

Several epidemiological studies (references in IARC 2008[2] and Wickliffe et al. 2007[69]) examining human sensitivity to butadiene following occupational exposures have found an association between somatic mutations and exposures. They also found an association between specific polymorphisms in biotransformation and DNA repair genes and increased genetic damage. These studies addressed the role of polymorphisms in biotransformation genes such as the GSTs GSTT1 and GSTM1, and the gene EPHX1, coding for the principal detoxifying enzyme mEH, and in DNA repair genes involved in nucleotide excision repair (NER) and base excision repair (BER). Polymorphisms in biotransformation and DNA repair proteins may modulate genetic susceptibility[69]. Vacek et al. (2010[70]) studied production and accumulation of the metabolite 1,2:3,4-diepoxybutane in exposed and non-exposed males and females of the Czech cohort of workers in the SBR industry (described by Albertini et al. 2001[64]) by measuring THbVal adducts, and found that exposed men had significantly higher THbVal concentrations than non-exposed men, but exposed and non-exposed women did not differ significantly. THbVal concentrations were significantly correlated with mean 8-h TWA exposures to butadiene for both males and females. However, the rate of increase with increasing butadiene exposure was significantly lower for females, and the size of the differences increased with exposure. The authors concluded that apparently females absorb or metabolize less butadiene than males per unit exposure.

Tan et al. (2010[71]) studied micronucleus frequencies in peripheral lymphocytes of butadiene-exposed workers in a polybutadiene latex production plant in Ningbo, China, and reported that (1) the frequency in workers was significantly higher than in controls, (2) male workers had lower frequencies than female workers, and (3) workers who carried the genotypes of GSTM1 (+), CYP2E1 (c1c2/c2c2) and the mEH intermediate group had significantly higher frequencies than those carrying the genotypes of GSTM1 (-), CYP2E1 (c1c1) or the mEH high group. The same group studied the same workers with respect to polymorphism of NER (Wang et al. 2010[72]) using micronucleus frequencies in peripheral lymphocytes, and reported multiple NER polymorphisms (an ADPRT and several XRCC1 genotypes) and a XRCC1 haplotype to be associated with differential levels of frequencies.

To investigate the role of genetic polymorphisms in mEH or NER in the mutagenicity of butadiene, experiments were conducted in which mice lacking

mEH (Ephx1-null) or NER activity (Xpc-null) were exposed to butadiene by inhalation or to epoxybutene by intraperitoneal injection. Xpc-null mice were significantly more sensitive to epoxybutene exposure, exhibiting an average 2.8-fold increase in hprt mutant frequency relative to those of exposed wild-type mice[69,73]. This study with the Ephx1-null mice supports the hypothesis that humans with a diminished mEH activity are more susceptible to relatively high levels of butadiene exposure, whereas the study with the Xpc-null mice provides initial insights into the recognition and repair pathways involved in maintaining genomic integrity in vivo.

Other data

Diepoxybutane, the most potent metabolite of butadiene, is a bifunctional alkylating agent that exhibits both inter-strand and intra-strand DNA cross-linking ability, and DNA-protein cross-linking ability. Diepoxybutane also generates reactive oxygen species that can damage DNA or produce H_2O_2. Apoptosis in reponse to diepoxybutane exposure has also been observed in Big Blue Rat cultured cells, mouse L929 cultured cells, as well as in human CD34+ bone marrow cells.

Fred et al. (2008[74]) studied whether the large differences in outcome of cancer tests with butadiene could be predicted quantitatively on the basis of the concentration over time in blood (area under the curve: AUC) of the epoxide metabolites, their mutagenic potency, and a multiplicative cancer risk model which has earlier been used for ionizing radiation. Published data on Hb adduct levels from inhalation experiments with butadiene were used for the estimation of the AUC of the epoxide metabolites in the cancer tests. The estimated AUC of the epoxides were then weighed together to a total genotoxic dose, by using the relative genotoxic potency of the respective epoxide interferred from in vitro hprt mutation assays using epoxybutene as standard. The tumour incidences predicted with the risk model on the basis of the total genotoxic dose correlated well with the earlier observed tumour incidences in the cancer tests. The total genotoxic dose that leads to a doubling of the tumour incidences was estimated to be the same for rat and mouse, 9 to 10 mmol/L.h epoxybutene-equivalents.

Conclusion

Butadiene, taken up by inhalation, is metabolized into DNA-reactive epoxides (stereoisomers of epoxybutene, epoxybutanediol and diepoxybutane). Both in vitro and in vivo animal studies have demonstrated the presence of butadiene

metabolites after exposure. Metabolite-specific Hb adducts have been found in workers. Both animal and in vitro studies indicate that (combinations of) metabolites from butadiene are clastogenic and may bind to DNA. In human studies, no explicit butadiene-induced genotoxic effects have been observed: results on cytogenetic endpoints and on hprt mutations in workers exposed to butadiene are not conclusive. This may be due to interindividual differences in metabolic activity and/or in DNA-repair capacities. Although the genotoxicity data indicate that diepoxybutane is the most genotoxic epoxide formed from butadiene, the relative contribution of all epoxide metabolites to the mutagenicity and carcinogenicity of butadiene is not known. The enzymes involved in the formation and further biotransformation of epoxides are polymorphic in human populations, but the extent to which the variabilities of these enzymes modulate the carcinogenicity of butadiene is not known (IARC 2008[2]).

2.8 Toxicity profile

In Hurst (2007[6]), the DECOS report of 1990[1], and the evaluation of the Agency for Toxic Substances and Disease Registry (ATSDR, 2009[40]) overviews of the toxicity of butadiene were presented, which are summarized below.

Human data

Irritation of eyes, nasal passages, throat, and lungs was noted in workers exposed to butadiene during early manufacture of rubber (Wilson 1944, in Hurst, 2007[6]).

De Jong et al. 1983 (cited in Decos 1990[1]) reported the following short-term effects of different butadiene at concentrations in air to industrial workers (exposure period is not mentioned):

- 2,200 mg/m^3: no effects
- 4,400-8,800 mg/m^3: slight irritation of the eyes and bronchi
- Volunteers exposed for 8 hours to a concentration of 17,600 mg/m^3 butadiene showed no other effects than irritation of the eyes and bronchi.

Animal data

Lethality* as a consequence of acute exposure in animals have been reported to vary between 3.2 and 5.5 g butadiene per kg bw (LD_{50}s for orally exposed mice

* LC_{50}/LD_{50}: lethal concentration / lethal dose at which 50% of the exposed animals die within 24 h.

and rats), and between 270 and 550 g butadiene per m^3 (LC_{50}s for inhalatory exposed mice, rats and rabbits).

The clinical signs of intoxication by butadiene following inhalation exposure included hyperventilation, twitching, excitation, anaesthesia and narcosis.

Irritation of the mucous membranes resulting in conjunctivitis, nasal and bronchial irritation leading to respiratory obstruction were the outcome of exposure of mice and rats, for unspecified times, to atmospheres containing 198,000-308,000 mg/m^3 butadiene.

Ophthalmoscopic examination of the rabbit eye revealed no signs of injury during or following exposure to atmospheres containing up to 14,740 mg/m^3 butadiene for 7.5 h/day, 6 days/week during 8 months. A similar result was obtained in a limited study performed concurrently on dogs (Shell 1986, in DECOS[1]).

Early studies noted that inhalation of high concentrations of butadiene was anaesthetic in animals, as a concentration of 550,000 mg/m^3 was lethal to rabbits within 30 minutes. A 50% mortality was observed in rats and mice after exposure to 269,000 mg/m^3 and 285,000 mg/m^3 after 4- and 2-h exposures, respectively. These acute exposures resulted in irritation of eye, respiratory tract and skin, as well as in effects on the central nervous system (reviewed in Hurst 2007[6]).

Himmelstein et al. 1997 (in Hurst 2007[6]) reviewed the toxic effects (other than cancer) in animals. Higher inhaled concentrations butadiene were related to biochemical alterations, including glutathione depletion in liver, lung and heart. This depletion was noted to be more complete and at lower inhaled concentrations in mice than in rats, and was correlated with increased concentrations of the metabolites butadiene monoepoxide and butadiene diepoxide.

Reproductive and developmental studies of the US National Toxicology Program (NTP) showed mice being more sensitive to butadiene inhalation than rats. Exposure of rats to 2,200 mg/m^3 resulted in decreased weight gain during pregnancy, but no fetal developmental toxicity was observed. In Swiss CD-1 mice exposure to 440 and 2,200 mg/m^3 resulted in anomalies including extra ribs and decreased ossification of sternebrae in fetuses. Additional NTP studies showed abnormal sperm head morphology in male mice exposed to concentrations of 2,200-11,000 mg butadiene/m^3 and an increased ratio of dead to total implanted fetuses in dominant lethal assays in female mice. These observations are indicative for butadiene, or its metabolites, being mutagenic to germ cells in mice at high concentrations. Testicular atrophy was observed in B6C3F1 mice exposed to 1,375 mg/m^3 butadiene, and atrophy of ovaries was observed at 13.8

mg/m³ (LOAEL; a NOAEL* could not be derived)[37]. ATSDR (2009[40]) characterized the effect at this LOAEL to be a serious reproductive effect.

Fetal toxicity was observed following the mating of untreated female mice with males exposed to 27.5 mg/m³ butadiene for 10 weeks (6 h/day, 5 days/week). Observed effects included an increase in late fetal death, exencephaly and skull abnormalities. Early fetal death occurred in untreated female mice mated to males exposed to 144 mg/m³ for 4 weeks, 6 h/day, 5 days/week (ATSDR 2009[40]).

When exposed to concentrations up to 17,680 mg/m³ butadiene during days 6-15 of gestation (GD 6-15), Sprague-Dawley rats showed signs of dose-related maternal and fetal toxicity. Depressed body weight gain amongst dams was observed at ≥ 442 mg/m³, and fetal growth was significantly decreased in the 17,680 mg/m³ group. A significantly increased incidence of skeletal abnormalities (wavy ribs, irregular rib ossification) occured in the 2,210 mg/m³ group and major abnormalities (defects of the skull, spine, sternum, long bones and ribs) were observed in the 17,680 mg/m³ group. In mice, a 5-23% decrease in fetal body weight gain in males was observed after exposure of dams during GD 6-15 to 88-2,210 mg/m³ butadiene, and increased incidences of extra ribs and reduced ossification of sternebrae were found in fetuses from groups exposed to 442 mg/m³ and 2,210 mg/m³, respectively (ATSDR 2009[40]).

In butadiene-exposed mice, also toxicity of the haematopoetic system was observed. In two strains of male mice exposed to 2,750 mg/m³ for 6-24 weeks, macrocytic-megablastic anaemia was observed. In addition leukopenia and an increase in the number of erythrocyte micronuclei were observed (IARC 1999[75], Hurst 2007[6]).

Conclusion

At the time that the industrial manufacture of butadiene was started (thus occupational exposure to relatively high concentrations), irritation of eyes and respiratory tract was noted in humans. Repeated dose toxicity resulted in biochemical alterations and haematopoietic disturbance. At high concentrations butadiene is lethal to animals. A LOAEL of 13.8 mg/m³ was derived from a chronic study in mice, based on ovarian atrophy (LOAEL reproduction and overall LOAEL); at this dose level also respiratory adenomas and carcinomas were found in female mice. Developmental toxicity (late fetal death, exencephaly and skull abnormalities) was observed at 27.5 mg/m³ in mice (LOAEL).

* NOAEL: no observed adverse effect level.

2.9 Overall conclusion

The Committee agrees with the conclusion of the Subcommittee on the Classification of carcinogenic substances (see Annex I) that butadiene is a stochastic genotoxic carcinogen, expressing its genotoxicity through its reactive metabolites (1,2-epoxy-3-butene, 1,2:3,4-diepoxybutane and 3,4-epoxy-1,2-butanediol), which are alkylating agents. This conclusion is in line with similar conclusions by others (IARC 2008[2], 2009[7]; Kirman et al. 2010a[10], 2010b[11]; Albertini et al. 2010[12]).

Butadiene expresses its carcinogenicity at lower exposures compared to the lowest exposure at which toxic effects other than carcinogenicity become manifest.

Chapter 3

Risk assessment

3.1 Health risk to humans and selection of the suitable study for risk estimation in the occupational situation

Inhalation studies with animals exposed to butadiene showed increased tumour incidences in many tissue types.

However, to estimate health-based occupational cancer risk values, the Committee prefers to use human data above animal data. In case of exposure to butadiene many human data are published. The data mainly concern mortality due to lymphatic and/or haematopoietic tumours; no distinctly increased incidence of mortality was found for tumours in other tissues in the few studies in which this was investigated.

The human studies showed a dose-related association between butadiene exposure and tumour development[2,19,21-25,27,30]. Generally, these associations were still statistically significant when the data were corrected for age (leukaemia with cumulative exposure), peak exposure, and average intensity of exposure[24].

Eight epidemiological studies on leukaemia mortality among workers exposed to butadiene were of interest. The papers concern Delzell et al. (1996[18], 2001[25]), Cheng et al. (2007[24]), Graff et al. (2005[22]), Macaluso et al. (1996[27]), Matanoski et al. (1997[19]), Sielken et al. (2007[30]) and Sielken and Valdez-Flores (2011[31]).

Most of the studies found cumulative exposure to butadiene to fit best with the observed extra leukaemia deaths following occupational exposure to this substance. Only Sielken et al. (2007[30]) and Sielken and Valdez-Flores (2011[31]) used the cumulative number of butadiene peaks during the occupational exposure period as a co-variable to estimate the risks, which resulted in relatively low risks compared with the other studies. It is difficult to understand that the relationship between cumulative butadiene exposure, and risk of leukaemia found in the other studies, would only be the result of exposure to butadiene peaks, the more because Sielken and Valdez-Flores assigned to all butadiene peaks > 100 ppm (221 mg/m^3) the same weight, without accounting for peak height or peak duration. Dominant influence of butadiene peaks is also questionable in view of the results of Graff et al. (2005[22]), who found significant exposure-response relationships for cumulative (occupational) exposures to both < 100 ppm butadiene, and ≥ 100 ppm butadiene. Therefore the Committee decided not to use assumptions on peak exposure.

Reviewing all studies, Cheng et al. (2007[24]) provided the most extensive set of quantitative data, and was most transparent in the methods used regarding exposure data and exposure-response modelling, including corrections for co-exposure to STYR and DMDTC. In Figure 2 the data on relative risk and cumulative exposure (see Table 2) from this study are depicted. These exposure and response data were used to estimate health-based occupational cancer risk values.

3.2 Calculation of the health-based occupational cancer risk values

According to the 'Guideline for the calculation of carcinogenic risks' of the Health Council of The Netherlands[76], the Committee used a survival analysis, also called 'life-table' analysis, in estimating the cancer risk values. Survival analysis is a statistical methodology to describe mortality or survival rates (expressed as the number of deaths per 100,000 person-years) in populations during a specified time. By comparing mortality rates between an exposed population and a non-exposed population, the number of extra deaths that corresponds to a certain exposure level can be estimated. This 'number of extra deaths' serves as a point of departure to estimate cancer risk values.

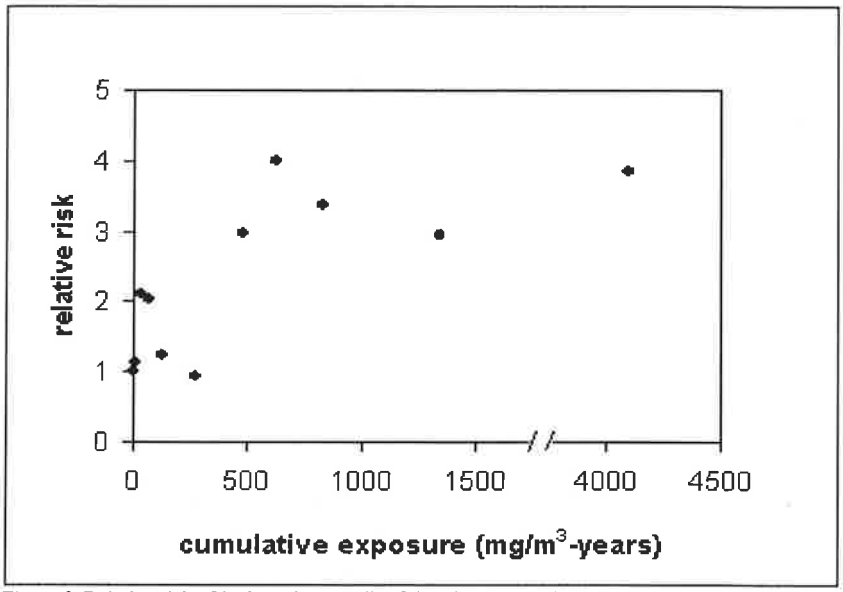

Figure 2 Relative risk of leukaemia mortality following occupational exposure to 1,3-butadiene, according to Cheng et al. (2007[24]).

In case of leukemia and occupational butadiene exposure, the Committee used the following principles and assumptions:

1 The Committee calculated leukaemia mortality of the general population on the basis of national data on leukaemia mortality in five-year age bands, obtained through Statistics Netherlands (Centraal Bureau voor de Statistiek). Mortality data for the years 2000 to 2010 were used, separated by age and sex. Rates for women and men were averaged, so that the calculations would describe the average risk for the population. To soften the transitions between age categories, the mortality data were 'smoothed'. These 'modelled' mortality data were employed in the Committee's analysis.
2 For the occupational exposure to butadiene, it is assumed that exposure of the cohort starts at the age of 20, and lasts until the age of 60 years. Every year, the cohort reduces in size, through death as a result of the cause of death under study and other causes; the cohort is followed until it reaches the age of 100 years[76].
3 Assuming a given average annual exposure to butadiene, every year that a person in the cohort is exposed is another year contributing to his/her cumulative exposure. This approach employs cumulative exposure because

studies of workers exposed to high levels of butadiene always work with cumulative exposure; the formulae employed are also based on cumulative exposure. Using this cumulative exposure, which is recalculated for each year, and the assumed exposure-response relationship between exposure to butadiene and death from leukaemia, the number of extra deaths is calculated for each year that the cohort ages. Using this information, first the additional risk of death per year associated with exposure to butadiene can be calculated, and then the lifelong additional risk of death associated with exposure.

Leukaemias included in the analysis are listed in the 10th International Code of Diseases of the WHO, categories C81-C96 ("*malignant neoplasms, stated or presumed to be primary, of lymphoid, haematopoietic and related tissue; excluded: secondary and unspecified neoplasm of lymph nodes*"; WHO 2003[77]). In applying this approach the Committee extrapolates data on leukaemia as reported by Cheng et al. (2007[24]) to include more forms of lymphohaematopoietic cancers. The Committee is of the opinion that there is sufficient information indicating the risk of various lymphohaematopoietics cancers following butadiene exposure (see, e.g., IARC 2008[2]). The diagnosis and classification of lymphatic and haematopoietic malignancies is very complex, and has undergone several changes in the course of time (as outlined in section 2.4). Thus, limiting the risk evaluation to leukaemia only would certainly result in an underestimation of the risk of developing cancer following butadiene exposure. The exposure-response data published for myeloid (implicitly also covered in the exposure-response of leukemia) and lymphoid neoplasms are more limited than for leukaemia. The Committee noted that the exposure-response association as published in Cheng et al. (2007[24]) is not noticeably different from the published association for leukaemia, albeit that the slope-factor is lower. Given these uncertainties, the Committee prefers to use the leukaemia data of Cheng c.s., 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 of the risk, but prefers this rather than ending up with an underestimation by limiting the risk to leukaemia only.

Results of the analysis

First, using the data by Cheng et al. (2007[24]; see Table 2), the Committee derived the model with the best fit regarding exposure-response relationships. Using the software programme SAS, and with PROC NLMIXED the following two

relationships with the best fits were obtained (RR = 1 means no difference in mortality when compared to the general population):

- RR = 1 + 0.001159 x (cumulative exposure) (linear additive model)
- RR = 1 + 0.005934 x (cumulative exposure)$^{0.7626}$ (exponential model)

Of these two models the linear additive model has a slightly better fit*, and hence this model was chosen for the next step, the survival analysis.

The survival analysis was performed using the software R (in Windows). For the derivation of health-based calculated occupational cancer risk values (HBC-OCRV), an additional risk of one extra cancer death due to occupational exposure per 250 (4 x 10^{-3}) and 25,000 (4 x 10^{-5}) is taken into account. The results are shown in Table 4.

Table 4 Exposure-response modelling and survival analysis.

Study	Cheng et al. 2007[24]
Model	RR = 1 + 0.001159 x (cum. exp.)
Original unit	butadiene ppm-years
Mean exposure at risk 4x10^{-3}	4.7 ppm = 10 mg/m^3
Mean exposure at risk 4x10^{-5}	0.047 ppm = 0.1 mg/m^3

Health-based calculated occupational cancer risk values (HBC-OCRVs)

The Committee calculates that 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 (5 ppm)
- 4 per 100,000 (4x10^{-3}) deaths in the general population, at 40 years of occupational exposure, equals to 0,1 mg 1,3-butadiene per m^3 (5 ppm).

The recommended values are expressed as 8-hour time-weighted average concentrations.

Other (toxic) effects have been reported in experimental animals: the lowest overall LOAEL was 13.8 mg/m^3, based on ovarian atrophy observed in mice in the two-years carcinogenicity/toxicity inhalation study of the US National Toxicology Program (NTP 1993[37], Table 5). The Committee performed a benchmark dose (BMD) analysis on the data of this study using the BMD

* The linear additive model has an AIC of 50.3, the exponential model has an AIC of 51.9. The AIC value (Akaike's Information Criterion; Akaike 1974[78], 1980[79]) is -2L+2p, where L is the log-likelihood at the maximum likelihood estimates for the parameters, and p is the number of model parameters estimated. It is used to compare different types of models which use a similar fitting method, The model with the lowest AIC is presumed to be the better model.

Table 5 Inhalation study with B6C3Fl mice exposed to 1,3-butadiene, 6 h/day, 5 days/week during 103 weeks (NTP 1993[37])

Exposure (mg/m³)	0	13.8	44.2	138	442	1381
Animals with ovarian atrophy[a]	4/49	19/49	32/48	42/50	43/50	69/79
Percentage animals affected	8	39	67	84	86	87

[a] animals with ovarian atrophy / total number of animals.

software of the US-EPA (US-EPA 2012[80]). 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/m³. 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/m³. This value is practically equal to the 4 x 10^{-5} risk HBC-OCRV of 0.1 mg/m³ that the Committee derived above. Hence this HBC-OCRV is not expected to result in effects other than carcinogenicity.

3.3 Dermal uptake of 1,3-butadiene

To decide whether a skin notation should be recommended to the substance, the Committee uses the ECETOC criteria for assigning a skin notation[81].

Butadiene is a gas with a boiling point of -4.4°C. As the vapour pressure at 21°C is 240 kPa, the compound is not expected to give rise to skin exposure by direct contact. Therefore, the Committee indicates no skin notation for butadiene on the basis that exposure to gases requires a different protection regime.

3.4 Risk values derived by SCOEL

The Committee noted that the European Scientific Committee on Occupational Exposure Limits (SCOEL) presented also data on extra cancer risks (SCOEL 2007[8]). At occupational exposure of 5 ppm (11 mg/m³) SCOEL estimated the extra risk of leukaemia mortality at -0.05-11.7 deaths between the age of 25-85 years, per 1,000 males occupationally exposed during working life from 25-65 years. The Committee, however, did not use these extra cancer risk data for several reasons, including (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.

3.5 Existing cancer risk values and occupational exposure limits

Table 6 summarizes the risk values of a number of (inter)national organisations for dying from leukaemia following occupational exposure to butadiene.

Table 6 Risk values of other organisations for dying from leukaemia following occupational exposure to 1,3-butadiene

Organisation[a]	1,3-Butadiene concentration	Risk level	Reference
France (ANSES), 2011[82]	0.08 mg/m^3	1 x 10^{-4}	Health Canada 2000[81], BAuA 2010[80]
	0.008 mg/m^3	1 x 10^{-5}	
	0.0008 mg/m^3	1 x 10^{-6}	
Germany (BAuA), 2010[83]	5 mg/m^3 (2 ppm)	4 x 10^{-3}[b]	Graff et al. 2005[22], Cheng et al. 2007[24]
	0.5 mg/m^3 (0.2 ppm)	4 x 10^{-4}[c]	
Canada (Health Canada), 2000[84]	7.8 mg/m^3	1 x 10^{-2}[d]	Delzell et al. 1996[18]

[a] ANSES, BAuA and Health Canada are (semi)governmental organisations responsible for independent scientific advice of their respective governments.
[b] "Akzeptanzrisiko"
[c] "Toleranzrisiko"
[d] Tumorigenic concentration for 1% of the occupationally exposed people (TC_{01})
ANSES: Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail, France.
BAuA: Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, Germany.

Existing occupational exposure levels for butadiene, other than the ones summarized in Table 6, are presented in Table 7.

Like the Committee, the organisations cited in Table 5 used mortality data from leukaemia of approximately 17,000 workers in the North-American butadiene industry as reported by a number of investigators (Delzell et al. 1996[18], 2001[25], Graff et al. 2005[22], Cheng et al. 2007[24]). The risk values derived are all in the same order of magnitude as the values derived above (Section 3.2) by the Committee. Differences between the various risk values can be attributed to (1) the epidemiological dataset used, (2) the types of leukaemia that are included, (3) the model applied to estimate an exposure-response relationship, (4) the life tables applied, and (5) the age to which mortality is analysed.

Table 7 Existing occupational exposure limits (OELs) for 1,3-butadiene.

Country - organisation	OEL (mg/m^3)	Type of OEL	Note
The Netherlands (Ministry of Social Affairs and Employment)	46.2	TWA	Car
Denmark	22	TWA	Car
Norway	2.2	TWA	Car
Sweden	1	TWA	Car
	10	STEL	
Finland	2.2	TWA	Car
United Kingdom	22	TWA	Car
USA			
- ACGIH	4.4 (TLV)	TWA	Car
- OSHA	2.2 (PEL)	TWA	Car
	11 (PEL)	STEL	-
- NIOSH	Lowest feasible concentration	REL	Car
	4400 (STEL)	IDLH	-

AGCIH	American Conference of Governmental Industrial Hygienists
NIOSH	National Institute for Occupational Safety and Health
IDLH	immediately dangerous to life or health
OSHA	Occupational Safety and Health Administration
PEL	permissible exposure limit
REL	recommended exposure limit
STEL	short-term exposure limit
TLV	threshhold limit value
TWA	time-weighted average
Car	carcinogen / suspected carcinogen

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Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Safety (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, an 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in Annex B.

Annex **B**

The Committee

- R.A. Woutersen, *chairman*
  Toxicologic Pathologist, TNO Innovation for Life, Zeist, and Professor of Translational Toxicology, Wageningen University and Research Centre, Wageningen
- D.J.J. Heederik
  Professor of Risk Assessment in Occupational Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R. Houba
  Occupational Hygienist, Netherlands Expertise Centre for Occupational Respiratory Disorders, Utrecht
- H. van Loveren
  Professor of Immunotoxicology, Maastricht University, Maastricht, and National Institute for Public Health and the Environment, Bilthoven
- G.J. Mulder
  Emeritus Professor of Toxicology, Leiden University, Leiden
- T.M. Pal
  Occupational Physician, Netherlands Centre for Occupational Diseases, University of Amsterdam, Amsterdam
- A.H. Piersma
  Professor of Reproductive Toxicology, Utrecht University, Utrecht, and National Institute for Public Health and the Environment, Bilthoven

- H.P.J. te Riele
  Professor of Molecular Biology, VU University Amsterdam, and Netherlands Cancer Institute, Amsterdam
- I.M.C.M. Rietjens
  Professor of Toxicology, Wageningen University and Research Centre, Wageningen
- R.C.H. Vermeulen
  Epidemiologist, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- P.B. Wulp
  Occupational Physician, Labour Inspectorate, Groningen
- B.P.F.D. Hendrikx, *advisor*
  Social and Economic Council, The Hague
- A.J. Baars, *scientific secretary*
  Health Council of the Netherlands, The Hague

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Letter of submission

Subject	: Submission of the advisory report *1,3-Butadiene*
Your Reference	: DGV/MBO/U-932342
Our reference	: U-7739/JR/fs/459-K68
Enclosed	: 1
Date	: May 31, 2013

Dear Minister,

I hereby submit the advisory report on the effects of occupational exposure to *1,3-butadiene*.

This advisory report is part of an extensive series in which concentration levels in the air are estimated, which correspond to an excess risk of cancer mortality by occupational exposure of 4 per1,000 or 4 per 100,000 deaths in the general population.

The advisory report in question was prepared by the Health Council's Dutch Expert Committee on Occupational Safety (DECOS) and assessed by the Standing Committee on Health and the Environment.

I have today sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their consideration.

Yours sincerely,

(signed)
Professor. W.A. van Gool,
President

Annex **D**

Comments on the public review draft

A draft of the present report was released in 2012 for public review. The following organisations and persons have commented on the draft document:

- Th.J. Lentz, National Institute for Occupational Safety and Health, Cincinnati (OH), USA
- G. Wallace, The European Chemical Industry Council (CEFIC), Lower Olefins Sector Group, Brussels, Belgium
- W.F.J.P.M. ten Berge, occupational toxicologist, Westervoort, The Netherlands

Annex **E**

Abbreviations

ATSDR	Agency for Toxic Substances and Disease Registry (USA)
AUC	area under the curve (in a blood concentration vs. time curve of a substance)
BER	base excision repair
BMD	benchmark dose
BMDL	benchmark dose at the lower 95% confidence level
butadiene	1,3-butadiene (CAS no. 106-99-0)
CA	chromosomal abberation
CI	confidence interval (95% unless otherwise stated)
CYP	cytochromes P-450
DECOS	Dutch Expert Committee on Occupational Exposure Standards
DHBMA	1,2-dihydroxybutyl mercapturic acid
DMDTC	dimethyldithiocarbamate
EPA	Environmental Protection Agency (USA)
GSH	glutathione
GST	glutathione S-transferase
Hb	haemoglobin
HBC-OCRV	health-based calculated occupational cancer risk value
hprt	hypoxanthine guanine phosphoribosyltransferase
IARC	International Agency for Research on Cancer
JHU	Johns Hopkins University (USA)

LC	lethal concentration
LD	lethal dose
LOAEL	lowest observed adverse effect level
(m)EH	(microsomal) epoxide hydrolase
MF	mutation frequency
MHBMA	monohydroxy-3-butenyl mercapturic acid
MHbVal	N-(2-hydroxy-3-butenyl)valine
NER	nucleotide excision repair
NIOSH	National Institute for Occupational Safety and Health (USA)
NOAEL	no observed adverse effect level
NTP	National Toxicology Program (USA)
OR	odds ratio: the ratio of the odds of an event occurring in one group to the odds of it occurring in another group (in epidemiology generally used in case-control studies)
PyrVal	N,N-(2,3-dihydroxy-1,4-butadiyl)valine
RR	relative risk: the ratio of the probability of an event occurring in an exposed group versus a non-exposed group (in epidemiology generally used in cohort studies)
SBR	styrene-butadiene rubber
SCE	sister chromatid exchange
SCOEL	Scientific Committee on Occupational Exposure Limits of the European Union
SMR	standardized mortality ratio
STEL	short term exposure limit
STYR	styrene
THbVal	N-(2,3,4-trihydroxybutyl)valine
TWA	time weighted average
UAB	University of Alabama at Birmingham (USA)

Annex **F**

Human epidemiological studies

(taken from IARC, 2008[2], and completed with additional information)

F.1 1,3-Butadiene monomer production

SMR = standardized mortality ratio; CI = confidence interval; TWA = time-weighted average

Table F.1.1 USA - Ward et al. 1995[13], 1996 (in IARC 2008[2]).

Cohort description	Exposure asessment	Organ site	Exposure categories	No. of deaths	SMR (95% CI)	Adjustment for potential confounders	Comments
364 male workers in three units (case-control; control = county mortality rate)	Employment in butadiene departments, no benzene or ethylene oxide present	All cancers	-	48	1.1 (0.8-1.4)	Age, time period; county reference rates	All 4 cases of lympho- and reticulosarcomas had been employed ≥ 2 years (SMR 8.3, 95% CI 1.6-14.8), as had those of stomach cancer (SMR 6.6, 95% CI 2.1-15.3); all occurred in the rubber reserve plant.
		Lymphatic and haematopoietic		7	1.8 (0.7-3.6)		
		Lympho- and reticulosarcoma		4	5.8 (1.6-14.8)		
		Leukaemia		2	1.2 (0.2-4.4)		

Table F.1.2 USA - Tsai et al. 2001[15].

Cohort description	Exposure asessment	Organ site	Exposure categories	No. of deaths	SMR (95% CI)	Adjustment for potential confounders	Comments
614 male workers	Employed ≥ 5 yrs in butadiene production; most 8-h TWAs for butadiene < 22 mg/m³	All cancers	-	16	0.6 (0.3-0.9)	Age, race, calendar year; reference county-specific rates	A concurrent morbidity study failed to show differences in haematological values between butadiene-exposed and unexposed workers within the complex
		Lymphatic and haemopoietic	-	3	1.1 (0.3-1.5)		

Table F.1.3 USA – Divine and Hartman 2001[14].

Cohort description	Exposure asessment	Organ site	Exposure categories	No. of deaths	SMR (95% CI)	Adjustment for potential confounders	Comments
2,800 male workers employed ≥ 6 months in 1943-96 (case-control; control = general population mortality rate)	Industrial hygiene sampling data	All cancers	Employed	333	0.9 (0.8-1.0)	Age, time period, age at hire	No increasing trend by duration of employment; no increasing trend by exposure group; lymphatic haematopoietic cancers and lymphosarcoma significantly increased in the highest exposure category; elevations were found in workers employed <1950, and were highest in short-term workers
			employed < 5 yr	170	1.0 (0.8-1.1)		
			employed 5-19 yr	55	0.8 (0.6-1.1)		
			employed ≥ 20 yr	108	0.8 (0.7-1.0)		
		Lympho-haemato-poietic	Employed	50	1.4 (1.1-1.9)		
			employed < 5 yr	26	1.6 (1.0-2.3)		
			employed 5-19 yr	8	1.2 (0.5-2.4)		
			employed ≥ 20 yr	16	1.3 (0.8-2.2)		
			High exposure < 5 yr	20	1.8 (1.1-2.8)		
			High exposure ≥ 5 yr	14	1.5 (0.8-2.5)		
			First employed 1942-1949	46	1.5 (1.1-2.1)		
			First employed ≥ 1950	4	0.7 (0.2-1.8)		

Non-Hodgkin lymphoma	Employed	19	1.5 (0.9-2.3)
	employed < 5 yr	12	1.3 (0.3-3.7)
	employed 5-19 yr	3	0.9 (0.3-2.3)
	employed ≥ 20 yr	4	2.0 (0.9-3.9)
	High exposure < 5 yr	8	1.1 (0.3-2.9)
	High exposure ≥ 5 yr	4	1.6 (0.9-2.6)
	First employed 1942-1949	17	1.6 (0.9-2.6)
	First employed ≥ 1950	2	0.9 (0.1-3.2)
Leukaemia	Employed	18	1.3 (0.8-2.0)
	employed < 5 yr	9	1.4 (0.6-2.6)
	employed 5-19 yr	2	0.7 (0.1-2.6)
	employed ≥ 20 yr	7	1.5 (0.6-3.1)
	High exposure < 5 yr	8	1.9 (0.8-3.7)
	High exposure ≥ 5 yr	5	1.4 (0.4-3.2)
	First employed 1942-1949	18	1.5 (0.9-2.4)
	First employed ≥ 1950	0	0 (0-178)

F.2 Styrene-butadiene rubber production

SMR = standardized mortality ratio; RR = relative risk; CI = confidence interval; DMDTC = dimethyldithiocarbamate; NR = not reported; SE = standard error; TWA = time-weighted average

Table F.2.1 USA – McMichael et al. 1976 (in IARC 2008[2]).

Cohort description	Exposure asessment	Organ site	Exposure categories	No. of cases	RR (99.1% CI)	Adjustment for potential confounders	Comments
Case-cohort of 6,678 male rubber workers	Employment for > 2 yr in SBR production based on work histories	All lymphatic and haematopoetic	≥ 5 yr in synthetic plant	51	6.2 (4.1-12.5)	Age	No information on exposure to specific compounds
		Lymphatic leukaemia		14	3.9 (2.6-8.0)		

Table F.2.2 USA – Meinhardt et al. 1982 (in IARC 2008[2], overlapping with Delzell et al. 1996[18]).

Cohort description	Exposure asessment	Organ site	Exposure categories	No. of deaths	SMR (95% CI)	Adjustment for potential confounders	Comments
2,756 white men employed ≥ 6 months (plant A: 1,662 men; plant B: 1,094 men)	Duration and time of employement	Lymphatic and haematopoetic	Plant A	9	1.6 (NR)	Age, time period, race	-
		Lymphosarcoma and reticulosarcoma	Plant A, total	3	1.8 (NR)		
			Plant A, working 1943-1945	3	2.1 (NR)		
			Plant B, total	1	1.3 (NR)		
		Leukaemia	Plant A, total	5	2.0 (NR)		
			Plant A, working 1943-1945	5	2.8 (NR)		
			Plant B, total	1	1.0 (NR)		

Table F.2.3 USA and Canada – Delzell et al. 1996[18] (includes data from Matanoski et al. 1990[16], 1993[17], 1997[19], Santos-Burgoa et al. 1992[20], and Meinhardt et al. 1982, Matanoski & Schwartz 1987, Lemen et al. 1990, in IARC 2008[2]).

Cohort description	Exposure asessment	Organ site	Exposure categories	No. of cases	RR (95% CI)	Adjustment for potential confounders	Comments
15,649 workers employed for at least one year in eight production plants in 1943-1991	8,281 unique combinations of work area/ job title, grouped in 308 work areas with similar exposure	All cancers	Five main process groups and seven subgroups	950	0.9 (0.9-1.0)	Age, race, calendar time	Among 'ever hourly paid' workers, 45 leukaemia deaths, (SMR 1.4, 95% CI 1.0-1.9); SMR for hourly workers having worked for > 10 years and hired ≥ years ago: 2.2 (95% CI 1.5-3.2), based on 28 leukaemia deaths
		Lymphosarcoma		11	0.8 (0.4-1.4)		
		Other lymphopoetic		42	1.0 (0.7-1.5)		
		Leukaemia	Polymerization	48	1.3 (1.0-1.7)		
			maintenance	15	2.5 (1.4-4.1)		
			labour	13	2.7 (1.4-4.5)		
			laboratories	10	4.3 (2.1-7.9)		

Table F.2.4a USA and Canada - Macaluso et al. 1996[27] (overlapping with Delzell et al. 1996[18]).

Cohort description	Exposure asessment	Organ site	Exposure categories mg/m^3-years	No. of deaths	SMR (95% CI)	Adjustment for potential confounders	Comments
12,412 subjects	Retrospective quantitative estimates of exposure to butadiene, styrene and benzene by work area	Leukaemia	0	8	0.8 (0.3-1.5)	Age, race, co-exposure to styrene and benzene	Including seven decendents for whom leukaemia was listed as contributory cause of death
			< 2.2	4	0.4 (0.4-1.1)		
			2.2 – 43	12	1.3 (0.7-2.3)		
			44 - 175	16	1.7 (1.0-2.7)		
			≥ 176	18	2.6 (1.6-4.1)		
			p-trend	-	= 0.01		

Table F.2.4b USA and Canada - Macaluso et al. 1996[27] (overlapping with Delzell et al. 1996[18]).

Cohort description	Exposure asessment	Organ site	Exposure categories mg/m³-years	No. of deaths	Mantel-Haenszel rate ratio	Adjustment for potential confounders	Comments
12,412 subjects	Retrospective quantitative estimates of exposure to butadiene, styrene and benzene by work area	Leukaemia	0	8	1.0	Race, cumulative exposure to styrene	Including seven decendents for whom leukaemia was listed as contributory cause of death
			< 2.2	4	2.0 (NR)		
			2.2 - 43	12	2.1 (NR)		
			44 - 175	16	2.4 (NR)		
			≥ 176	18	4.5 (NR)		
			p-trend	-	= 0.01		

Table F.2.5 USA and Canada - Matanoski et al. 1997[19] (overlapping with Delzell et al. 1996[18]).

Cohort description	Exposure asessment	Organ site	Exposure categories	No. of cases	RR (95% CI)	Adjustment for potential confounders	Comments
Nested case-control study from a cohort of 12,113 employees at SBR plant	Estimated cumulative exposure and average intensity of exposure to butadiene	Hodgkin lymphoma	Average intensity of exposure to butadiene, 2.2 mg/m³ compared with 0 mg/m³	8	1.7 (1.0-3.0)	Birth year, age at hire before 1950, race, length of employment	Non-Hodgkin lymphoma and multiple myeloma were not associated with exposure to butadiene
		Leukaemia		26	1.5 (1.1-2.1)		

Table F.2.6 USA and Canada - Sathiakumar et al. 1998 (in IARC 2008[2], same as Delzell et al. 1996[18]).

Cohort description	Exposure asessment	Organ site	Exposure categories	No. of deaths	SMR (95% CI)	Adjustment for potential confounders	Comments
12,412 subjects	Retrospective quantitative estimates of exposure to butadiene, styrene and benzene by work area	Non-Hodgkin lymphoma	Hourly workers ≥ 10 years worked and ≥ years since hire	14	1.4 (0.8-2.3)	Age, race, calendar time	No pattern by duration of employment, time since hire, period of hire or process group

Table F.2.7 USA and Canada - Delzell et al. 2001[25].

Cohort description	Exposure asessment	Organ site	Exposure categories butadiene mg/m³-years	No. of deaths	Poisson regression estimated relative rates (95% CI)	Adjustment for potential confounders	Comments
13,130 men employed for at least one year during 1943-1991 at six SBR plants	Quantitative estimates	Leukaemia	0	7	1.0	Age, years since hire	The association of risk for leukaemis with butadiene was stronger for mg/m³-years due to exposure intensities > 221 mg/m³
			> 0 - < 191	17	1.2 (0.5-3.0)		
			191 - < 800	18	2.0 (0.8-4.8)		
			≥ 800	17	3.8 (1.6-9.1)		
			p-trend	-	< 0.001		
			0	7	1.0	Age, years since hire, co-exposure to other agents	
			> 0 - < 191	17	1.3 (0.4-4.3)		
			191 - < 800	18	1.3 (0.4-4.6)		
			≥ 800	17	2.3 (0.6-8.3		
			p-trend	-	= 0.250		
			Exposure intensity < 221 mg/m³				
			0	7	1.0	Age, years since hire	
			> 0 - < 191	17	1.1 (0.5-2.7)		
			191 - < 213	17	2.8 (1.2-6.8)		
			≥ 213	18	3.0 (1.2-7.1)		
			p-trend	-	= 0.25		
			Exposure intensity > 221 mg/m³				
			0	7	1.0	Age, years since hire	
			> 0 - < 103	17	2.1 (0.9-5.1)		
			103 - < 519	17	2.8 (1.2-6.7)		
			≥ 519	18	5.8 (2.4-13.8)		
			p-trend	-	= 0.01		

Table F.2.8 USA and Canada - Graff et al. 2005[22].

Cohort description	Exposure asessment	Organ site	Exposure categories butadiene mg/m³-years	No. of deaths	Poisson regression estimated relative rates (95% CI)	Adjustment for potential confounders	Comments
16,579 men working at six plants ≥ 1 year by 1991 and followed up through to 1998	Same as Delzell et al. 2001; cumulative exposure estimates for butadiene, styrene and DMDTC	Leukaemia	0	10	1.0	Age, years since hire	SMR analyses with external reference rates (national and state-specific) also conducted and results for leukaemia consistent with those of internal analysis using Poisson regression models
			> 0 - < 75	7	1.4 (0.7-3.1)		
			75 - < 408	18	1.2 (0.6-2.7)		
			408- < 939	18	2.9 (1.4-6.4)		
			≥ 939	18	3.7 (1.7-8.0)		
			p-trend	-	< 0.001		
		Leukaemia	0	10	1.0	Age, years since hire, other agents	
			> 0 - < 75	17	1.4 (0.5-3.9)		

	75 - < 408	18	0.9 (0.3-2.6)	
	408- < 939	18	2.1 (0.7-6.2)	
	≥ 939	18	3.0 (1.0-9.2)	
	p-trend	-	= 0.028	
Chronic lympho-cytic leukaemia	< 75	7	1.0	
	75 - < 939	11	1.5 (0.6-4.0)	
	≥ 939	7	3.9 (1.3-11.0)	
	p-trend	-	= 0.014	
Chronic myelo-genous leukaemia	< 75	3	1.0	
	75 - < 939	8	2.7 (0.7-10.4)	
	≥ 939	5	7.2 (1.7-30.5)	
	p-trend	-	= 0.007	
Other leukaemia	< 75	5	1.0	
	75 - < 939	5	1.1 (0.3-3.9)	
	≥ 939	4	4.0 (0.3-15.0)	
	p-trend	-	= 0.060	

Table F.2.9 USA and Canada - Sathiakumar et al. 2005[23].

Cohort description	Exposure asessment	Organ site	Exposure categories	No. of deaths	SMR (95% CI)	Adjustment for potential confounders	Comments
17,924 male workers employed ≥ 1 year before 1992 followed through to 1998	Same as Delzell et al. 1996	All cancer	Hourly workers	1,608	0.92 (0.88-0.97)	Age, race, calendar period	Leukaemia excesses in production mainly due to chronic lymphatic leukaemia: polymerization (8 cases, SMR 4.0, 95% CI 2.1-9.8), coagulation (5 cases, SMR 6.1, 95% CI 2.0-14.2), and finishing (7 cases, SMR 3.4, 95% CI 1.4-7.1); myelogenous leukaemia particularly high in maintenance labour (acute, 5 cases, SMR 3.0, 95% CI 1.0-6.9) and laboratory (total 6 cases, SMR 3.3, 95% CI 1.2-7.2, chronic 3 cases, SMR 5.2, 95% CI 1.1-15.3)
		Lymphohae matopoietic	Hourly workers	162	1.1 (0.9-1.2)		
		Hodgkin lymphoma	Hourly workers	12	1.1 (0.6-2.0)		
		Multiple myeloma	Hourly workers	26	1.0 (0.6-1.4)		
		Non-Hodgkin lymphoma	All workers	53	1.0 (0.8-1.3)		
			Hourly workers	49	1.1 (0.8-1.5)		
		Chronic lympho-cytic leukaemia	All workers	16	1.5 (0.9-2.5)		
			Hourly workers	15	1.7 (0.9-2.8)		
		Leukaemia	All workers	71	1.2 (0.9-1.5)		
			Hourly workers	63	1.2 (0.9-1.6)		
			≥ 20 years since hire 10 years worked				
			Production				
			polymerization	18	2.0 (1.2-3.2)		
			coagulation	10	2.3 (1.1-4.3)		
			finishing	19	1.6 (0.9-2.4)		
			labour maintenance	15	2.0 (1.1-3.4)		
			laboratories	14	3.3 (1.8-5.5)		

Table F.2.10 USA and Canada - Delzell et al. 2006 (in IARC 2008[2]).

Cohort description	Exposure asessment	Organ site	Exposure categories butadiene mg/m³-years	No. of cases	RR (95% CI)	Adjustment for potential confounders	Comments
Same as Graff et al. 2005	Work histories and exposure data as Delzell et al. 2001; exposure estimation procedures as Macaluso et al. 2004	Non-Hodgkin lymphoma	0	11	1.0	Age, years since hire, other agents	-
			> 0 - < 75	16	1.0 (0.4-2.6)		
			75 - < 408	10	0.4 (0.1-1.2)		
			408 - < 939	12	0.9 (0.3-2.7)		
			≥ 939	9	0.7 (0.2-2.3)		
		Non-Hodgkin lymphoma and chronic lymphocytic leukaemia combined	0	12	1.0		
			> 0 - < 75	18	0.9 (0.4-2.1)		
			75 - < 408	14	0.4 (0.2-1.1)		
			408 - < 939	17	1.0 (0.4-2.7)		
			≥ 939	14	0.9 (0.3-2.7)		
		Lymphoid neoplasms	0	24	1.0		
			> 0 - < 75	28	0.9 (0.5-2.0)		
			75 - < 408	25	0.7 (0.3-1.6)		
			408 - < 939	21	1.3 (0.6-3.1)		
			≥ 939	22	1.5 (0.6-3.8)		
		Myeloid neoplasms (erythroleu-kaemia, myelofibrosis, myelodysplasia, polycythemia vera, myeloproliferative disease	< 75	19	1.0		
			75 - < 408	15	0.8 (0.3-1.7)		
			408 - < 939	11	1.6 (0.6-4.1)		
			≥ 939	11	2.4 (0.9-6.8)		

Table F.2.11 USA and Canada - Cheng et al. 2007[24].

Cohort description	Exposure asessment	Organ site	Exposure categories	No. of deaths	Cox regression coefficient (ß) for exposure response, SE, and p-value	Adjustment for potential confounders	Comments
Same as Sathiakumar et al. 2005 (case-control; control = state-specific US and Canadian male mortality rate)	Same as Delzell et al. 2001	Leukaemia	*Cumulative butadiene mg/m³-years* Continuous	81	ß = 3.0×10^{-4} SE = 1.4×10^{-4} P = 0.04 (0.1×10^{-4} - 5.8×10^{-4})	Age, year of birth, plant, years since hire, DMDTC	Lymphoid neoplasms associated with butadiene mg/m³-years and myeloid neoplasms with butadiene peaks, neither trend significant after adjusting for

Mean scored deciles	ß = $5.8x10^{-4}$ SE = $2.7x10^{-4}$ P = 0.03 ($0.5x10^{-4}$ – $11.1x10^{-4}$)	covariates; DMDTC as a continuous variable not associated with leukaemia, risk estimates for quartiles of exposure to DMDTC significantly increased without monotonic trend
Total number of peaks		
Continuous	ß = $5.6x10^{-5}$ SE = $2.4x10^{-5}$ P = 0.02 ($0.8x10^{-5}$ - $10.4x10^{-5}$)	
Mean scored deciles	ß = $7.5x10^{-5}$ SE = $3.7x10^{-5}$ P = 0.04 ($0.3x10^{-5}$ – $14.7x10^{-5}$)	
Average intensity		
Continuous	ß = $3.6x10^{-3}$ SE = $2.1x10^{-3}$ P=0.09 ($-0.5x10^{-3}$ - $7.7x10^{-3}$)	
Mean scored deciles	ß = $3.8x10^{-3}$ SE = $3.7x10^{-3}$ P=0.40 ($-3.5x10^{-3}$ - $11.0x10^{-3}$)	

Table F.2.12a USA and Canada - Sielken et al. 2007[30].

Cohort description	Exposure asessment	Organ site	Exposure categories Cumulative butadiene mg/m³-years	No. of deaths	SMR	Adjustment for potential confounders	Comments
Same as Sathiakumar et al. 2005 (case-control, control = state specific US and Canadian male mortality rate)	Same as Sathiakumar et al. 2005	Leukaemia	All	68	1.24	Age, year since hire, calendar year, race	Data continued in Tables F.2.12b and F.2.12c
			≤ 2,957	65	1.21		
			≤ 2,210	62	1.17		
			≤ 1,105	58	1.16		
			≤ 884	54	1.11		
			≤ 663	50	1.08		
			≤ 442	5	1.05		
			≤ 221	38	1.06		

Table F.2.12b USA and Canada - Sielken et al. 2007[30].

Covariate considered for inclusion in the Poisson regression model	Slope of linear rate ratio model (SE)	Maximum log-likelihood (covariate included)	Maximum log likelihood (covariate excluded)	Chi-square statistic	p-value	Comments
Age	1.68×10^{-3} (8.21×10^{-4})	-83.96	-120.60	73.28	4.6×10^{-15} [a]	The likelihood and estimated slope after one non-exposure or exposure covariate is added to the Poisson regression model with the rate ratio being a linear function of cumulative butadiene mg/m³-years
Years since hire	1.52×10^{-3} (7.75×10^{-4})	-84.15	-109.62	50.95	2.3×10^{-10} [a]	
Calendar year	3.28×10^{-3} (1.31×10^{-3})	-87.32	-98.83	23.01	0.00013 [a]	
Race	3.56×10^{-3} (1.46×10^{-3})	-41.49	-41.51	0.05	0.82	
Butadiene peaks (peak-years)	5.77×10^{-4} (5.49×10^{-4})	-68.75	-80.50	23.51	0.00027 [a]	
Butadiene > 221 mg/m³-year	7.33×10^{-4} (1.51×10^{-3})	-49.01	-52.37	6.72	0.24	
Butadiene ≤ 221 mg/m³-year	7.53×10^{-4} (8.98×10^{-4})	-53.19	-55.64	4.90	0.43	

[a] 1% significance level

Table F.2.12c USA and Canada - Sielken et al. 2007[30].

Covariate considered for inclusion in the Poisson regression model	Slope of linear rate ratio model (SE)	Maximum log-likelihood (covariate included)	Maximum log-likelihood (covariate excluded)	Chi-square statistic	p-value	Comments
Years since hire	1.54×10^{-3} (7.78×10^{-4})	-171.16	-176.01	9.71	0.046[a]	The likelihood and estimated slope after age has been added as a categorial covariate and one additional non-exposure or exposure covariate is added to the Poisson regression model with the rate ratio being a linear function of cumulative butadiene mg/m³-years
Calendar year	1.65×10^{-3} (8.15×10^{-4})	-189.14	-191.45	4.61	0.33	
Race	1.57×10^{-3} (8.35×10^{-4})	-107.72	-107.80	0.15	0.70	
Butadiene peaks (peak-years)	1.89×10^{-4} (3.60×10^{-4})	-155.77	-167.27	22.99	0.00034[b]	
Butadiene > 221 mg/m³-year	6.08×10^{-5} (4.67×10^{-4})	-127.77	-132.73	9.91	0.078	
Butadiene ≤ 221 mg/m³-year	6.67×10^{-4} (8.68×10^{-4})	-135.71	-137.65	3.88	0.57	

[a] 5% significance level
[b] 1% significance level

Table F.2.13 USA and Canada - Sathiakumar and Delzell 2009[28].

Cohort description	Exposure asessment	Organ site	Exposure categories	No. of cases	SMR (95% CI)	Adjustment for potential confounders	Comments
4.863 women working at six plants ≥ 1 year by 1991 and followed up through to 2002	Retrospective quantitative estimates of exposure to butadiene and styrene by work area	Leukaemia	SBR-related operations, ever hourly	0	0 (0-1.4)	Age, years since hire, every hourly status (see also 'Comments')	There was generally a high correlation between the exposures to butadiene (in mg/m^3-years) and styrene (in mg/m^3-years); attempts to discriminate between the two did not result in any significant difference. Adjustment for smoking reduced the association between exposure category and lung canceer by 8-11%
			SBR-related operations, never hourly	1	0.7 (0-4.1)		
			Residual operations, ever hourly	2	1.2 (0.1-4.2)		
			Residual operations, never hourly	0	0 (0-1.9)		
			Administration, never hourly	7	1.1 (0.5-2.4)		
		Non-Hodgkin lymphoma	SBR-related operations, ever hourly	4	1.4 (0.4-3.7)		
			SBR-related operations, never hourly	1	0.6 (0-3.5)		
			Residual operations, ever hourly	4	2.2 (0.6-5.8)		
			Residual operations, never hourly	1	0.4 (0-2.5)		
			Administration, never hourly	7	1.0 (0.4-2.0)		
		Multiple myeloma	SBR-related operations, ever hourly	2	1.0 (0.1-3.6)		
			SBR-related operations, never hourly	1	1.4 (0-7.7)		
			Residual operations, ever hourly	1	0.8 (0-4.2)		
			Residual operations, never hourly	0	1.0 (0-5.3)		
			Administration, never hourly	3	0.9 (0.2-2.6)		
		Hodgkin lymphoma	SBR-related operations, ever hourly	0	0 (0-9.1)		

	SBR-related operations, never hourly	0	0 (0-23.3)
	Residual operations, ever hourly	0	0 (0-18.4)
	Residual operations, never hourly	0	0 (0-19.6)
	Administration, never hourly	1	1.4 (0-7.5)
Breast	SBR-related operations, ever hourly	11	0.7 (0.4-1.3)
	SBR-related operations, never hourly	8	0.9 (0.4-1.8)
	Residual operations, ever hourly	7	0.8 (0.3-1.6)
	Residual operations, never hourly	9	0.8 (0.3-1.4)
	Administration, never hourly	40	1.1 (0.8-1.5)
Ovary	SBR-related operations, ever hourly	5	1.2 (0.4-2.8)
	SBR-related operations, never hourly	2	0.8 (0.1-2.9)
	Residual operations, ever hourly	2	0.8 (0.1-2.8)
	Residual operations, never hourly	5	1.4 (0.5-3.4)
	Administration, never hourly	10	1.0 (0.5-1.8)
Lung	SBR-related operations, ever hourly	34	1.7 (1.1-2.4)
	SBR-related operations, never hourly	12	1.1 (0.6-1.9)
	Residual operations, ever hourly	15	1.6 (0.9-2.6)

	Residual operations, never hourly	16	1.1 (0.6-1.8)
	Administration, never hourly	36	0.8 (0.6-1.1)
Bladder	SBR-related operations, ever hourly	2	1.9 (0.2-6.8)
	SBR-related operations, never hourly	0	0 (0-8.6)
	Residual operations, ever hourly	4	5.2 (1.4-13.4)
	Residual operations, never hourly	0	0 (0-5.8)
	Administration, never hourly	3	1.4 (0.3-4.2)

Table F.2.14 USA and Canada - Sielken and Valdez-Flores 2011[31].

Cohort description		Exposure assessment				Organ site
Same as Sathiakumar et al. 2005, 2007		Same as Sathiakumar et al. 2005, 2007				Leukaemia
Covariate considered for inclusion in the Cox proportions hazard model	Slope of cumulative BTD mg/m³-years in the log-linear rate ratio model (SE)	Maximum log likelihood (covariate included)	Maximum log likelihood (covariate excluded)	Chi-square statistic	p-value	Comments
None [a]	$2.90x10^{-4}$ ($1.03x10^{-4}$)	not applicable				Total leukaemia: increase in the maximum log-likelihood when one of the non-exposure or exposure covariates is added to the Cox proportional hazards model with the rate ratio being a log-linear function of cumulative BTD mg/m³-years.
Years since hire [b]	$2.92x10^{-4}$ ($1.04x10^{-4}$)	-689.90	-692.08	4.36	0.3591	
Calendar year [b]	$2.84x10^{-4}$ ($1.03x10^{-4}$)	-689.48	-692.08	5.20	0.2672	
Race [c]	$2.59x10^{-4}$ ($1.16x10^{-4}$)	-691.88	-692.08	0.40	0.5286	
Plant [d]	$3.88x10^{-4}$ ($1.16x10^{-4}$)	-687.93	-692.08	8.31	0.1399	
STYR (mg/m³-years)	$2.15x10^{-4}$ ($1.31x10^{-4}$)	-688.45	-692.08	6.64	0.2491	
DMDTC (mg/m³-year)	$1.79x10^{-4}$ ($1.23x10^{-4}$)	-681.39	-692.08	21.68	0.0006 [e]	
Number of BTD high intensity tasks	$2.01x10^{-4}$ ($1.30x10^{-4}$)	-679.23	-692.08	23.49	0.0003 [e]	
Number of STYR high intensity tasks	$1.13x10^{-4}$ ($1.40x10^{-4}$)	-679.77	-692.08	24.83	0.0002 [e]	
BTD ≤ 221 mg/m³-year	$2.03x10^{-4}$ ($1.36x10^{-4}$)	-688.49	-692.08	7.18	0.2078	
BTD > 221 mg/m³-year	$1.39x10^{-4}$ ($1.57x10^{-4}$)	-684.63	-692.08	14.90	0.0108 [f]	
STYR ≤ 215 mg/m³-year	$2.18x10^{-4}$ ($1.32x10^{-4}$)	-685.90	-692.08	11.54	0.0417 [f]	
STYR > 215 mg/m³-year	$1.59x10^{-4}$ ($1.40x10^{-4}$)	-678.64	-692.08	27.82	$3.9x10^{-5}$ [e]	

BTD: 1,3-butadiene; STYR: styrene; DMDTC: dimethyldithiocarbamate.

[a] Cox model with only cumulative BTD mg/m³-years
[b] Categories for years since hire and calendar year were based on quintiles of leukaemia decendents.
[c] Race was categorized as black and others.
[d] Covariates for cumulative exposures were partitioned as controls and quintiles of exposed leukaemia decendents.
[e] 1% significance level.
[f] 5% significance level.

Annex **G**

Animal studies

G.1 Carcinogenicity studies with 1,3-butadiene

h = hour; d = day; w = week; m = month; y = year; M = male; F = female; freq = frequency; X_{po} = duration of exposure; X_{pe} = duration of the experiment; bw = body weight; ip = intraperitoneal; sc = subcutaneous

Table G.1.1 Rat - IARC 2008[2], DECOS 1990[1], Owen & Glaister 1990[39].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Rat Sprague-Dawley	0, 2,200, 17, 600 mg/m³ (whole-body inhalation)	6 h/d, 5d/w	M/F (100/sex)	M: 111 w F: 105 w	M: 111 w F: 105 w	M: 45, 50, 32 F: 46, 32, 24	M: 84, 70, 87 F: 97, 98, 94	M: 3/100, 1/100 and 10/100 pancreatic exocrine adenoma ($p \leq 0.001$); 0/100, 3/100 and 8/100 interstitial-cell tumour of testis (p for trend ≤ 0.001) F: 0/100, 2/100 and 10/100 follicular-cell adenoma of thyroid gland (p for trend ≤ 0.01); 1/100, 4/100 and 5/100 sarcoma of uterus (p for trend ≤ 0.05); 0/100, 0/100 and 4/100 carcinoma of Zymbal gland (p for trend ≤ 0.01); 18/100, 15/100 and 26/100 mammary adenocarcinoma

Table G.1.2 Mouse - IARC 2008[2], DECOS 1990[1].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Mouse B6C3F1	0, 1,380, 2,760 mg/m³ (whole-body inhalation)	6 h/d, 5 d/w	M/F (50/sex)	60/61 w	61 w[a]	M: 49, 11, 7 F: 46, 15, 30	see comments	M: 0/50, 16/49, 7/49 heart haemangiosarcoma; 0/50, 23/50, 29/50 malignant lymphoma; 2/50, 14/49, 15/49 lung alveolar/ bronchiolar adenoma/ carcinoma; 0/49, 7/40, 1/44 forestomach papilloma/carcinoma; brain glioma in 1 low dose and 2 high dose males F: 0/50, 11/48, 18/49 heart haemangiosarcoma; 1/50, 10/49, 10/49 malignant lymphoma; 3/49, 12/48, 23/49 lung alveolar/ bronchiolar adenoma/ carcinoma; 0/49, 5/42, 10/49 forestomach papilloma/carcinoma; 0/50, 2/47, 5/49 hepatocellular adenoma/ carcinoma; 0/50, 2/49, 6/49 mammary acinar-cell carcinoma; 0/49, 6/45, 12/48 ovarian granulosa-cell tumours All incidences (except glioma) in treated animals were statistically significantly increased

[a] terminated because of high incidence of deaths (mainly due to malignant lymphomas).

Table G.1.3 Mouse - IARC 2008[2], NTP 1993[37], Melnick et al. 1990[38].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Mouse B6C3F1	0, 14, 44, 138, 440, 1,380 mg/m^3 (whole-body inhalation)	6 h/d, 5 d/w	M/F (70/sex; highest dose group 90/sex)	up to 2 y	2 y	M: 35/70, 39/70, 24/70, 22/70, 3/70, 0/90 F: 37/70, 33/70, 24/70, 11/70, 0/70, 0/90	M: 44, 40, 45, 48, 49, 62 F: 35, 47, 43, 48, 49, 72	M: 4, 3, 8, 11, 9, 69 lymphoma; 0, 0, 1, 5, 20, 6 heart haemangiosarcoma; 22, 23, 20, 33, 42, 12 lung alveolar/bronchiolar adenoma/carcinoma; 1, 0, 1, 5, 12, 13 forestomach papilloma/carcinoma; 6, 7, 11, 24, 33, 7 Harderian gland adenoma/adenocarcinoma; 31, 27, 35, 32, 40, 12 hepatocellular adenoma/carcinoma; 0, 0, 0, 0, 5, 0 preputial gland adenoma/carcinoma F: 10, 14, 18, 10, 19, 43 lymphoma; 0, 0, 0, 1, 20, 26 heart haemangiosarcoma; 4, 15, 19, 27, 32, 25 lung alveolar/bronchiolar adenoma/carcinoma; 2, 2, 3, 4, 7, 28 forestomach papilloma and carcinoma; 9, 10, 7, 16, 22, 7 Harderian gland adenoma/adenocarcinoma; 17, 20, 23, 24, 20, 3 hepatocellular adenoma/carcinoma; 0, 2, 2, 6, 13, 13 mammary gland adenocarcinoma; 1, 0, 0, 9, 11, 6 ovarian benign and malignant granulosa-cell tumours

Table G.1.4 Mouse - IARC 2008[2], NTP 1993[37], Melnick et al. 1990[38].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Mouse B6C3F1	0, 440 mg/m³ for 40 w, 690 mg/m³ for 52 w; 1,380 mg/m³ for 13 or 26 w (whole-body inhalation)	6 h/d, 5d/w	M (50; 70 controls)	13, 40 or 52 w	104 w	35, 9, 1, 5, 0	44, 49, 50, 49, 49	4, 12, 15, 24, 37 lymphoma; 0, 15, 33, 7, 13 hearth haemangiosarcoma; 22, 35, 32, 27, 18 lung alveolar/ bronchiolar adenoma/ carcinoma; 1, 6, 13, 8, 11 forestomach squamous-cell papilloma/ carcinoma; 6, 27, 28, 23, 11 Harderian gland adenoma/adenocarcinoma; 0, 1, 4, 5, 3 preputial gland adenoma/carcinoma; 0, 5, 3, 1, 1 renal tubular adenoma; two neuroblastoma and three glioma at 1380 mg/m3 for 13 or 26 w

Table G.1.5 Mouse - IARC 2008[2].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Mouse B6C3F1/ NIH Swiss	B6C3F1: 0, 2,760 mg/m³ for 12 or 52 w; NIH Swiss: 2,760 mg/m³ (whole-body inhalation)	6 h/d, 5d/w	M (50-60)	12 or 52 w	52 w	not specified		1/60, 10/48, 34/60 thymic lymphoma for B6C3F1 mice and 8/57 thymic lymphoma for Swiss mice; 5/60 heart haemangiosarcoma in B6C3F1 and 1/57 heart haemangiosarcoma in NIH Swiss mice treated for 52 weeks; their hypothesis that the high incidence of lymphoma was partially caused by activation of an endogenous retrovirus in B6C3F1 mice was confirmed (NIH Swiss does not express this virus)

Table G.1.6 Mouse - IARC 2008[2].

Species	Dose	Freq	Sex (no./ group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Mouse B6C3F1	0, 2,200, 11,000, 22,000 mg/m³ (whole-body inhalation)	single 2 h	M/F (60/sex)	single	2 y	M: 28/60, 34/60, 44/60, 34/60 F: 45/60, 36/60, 38/60, 48/60	comparable to control	-

G.2 Carcinogenicity studies with 1,2-epoxybutene (epoxybutene)

Table G.2.1 Mouse - IARC 2008[2].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Mouse Swiss	untreated, 100 mg epoxybutene (dermal)	3x/w	M (30)	lifetime	lifetime	not specified	4	3 skin papilloma; 1 squamous-cell carcinoma (according to IARC similar incidence as in untreated group)

G.3 Carcinogenicity studies with 1,2:3,4-diepoxybutane (diepoxybutane)

Table G.3.1 Rat - IARC 2008[2], DECOS 1990[1].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Rat Sprague-Dawley	0, 1 mg D, L-diepoxy-butane in 0.1 ml tricaprylin (sc)	1x/w	F (50)	550 d	550 d	not specified	not specified	0: 9 local fibrosarcoma; 1: 1 breast adenocarcinoma

Table G.3.2 Rat - IARC 2008[2].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Rat Sprague-Dawley	5 mg/ml diepoxybutane in 0.5 ml tricaprylin (gavage)	1x/w	F (5)	363 d	363 d	not specified	not specified	no gastric tumours

Table G.3.3 Rat - IARC 2008[2].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Rat Sprague-Dawley	0, 8.8, 17.6 mg/m^3 D,L-diepoxybutane (inhalation)	6 h/d; 5d/w	F (56)	6 w	up to 18 m	reduced survival, not further specified	not specified	0/47, 12/48, 24/48 nasal mucosal tumours (principally squamous-cell carcinoma); multiple tumours in 3 rats at 17.6 mg/m^3

Table G.3.4 Mouse - IARC 2008[2], DECOS 1990[1].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Mouse Swiss	0, 100 mg D,L-diepoxybutane or meso-diepoxybutane in acetone (dermal)	3x/w	M (30; 120 control)	lifetime	lifetime	reduced survival	not specified	control: 8 skin papilloma; no carcinoma D,L: 2 skin papilloma; 1 squamous-cell carcinoma meso: 6 skin papilloma; 4 squamous-cell carcinoma

Table G.3.5 Mouse - IARC 2008[2].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Mouse Swiss	0, 3 or 10 mg D,L-diepoxy-butane or meso-diepoxybutane in acetone (dermal)	3x/w	F (30, 60 control)	lifetime	lifetime	not specified	not specified	control: none D,L: 10 skin papilloma and 6 squamous-cell carcinoma at 3 mg and 1 skin papilloma at 10 mg meso: 1 skin papilloma at 3 mg and 5 skin papilloma and 4 squamous-cell carcinoma at 10 mg

Table G.3.6 Mouse - IARC 2008[2].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Mouse strain A	0, 1.7-192 mg/kg bw L-diepoxybutane in water or tricaprylin (ip)	3x/w	M/F (15/sex)	12 w	39 w	not specified	incidence: 40-78% for L-diepoxybutane versus 27-37% for controls	lung tumours

Table G.3.7 Mouse - IARC 2008[2].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Mouse Swiss	0.1 or 1.1 mg D,L-diepoxy-butane in tricaprylin (sc)	1x/w	M (30-50)	401-589 d	401-589 d	not specified	not specified	0: no tumour in 110 mice 0.1: 5/50 local fibrosarcoma; 2/50 breast adenocarcinoma 1.1: 5/30 local sarcoma

Table G.3.8 Mouse - IARC 2008[2].

Species	Dose	Freq	Sex (no./group)	Xpo	Xpe	No. survivors	No. animals with tumours	Specified tumours / comments
Mouse B6C3F1	0, 8.8, 17.6 mg/m^3 D,L-diepoxy-butane (inhalation)	6 h/d, 5 d/w	F (56)	6 w	up to 18 m	reduced survival (due to nasal lesions)	not specified	18 months: 0/40, 2/42, 5/36 ($p < 0.05$) Harderian gland tumours; tumours in nasal mucosa, reproductive organs, lymph nodes, bone, liverpancreas and lung were not statistically significantly increased

Annex **H**

DNA base-adducts formed from 1,3-butadiene metabolites in vitro

Data from IARC 2008[2], unless indicated otherwise.

BD = 1,3-butadiene; CD = circular dichroism; DEB = diepoxybutane; dG = deoxyguanine; dGMP = desoxyguanosine monophosphate; EB = epoxybutene; EBD = epoxybutanediol; FAB = positive ion fast atom bombardment; G = guanosine; HMVK = hydroxymethylvinyl ketone; HPLC = high-performance liquid chromatography; LC = liquid chromatography; NMR = nuclear magnetic resonance; MS = mass spectrometry; MS/MS = tandem mass spectrometry; THBG = trihydroxybutylguanine; UV = ultraviolet.

Target	Butadiene metabolite	Adducts formed	Analytical methods	Reference
2'-Deoxy-adenosine	EB	(R)-N6-(1-Hydroxy-3-buten-2-yl)deoxyadenosine; (S)-N6-(1-hydroxy-3-buten-2-yl)deoxyadenosine	NMR, MS, CD	Nechev et al. (2001)
2'-Deoxy-guanosine	EB	(R)-N2-(1-Hydroxy-3-buten-2-yl)deoxyguanosine; (S)-N2-(1-hydroxy-3-buten-2-yl)deoxyguanosine	NMR, MS, CD spectra	Nechev et al. (2001)
2'-Deoxy-guanosine	EB	N7-(2-Hydroxy-3-butenyl)guanine (G1) (equal amounts); N7-(1-(hydroxymethyl)-2-propenyl)guanine (G2) (equal amounts)	LC/MS, NMR	Boogaard et al. (2001, 2004)
Single- and double-stranded calf thymus DNA	EB	N7-(2-Hydroxy-3-buten-1-yl)guanine (G1);N7-(1-hydroxy-3-buten-2-yl)guanine (G2); diastereomers of N3-(2-hydroxy-3-buten-1-yl)deoxyuridine; N6-(2-hydroxy-3-buten-1-yl)deoxyadenosine; N3-(2- hydroxy-3-buten-1-yl)adenine (A1); N3-(1-hydroxy-3-buten-2-yl)adenine (A2)	HPLC, UV, FAB-MS	Selzer & Elfarra (1999), Elfarra et al. (2001)

Calf thymus DNA	EB	N7-(2-Hydroxy-3-butenyl)guanine (G1); N7-(I-[hydroxymethyl[-2-propenyl)guanine (G2); N3-(2-hydroxy-3-butenyl)adenine (A1); N3-(1-hydroxymethyl-2-propenyl)adenine (A2)	HPLC, UV	Boogaard et al. (2004)
2'-Deoxy-guanosine	EBD	N7-(1-[Hydroxymethyl[-2,3-dihydroxypropyl) guanine (major); (G3) N7-(2,3,4-trihydroxybut-1-yl)guanine (minor) (G4)	LC/MS, NMR	Boogaard et al. (2001)
Deoxy-adenosine-5'-monophosphate	EBD	N6-2,3,4-Trihydroxybutyladenine; N1-trihydroxybutyladenine	-	Zhao et al. (1998)
2'-Deoxy-guanosine-5'-phosphate, calf thymus DNA	EBD	N7-(2,3,4-Trihydroxybut-1-yl)guanine (G4)	HPLC, UV	Koivisto et al. (1999)
Salmon testis DNA	DEB	N6-2,3,4-Trihydroxybutyladenine; N1-trihydroxybutyladenine	-	Zhao et al. (1998)
2'-Deoxy-adenosine; calf thymus DNA	DEB	N6,N6-(2,3-Dihydroxybutan-1,4-diyl)-2'-deoxyadenosine; 1,N6-(2-hydroxy-3-hydroxymethylpropan-1,3-diyl)-2'-deoxyadenosine; 1,N6-(1-hydroxymethyl-2-hydroxypropan-1,3-diyl)-2'-deoxyadenosine	UV, NMR, MS/MS	Seneviratne et al. (2010[85])
2'-Deoxy-guanosine	DEB	Diastereomeric pairs of N-(2-hydroxy-1-oxiranylethyl)-2'-deoxyguanosine (P4-1 and P4- 2); 7,8-dihydroxy-3 -(2-deoxy-ß-D-erythro-pentofuranosyl)-3,5,6,7,8,9-hexahydro-1,3-diazepino[1,2-a]purin-11(11H)one (P6); 1-(2-hydroxy-2-oxiranylethyl)-2'deoxyguanosine (P8 and P9); 1-(3-chloro-2-hydroxy-1-[hydroxymethyl]propyl)-2'-deoxyguanosine (1AP9 and 2AP9); 4,8-dihydroxy-1-(2-deoxy-ß-D-erythro-pentofuranosyl)-9-hydroxymethyl-6,7,8,9-tetrahydro-1H-pyrimido(2,1-b) purinium ion (1BP4 and 2BP4); 6-oxo-2-amino-9-(2-deoxy-ß-D-erythropentofuranosyl)-7-(2-hydroxy-2-oxiranylethyl)-6,9-dihydro-1H purinium ion (P5 and P5')	HPLC, MS, NMR	Zhang & Elfarra (2003)
2'-Deoxy-guanosine	DEB	7-Hydroxy-6-hydroxymethyl-5,6,7,8-tetrahydropyrimido (1,2-a)purin-10(1H)one (H2); 2-amino-1-(4-chloro-2,3-dihydroxybutyl)1,7-dihydro-6H-purine-6-one (H4); 2-amino-1-(2,3,4-trihydroxybutyl)-1,7-dihydro-6H-purin-6-one (H1'/H5'); 7,8-dihydroxy-1,5,6,7,8,9-hexahydro-1,3-diazepino(1,2a)purin-11(11H)one (H2'); 5-(3,4-dihydroxy-1-pyrrolidinyl)-2,6-diamino-4(3H)pyrimidinone (H3'); 2-amino-7-(3-chloro-2,4-dihydroxybutyl)-1,7-dihydro-6H-purin-6-one (H3); 2-amino-7-(2,3,4-trihydroxybutyl)-1,7-dihydro-6H-purin-6-one (H4')	HPLC, MS, NMR	Zhang & Elfarra (2004)
2'-Deoxy-guanosine	DEB	Diastereomeric pairs of N-(2-hydroxy-1-oxiranylethyl)-2'-deoxyguanosine (P4-1 and P4-2); 7,8-dihydroxy-3-(2-deoxy-ß-D-erythro-pentofuranosyl)-3,5,6,7,8,9-hexahydro-1,3-diazepino(1,2-a)purin-11(11H)one (P6); 1-(2-hydroxy-2-oxiranylethyl)-2'deoxyguanosine (P8 and P9); 6-oxo-2-amino-9-(2-deoxy-ß-D-erythro-pentofuranosyl)-7-(2-hydroxy-2-oxiranylethyl)-6,9-dihydro-1H purinium ion (P5 and P5')	HPLC, UV, MS, NMR	Zhang & Elfarra (2005)

2'-Deoxy-guanosine	DEB	7,7'-(2,3-Dihydroxy-1,4-butanediyl)bis(2-amino-1,7-dihydro-6H-purin-6-one) (bis-N7G-BD); 2'-deoxy-1-(4-[2-amino-1,7-dihydro-6H-purin-6-on-7-yl]-2,3-dihydroxybutyl)-guanosine (N7G-N1dG-BD); 2-amino-9-hydroxymethyl-4-(4-acetyloxy-2,3-dihydroxybutyl)-8,9- dihydro-7H-(1,4)oxazepino(4,3,2-gh)purin-8-ol (PA1); 2-amino-9-hydroxymethyl-4-{4-[2-amino-9- or 7-(4-acetyloxy-2,3-dihydroxybutyl)-1,7-dihydro-6H-purin-6-on-7- or 9-yl]-2,3- dihydroxybutyl}-8,9-dihydro-7H-(1,4)-oxazepino(4,3,2-gh)purin-8-ol (PA2); 2-amino-7,9-bis(4-acetyloxy-2,3-dihydroxybutyl)-1,7-dihydro-6H-purin-6-one (PA3); 9,9'-bis(4-acetyloxy-2,3-dihydroxybutyl)-7,7'-(2,3-dihydroxy-1,4-butanediyl)bis(2-amino-1,7-dihydro-6H-purin-6-one) (PA4)	HPLC, UV, MS, NMR	Zhang & Elfarra (2006)
2'-Deoxy-adenosine	DEB	(R,R)-N6-(2,3,4-Trihydroxybut-1-yl)deoxyadenosine; (S,S)-N6-(2,3,4-trihydroxybut-1-yl)deoxyadenosine	NMR, MS, CD	Nechev et al. (2001)
2'-Deoxy-guanosine	DEB	(R,R)-N2-(2,3,4-Trihydroxybut-1-yl)deoxyguanosine; (S,S)-N2-(2,3,4-trihydroxybut-1-yl)deoxyguanosine	NMR, MS, CD spectra	Nechev et al. (2001)
2'-Deoxy-guanosine	DEB	N7-(2,3,4-Trihydroxybutyl)guanine (G4; major); N7-(1-(hydroxymethy l)-2,3-dihydroxypropyl)guanine (G3; minor)	LC-MS, NMR	Boogaard et al. (2001, 2004)
Guanosine	(±)-DEB	(±)-N7-(2,3,4-Trihydroxybutyl)guanine	LC-MS/MS	Oe et al. (1999)
Guanosine	meso-DEB	meso-N7-(2.3,4-Trihydroxybutyl)guanine (G4)	LC/MS-MS	Oe et al. (1999)
2'-Deoxy-guanosine-5'-phosphate; calf thymus DNA	RR/SS DEB	N7-(2-Hydroxy-3,4-epoxy-1-yl)-5'dGMP	HPLC, UV	Koivisto et al. (1999)
Calf thymus DNA	Racemic DEB	1-(Aden-1-yl)-4-(guan-7-yl)-2,3-butanediol (N1A-N7G-BD; 1); 1-(aden-3-yl)-4-(guan-7-yl)-2,3-butanediol (N3A-N7G-BD; 2); 1-(aden-7-yl)-4-(guan-7-yl)-2,3-butanediol (N7A-N7G-BD; 3); 1-(aden-N6-yl)-4-(guan-7-yl)-2,3-butanediol (N6A-N7G-BD; 4)	MS/MS, HPLC, UV	Park et al. (2004)
Guanosine; calf thymus DNA	DEB	1,4-bis-(Guan-7-yl)-2,3-butanediol (bis-N7G-BD); N7-(2',3',4')trihydroxybutylguanine (N7-THBG)	UV, MS, NMR	Park & Tretyakova (2004)
Guanosine	meso-DEB	meso-1,4-bis-(Guan-7-yl)-2,3-butanediol	UV, MS, NMR	Park et al. (2005)
2'-Deoxy-guanosine; calf thymus DNA	HMVK	Diasteromeric pair of HMVK-derived 1,N2-propanodeoxy-guanosine C-6 adducts, as well as a diastereomeric pair of C-8 HMVK-derived 1,N2 -propanodeoxyguanosine adducts	UV, MS, NMR	Powley et al. (2003)

Annex I

Evaluation of the Subcommittee on the Classification of carcinogenic substances

I.1 Scope

On request of the Dutch Expert Committee on Occupational Safety of the Health Council, the Subcommittee on the Classification of carcinogenic substances evaluates the carcinogenic properties of 1,3-butadiene.

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the carcinogenic properties of substances, and to propose a classification with reference to an EU-directive (see Annex J). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substance in question.

The members of the Subcommittee on the Classifaction of carcinogenic substances are listed at the end of this Annex. This evaluation is based on the data summarized in Chapter 2 of the present report.

I.2 Carcinogenicity of 1,3-butadiene

1,3-Butadiene (butadiene) was classified previously as a human carcinogen by the International Agency for Research on Cancer (category 1; IARC 2008, 2009) and by the European Commission (category 1A; EU-RAR 2002).

Many human studies show an elevated risk of leukaemia or other cancers of the lymphohaematopoietic system following exposure to butadiene. Only three of these studies have been conducted on workers employed in butadiene manufacturing facilities, where exposure is to butadiene monomer alone. Most studies, however, have been done on workers exposed to butadiene during styrene-butadiene rubber (SBR) production. Although a relative large number of studies has been reported (fully reviewed in DECOS 1990, IARC 2008, ATSDR 2009), many of these studies update previously reported findings and thus relate to the same or overlapping cohort populations.

Compared to exposure to butadiene alone in the monomer production sites, multiple chemical exposures of SBR workers makes interpretation of the results more difficult. In addition, have worked in both the butadiene manufacturing industry and in the SBR industry, which makes the interpretation of these studies even more complicated.

In two of the butadiene monomer industry studies a slight overall excess of mortality from leukaemia was observed, whereas in the third study a small deficit in mortality from leukaemia was observed. The increased 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 leukaemia was observed with duration of exposure or cumulative exposure.

Two studies of SBR workers by researchers at the University of Alabama at Birmingham, USA (Delzell et al. 2001, Cheng et al. 2007) were considered to be the most informative. In these study the mortality rates of approximately 17,000 workers from eight facilities in the USA and Canada were examined, and included earlier studies of some of the facilities. Limiting factors in the evaluations were that the diagnosis and classification of lymphatic and haematopoietic malignancies are very complex and have undergone several changes over the course of time. Although overall mortality from leukaemia was only slightly elevated in the most recent update of this cohort, larger increases of mortality from leukaemia (chronic lymphocytic and chronic myelogenous leukaemia) were seen in workers in the most highly exposed areas of the plants and among hourly paid workers, especially those who had been hired in the early years and had longer (≥10 years) employment. Furthermore, a significant exposure-response relationship between cumulative butadiene exposure and mortality from leukaemia was observed, and the most recent analyses indicate that the exposure-response relationship for butadiene and leukaemia was independent of exposure to styrene and dimethyldithiocarbamate (Cheng et al.

2007, Sielken et al. 2007, Graff et al. 2009, Sathiakumar and Delzell 2009, Sathiakumar et al. 2009).

The Subcommittee concludes that the human studies provide limited evidence regarding the carcinogenicity of butadiene: there is a positive association between exposure to butadiene and cancer, but coincidences, bias and confounders cannot fully be excluded.

Several studies with mice showed increased tumour formation in various organs in both sexes at exposures to approximately 1 ppm (2.2 mg/m^3) butadiene. This was not observed in rats at exposures up to 1,000 ppm (2,200 mg/m^3), likely due to the crucial role of oxidative metabolism: butadiene requires metabolic activation to generate electrophilic epoxides in which important species differences exist (mice are more efficient in the production of epoxide metabolites of butadiene, while rats and humans are more efficient in the hydrolytic detoxification of these metabolites) (reviewed in IARC 2008, EU-RAR 2002, ATSDR 2009, Kirman et al. 2010b). Although carcinogenicity has been observed in one animal species only, positive results have been obtained in several studies, including a number of studies by the US National Toxicology Program. The Subcommittee concludes that the animal studies provide sufficient evidence for the carcinogenicity of butadiene.

Many tests on mutagenicity, genotoxicity and mechanism of action clearly indicate that butadiene is a genotoxic compound in humans and in experimental animals, requiring metabolic activation to generate electrophilic and DNA-reactive epoxides (stereoisomers of epoxybutene, epoxybutanediol and diepoxybutane; reviewed in IARC 2008, EU-RAR 2002, ATSDR 2009, Albertini et al. 2010, Kirman et al. 2010a, 2010b). The Subcommittee considers butadiene therefore as a stochastic genotoxic carcinogen, and advises to calculate health based occupational cancer risk values.

I.3 Recommendation for classification

Based on the available data, the Subcommittee recommends classifying 1,3-butadiene in category 1A ('the compound is known to be carcinogenic to man'), and considers the substance as a stochastic genotoxic carcinogen.

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Meeting date: 3 December 2010

Annex **J**

Carcinogenic classification of substances by the Committee

The committee expresses its conclusions in the form of standard phrases:

Category	Judgement of the committee (GR_{GHS})	Comparable with EU Category	
		67/584/EEC before 12/16/2008	EC No 1272/2008 as from 12/16/2008
1A	*The compound is known to be carcinogenic to man.* • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, the mechanism of action is not known.	1	1A
1B	*The compound is presumed to be carcinogenic to man.* • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, the mechanism of action is not known.	2	1B
2	*The compound is suspected to be carcinogenic to man.*	3	2
(3)	*The available data are insufficient to evaluate the carcinogenic properties of the compound.*	Not applicable	Not applicable
(4)	*The compound is probably not carcinogenic to man.*	Not applicable	Not applicable

Source: Health Council of The Netherlands. Guideline to the classification of carcinogenic compounds. The Hague: Health Council of the Netherlands, 2010; publication no. A10/07E.