ECHA
EUROPEAN CHEMICALS AGENCY

ANNEX 1
in support of the Committee for Risk Assessment (RAC) for evaluation of limit values for isoprene at the workplace

ECHA/RAC/OEL-O-0000007102-87-01/F

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Scope of the task and literature search

ECHA has been tasked by the European Commission to evaluate the exposure to isoprene to assess the option of an airborne occupational exposure limit, other limit values (BLV/BGV) and notations.

This report is based on international assessments such as DFG (2009), IARC (1999), OECD (2005), BG Chemie (2000) and AGS (2012). This has been complemented by a literature search (July 2021) of published papers from the last ten years.

ECHA evaluation and recommendation

The table below presents the outcome of the scientific evaluation to derive limit values for isoprene.

Derived Limit Values

OEL as 8-hour TWA:	8.5 mg/m^3 (3 ppm)
STEL:	-
BLV:	-
BGV:	-

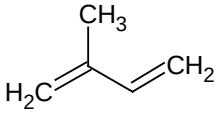
Notations

Notations:	none

1. Chemical Agent Identification and Physico-Chemical Properties

Isoprene is an organic substance. It is a colourless and volatile liquid which is soluble in most hydrocarbons and insoluble in water.[1]

Table 1: Identity and physico-chemical properties[2]

Endpoint	Value
IUPAC Name	2-methyl-1,3-butadiene
Synonyms	isoprene, isopentadiene, β-methylbivinyl
EC No	201-143-3
CAS No	78-79-5
Chemical structure	
Chemical formula	C5H8
Appearance	colourless liquid
Boiling point	34 °C
Density	0.679 g/cm3 (20 °C)
Vapour pressure	63.397 kPa (21.1 °C)
Partition coefficient (log Pow)	2.42 (20 °C)
Water solubility	642 mg/L (25 °C)
Viscosity	0.21 mPa·s
Conversion factor	1 ppm = 2.83 mg/m^3 (25 °C) [3] 1 mg/m^3 = 0.36 ppm

[1] Kirk-Othmer Encyclopedia of Chemical Technology. (2014)

[2] Phys-chem values obtain from corresponding registration data

[3] The conversion factor is derived from the assumption of ideal gas behaviour as

$$concentration\left[\frac{mg}{m^3}\right] = 68.12\frac{g}{mol}\cdot\frac{1.013\cdot 10^5 Pa\cdot 1m^3}{8.314\cdot\frac{Pa\cdot m^3}{mol\cdot K}\cdot 298.15K}\cdot 10^{-3}\cdot concentration[ppm]$$

2. EU Harmonised Classification and Labelling - CLP (EC) 1272/2008

Table 2: EU classification: Summary of harmonised classification and labelling for isoprene

Index No	EC No	CAS No	Annex VI of CLP hazard class and category	Hazard statement code
601-014-00-5	201-143-3	78-79-5	Flam. Liq. 1* Muta. 2 Carc. 1B Aquatic Chronic 3	H224 H341 H350 H412

* Isoprene is classified as a Flammable Liquid Category 1 (H224: Extremely flammable liquid and vapor) and is reactive (it can produce poisonous gases in a fire or react violently with oxidising agents). The lower and upper explosive limits are 2 and 9% respectively. Containers may also explode. Vapour is heavier than air and may travel some distance to cause fire or explosion far from the source and flash back.

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

3.1 Directive 98/24/EC and Directive 2004/37/EC

There are currently no binding or indicative occupational exposure limit values for isoprene under Directives 98/24/EC or 2004/37/EC.

3.2 REACH Registrations

Table 3: REACH Registrations and tonnage

Substance(s)		Tonnage (tonnes/annum	
name	EC number	Full registration	intermediate use
isoprene	201-143-3	1000-10000 (<5 registrations)	>100000 (67 registrations)

3.3 Authorised uses under Annex XIV of REACH

Isoprene is not listed in Annex XIV of REACH.

3.4 Restricted uses under Annex XVII of REACH

Isoprene is not listed in Annex XVII of REACH.

3.5 Plant Protection Products Regulation (EC) 1107/2009

Isoprene is not approved for use as an active substance in plant protection products in accordance with Article 4 of Regulation (EC) No 1107/2009.

3.6 Human and Veterinary Medicinal Products Directives 2001/83/EC and 2004/28/EC respectively

There are no authorisations for use of isoprene in human or veterinary medicines.

3.7 Biocidal Products Regulation (EU) 528/2012

Isoprene is not approved for use as an active substance in biocidal products in accordance with Article 5.1(a) of Regulation (EU) No 528/2012.

3.8 Other legislations

According to Annex II of the EU Regulation (EC) No 1223/2009[4] on cosmetic products, Isoprene (stabilized); (2-methyl-1,3-butadiene) (EC 201-143-3) is prohibited in cosmetic products.

4. Existing Occupational Exposure Limits

There is no OEL for isoprene established at EU level. However, some EU Member States have established an OEL for 8 hours, and in some cases also a STEL value. Table 4 presents OEL values for EU Member states as well as one value from outside the EU.

The list should not be considered as exhaustive.

Table 4: Existing Occupational Exposure Limits (OELs) indicated as 8-h Time-Weighted Average (TWA) and Short-term exposure (15 min) for isoprene

Country	TWA (8 hrs)		STEL (15 min)		Remarks
	ppm	mg/m³	ppm	mg/m³	
Germany (AGS)	3	8.4	24	67.2	
Germany (DFG)	3	8.5	24	68	
Latvia		40			
Poland		100		300	
Switzerland	3	8.5	24	68	

Source: Gestis database (searched July 2021): International limit values for chemical agents (Occupational exposure limits, OELs) (https://www.dguv.de/ifa/gestis/gestis-internationale-grenzwerte-fuer-chemische-substanzen-limit-values-for-chemical-agents/index-2.jsp)

Biological limit values

No values found.

[4] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1223&from=EN

5. Occurrence, Use and Occupational Exposure

5.1 Occurrence

In humans, isoprene is produced endogenously at a rate of 0.15 µmol/kg body weight per hour (Taalman, 1996). The mean endogenous blood concentration of isoprene has been reported as 2.52 µg/L, but it can range from 1.0 to 4.8 µg/L (Cailleux et al., 1992). The endogenous formation of isoprene in animals seems to be markedly lower than in humans. For further details on endogenous formation, see Section 7.1.1. As described in Section 9.2.1.1, by application of physiological toxicokinetics modelling, DFG (2009) estimated a mean endogenous isoprene concentration of 5.2 ± 4.0 nmol/l in the venous blood.

Isoprene is produced and emitted by many species of trees (major producers are oaks, poplars, eucalyptus, and some legumes). About half the annual isoprene emissions from vegetation comes from tropical broadleaf trees and the remainder primarily from shrubs. In deciduous forests, isoprene makes up approximately 80% of hydrocarbon emissions. Microscopic and macroscopic algae also produce isoprene.

Isoprene is emitted from automobile exhausts, gasoline, biomass combustion, and wood pulping, and it is the by-product of ethylene production by naphtha cracking. Isoprene is present in some food such as roasted coffee and beer and ales. In addition, tobacco smoke also contains isoprene, and it is the main source of isoprene indoors. (Alwis et al., 2016, Biren et al., 2020, Taalman, 1996).

5.2 Production and Use Information

There are more than 70 REACH registrants of isoprene in the EU.

Production

Isoprene is obtained by extractive distillation from an isoprene concentrate stream produced by the ethylene production process. In the pyrolysis furnaces of the ethylene production process, paraffinic feedstocks such as ethane, propane, naphthas or gas oils, are subjected to high temperatures in the presence of steam. These conditions result in the partial conversion or cracking of the hydrocarbon feedstock components and formation of unsaturated hydrocarbons. Ethylene and propylene are the primary products, but other olefins, diolefins, aromatics and cyclics are also produced, including a relatively small amount of isoprene. The ethylene process compresses and separates the pyrolysis furnace effluent into product streams. Isoprene produced in the cracking furnace is contained in one of these product streams, the pyrolysis gasoline. Isoprene concentrates thus produced from the pyrolysis stream has a typical isoprene content of 40%. This concentrate is then processed in an extractive distillation unit that uses a solvent such as acetonitrile to facilitate isolation of the contained isoprene as a 99% purity product (OECD, 2005).

Isolation of isoprene from the ethylene process co product streams as described above is the primary source of isoprene. Only this method of production is practiced in the United States and Western Europe (OECD, 2005).

The registrations for isoprene indicate approximately 400,000 tonnes/year, from 70+ registrants, of which the vast majority is imported from outside the EU.

Uses

Isoprene is used as a chemical intermediate to manufacture primarily polymers, which occurs in closed production systems. Greater than 95% of high-purity isoprene is used as a monomer to manufacture elastomers such as polyisoprene, styrenic thermoplastic elastomer block copolymers (styrene-isoprene-styrene [SIS]), and butyl rubber. Asghar and Masoon (2020) indicate that the isoprene market is increasing due to its increasing

applications in tires, conveyor belts, hoses, moulded rubber, and also in medical equipment such as gloves and balloons. The growth of the isoprene industry is directly related to the growth of the synthetic rubber industry. The growing demand for fuel efficiency and eco-friendly tires is driving the growth of the tire industry and in turn the growth of the isoprene market.

The remaining amount of isoprene is used to manufacture specialty chemicals, intermediates, and derivatives, which are then used in the production of vitamins, pharmaceuticals, flavourings and perfumes, and epoxy hardeners, and fuels.

5.3 Occupational exposure

In the EU, isoprene is mostly used at industrial sites in closed systems for polymer production processes, although ancillary activities (sampling, transfers, pelletising etc.) do lead to some levels of worker exposure. Other uses, such as industrial use as an intermediate, and industrial and professional uses as a fuel, also lead to some levels of worker exposure. Potential occupational exposure to isoprene occurs through inhalation and dermal contact, but no measured data on exposures was found. In the REACH registration dossiers exposures have been modelled using ECETOC's Targeted Risk Assessment (TRA) Workers[5], indicating exposures for inhalation up to 7 mg/m³ (with the highest indicated for industrial use as fuel), and dermal up to 8 mg/kg bw/day (with the highest indicated for activities such as maintenance and cleaning).

Air-monitoring data were collected at three U.S. facilities that produced isoprene monomers or polymers; 98.5% of the samples showed concentrations of less than 10 ppm (27.86 mg/m^3), and 91.3% of less than 1 ppm (2.79 mg/m^3) (NTP, 2011). Similar up-to-date studies within the EU were not found.

5.4 Routes of exposure and uptake

The greatest potential for exposure to isoprene in the environment is in the air compartment because of its high vapour pressure. Partitioning to air from aquatic and terrestrial compartments would occur rapidly due to isoprene's physicochemical characteristics. As such, isoprene has an overall low potential for exposure in environmental compartments other than air. However, its persistence in air is short lived as a result of degradation processes, which suggests that exposure to isoprene will be limited in the environment (OECD, 2005).

5.4.1 Worker exposure

Potential occupational exposure to isoprene through inhalation (primarily) and dermal contact could occur at workplaces where isoprene or synthetic rubber is produced or used.

5.4.2 General population

As indicated earlier isoprene is produced and emitted by vegetation, although its persistence in air is short lived as a result of degradation processes. Wagner and Kuttler (2014) measured isoprene concentrations at various sites in Essen, Germany using two compact online GC-PID systems. During the measurement period in the summer of 2012, the average hourly isoprene concentrations reached 0.13 to 0.17 ppb (0.004 to 0.005 mg/m^3) between 10 and 20 LST (local standard time, UTC + 1 h), and is strongly influenced by meteorological conditions such as wind speed and atmospheric chemistry. Wang et al (2013) measured the daily and daytime average concentrations of isoprene

[5] https://www.ecetoc.org/targeted-risk-assessment-tra/

in the summer at the subtropical urban site (in Taipei) as 0.72 and 1.26 ppbv (0.002 and 0.0035 mg/m^3), respectively, which were higher than the measurements in many urban and rural areas in temperate zones. The results also revealed that biogenic isoprene in the summertime overwhelmed anthropogenic isoprene, although the traffic is usually heavy in the city.

There are no direct sales of isoprene to consumers. However, isoprene is used in the production of polymers used in paint resins, tires, footwear, adhesives, and motor oil viscosity improvers. An unknown percentage of unreacted monomers is present in the end-products. Possible migration rate is also unknown, although some studies indicate that exposure to isoprene is considered to be negligible (OECD, 2005).

6. Monitoring Exposure

6.1 External exposure

No official validated method (e.g., from OHS institutes) has been found, however, several peer reviewed articles deal with the possibilities to measure isoprene in air.

Some publications deal with adequate analytical and sampling techniques. For instance, (Butterfield et al., 2020) and (Eijk and Kotzias, 1994) identify adequate sampling tubes and sampling volumes for isoprene, and at times the analytical technique used but do not provide data on the analytical results (e.g. limits of detection) or uncertainties. Maceira et al. (2017) and Sacco (2009) provide further data on the analytical methods including some analytical parameters such as quantification/detection limits and reproducibility or the expanded uncertainty for the measurements .

The findings of these papers are summarized in the table below:

Table 5: Analytical methods and techniques for isoprene

Sampling methods / desorption	Analytical technique	LOQ and sampling volume and time	Reference
Carbopack-X tubes (active) Thermal desorption	GC/FID[1]	n/a	(Butterfield et al., 2020)
Carbotrap B tubes (active) Thermal desorption	GC/FID[1]	n/a	(Eijk and Kotzias, 1994)
Tenax TA/Carbograph 1TD tubes (active) Thermal desorption	GC/MS[2]	83 ng/m^3 2.64 L (2 hours at 22 mL min^{-1})	(Maceira et al., 2017)
Carbotrap B/Carbopack X/Carboxen 569 tubes (active) Thermal desorption	GC/MS[2]	8 ng/m3 6 L (2 hours at 50 mL min^{-1})	(Maceira et al., 2017)
Radiello diffusive sampler loaded with Carbopack-X	GC/MS[2]	ppb range	(Sacco, 2009)

Sampling methods / desorption	Analytical technique	LOQ and sampling volume and time	Reference
Thermal desorption		No sampling volume (passive sampling) Recommended sampling time: 8 hours (whole shift)	

(1) GC/FID: Gas chromatography–flame-ionization detection

(2) GD/MS: Gas chromatography–mass spectrometry

The methods found indicate that it should be possible to measure isoprene in air at relatively low concentrations.

6.2 Biomonitoring of exposure (internal exposure)

In humans, isoprene is produced endogenously at a rate of 0.15 µmol/kg body weight per hour (Taalman, 1996). The mean endogenous blood concentration of isoprene has been reported as 2.52 µg/L, but it can range from 1.0 to 4.8 µg/L (Cailleux et al., 1992). When running a physiological toxicokinetic (PT) model, the rate of endogenous isoprene formation was estimated as 13.1 ± 10 µmol/h for a person weighing 70 kg (corresponding to 0.19 µmol/h kg bw) (DFG, 2009; see Section 9.2.1.1). It has been estimated that about 90% of endogenous isoprene is metabolized and 10% is exhaled unchanged (Biren et al., 2020, Csanady and Filser, 2001). For further details, see Section 7.1.1.

Whilst isoprene appears to be ubiquitous in exhaled human breath, isoprene has not been shown to increase in people with increased isoprene exposure (Buszewski et al., 2009, Alwis et al., 2016).

In the last years, biomarkers of exposure to isoprene have been proposed. In particular Alwis et al. (2016) studied urinary mixture of N-acetyl-S-(1-[hydroxymethyl]-2-methyl-2-propen-1-yl)-L-cysteine and N-acetyl-S-(2-hydroxy-3-methyl-3-buten-1-yl)-L-cysteine) (IPM1) and N-acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine (IPM3) as potential biomarkers of exposure to isoprene. IPM3 was identified as a major urinary isoprene metabolite and isoprene exposure biomarker for humans. (Biren et al., 2020) used IPM3 as biomarkers of isoprene exposure to assess the isoprene exposure on US general population.

In principle, also hemoglobin adducts of the diepoxide could be used, but there is no published procedure so far.

6.2.1 Background levels

Isoprene exposure of the general population in the US was assessed (Biren et al., 2020) as part of the 2015-2016 National Health and Nutrition Examination Survey. The study concluded that the levels of IPM3 in urine were significantly higher in smokers (39.8 µg/g creatinine) versus non-smokers (3.05 µg/g creatinine). The study also looked for possible correlations based on sex, race, body mass index and diet.

No studies on background levels (of any biomarker) on European general populations have been found.

6.2.2 Occupational exposure

No studies showing correlation between internal and external exposure to isoprene have been found.

6.2.3 Biomonitoring analytical methods

The US Centre for Disease Control and Prevention (CDC) developed analytical methods to measure the isoprene biomarkers IMP3 and IMP1 in urine.

Analytical techniques and detection limits were reported by Alwis et al. (2016).

Table 6: Analytical methods and techniques for isoprene biomarkers

Method /reference	Analytical technique	Biomarker	Limit of detection (LD) or quantification (LQ)
(Alwis et al., 2016).	UPLC/ESI-MSMS[1]	IPM3	0.5 ng/ml
(Alwis et al., 2016)	UPLC/ESI-MSMS[1]	IPM1	9.5 ng/ml

(1) Ultra high performance liquid chromatography coupled with electrospray ionization triple quad tandem mass spectrometry

7. Health Effects

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

7.1.1 Human data

Endogenous formation

It is well known that isoprene is formed endogenously in humans. The process is however a bit unknown, but dimethylallyl pyrophosphate (a cholesterol precursor) has been identified as a probable source (Deneris et al., 1984, Deneris et al., 1985). Other potential routes involve degradation of farnesyl or geranyl residues of prenylated proteins, or peroxidation of squalene (Stein and Mead, 1988, Zhang and Casey, 1996). In a study on healthy female and male volunteers, isoprene blood concentrations of 37 ± 25 nmol/l (range 15-70 nmol/l) were reported (Cailleux et al., 1992). In the study by (Cailleux et al., 1992), in 33 mechanically ventilated patients, the measured mean blood concentrations were 10.29±6.17 nmol/l in venous blood, and 6.68±4.71 nmol/l in arterial blood.

Excretion

It has been estimated that about 90% of endogenous isoprene is metabolized and 10% is exhaled unchanged (Biren et al., 2020, Csanady and Filser, 2001). The blood:air partition coefficient of isoprene has been reported to be very low, 0.75, meaning that isoprene has a low affinity for blood, and excretion with the exhaled air is relevant.

DFG (2009) presents a summary of several studies measuring isoprene levels in exhaled air of healthy volunteers. There are large inter-individual differences in the measured levels. In the study by Turner et al. (2006), the mean value was 118 ± 68 ppb (range 0-474 ppb) (Turner et al., 2006). No significant differences related to age or sex were identified in a study involving 66 women and 60 men (DeMaster and Nagasawa, 1978). Lechner et al. (2006) did however present a study in which the exhaled isoprene concentration of women seemed to be slightly lower than that of men. The isoprene concentrations in the exhaled air of children were reported to be lower than of adults, being very low in newborns, and lower in younger children than in school age children (Nelson et al., 1998, Taucher et al., 1997).

7.1.2 Animal data

Endogenous formation

The endogenous formation of isoprene in animals seems to be markedly lower than in humans. In the study by Cailleux et al. (1992), <1 nmol/l isoprene was detected in the blood of several animal species (rat, rabbit, dog, pony, cow, sheep). In a study with pigs, the venous blood concentrations were 0.2-1.3 nmol/l and arterial blood concentrations 0.3-0.7 nmol/l. (Miekisch et al., 2001).

Absorption, distribution, metabolism and excretion

The absorption of isoprene decreases at increased dose levels, meaning that the excretion of unmetabolized isoprene increases when the exposure doses are high. Saturation kinetics of isoprene metabolism has been described in rats and mice. According to Peter et al. (1990), at inhalation exposure concentrations up to 300 ppm, linear pharmacokinetics apply. When the atmospheric concentrations increase to about 1000 ppm (for rats) or 2000 ppm (for mice) saturation of the isoprene metabolism is practically complete (Peter et al., 1990).

In a study with rats, inhalation of 0, 8, 260, 1480 or 8200 ppm (0, 22.6, 736, 4188, 23206 mg/m^3) of ^{14}C radiolabelled isoprene took place for 6 hours. Next, information on ^{14}C levels in urine, faeces, exhalation air, and content remaining in the body was collected. At the end of the exposure period, 19%, 9.1%, 5.8% and 4.5% of the total inhaled dose at the dose levels 8, 260, 1480 or 8200 ppm, respectively, remained in the body. It was estimated that of the inhaled isoprene dose, 25.3% was metabolized at 8 ppm, 12.0% at 260 ppm, 4.7% at 1480 ppm, and 3.6% at 8200 ppm. Over 75% of the quantity attributed to metabolism was excreted in the urine at all doses. At the top dose, 95.5% was excreted without biotransformation. A mean half-life of 10.2 hours was calculated for ^{14}C in urine, not impacted by the exposure duration. Levels of ^{14}C (related to isoprene itself, and to diol-, monoepoxide- and diepoxide metabolites) were detected in the nose, lungs, liver, kidney and fat, with the highest levels detected after six hours in fat tissue. (Dahl et al., 1990, Dahl et al., 1987, BGChemie, 2000). Gargas et al. (1989) determined liquid/tissue:air partition coefficients for isoprene: blood:air 1.87 ± 0.10; fat:air 72.0 ± 2.4; liver:air 3.12 ± 0.87; muscle:air 2.04 ± 0.27. The determinations were made based on experiments using blood and fat, liver, and muscle tissue homogenates from rats.

In mice, a steady state was reached in blood 15-30 minutes after the start of inhalation exposure to ^{14}C radiolabelled isoprene at 20, 200 or 2000 ppm (57, 566, 5660 mg/m^3) up to 6 hours. The blood concentrations at these doses were 24.8 ng/ml, 830 ng/ml, or 6800 ng/ml, respectively. In urinary measurements it was noted that 52-73% of the radioactivity associated with isoprene metabolites was excreted (Bond et al., 1991), being clearly lower than in the rat study (Dahl et al., 1990, Dahl et al., 1987). At the end of the exposure, the ^{14}C retained in the body was calculated as 5.9% (20 ppm), 8.9% (200 ppm), and 3.8% (2000 ppm).

After a single intraperitoneal injection of 64 mg/kg bw ^{14}C isoprene to rats and mice, 50% was exhaled in unchanged form and about 32% was excreted as metabolites in the urine (main metabolites in rats being: 53% 2-hydroxy-2-methyl-3-butenoic acid, 23% 2-methyl-3-buten-1,2-diol, and 13% the C-1 glucuronide of 2-methyl-3-buten-1,2-diol) (Buckley et al., 1999).

The formation of ^{14}C isoprene haemoglobin adducts occurred linearly (24 h after the last injection) in mice and rats given single or three consecutive intraperitoneal injections of ^{14}C isoprene at dose levels up to 30 mg/kg bw/day (Sun et al., 1989). Twenty-four hours after the end of a 6-hour inhalation exposure of mice with ^{14}C isoprene (20, 200, 2000 ppm), corresponding haemoglobin adduct concentrations of 11, 90, and 170 pmol/mg globin were detected. At the two highest doses, no linear relationship between retention and exposure concentration was identified. Furthermore, the formation of haemoglobin adducts was not linearly corresponding with the retained ^{14}C isoprene concentrations (Bond et al., 1991). The formation of haemoglobin adducts can be considered as a biomarker of exposure to reactive metabolites of isoprene with no toxicological impact

7.1.3 *In vitro* data

The metabolism of isoprene has been investigated in detail in microsomal preparations (reviewed in BG Chemie, 2000). The formation of metabolites was analysed in microsomal preparations of livers from mice, rats, hamsters, and rabbits after incubation with 0.5 M isoprene solution. First, isoprene was metabolized by microsomal mono-oxygenases to monoepoxides. The formation of the main metabolite 3,4-epoxy-3-methyl-1-butene started within a few minutes. It was then further hydrolysed to trans-3-methyl-1-butene-3,4-diol. The second monoepoxide metabolite 3,4-epoxy-2-methyl-1-butene (hydrolysed to trans-2-methyl-1-butene-3,4-diol) was formed in smaller amounts (14-25% of the main monoepoxide). Epoxide hydrolase is considered the relevant enzyme for the hydrolysis. The monoepoxides may be oxidised by cytochrome P450 enzymes into the mutagenic diepoxide, 2-methyl-1,2,3,4-diepoxybutane. The rate of formation of 3,4-epoxy-3-methyl-1-butene in animal microsomal preparations was estimated to be five to eight times higher than that of 3,4-epoxy-2-methyl-1-butene in studies with microsomes from transfected cell lines. The metabolism was also studied in human liver microsomes, the results indicating that the rate of 3,4-epoxy-3-methyl-1-butene formation was four times higher than that of 3,4-epoxy-2-methyl-1-butene. The formation of 2-methyl-1,2,3,4-diepoxybutane followed similar reaction rates for each of the monoepoxides in human samples. (BGChemie, 2000, Bogaards et al., 1996, DelMonte et al., 1985, Gervasi et al., 1985, Gervasi and Longo, 1990, Longo et al., 1985).

7.1.4 Toxicokinetic modelling

A physiological toxicokinetic (PT) five-compartment model was developed to for inhaled and endogenously formed isoprene (Csanady and Filser, 2001, Filser et al., 1996). The approach is reviewed in detail by DFG (2009).

In the study by Bogaards et al. (2001), interspecies differences in enzyme activity potentially affecting the formation of isoprene diepoxide metabolites were investigated by incorporation of kinetic parameters into a physiologically-based pharmacokinetic (PBPK) model. The enzyme kinetic parameters were determined *in vitro* in liver samples. When comparing enzymatic activities, only marginal differences were found between species (rat, mouse, human) regarding the cytochrome P450-mediated oxidation of isoprene and isoprene monoepoxides. However, major differences were found regarding the hydrolysis and conjugation of isoprene epoxides, both involved in the detoxification of the critical epoxides. The hydrolysis capacity of isoprene was found to be much higher in humans than in mice and rats, suggesting a lower susceptibility of humans to isoprene exposure. For the conjugation of epoxides with glutathione S-transferase the reversed order was observed. After incorporation of the *in vitro* metabolism data in a PBPK model, the predicted isoprene diepoxide levels in mouse liver were slightly higher than in rat, but, on average, much lower in humans (about 20-fold lower in humans when compared to mice and about 15-fold lower in humans when compared to rats). However, considering the intra-individual variations of enzyme activities in humans, the authors estimated that for a 'worst-case' scenario of an individual presenting both an extensive oxidation by cytochrome P450 and a low detoxification by epoxide hydrolase, isoprene diepoxide concentrations were predicted similar or even higher than those predicted for the mouse and rat. Nevertheless, on average, especially the higher activity of the mitochondrial epoxide hydrolase in humans compared to mice results in lower predicted diepoxide levels in humans (Bogaards et al., 2001).

7.1.5 Summary

It is well established that isoprene is formed endogenously in humans. As the blood:air partition coefficient of isoprene is low, excretion with the exhaled air is relevant. There are several studies showing data on isoprene measurements in the exhaled air of humans not exogenously exposed to isoprene. Data on isoprene levels in human blood

have also been reported. In animals, the endogenous formation of isoprene is markedly lower than in humans.

The metabolism of isoprene has been investigated in detail in microsomal preparations *in vitro*. In the first step, in the presence of mono-oxygenases monoepoxides are formed, the main metabolite being 3,4-epoxy-3-methyl-1-butene. The monoepoxides are then further metabolised to diepoxides (e.g., 2-methyl-1,2,3,4-diepoxybutane). Formation of haemoglobin adducts has been observed in rats and mice. Significant interspecies differences in epoxide hydrolase activity have been identified. The low expression of epoxide hydrolase in mice is likely to be an important reason for higher toxicity compared to humans.

In animal studies, lowered proportions of the administered doses are absorbed as the dose increases, and an increasing proportion is excreted without biotransformation due to metabolic saturation.

7.2 Acute toxicity

7.2.1 Human data

7.2.1.1 Acute oral toxicity

There are no human data on acute toxicity of isoprene via the oral route.

7.2.1.2 Acute dermal toxicity

There are no human data on acute toxicity of isoprene via the dermal route.

7.2.1.3 Acute inhalation toxicity

There are no human data on fatalities after short-term inhalation exposure to isoprene. Irritation and similar effects after short-term inhalation exposure are described in Section 7.4.1.

7.2.2 Animal data

7.2.2.1 Acute oral toxicity

Oral administration of isoprene does not cause acute toxicity. The acute oral LD_{50}-value has been reported to be 2043-2210 mg/kg bw in male Wistar rats (DFG, 2009, ECHA, 2021, OECD, 2005).

7.2.2.2 Acute dermal toxicity

Dermal application of isoprene showed no deaths in acute dermal studies. LD_{50}-values >679 mg/kg bw have been reported (ECHA, 2021).

7.2.2.3 Acute inhalation toxicity

Acute inhalation studies were summarised by DFG (2009). The lowest reported LC_{50}-value reported for rats is about 65000 ppm (183950 mg/m^3; 4 hours of inhalation exposure) and in mice 50000 ppm (141500 mg/m^3; 2 hours exposure).

7.2.3 *In vitro* data

No data available.

7.2.4 Summary

The acute toxicity of isoprene is low.

7.3 Specific target organ toxicity / Repeated dose toxicity

7.3.1 Human data

For repeated dose toxicity some human studies in the rubber industry were reviewed by DFG (2009) but due to concomitant exposure to several chemicals they were not suitable for assessing repeated dose toxicity effects of isoprene (Mamedov and Aliev (1985a) Mamedov and Aliev (1985b) Mitin Iu (1969)).

7.3.2 Animal data

Oral exposure

Administration of 200 mg isoprene/kg bw/day on day 1, and 45 mg isoprene/kg bw/day on the following four days to male Wistar rats for five days did not cause deaths or other adverse effects (BGChemie, 2000).

Inhalation exposure

No adverse effects were observed in male/female F344/N rats after two weeks of inhalation exposure at dose levels of 0, 438, 875, 1750, 3500 or 7000 ppm (1240, 2480, 4950, 9900, 19810 mg/m^3) isoprene (Melnick et al., 1990, NTP, 1995).

Increased thymus cell proliferation was reported after 30 days (4 h/day) of daily isoprene exposure (98 mg/m^3, about 35 ppm) in male rats. At a higher dose level (1016 mg/m^3, about 364 ppm) decreased thymus cell count and mitotic index were reported. After exposure for four months (10.8 and 116 mg/m^3; about 3.9 and 41.6 ppm), there was an increase in thymus weight, cell count, mitotic index in the thymus. Changes in lymphocyte count were also reported. All these parameters were normalised one month after the end of the 4-months exposure period, but cell proliferation in the thymus was then increased (Mamedov 1979, reviewed in BG Chemie 2000).

A sub-chronic inhalation study was performed by NTP (Melnick et al., 1994, NTP, 1995). Male and female F344/N rats were exposed to isoprene at doses of 0, 70, 220, 700, 2200 and 7000 ppm (198, 623, 1980, 6230, 19800 mg/m^3) 6 h/day, 5 days/week for 13 weeks. In females, reduced numbers of neutrophils were detected at all dose levels, and in males at the top dose. However, no effects on leukocyte count or bone marrow cellularity counts were seen, and the authors considered that the observed effect may have been related to shifting neutrophils from the circulating pool to the marginal pool. No other treatment-related effects were identified (Melnick et al., 1994, NTP, 1995).

Following the same dose levels and dosing schemes as in the 13-week NTP study described above, 40 male F344/N rats/dose were exposed to isoprene for 6 months (26 weeks). At the end of the exposure period, an increased incidence of Leydig cell hyperplasia in the testes was observed in the highest dose (7000 ppm; 19810 mg/m^3) group. After a 6-months recovery period, increased Leydig cell hyperplasia was detected in rats of all isoprene doses (LOAEC 70 ppm; 200 ppm). In addition, Leydig cell adenomas were found in the high-dose group of 7000 ppm. No changes in haematological parameters, body weight or body weight gain were reported. Details on tumour findings are described in Section 7.7.2. (Melnick et al., 1994, Melnick et al., 1996, NTP, 1995)

Renal tubular hyperplasia and fibrotic changes in the spleen were reported in male F344/N rats at 700 and 7000 ppm (1980 and 19810 mg/m^3) (isoprene in a carcinogenicity study by NTP (105 weeks of exposure, 6 h/day, 5 days/week; doses 0, 220, 700, 7000 ppm (623, 1980, 19800 mg/m^3)). In the high-dose group, increased hyperplasia in the parathyroid gland of males was identified. In females, hyperplasia in the bile duct and purulent inflammation in the nose were reported at 7000 ppm. Isoprene exposure did not affect body weight, body weight gain or survival rate (NTP, 1999).

Exposure to isoprene for two weeks (0, 438, 875, 1750, 3500, 7000 ppm (1240, 2480, 4950, 9900, 19810 mg/m^3) 6 h/day, 5 days/week) caused decreased haemoglobin concentrations, haematocrit values and erythrocyte numbers, as well as epithelial hyperplasia in in the forestomach of male and female B6C3F$_1$ mice at all dose levels. Furthermore, the relative liver weights were increased, and absolute thymus weights decreased in both sexes in all groups. In addition, an increase in cytoplasmic vacuolisation in the liver and decrease in body weight, absolute and relative spleen weight, and absolute testis weight were reported in male mice of all dose groups. (Melnick et al., 1990, NTP, 1995)

Bronchial irritation, pulmonary emphysema, bone marrow hyperplasia and signs of increased erythrocyte turnover were reported in mice exposed to 60000 mg/m^3 (21500 ppm) isoprene 2 h/day for 20 days (no further details available) (BGChemie, 2000).

In a 13-week NTP study male and female B6C3F$_1$ mice were exposed to 0, 70, 220, 700, 2200 or 7000 ppm (198, 623, 1980, 6230, 19800 mg/m^3) isoprene 6 h/day, 5 days/week. At doses of 700 ppm and above, decreases in haematocrit, haemoglobin, erythrocyte count, and absolute spleen weight were reported. Additional findings in both female and male animals at the same dose levels included increased mean cell volume, macrocytic anaemia, and epithelial hyperplasia in the forestomach. At 2200 ppm and above, male relative liver weight increased and relative testis wight decreased. At 7000 ppm, decreased relative spleen weight and increased oestrus cycle length were reported in female mice. In males, degeneration of the olfactory epithelium, testicular atrophy, and decreased sperm concentrations and spermatid heads were observed. Isoprene exposure did not affect body weight gain or mortality. (Melnick et al., 1994, NTP, 1995).

In addition to the 13-week study, NTP also conducted a 6-month (26 weeks) isoprene inhalation study in male mice, with the same dose regime (0, 70, 220, 700, 2200 or 7000 ppm (198, 623, 1980, 6230, 19800 mg/m^3) isoprene 6 h/day, 5 days/week). The exposure period was followed by 6 months of recovery, after which the remaining animals were examined. No adverse effects were observed at the lowest dose level at the end of the exposure period. At 220 ppm and above, decreased grip strength of fore- and hindlimbs were reported (reversible at follow up). Findings at 700 ppm included macrocytic anaemia (reversible) and epithelial hyperplasia of the forestomach, which was still observed 6 months after the end of the exposure. At 7000 ppm, increased mortality, degeneration of the nasal olfactory epithelium and of the white matter of spinal cord, impaired hindlimb function, and atrophy of skeletal muscles and testes were reported. Six months after exposure, degeneration of the white matter of the spinal cord was observed at all dose levels, starting at 70 ppm, and degeneration of the olfactory epithelium at 220 ppm and above. No NOAEC could be identified, and 70 ppm is the LOAEC (neurological effects). Details on tumour findings are described in Section 7.7.2. (Melnick et al., 1994, Melnick et al., 1996, NTP, 1995)

In the study by Placke et al. (1996), female B6C3F$_1$ mice inhaled isoprene (0, 10, 70 ppm (0, 28, 200 mg/m^3), 8 h/day, 5 days/week) for 80 weeks. For male mice, the dose levels were 0, 10, 70, 220, 700, 2200 ppm (28, 198, 623, 1980, 6230 mg/m^3). Proliferation of haematopoietic cells in the spleen and bone marrow myeloid hyperplasia were reported for both sexes at all dose levels. Slight metaplasia of the olfactory epithelium to respiratory epithelium was seen at 70 ppm in females, and at 280 ppm and above in males. In male mice the survival rate was around 50% at 280 ppm and above. Decreased absolute and relative testis weight was reported at 280 ppm and above. Details on tumour findings are described in Section 7.7.2. (Placke et al., 1996)

7.3.3 *In vitro* data

No relevant data available.

7.3.4 Summary

Isoprene has been shown to cause systemic effects after inhalation exposure. In general, mice seem to be more sensitive than rats. Proliferation of haematopoietic cells in the spleen and bone marrow myeloid hyperplasia were reported for both sexes starting at 10 ppm after long-term (80 weeks) exposure. In a study with 26 weeks of exposure, followed by 6 months of recovery, degeneration of the white matter of the spinal cord was observed in mice at doses of 70 ppm and above, and degeneration of the olfactory epithelium at 220 ppm and above. 10 ppm can be considered as a LOAEC for non-cancer effects in test animals after repeated isoprene exposure.

7.4 Irritancy and corrosivity

7.4.1 Human data

BG Chemie (2000) and DFG (2009) refer to data from an unpublished report of a study in human volunteers (Bayer, 1972). One woman and two men inhaled isoprene at 278–27800 mg/m^3 (about 100–10 000 ppm) for 5 minutes. Isoprene concentrations of 278 mg/m^3 (about 100 ppm) were at the limit of odour perception, 695 mg/m^3 (about 250 ppm) clearly perceptible and 2780 mg/m^3 (about 1000 ppm) very perceptible. In addition, headache and pronounced headache occurred at 13 900 mg/m^3 (about 5000 ppm) and 27 800 mg/m^3 (about 10 000 ppm), respectively. Furthermore, at the highest concentration there was a marked irritation of the bronchi (no further details of the nature of irritation were reported).

BG Chemie (2000) and DFG (2009) also refer to a Russian study (published in Russian) among 10 human volunteers (Gostinskii, 1965). Inhalation of isoprene at 160 mg/m^3 (about 57 ppm) produced mild mucosal irritation in nose, larynx and pharynx. The odour threshold was cited as being 10 mg/m^3 (about 3.6 ppm) (no further details were reported).

There are no human data on skin or eye irritation or on corrosivity.

Studies conducted in rubber production in general were not considered useful due to concomitant exposure to several chemicals and lack of knowledge of quantitative exposure levels to specific chemicals.

7.4.2 Animal data

Isoprene has been indicated as slightly irritating to the skin (ECHA, 2021). Reversible erythema was observed in a rabbit study (OECD, 2005). There are very limited data on eye irritation, but one study indicates a potential to irritate the eyes (OECD, 2005).

In mice, exposure at high dose levels resulted in some studies in decreased respiration rates and respiratory minute volume. The RD_{50} value, reflecting respiratory irritation, was calculated as 57200 ppm 161900 mg/m^3 for mice (Wilkins et al., 2001, Wolkoff et al., 2000).

7.4.3 *In vitro* data

No data available.

7.4.4 Summary

Isoprene may cause slight irritation, but there are no indications of corrosive properties.

7.5 Sensitisation

7.5.1 Human data

7.5.1.1 Respiratory sensitisation

There are no human data on respiratory sensitisation.

7.5.1.2 Skin sensitisation

There are no human data on skin sensitisation.

7.5.2 Animal data

7.5.2.1 Respiratory sensitisation

There are no animal data on respiratory sensitisation.

7.5.2.2 Skin sensitisation

There are no animal data on skin sensitisation.

7.5.3 *In vitro* data

No data available.

7.5.4 Summary

There are no data available on sensitisation.

7.6 Genotoxicity

7.6.1 Human data

There are no human data on genotoxicity.

7.6.2 Animal data

Positive results have been reported in several *in vivo* studies. Increased numbers of micronuclei have been detected in peripheral blood erythrocytes of male mice exposed for 12 days to isoprene at 700 ppm (1980 mg/m^3) and above (NTP, 1999, Placke et al., 1996, Shelby, 1990, Shelby and Witt, 1995, Tice, 1988, Tice et al., 1988). Also, after sub-chronic exposure micronuclei were induced in peripheral blood erythrocytes of exposed mice. Effects were seen in males at 700 ppm and above (NOAEC 220 ppm) and in females at 220 ppm and above (NOAEC 70 ppm; 200 mg/m^3) after 13 weeks of exposure in the NTP study (NTP, 1999), and in male mice at 2200 ppm (6230 mg/m^3) after 40 weeks of inhalation exposure (NOAEC 140 ppm) (Placke et al., 1996). However, in a four-week inhalation study with isoprene doses up to 7000 ppm (19800 mg/m^3), micronuclei were not induced in lung fibroblasts of male and female rats (NTP 1999).

Increased sister chromatid exchange frequencies were detected in the bone marrow of male mice upon 12 days of inhalation exposure at doses of 220 ppm (630 mg/m^3) (Shelby, 1990) or 438 ppm (1240 mg/m^3) (Tice, 1988, Tice et al., 1988). At 700 ppm (1980 mg/m^3) and above, there was no further increase in the sister chromatid exchange frequencies.

Inhalation exposure to isoprene (doses up to 7000 ppm; 19800 mg/m^3) for 12 days did not cause an induction of chromosomal aberrations in the bone marrow of the exposed male mice (Shelby, 1990, Shelby and Witt, 1995, Tice, 1988, Tice et al., 1988).

In studies by Hong et al. (1997) and Sills et al. (Sills et al., 2001, Sills et al., 1999), the occurrence of mutations were studied in tumours in the Harderian gland, lung and forestomach found in mice upon exposure to 2200 ppm (6230 mg/m^3) isoprene for 26 weeks, followed by a recovery period of 26 weeks. The results showed increased frequencies of K-ras and H-ras mutations.

7.6.3 *In vitro* data

Isoprene has been tested for mutagenicity in several bacterial studies (Ames tests). No mutations have been reported in the presence or absence of metabolic activation (DeMaster and Nagasawa, 1978, Kushi et al., 1985, Mortelmans et al., 1986, NTP, 1995, NTP, 1999, NTP, 1983). No increase in the incidence of chromosomal aberrations was observed in Chinese hamster ovary (CHO) cells exposed to isoprene (NTP, 1995, 1999). No effects were seen in sister chromatid exchange tests with isoprene in CHO cells (NTP 1995, 1999).

Isoprene-induced DNA damage was observed in the Comet assay performed in peripheral blood mononuclear cells (PBMCs) or human leukaemia cells (HL60) with metabolic activation. No effects were seen in the absence of metabolic activation. In contrast, the isoprene mono-epoxide 3,4-epoxy-3-methyl-1-butene (EPOX I) alone induced DNA damage in both PBMCs and human leukaemia cells (HL60) in the absence of added metabolic activation. (Fabiani et al., 2007)

The study by Gervasi et al. (1985) reported no mutations in Ames tests with the monoepxides 1,2-epoxy-2-methyl-3-butene or 1,2-epoxy-3-methyl-3-butene (=3,4-epoxy-3-methyl-1-butene, EPOX I) without metabolic activation. The diepoxide 1,2:3,4-epoxy-2-methylbutane, on the other hand, caused mutagenic effects in Salmonella typhimurium TA100 without metabolic activation.

7.6.4 Summary

There are no data on human genotoxicity. Animal studies show genotoxic effects of isoprene, mainly observed as increased frequencies of micronuclei in peripheral blood erythrocytes and of sister chromatid exchange frequencies in bone marrow of mice upon inhalation exposure. In bacterial tests with isoprene or monoepoxide metabolites, 1,2-epoxy-2-methyl-3-butene and 1,2-epoxy-3-methyl-3-butene did not induce genotoxic effects, whereas the diepoxide 1,2:3,4-diepoxybutane caused mutagenicity. DNA damage has been reported in one study in peripheral blood mononuclear cells (PBMCs) or human leukaemia cells (HL60) with metabolic activation. Furthermore, the mono-epoxide EPOX I alone induced DNA damage in both cell types without metabolic activation.

7.7 Carcinogenicity

7.7.1 Human data

IARC (1999) did not identify any human studies having assessed carcinogenicity of isoprene. Based on *sufficient evidence* in experimental animals for the carcinogenicity of isoprene, IARC evaluated isoprene as *possibly carcinogenic to humans (Group 2B).* Following IARC (1999) assessment Rice and Boffetta (2001) assessed the outstanding issues and research priorities in epidemiology for 1,3-Butadiene, isoprene and chloroprene. For isoprene they concluded that while no epidemiologic studies are available on cancer risk from occupational exposure to isoprene, such studies could be conducted within the framework of existing or future studies of styrene-butadiene rubber workers, assuming that isoprene exposure can be disentangled from butadiene and styrene exposure, and they welcomed a feasibility study on this. Furthermore, Rice and Boffetta (2001) noted that the other industry in which isoprene exposure occurs is the chemical industry. The methodological problems of a possible study of isoprene-exposed chemical workers, however, would be similar to those described above with respect to the synthetic rubber industry.

Since the Rice and Boffetta (2001) assessment several cancer follow-ups have been published in North American rubber industry worker cohorts (Sathiakumar et al. (2005), Sathiakumar et al. (2009), Sathiakumar et al. (2015), Sathiakumar et al. (2019), Sathiakumar et al. (2021), Sielken and Valdez-Flores (2011)), in pooled European

rubber industry cohorts (Boniol et al. (2016), Boniol et al. (2017b)) and a cross-sectional study among workers of a petrochemical plant producing acrylonitrile butadiene styrene copolymer in Iran (Sadeghi-Yarandi et al. (2020)). However, these studies focused on either risk from rubber industry as such or risk from butadiene (and styrene) while no assessment of cancer risk from isoprene exposure was performed. In a further meta-analysis Boniol et al. (2017a) combined data from 46 cohort and 59 case-control studies having assessed risk of cancer in the rubber manufacturing industry (both studies published before and after the above IARC assessment). The risk estimate was increased for bladder cancer (SRR = 1.36; 95% CI 1.18 - 1.57), leukaemia (SRR = 1.29; 95% CI 1.11 - 1.52), lymphatic and haematopoietic system (SRR = 1.16; 95% CI 1.02 - 1.31) and larynx cancer (SRR= 1.46; 95% CI 1.10 - 1.94). For lung cancer, a borderline statistically significant increased risk was identified (SRR = 1.08; 95% CI 0.99 - 1.17). No association was found for stomach cancer (SRR = 1.06; 95% CI 0.95 - 1.17). In further stratified analyses, risks of cancer were not increased for workers employed after 1960 for bladder cancer (SRR = 1.06; 95% CI 0.66 - 1.71), lung cancer (SRR = 0.94; 95% CI 0.68 - 1.29) or leukaemia (SRR = 0.92; 95% CI 0.62 - 1.36). However, only risk estimates for rubber industry work as such were reported, no risk estimates for isoprene or any other specific chemical exposure could be assessed. SCOEL (2016) assessed rubber fumes and dusts and identified that workers in the rubber trade usually experience concomitant exposure to a high number of a wide variety of chemicals.

No isoprene exposed chemical industry or other cohort studies were identified. Neither were cancer case-control studies identified that would have assessed risk from isoprene exposure.

7.7.2 Animal data

The most relevant animal data are summarised below. Extensive overviews of animal studies and tumour incidences can be found in DFG (2009) and BGChemie (2000).

An increased incidence of testicular interstitial cell hyperplasia over the control group, was observed in male F344/N rats exposed to isoprene via inhalation for 26 weeks (6h/day, 5 days/week) at 220 ppm (620 mg/m^3) (3/10 animals) and 2200 ppm (6230 mg/m^3) (3/10 animals), reaching statistical significance at the highest dose level group, with all animals exposed to 7000 ppm (19800 mg/m^3) being affected (10/10) (NOAEC 70 ppm; 200 mg/m^3). Following a 26-week recovery period, the incidence/severity of hyperplastic lesions was marginally increased in the exposed groups, over the substantially affected control group (Table 7). An increase with increasing isoprene doses, in benign testicular interstitial cell tumours was also observed. (Melnick et al., 1994, Melnick et al., 1996, NTP, 1995)

Table 7: Incidences of testicular lesions/tumours in male F344/N rats exposed to isoprene for 26 weeks (10 rats/group), followed by a 26-week recovery (30 rats/group) (Melnick et al., 1994)

Doses (ppm)	0	70	220	700	2200	7000
After 26-week exposure						
Interstitial cell hyperplasia	1/10[a]	1.10	3/10	1/10	3/10	10/10*
After 26-week recovery						
Interstitial cell hyperplasia	25/30	29/30	28/30	30/30**	29/30	29/30
Interstitial cell adenoma	3/30	3/30	4/30	7/30	8/30	9/30

[a] number of lesion-bearing animals/number of animals examined
Fisher exact test: *p≤0.01, **p≤0.05

In a 2-year inhalation study of NTP (1999), groups of 50 male and 50 female F344/N rats inhaled isoprene 6 h/day, 5 days per week for 105 weeks at concentrations of 0, 220, 700 or 7000 ppm (0, 620, 1980, 19800 mg/m^3). An exposure-related increased incidence of mammary gland fibroadenomas, compared to controls, was found in both male and female rats, exposed to 220 ppm and above (incidence exceeding the range of historical controls; statistically significance reached at 7000 ppm in male rats and at 220 ppm and above in female rats). Significant, exposure-related increases in the occurrences of renal tubule adenomas and interstitial cell tumours of the testes, compared to the controls, were reported in male animals at 700 ppm isoprene and above. In addition, four mammary gland carcinomas, rarely occurring in chamber control male rats, were observed only in the groups exposed to isoprene. The carcinoma incidence was not increased in exposed female rats. Single occurrences of rarely occurring female brain tumours (e.g., malignant astrocytoma, malignant glioma, malignant medulloblastoma) were regarded as potentially substance related (Table 8). Isoprene exposure did not affect body weight or survival. (NTP, 1999)

Table 8: Incidences of adenomas/carcinomas in 50/sex/group of necropsied/microscopically examined F344/N rats, exposed to isoprene in the 2-year inhalation study (extracted in parts from NTP, 1999)

Doses (ppm)	0		220		700		7000	
Mammary gland	M	F	M	F	M	F	M	F
Fibroadenoma Multiple[a]	1	7	1	12	0	19**	7*	17**
Fibroadenoma (Includes Multiple)[b]	2 (4%)	19 (38%)	4 (8%)	35 (70%)	6 (12%)	32 (64%)	21 (42%)	32*** (64%)
Carcinoma	0	4	1	2	1	1	2	3
Kidney (Male only)								
Renal tubule Adenoma (incl. multiple)[c]	2		4		8**		15*	
Renal tubule Adenoma or carcinoma[d]	2(4%)		4(8%)		8(16%)****		15(30%)***	
Testis								
Interstitial Cell Adenoma, Bilateral[a]	20		29		37*		48*	
Interstitial Cell Adenoma (includes bilateral)[f]	33(66%)		37(74%)		44(88%)***		48(96%)***	

[a] Number of animals with neoplasm/lesion
[b] Overall rate; Number of animals with neoplasm per number of animals necropsied (n=50)
[c] Single sections and step sections data analysis (combined)
[d] Overall rate; Number of animals with neoplasm per number of animals with kidney examined microscopically (n=50)
[f] Overall rate; Number of animals with neoplasm per number of animals with testis examined microscopically (n=50)
Poly-3 test: *p≤0.01, **p≤0.05, ***p<0.001, ****p=0.002 p=0.047

In male B6C3F$_1$ mice, a 26-week isoprene inhalation study was also performed by NTP, using dose levels of 0, 70, 220, 700, 2200 and 7000 ppm (0, 198, 620, 1980, 6230, 19800 mg/m^3), followed by a recovery period of 26 weeks. At the end of the follow-up period, an increased incidence of Harderian gland adenomas over the control group, was reported at all dose levels (statistically significant at 700 ppm and above), while significant increases were also reported in the combined incidence of hepatocellular adenomas or carcinomas (at 700 ppm and above), alveolar/bronchial adenomas or carcinomas (at 2200 ppm and above), and squamous cell papillomas or carcinomas of the forestomach at 7000 pm (Table 9). Increased mortality at 2200 and 7000 ppm was observed at the end of the study period. (Melnick et al., 1994, Melnick et al., 1996, NTP, 1995)

Table 9: Incidences of adenomas/carcinomas in male B6C3F$_1$ mice exposed to isoprene for 26 weeks, followed by a 26-week recovery (reproduced in part from Melnick et al., 1994)

Doses (ppm)	0	70	220	700	2200	7000
Liver						
Hepatocellular adenoma	4/30[a]	2/30	6/29	15/30*	18/30*	16/28*
Hepatocellular carcinoma	4/30	1/30	3/29	5/30	4/30	9/28**
Adenoma or carcinoma	7/30	3/30	7/29	15/30**	18/30*	17/28*
Lung						
Alveolar/bronchial adenoma	2/30	2/30	1/29	4/30	10/30**	8/28**
Alveolar/bronchial carcinoma	0/30	0/30	0/29	1/30	1/30	3/28
Adenoma or carcinoma	2/30	2/30	1/29	5/30	10/30**	9/28*
Forestomach						
Squamous cell papilloma	0/30	0/30	0/29	1/30	2/30	5/28
Squamous cell carcinoma	0/30	0/30	0/29	0/30	2/30	1/28
Papilloma or carcinoma	0/30	0/30	0/29	1/30	4/30	6/28**
Harderian gland adenoma	2/30	6/30	4/29	14/30*	13/30*	12/28*

[a] number of lesion-bearing animals/number of animals examined
Fisher exact test: *p≤0.01, **p≤0.05

In a set of long-term studies, B6C3F$_1$ mice (50 male and/or female/group) were exposed to isoprene by inhalation for 80 weeks (8 h/day, 5 days/week), followed by a recovery period up to week 104 (Cox et al., 1996, Placke et al., 1996). The doses for females were 0, 10 and 70 ppm (0, 24, 200 mg/m^3), and for males 0, 10, 40, 280, 700, 2200 ppm (0, 24, 110, 790, 1980, 6230 mg/m^3). In addition, groups of male mice were exposed for 20 weeks (0, 280, 2200 ppm; the last one 4 h/day) and 40 weeks (0, 70, 140, 2200 ppm; corresponding to 0, 198, 400, 6230 mg/m^3). Also, these groups were examined 104 weeks after the beginning of the exposures. Significantly increased incidences of Harderian gland adenomas were observed in male mice at 70 ppm isoprene and above, already after 20 weeks of exposure. Other increased tumour types in male animals included hepatocellular adenomas at 140 ppm and above, histiocytic sarcomas at 280 ppm and above, and alveolar/bronchial adenomas and carcinomas at 700 ppm and above. (Table 10). In female mice, the incidences of Harderian gland adenomas and pituitary gland adenomas were significantly increased at 70 ppm. The pituitary gland findings were however not considered clearly substance related, as the incidence was below that of historical controls. Furthermore, there was a slight, but not statistically significant increase in the incidence of hemangiosarcomas in the spleen of females at 70 ppm. (Table 11). As different exposure durations were applied for the groups of male animals, the authors were able to investigate the effect of dose versus the effect of

exposure duration. It was considered that the dose level has a bigger impact than the exposure duration on the tumour frequencies. (Cox et al., 1996, Placke et al., 1996)

Table 10: Incidences of selected exposure-related neoplasms in a subset of male B6C3F$_1$ mice (50/group) exposed to isoprene for 80 weeks (8 h/day, 5 days/week) (extracted in parts from Placke et al., 1996 and Cox et al., 1996)

Exposure (ppm/weeks)	0/80	10/80	70/80	280/80	700/80	2200/80
Alveolar/bronchiolar Adenoma	11/50[a]	16/50	4/50	13/50	23/50*	30/50*
Alveolar/bronciolar Carcinoma	0	1	2	1	7*	7*
Hepatocellular Adenoma	11/50	12/50	15/50	24/50*	27/48*	30/50*
Hepatocellular Carcinoma	9	6	9	16	17	16
Harderian gland Adenoma	4/47	4/49	9/50	17/50*	26/49*	35/50*
Harderian gland Carcinoma	0	0	0	1	3	2
Histiocytic sarcoma	0/50	2/50	2/50	4/50	2/50*	2/50

[a]Fraction of animals found to have the reported tumour type (row heading) at necropsy
Fisher exact test: *p≤0.05

Table 11: Incidences of selected neoplasms in female B6C3F$_1$ mice (50/group) exposed to isoprene for 80 weeks (8 h/day, 5 days/week) (extracted in part from Placke et al., 1996)

Exposure (ppm/weeks)	0/80	10/80	70/80
Harderian gland Adenoma	2/49	3/49	8/49*
Harderian gland Carcinoma	0	0	0
Pituitary adenoma	1/49	6/46	9/49*
Hemangiosarcoma Spleen	1/50	1/49	4/50

Fisher exact test: *p<0.05

7.7.3 Summary

It has been clearly demonstrated that inhalation exposure with isoprene induces tumours in rats and mice. However, no isoprene exposed chemical industry or other cohort studies were found. While the epidemiological studies indicate a reduction of risk for more recently exposed populations in the rubber industry overall, no risk estimates have been established on exposure to isoprene itself.

7.8 Reproductive toxicity

7.8.1 Human data

There are no human data on reproductive toxicity.

7.8.2 Animal data

Fertility

There are no studies on the effects of isoprene on sexual function and fertility (e.g., screening study, extended one-generation reproductive toxicity study or 2-generation study).

In repeated dose toxicity studies some investigations on reproductive organs are normally included. No effects on testes weights, epididymides weights, sperm parameters, or the oestrus cycle were observed in F344/N rats after 13 weeks of inhalation exposure (6 h/day, 5 days/week) with isoprene (doses 70, 700, 7000 ppm (corresponding to 198, 1980, 19800 mg/m^3); NOAEC 7000 ppm) (Melnick et al., 1994, NTP, 1995). In mice, on the other hand, a NOAEC of 70 ppm isoprene was identified upon inhalation exposure for 13 weeks. Effects including decreases in the absolute weight of epididymides and cauda epididymides, sperm motility, sperm concentration, and number of spermatids and sperm heads per testis were observed at 700 ppm. In addition, at the highest dose level (7000 ppm) decreased absolute testis weight and testicular atrophy were reported. (Melnick et al., 1994, NTP, 1995).

In male F344/N rats exposed to 7000 ppm (19800 mg/m^3) isoprene by inhalation for 6 months (6 h/day, 5 days/week), hyperplasia was observed in the interstitial cells of the testes. After a 6-month recovery period, interstitial cell adenomas were found. (Melnick et al., 1994, Melnick et al., 1996, NTP, 1995). In the same reports, testicular atrophy and decreased absolute and relative testis weight was described for B6C3F$_1$ mice at 7000 ppm isoprene exposure for 6 months. No such effects were seen after a recovery period of 6 months (Melnick et al., 1994, Melnick et al., 1996, NTP, 1995).

The study by Doerr et al. (1995) showed that intraperitoneal administration of 7.34 mmol isoprene/kg body weight/day (~500 mg/kg bw/day) correlated with a reduction in the number of small and growing follicles in the ovaries of female B6C3F$_1$ mice.

Developmental toxicity

No statistically significant changes in body weight, or external, skeletal, or visceral effects were observed among the foetuses of pregnant Sprague Dawley rats exposed to isoprene by inhalation (280, 1400 or 7000 ppm; corresponding to 790, 3960, 19800 mg/m^3) during gestation days 6-19. Furthermore, there were no changes in pregnancy parameters or survival rate. No maternal toxicity was reported, and the NOAEC for maternal and developmental effects was thus 7000 ppm isoprene (NTP 1995).

Maternal toxicity, including decreased body weight gain and increased absolute and relative kidney weight was reported for pregnant CD-1 Swiss mice upon inhalation exposure to 7000 ppm (19800 mg/m^3) isoprene during gestation days 6-17. The NOAEC for maternal toxicity was 1400 ppm (3960 mg/m^3) (NTP 1995). In the foetuses, the occurrence of variations or reduced ossification (supernumerary ribs mainly) was increased in the dose groups 1400 ppm and 7000 ppm. In addition, the body weight of male foetuses was decreased at 1400 and 7000 ppm. In female foetuses there was a concentration-dependent decrease in the body weight of female foetuses at all doses (280, 1400, 7000 ppm). However, DFG (2009) considered that the NOAEC can be identified as 280 ppm, as at that dose level, the number of living foetuses per litter was on average markedly higher in the other dose groups. This may be an important reason for the observed lower body weight of foetuses. Furthermore, DFG (2009) noted that the NOAEC for maternal toxicity effects (1400 ppm) was surprisingly high, as haematotoxic and hepatotoxic effects would have been expected at lower concentrations, but no details on such investigations were presented in NTP (1995).

In the study by Komatsu (1971, reviewed in DFG 2009), pregnant Wistar rats administered doses of 0, 22, 379 or 1895 mg isoprene/kg bw/day on gestation days 9-12. No effects on body weight gain were reported but there was some increase in the resorption frequencies, being 0%, 4.8%, 3.1% and 6.3%, respectively. Some differences

in body weight or reduced sternal ossification of surviving foetuses were observed, but these were not dose dependent. DFG (2009) considered that the study cannot be used for the evaluation of developmental toxicity of isoprene, due to uncertainties for example related to oral administration of a volatile substance.

7.8.3 Summary

There are limited data on effects of isoprene on sexual function and fertility. In repeated dose studies some effects on testes have been reported at high dose level (7000 ppm). Regarding developmental effects, some minor findings (foetal weight, reduced ossification) have been reported.

8. Other considerations

8.1 Mode of action (MoA) considerations

Isoprene is classified as Carc. 1B under CLP, and there are several *in vivo* studies showing tumour formation after long-term inhalation exposure. There are no human epidemiological studies on the carcinogenicity of isoprene.

Genotoxicity studies show effects *in vivo*, but ambiguous results *in vitro*. The genotoxic effects have been linked to the formation of methyl-1,2:3,4-diepoxybutane, a mutagenic diepoxide metabolite (BGChemie, 2000, DFG, 2009). No mutagenic effects or induction of chromosome aberrations or sister chromatid exchanges have been detected under *in vitro* conditions with isoprene exposure. The monoepoxide metabolites 1,2-epoxy-2-methyl-3-butene and 1,2-epoxy-3-methyl-3-butene have not been shown to cause genotoxic effects.

Increased frequencies of K-ras and H-ras mutations have been detected in Harderian gland tumours found in isoprene-exposed mice 26 weeks after the beginning of the inhalation exposure period (Hong et al., 1997, Sills et al., 2001, Sills et al., 1999).

Based on the available information, isoprene is considered a genotoxic carcinogen with genotoxic effects seen *in vivo*, but not *in vitro,* indicating that metabolism plays an important role. The effects could thus be identified as non-threshold effects (see 9.1.2). However, it is important to note that isoprene is endogenously formed in humans, at an estimated rate of 0.2 µmol/kg bw/hour. Approximately 10% of the endogenous isoprene is exhaled in unchanged form and the rest is metabolised to monoepoxides and further to the diepoxide methyl-1,2:3,4-diepoxybutane (Hong et al., 1997, Sills et al., 2001, Sills et al., 1999). The endogenous formation of isoprene in rats and mice is significantly lower than in humans. Therefore, it is difficult to derive an exposure-risk relationship from animal data that would account for the cancer risk in humans.

Repeated dose studies show some neurological effects in mice (hindlimb dysfunction and degeneration of the white matter of the spinal cord).

It is noted that the monoepoxides of isoprene can form haemoglobin adducts, but the potency of the effects seems to be significantly lower than for example with the related substance butadiene. This mechanism is considered to have only a minor impact on the toxicity of isoprene.

8.2 Lack of specific scientific information

No specific information gaps were identified.

8.3 Groups at Extra Risk

No groups at extra risk were identified.

9. Evaluation and recommendations

9.1 Cancer risk assessment

9.1.1 Published approaches for cancer risk assessment

9.1.1.1 DFG and AGS

DFG (2005) considered isoprene a genotoxic carcinogen with genotoxicity mediated by a metabolite. DFG also noted that isoprene is endogenously produced in the human body. AGS (2012) agreed on the approach. The approach taken is described in Section 9.2.1.1 and the AGS (2012) additional estimation of the residual cancer risk is described in Section 9.2.1.2.

9.1.1.2 Japanese Society for Occupational Health

The Committee for Recommendation of Occupational Exposure Limits of the Japan Society for Occupational Health (JSOH) classifies the occupational carcinogens based primarily on the epidemiological evidence, but the results of the animal experiments and their extrapolation to human are also considered (JSOH, 2018). The classification is made by strength of the evidence but does not reflect the carcinogenic potency. For isoprene, an earlier carcinogenicity classification of group 2B continued to be indicated in the recent assessment (Azuma et al., 2017). The JSOH classification is using categories similar to IARC and group 2B refers to substances “with less sufficient evidence (possibly carcinogenic to humans)” (JSOH 2018).

It is noted that according to the JSOH (2018) methodology “only when scientifically reasonable information is available, JSOH will estimate a reference value corresponding to an individual excess lifetime risk of cancer due to exposure to a Group I (=carcinogenic to humans) carcinogen”.

For isoprene Azuma et al, (2017) derived an OEL thus following a threshold approach (see Section 9.2.1).

9.1.1.3 American Industrial Hygiene Association

The Workplace Environmental Exposure Level (WEEL) Committee of American Industrial Hygiene Association (AIHA) noted that isoprene is clearly carcinogenic in animal assays (AIHA, 2004). The Committee noted, however, that neurotoxic effects have been observed at lower inhalation exposure levels than carcinogenic effects and derived an OEL based on those effects (see Section 9.2.1.3).

9.1.2 Cancer risk assessment

There are no epidemiological studies directly assessing the cancer risk of isoprene. However, isoprene carcinogenicity has been clearly demonstrated in rats and mice. While isoprene itself is not DNA reactive and not mutagenic *in vitro*, the isoprene-derived diepoxide and perhaps one of the monoepoxides generated *in vivo* are mutagenic in bacterial or mammalian test systems, respectively, and are thus most likely the critical metabolites associated with tumour formation. Therefore, isoprene has to be regarded as a genotoxic carcinogen, and in principle additional cancer incidence estimates could be derived by linear extrapolation from the mice carcinogenicity data, with mice being the most sensitive species. Nevertheless, with regard to quantitative risk assessment, two major aspects need to be considered, namely species differences in metabolism and endogenous levels of isoprene.

It appears that especially mice but also rats are more susceptible towards isoprene when compared to humans, most likely due to differences in metabolism. Aspects on metabolism are discussed in section 7.1.

Endogenous blood levels of isoprene are significantly (about 30-fold) lower in rats than in humans. Approximately 10% of the endogenous isoprene is exhaled in unchanged form by humans and the rest is metabolised to monoepoxides and further to diepoxide. In mice studies, no exhaled isoprene has been detected.

Therefore, it is not possible to derive an exposure risk relationship from animal data that would account for the cancer risk in humans that is due to endogenous formation of isoprene. As further described in section 9.2.2, it is recommended to set an OEL so that exposure at work would not contribute significantly to the body burden of the toxic metabolite that is due to the endogenous isoprene formation.

9.2 Derived Occupational Exposure Limit (OEL) Values

9.2.1 Published approaches to establishing OELs

9.2.1.1 DFG

The report by DFG (2009) established a MAK value of 3 ppm (8.5 mg/m^3) for isoprene. DFG concluded that isoprene is a genotoxic carcinogen. As the mutagenic effects are caused by the metabolite methyl-1,2:3,4-diepoxybutane, but there are no data on internal exposure to metabolites, DFG considered that it was not possible to derive a MAK value from toxicity data. Instead, DFG established its MAK value based on data on the area under the isoprene concentration-time curve (AUC) in the blood, which was considered a meaningful parameter for isoprene exposure. In addition, data on the endogenous production of isoprene was taken into account.

To estimate the AUC, a physiological toxicokinetic (PT) model for inhaled and endogenously formed isoprene was applied (Csanady and Filser, 2001, Filser et al., 1996). For the estimation of endogenously formed isoprene in the general population, measured data on isoprene concentrations in exhaled air was collected from several publications. When running the PT model with the exhalation concentrations for an adult (body weight 70 kg) the rate of endogenous isoprene formation was estimated as 13.1 ± 10 µmol/h (corresponding to 0.19 µmol/h kg bw). The concentration in venous blood was estimated as 5.2 ± 4.0 nmol/l, and the AUC (0-80 years) as 3.6 ± 2.8 mmol x h/l. Next, the additional AUC for a situation with occupational exposure at 10 ppm (ml/m^3) for 40 years (8 h/day, 5 days/week, 48 weeks/year) was estimated from the model, ending up with a value of 9.8 mmol x h/l. The expected venous blood concentration after 8 h of exposure was 133 nmol/l. The average concentration in blood at the end of an 8-h exposure to 10 ppm at work was calculated to be 26-fold higher compared to non-exposed (133 nmol/l versus 5.2 nmol/l), whereas the AUC would be only 2.7-fold higher (9.8 mmol x h/l versus 3.6 mmol x h/l). This is because exposure at work accounts for about 1/10 of the time during a life span of 80 years.

Finally, DFG considered that “The AUC after exposure to an isoprene concentration of about 3 ml/m^3 [3 ppm] for eight hours daily over 40 years is the same as the AUC for lifelong exposure at the level of the standard deviation of the mean endogenous isoprene concentration […]. Therefore, exposure to 3 ml/m^3 makes no significant contribution to the cancer risk.”.

9.2.1.2 AGS

AGS (2012) agreed to apply an 8-hour TWA limit value of 3 ppm, following the arguments presented by DFG (2009). In addition to the DFG approach, for non-cancer effects AGS identified a LOAEC of 10 ppm in an 80-week inhalation study with mice. It was considered that there is probably no need for an assessment factor for inter/intraspecies variations because mice are considered to have a higher epoxide

burden than humans. Following this approach, application of only a factor of 3 (for LOAEC-NAEC extrapolation) leads to a limit value of 3 ppm. Application of an additional standard assessment factor of 5 (for inter/intraspecies variations) would result in a value of 0.66 ppm, which AGS considered unrealistic in perspective of the endogenous isoprene concentrations.

At the level of the 8 h TWA (3 ppm), the residual cancer risk was estimated by AGS as 4:1000 using data obtained in three chronic studies in rodents (NTP, 1995, NTP, 1999, Cox et al., 1996). By application of a benchmark dose approach, it was estimated that the testicular cancer risk would be 4:1000 at 5.6 ppm. For mammary gland adenomas and pituitary gland adenomas, the risk would be 4:1000 at 3.0 ppm and 2.6 ppm respectively, estimated with a T25 approach.

AGS (2012) considered that extrapolation to lower residual risk values would not be relevant since the 3 ppm limit value is based upon endogenous (and not workplace) isoprene exposure. AGS concluded that as the proposed limit value, derived from non-carcinogenic data, is the same as the concentration of the estimated tolerable residual cancer risk (4:1000), and since the value is in the range of the load caused by endogenous isoprene, the limit value of 3 ppm can be used.

It is noted that the interpretation of the residual cancer risk calculations by AGS is complicated by the far lower endogenous levels of isoprene and higher levels of toxic isoprene-derived epoxides in rodents compared to humans, which leads to overestimation of the human cancer risk with a factor that cannot be estimated.

9.2.1.3 Japanese Society for Occupational Health

The Committee for Recommendation of Occupational Exposure Limits of the Japan Society for Occupational Health (JSOH) recently proposed an OEL for isoprene (Azuma et al. 2017). JSOH considered a NOAEL of 10 ppm (28 mg/m^3) in mice based on observed pathological changes: increase in spinal degeneration at or above 70 ppm (200 mg/m^3) within a recovery period in male after 26-week inhalation (70-7000 ppm) (200-19800 mg/m^3) (Melnick et al. (1994) and NTP (1995)) and increased incidence of Harderian gland adenoma at or above 70 ppm, and local metaplasia in airway and olfactory epithelium at or above 140 ppm (male) and 70 ppm (female) in 40- and 80-week inhalation studies (Placke et al. (1996) and Cox et al. (1996)). An uncertainty factor of 3 was applied to the NOAEL considering that epoxide biotransformation was lower in humans than mice and an OEL Mean of 3 ppm was proposed.

The OEL Mean is defined “as the reference value to the mean exposure concentration at or below which adverse health effects caused by the substance do not appear in most workers working for 8 hours a day, 40 hours a week under a moderate work-load” (JSOH 2018).

9.2.1.4 American Industrial Hygiene Association

The Workplace Environmental Exposure Level (WEEL) Committee of American Industrial Hygiene Association (AIHA) revised the WEEL recommendation in 2004 (AIHA, 2004). The Committee noted that there was clear evidence of carcinogenicity in mice and rats at concentrations above 220 ppm (630 mg/m^3), and 200 ppm (570 mg/m^3), respectively. There was also no NOAEC identified for haematological effects in a subacute study in mice (LOAEC 438 ppm; corresponding to 1240 mg/m^3). The Committee further noted that a NOAEC was not found for decreased hindlimb grip-strength or spinal cord and sciatic nerve degeneration in a 6-month NTP study in mice (lowest exposure concentration of 70 ppm (200 mg/m^3) and the neurological effects were dose-related. As the LOAEC for these neurological effects was lower than the NOAEC observed for carcinogenicity and LOAEC for haematological effects, the Committee decided to set the WEEL (8-hour TWA) based on the LOAEC of 70 ppm for neurological effects and derived a WEEL of 2 ppm. It is noted that the documentation available does not further describe how the WEEL of 2 ppm was extrapolated from the LOAEC of 70 ppm.

9.2.2 Occupational Exposure Limits (OELs) - 8h TWA

The carcinogenicity of isoprene is recognised as the critical health effect. For the setting of an OEL, it is not possible to derive an exposure-risk relationship from animal data that would account for the human cancer risk that is due to endogenous formation of isoprene. This is due to significant endogenous formation of isoprene and its toxic diepoxide metabolite in humans, whereas endogenous formation is much lower in rats and mice (see Section 9.1.2.).

Therefore, it is proposed to follow a similar approach as DFG (2009), meaning the identification of an exposure level, which could be expected to be within the statistical range of the total internal isoprene levels. As explained in Section 9.2.1.1, using a PT-model, the additional AUC for isoprene in the blood was estimated for a situation with occupational exposure at 10 ppm for 40 years (8 h/day, 5 days/week, 48 weeks/year). When running the PT model with the exhalation concentrations for an adult person, the life-long AUC (0-80 years) was estimated as 3.6 ± 2.8 mmol x h/l. The additional AUC for a situation with 40 years of occupational exposure at 10 ppm was estimated to be approximately 9.8 mmol x h/l (DFG, 2009). From this, it can be estimated that occupational exposure to one third of that concentration, i.e., 3 ppm, would be approximately at the same level as the standard deviation of the AUC for life-long endogenous isoprene formation (3.6 ± 2.8 mmol x h/l). If the work-life long isoprene exposure level was 3 ppm, the resulting isoprene levels are still within the range of endogenous formation and only little additional cancer risk is expected, provided that the proposed OEL is complied with. Based on this an 8 h TWA of 8.5 mg/m^3 (3 ppm) isoprene is proposed.

Since the LOAEL of 10 ppm for spleen and bone marrow toxicity in mice is supposed to be due to the toxic epoxides of isoprene as well and considering the pronounced species differences between mice and humans described above, the proposed OEL is considered to be protective in humans also with respect to chronic toxicity.

With respect to reproductive toxicity, some effects on testes have been reported in repeated dose studies at high dose levels. Regarding developmental effects, some minor findings (foetal weight, reduced ossification) have been reported at high doses. In rats, the NOAEC for developmental toxicity with isoprene was 7000 ppm (highest tested concentration). In mice, decreased foetal weight of male foetuses and an increase of variations or reduced ossification was found, resulting in a NOAEC of 280 ppm (NTP, 1995). The proposed OEL of 3 ppm is at least 90-fold lower compared to the most sensitive species (mice). Therefore, no extra risk during pregnancy is expected.

9.2.3 Short Term Exposure Limits (STELs)

No STEL is proposed. The key effects are related to long-term exposure.

9.2.4 Biological Limit Value (BLV)

No BLV is proposed. The body burden of isoprene is due to endogenous production thus individual levels are variable and of similar magnitude as that caused by exposure at the proposed OEL of 3 ppm, thus biological exposure monitoring at such exposure levels would not be informative.

9.2.5 Biological Guidance Value (BGV)

No BGV is proposed.

9.3 Notations

No notations are proposed.

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