Committee for Risk Assessment
RAC

Opinion on scientific evaluation of occupational exposure limits for isoprene

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

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Telakkakatu 6, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

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OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON THE EVALUATION OF THE OCCUPATIONAL EXPOSURE LIMITS (OELs) FOR ISOPRENE

Commission request

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

Therefore, the Commission made a request on 11 December 2020 to ECHA in accordance with the Service Level Agreement (SLA) (Ares(2019)18725), to evaluate, in accordance with the Directive 2004/37/EC, isoprene which is classified as a carcinogen Category 1B in CLP legislation.

I PROCESS FOR ADOPTION OF THE OPINION

Following the above request from the European Commission RAC is requested to draw up an opinion on the evaluation of the scientific relevance of occupational exposure limits (OELs) for isoprene with a deadline of 30 September 2022.

Chemical name(s): isoprene

In support of the Commission's request, ECHA prepared a scientific report concerning occupational limit values for isoprene at the workplace. In the preparatory phase of making this report, a call for evidence was opened on 14 April 2021 to invite interested parties to submit comments and evidence on the subject by 13 July 2021. This scientific report was made publicly available[2] on 11 October 2021 and interested parties were invited to submit comments by 10 December 2021.

RAC developed its opinion on the basis of the scientific report submitted by ECHA. During the preparation of the opinion, the scientific report was further developed as an Annex to the RAC opinion.

The RAC opinion includes a recommendation to the Advisory Committee on Safety and Health at Work (ACSH) in line with the relevant Occupational Safety and Health legislative procedures.

II ADOPTION OF THE OPINION OF THE RAC

Rapporteurs, appointed by RAC: **Andrea Hartwig** and **Gerlienke Schuur**.

The opinion of RAC was adopted by **consensus** on **18 March 2022.**

[1] http://ec.europa.eu/social/main.jsp?langId=en&catId=148&newsId=2709&furtherNews=yes

[2] https://echa.europa.eu/documents/10162/431f6bc0-a4b3-a1a1-4e8f-6c8eb48ab64a

RAC Opinion of the assessment of the scientific relevance of OELs for isoprene

RECOMMENDATION

The opinion of RAC on the assessment of the scientific relevance of Occupational Exposure Limits (OELs) for isoprene is set out in the table below and in the following summary of the evaluation.

SUMMARY TABLE

The table presents the outcome of the RAC evaluation to derive limit values for the inhalation route and the evaluation for dermal exposure and a skin notation.

Derived Limit Values

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

Notations

Notations:	none

RAC OPINION

Background

This opinion concerns **isoprene** (See section 1 of Annex 1).

This evaluation takes previous reviews into account, in particular international assessments such as DFG (2009), IARC (1999), OECD (2005), BG Chemie (2000) and AGS (2012). This has been complemented by a literature search of published papers from the last ten years.

Key conclusions of the evaluation

- Isoprene (2-methyl-1,3-butadiene) is an intermediate in the chemical and rubber producing industry. Air-monitoring data were collected at three U.S. facilities (in the past) that produced isoprene monomers or polymers; 98.5% of the samples showed concentrations of less than 10 ppm (27.9 mg/m^3), and 91.3% of less than 1 ppm (2.8 mg/m^3) (NTP, 2011); similar data for Europe are missing.
- Isoprene also occurs endogenously, as a basic component of so-called isoprenoids, required for the synthesis of steroids and terpenes.
- Furthermore, isoprene is produced and emitted by many species of trees, accounting for around one-third of all hydrocarbons released into the atmosphere. Nevertheless, it is rapidly degraded, with environmental concentrations reaching low (ng/m^3) levels during the daytime.
- At the workplace, isoprene is easily taken up via inhalation, while dermal uptake is negligible. Isoprene itself is not genotoxic, but is readily metabolised to a genotoxic mono- and diepoxide, predominantly in the liver.
- Whilst no epidemiological studies are available which are suitable to assess the cancer risk to humans, carcinogenicity in rats and mice has been clearly demonstrated.
- Whilst acute toxicity is low, the most sensitive chronic toxicity endpoints of proliferation of haemopoietic cells in the spleen and bone marrow myeloid hyperplasia, were reported in both sexes in mice starting at 10 ppm after long-term exposure. Therefore, 10 ppm is considered the LOAEL for non-cancer effects in mice.
- The most critical adverse health effect is carcinogenicity, mediated presumably and predominantly by the isoprene-derived diepoxide. Due to differences in the epoxide hydrolase activity involved in the detoxification of DNA-reactive epoxides, mice especially, but also rats, appear to be more sensitive when compared to humans. Also, the endogenous production of isoprene, and thus also the steady-state levels of isoprene epoxides, is much lower in mice when compared to humans.
- For the setting of an OEL, it is difficult to derive an exposure-risk relationship from animal data that would reflect the cancer risk in humans, due to the endogenous formation of isoprene and its toxic diepoxide metabolite in humans, as well as pronounced interspecies differences in metabolism. Therefore, it is proposed to follow a similar approach to DFG (2009), i.e., the identification of an exposure level, expected to be within the statistical range of the total internal isoprene levels. Based on a physiological toxicokinetic (PT) model, the respective exposure level would be 3 ppm. Taking mice carcinogenicity data as a basis, this would correspond to an additional cancer risk of 4:1000 (AGS, 2012); however, due to the far lower endogenous levels of isoprene and the higher levels of toxic isoprene-derived epoxides in mice, cancer risks calculated from mice carcinogenicity data by linear extrapolation are likely to overestimate the human cancer risk.

- 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, the proposed OEL is considered to be protective in humans also with respect to chronic toxicity.

Carcinogenicity and mode of action (see sections 7.7 and 8.1 of Annex 1 for full discussion)

Epidemiological evidence

IARC (1999) classified isoprene as "possibly carcinogenic to humans" (2B). Within their evaluation, no human studies were identified as having assessed carcinogenicity of isoprene. Relevant exposure to isoprene occurs in the rubber industry. In the chemical industry, isoprene is used as an intermediate to manufacture respective polymers, mostly in closed production systems. Since the IARC evaluation, several cancer risk assessments and follow-ups have been reported for the North American rubber industry worker cohorts, in pooled European rubber industry cohorts as well as a cross-sectional study among workers of a petrochemical plant producing acrylonitrile butadiene styrene copolymer in Iran (see also SCOEL opinion on rubber fumes and dusts (2016). However, none of these studies assessed cancer risk from isoprene exposure alone. Instead, they focused on either risk from the rubber industry with simultaneous exposure to other carcinogens or on risk from butadiene (and styrene). Regarding the rubber manufacturing industry as such, a meta-analysis published by Boniol et al. (2017) revealed an elevated cancer risk with respect to bladder, leukaemia, the lymphatic and haematopoietic systems and the larynx, as well as borderline effects for lung cancer incidence. However, in the same study, a more refined analysis suggested that the risks of bladder cancer or leukaemia were not increased for workers employed after 1960. A recent update of the North American rubber industry cohort has taken place, which confirmed a positive exposure–response relationship between butadiene and leukaemia incidence among workers, most of whom had coexposure to styrene. Again, no assessment of cancer risk to isoprene was included (Sathiakumar et al., 2021).

Animal carcinogenicity studies

In contrast to the absent epidemiological evidence, it has been clearly demonstrated that inhalation exposure to isoprene induces tumours in rats and mice, and thus isoprene is classified as Carc. 1B under CLP. In a NTP (1999) carcinogenicity study, male and female F344/N rats inhaled isoprene 6 h/day, 5 days per week during 105 weeks at concentrations of 0, 220, 700 or 7000 ppm. The incidence of mammary fibroadenomas in females was significantly increased at 220 ppm and above. In males, increased occurrence of renal tubule adenomas and interstitial cell tumours of the testes were reported at 700 ppm and above. In a set of long-term studies, B6C3F1 mice 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). A significantly increased incidence of Harderian gland adenomas was observed in male mice at 70 ppm isoprene and above, already after 20 weeks of exposure. Other tumour types reported in male mice were 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. In female mice, the incidence of Harderian gland adenomas was increased at 70 ppm. For a more detailed description of tumor incidences in different studies see DFG, 2009, BG Chemie 2000 and Annex 1. Taken together, mice were more sensitive compared to rats, with regard to carcinogenicity.

Mode of action: Metabolism and genotoxicity

Concerning the mode of action, isoprene is considered to be a genotoxic carcinogen, with genotoxic effects seen *in vivo*, but not *in vitro,* indicating that metabolism plays an important role.

An estimated 90% of isoprene is metabolised in the liver and 10% extrahepatically (Csanády and Filser, 2001; Filser et al., 1996; DFG, 2009). In the presence of NADPH and liver microsomes of different test species, isoprene is metabolised by oxidation to two epoxides, namely 1,2-epoxy-2-methyl-3-butene and 1,2-epoxy-3-methyl-3-butene. The first metabolite is formed proportionally to a considerably greater extent and is subject to rapid, mainly non-enzymatic hydrolysis to form 1,2-dihydroxy-2-methyl-3-butene. The second metabolite, 1,2-epoxy-3-methyl-3-butene, is hydrolysed more slowly to form 1,2-dihydroxy-3-methyl-3-butene, mainly catalysed by the microsomal epoxide hydrolase, or oxidised to 1,2:3,4-diepoxy-2-methyl-butane (summarized in DFG, 2009, and Annex 1).

In the absence of metabolic activation, no mutations were observed in Ames tests with the monoepoxides 1,2-epoxy-2-methyl-3-butene or 1,2-epoxy-3-methyl-3-butene, while the diepoxide 1,2:3,4-epoxy-2-methylbutane caused mutagenic effects in Salmonella typhimurium TA100. Regarding mammalian cells in culture, isoprene-induced DNA damage was observed in the Comet assay performed in peripheral blood mononuclear cells (PBMCs) or human leukaemia cells (HL60) only in the presence of metabolic activation. In contrast to bacteria, the isoprene mono-epoxide 1,2-epoxy-2-methyl-3-butene alone induced DNA damage in both PBMCs and HL60 cells in the absence of added metabolic activation (Fabiani et al., 2007). Nevertheless, since 1,2-epoxy-2-methyl-3-butene is rapidly hydrolysed non-enzymatically, the isoprene-derived diepoxide may be considered most critical for carcinogenicity.

At high exposure levels via inhalation (2200 ppm isoprene for 26 weeks, followed by a recovery period of 26 weeks), increased frequencies of K-ras and H-ras mutations were found in tumours of the Harderian gland, lung and forestomach, considered to be an early event in tumour formation.

Besides the generation of DNA damage, formation of haemoglobin adducts has been observed in rats and mice (Sun et al., 1989), which may, however, be considered as a biomarker of exposure to reactive metabolites of isoprene with no toxicological impact.

Consequences for dose-response considerations

Isoprene is considered a genotoxic carcinogen, thus producing in principle non-threshold effects. However, as outlined below, differences in isoprene metabolism exist between animals and humans, and isoprene is endogenously formed in humans. For these reasons, it is difficult to derive an exposure-risk relationship from animal data that would quantitatively reflect the cancer risk in humans.

Chronic Toxicity and Cancer Risk Assessment (see section 9.1 of Annex 1 for full discussion)

The acute toxicity of isoprene is low. Regarding chronic toxicity, 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 in mice, 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. Therefore, the LOAEL for non-cancer effects is considered to be 10 ppm in mice. In a carcinogenicity study by NTP (105 weeks of exposure, 6 h/day, 5 days/week; doses 0, 220, 700 and 7000 ppm) in F344/N rats, renal tubular hyperplasia and fibrotic changes in the spleen were reported in males at 700 and 7000 ppm isoprene. 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).

The most critical effect is the carcinogenicity of isoprene. As stated above, there are no epidemiological studies directly assessing the cancer risk of isoprene. Instead, there have been meta-analyses pointing to an increased cancer incidence in exposed humans in the rubber industry, but focusing on butadiene and styrene. 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.

Species differences in metabolism

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

Tissue levels of isoprene diepoxide, the assumed major toxic metabolite of isoprene, are the result of the balance between three enzyme systems, namely cytochrome P450, epoxide hydrolase and glutathione S-transferase. Species differences between these enzyme systems may, therefore, be responsible for the susceptibility to toxic and carcinogenic effects resulting from isoprene exposure. When comparing enzymatic activities, only marginal differences were found between species 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. Thus, the hydrolysis capacity of isoprene epoxides was found to be much higher in humans, followed by rats and mice, suggesting a lower susceptibility of humans when compared to mice and rats. For the conjugation of epoxides with glutathione S-transferase the reversed order was observed. After incorporation of all the *in vitro* metabolism data in a PBPK model, the predicted isoprene diepoxide levels in liver in mice were slightly higher than in rats, 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, when taking into account the intra-individual variations of enzyme activities in humans, 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 to 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), expected to result in lower cancer risk.

Endogenous levels of isoprene

Similar considerations apply for the endogenous levels of isoprene as a basic component of so-called isoprenoids, required for the synthesis of steroids and terpenes. In humans, isoprene is generated at an estimated rate of 0.2 µmol/kg bw/hour. The mean endogenous blood concentration of isoprene has been reported as 5.2 +/- 4 nmol/L. Approximately 10% of the endogenous isoprene is exhaled in unchanged form and the rest is metabolised to monoepoxides and further to diepoxide (Hong et al., 1997; Sills et al., 2001; Sills et al., 1999). Endogenous blood levels of isoprene are significantly (about 30-fold) lower in rats than in humans; in mice, no exhaled isoprene could be detected.

Approach for setting an OEL

As a consequence of the pronounced species differences described above, it is proposed to follow a similar approach as DFG (2009) i.e., the identification of an exposure level,

which could be expected to be within the statistical range of the total internal isoprene levels. Based on a physiological toxicokinetic (PT) model, the respective exposure level was calculated to be 3 ppm.

Taking mice and rat carcinogenicity data as a basis, this would correspond to an additional cancer risk of 4:1000 (AGS, 2012); however, due to the far lower endogenous levels of isoprene and higher levels of toxic isoprene-derived epoxides especially in mice but also in rats, cancer risks calculated from animal carcinogenicity data by linear extrapolation are likely to overestimate the human cancer risk.

Derived Limit Values (see section 9.2 of Annex 1 for full discussion)

OEL - 8h-TWA

The carcinogenicity of isoprene is recognised as the critical health effect. As described above, for the setting of an OEL, it is difficult to derive an exposure-risk relationship from animal data that would account for the cancer risk in humans, due to species differences with respect to the endogenous formation of isoprene, which is far higher in humans compared to mice, as well as pronounced differences in metabolism, leading to higher levels especially of the isoprene-derived diol epoxide in mice. This metabolite is believed to be most critical for tumour formation. Therefore, mice being the most sensitive species, do not appear suitable for the estimation of cancer risk and the derivation of a health-based OEL.

Based on these considerations, RAC followed a similar approach to DFG (2009), meaning the identification of an exposure level, which will not exceed the statistical range of the total internal isoprene levels in humans. As described by DFG (2009) and explained in more detail in the Annex, Section 9.2.1.1, a physiological toxicokinetic (PT)-model was applied, estimating the additional Area Under the Curve (AUC) for isoprene in the blood 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 without additional occupational exposure, the life-long AUC (0-80 years) was estimated to be 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).

When taking carcinogenicity data from mice and rats as a basis and applying linear extrapolation, 3 ppm would account for an additional cancer risk of 4:1000 (AGS, 2012); however, for the species differences described above, this calculation would very likely overestimate the cancer risk for humans. Furthermore, since the resulting isoprene levels are still within the range of endogenous formation, only little additional cancer risk is expected, provided that the proposed OEL is complied with.

Based on these considerations, an 8 h TWA of 3 ppm (8.5 mg/m^3) 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, no further extrapolation factor is needed and the proposed OEL is considered to be protective in humans also with respect to chronic toxicity.

With respect to reproductive toxicity, there are only 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 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.

Short term limit value (STEL)

As systemic effects are the main effects, only the AUC and not the concentration are decisive for the effects due to the assumed genotoxic mechanism of action of isoprene. Therefore, provided that the 8 h OEL is complied with, no STEL is needed.

Biological monitoring, Biological guidance value and biological limit values (see sections, 6.2.1, 6.2.2, 9.2.4 and 9.2.5 of Annex 1 for full discussion)

In recent years, biomarkers of exposure to isoprene have been proposed. N-acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine (IPM3) as a major urinary isoprene metabolite and thus a biomarker of isoprene exposure and has been used to assess the isoprene exposure in the US general population. In principle, also hemoglobin adducts of the diepoxide could be used, but there is no published procedure so far. Since the body burden of isoprene due to endogenous formation and thus individual levels are variable and of similar magnitude as that caused by exposure at the proposed OEL of 3 ppm, biological exposure monitoring at such exposure levels would not be informative. Therefore, neither BGV nor BLV are proposed.

Groups at extra risk

There are no groups at extra risk.

Notations

No notations are proposed. With respect to dermal absorption, no data are available. As isoprene is highly volatile, the contribution of dermal exposure to the total exposure is assumed to be below 10%; therefore, no skin notation is needed.

ATTACHMENTS:

Annex 1 gives the detailed scientific grounds for the opinion.

RCOM (Annex 2): Comments received on the ECHA scientific report, and responses provided by ECHA and RAC (excluding confidential information).