TEXTE
54/2024

Final report

Toxicological basic data for the derivation of EU-LCI values for β-pinene, other terpenes, pentanols, 5-chloro-2-methyl-4-isothiazolin-3-one (CIT) and 2-methyl-4-isothiazolin-3-one (MIT)

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German Environment Agency

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Abstract: Toxicological basic data for the derivation of EU-LCI values for five substances/-groups from building products

The subject of this report is the preparation of substance reports for the derivation of EU-LCI values for the substances and substance groups mentioned in the title of this report. EU-LCI values are health-based reference concentrations for inhalation exposure of the general population. For their derivation, the toxicological data basis for the substances is researched, compiled and evaluated, and EU-LCI values are derived based on the guidance given in the ECA report No. 29 (EC, 2013). Already existing evaluations and values and the quintessential data for the derivation of the EU-LCI values for the substances are also presented according to the guidance of the ECA report in "fact sheets" and "data collection sheets".

The LCI values derived within the scope of this project are proposals. The final EU-LCI values will be determined by the EU-LCI Working Group. This Working Group is developing a harmonised European list of substances and their corresponding emission limits (EU-LCI values) from the varying evaluation lists of emissions from building products. The procedure of the EU-LCI Working Group in the derivation of these European reference values for building product emissions in indoor air has been harmonised with all stakeholders and published in the ECA report No. 29 (EC, 2013). All interested parties may keep themselves informed about the ongoing progress in the derivation of EU-LCI values on the website of the Working Group (https://ec.europa.eu/growth/sectors/construction/eu-lci/values_en). The German Environment Agency has continuously worked that the harmonisation initiative will be put forward by the European Commission. In November 2015, the Commission has mandated the EU-LCI Working Group to finalise the EU-LCI list. The substance dossiers prepared within the scope of this project will add in and accelerate this process.

This report is part of a series of evaluations for a number of other substances performed on behalf of the German Environment Agency (Umweltbundesamt) by the same authors in previous projects (e.g., Voss et al., 2021; 2023).

References

EC (2013) Harmonisation framework for health-based evaluation of indoor emissions from construction products in the European Union using the EU-LCI concept. Report No 29. EUR 26168 EN. Joint Research Centre, Institute for Health and Consumer Protection, Chemical Assessment and Testing Unit. Online: https://op.europa.eu/en/publication-detail/-/publication/d3d78842-bc95-4984-a2fe-2317731324bd. Accessed on 03.11.2023.

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Kurzbeschreibung: Toxikologische Basisdaten für die Ableitung von EU-LCI-Werten für fünf Stoffe/-gruppen aus Bauprodukten

Gegenstand des Berichts ist die Erstellung von Stoffberichten für die Ableitung von EU-LCI-Werten für die im Titel genannten Stoffe und Stoffgruppen. EU-LCI-Werte sind gesundheitsbasierte Referenzkonzentrationen für die inhalative Exposition der Allgemeinbevölkerung. Zur Ableitung wurden die toxikologischen Basisdaten für diese Stoffe recherchiert, zusammengestellt und bewertet und auf Basis der Vorgaben des ECA-Berichts Nr. 29 (EC, 2013) EU-LCI-Werte abgeleitet. Bereits bestehende Bewertungen und Richtwerte für diese Stoffe wurden gemäß den Vorgaben des ECA-Berichts in "data collection sheets" und die für die Ableitung der EU-LCI-Werte wesentlichen Daten in "fact sheets" zusammengestellt.

Bei den im Rahmen dieses Vorhabens abgeleiteten LCI-Werten handelt es sich um Vorschläge. Die endgültigen EU-LCI Werte werden von der EU-LCI Arbeitsgruppe festgelegt. Diese Arbeitsgruppe erarbeitet aus den verschiedenen Bewertungsstofflisten von Emissionen aus Bauprodukten eine harmonisierte europäische Liste mit Stoffen und den dazugehörigen Emissionsgrenzen (EU-LCI Werte). Die Vorgehensweise der EU-LCI-Arbeitsgruppe bei der Ableitung dieser europäischen Referenzwerten für Bauproduktemissionen in die Innenraumluft ist mit allen Stakeholdern abgestimmt und im ECA-Bericht Nr. 29 publiziert (EC, 2013). Über den aktuellen Fortschritt bei der Ableitung der EU-LCI-Werte können sich alle Interessierten auf der Website "The EU-LCI Working Group" informieren (https://ec.europa.eu/growth/sectors/construction/eu-lci/values_en). Das Umweltbundesamt hat in den letzten Jahren darauf hingearbeitet, dass die Europäische Kommission diese Harmonisierungsinitiative weiter voranbringt. Im November 2015 hat die Europäische Kommission das Mandat zur Fertigstellung der EU-LCI Liste an die EU-LCI-Arbeitsgruppe erteilt. Die im Rahmen dieses Forschungsvorhabens ausgearbeiteten Stoffdossiers unterstützen und beschleunigen diesen Prozess.

Dieser Bericht ist Teil einer Reihe von Bewertungen für eine Anzahl weiterer Stoffe, die von denselben Autoren im Auftrag des Umweltbundesamtes in früheren Projekten durchgeführt wurden (siehe etwa Voss et al., 2021; 2023).

Quellen

EC (2013) Harmonisation framework for health-based evaluation of indoor emissions from construction products in the European Union using the EU-LCI concept. Report No 29. EUR 26168 EN. Joint Research Centre, Institute for Health and Consumer Protection, Chemical Assessment and Testing Unit. Online: https://op.europa.eu/en/publication-detail/-/publication/d3d78842-bc95-4984-a2fe-2317731324bd. Zugriff am 03.11.2023.

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List of abbreviations

AD	Atopic dermatitis
AgBB	Ausschuss zur gesundheitlichen Bewertung von Bauprodukten (Committee for Health-related Evaluation of Building Products)
AGÖF	Arbeitsgemeinschaft Ökologischer Forschungsinstitute (Association of Ecological Research Institutes)
AGW	Arbeitsplatzgrenzwert (Occupational Exposure Limit)
BIT	Benzisothiazolinone
Bp.	Boiling point
BPC	Biocidal Products Committee
BPR	Biocidal Products Regulation
CAS	Chemical abstract service
CIT	5-Chloro-2-methyl-2H-isothiazol-3-one
CLP	Classification, labelling and packaging
CNS	Central nervous system
DFG	Deutsche Forschungsgemeinschaft (German Research Foundation)
DNEL	Derived no effect level
EC3	Effective concentration inducing a Stimulation Index (SI) of 3
ECHA	European Chemicals Agency
EEA	European Economic Area
EFSA	European Food Safety Authority
EU	European Union
F	Female(s)
FEEDAP	(EFSA) Panel on Additives and Products or Substances used in Animal Feed
GD	Gestation day
GLP	Good laboratory practice
HD	Humidifier disinfectant
HDLI	Humidifier disinfectant -associated lung injury
IUPAC	International union of pure and applied chemistry
LCI	Lowest concentration of interest
LLNA	Local lymph node assay
LO(A)EC/L	Lowest observed (adverse) effect concentration/level
LoD	Limit of detection
Log Pow	Logarithm of octanol/water partition coefficient
M	Male(s)
M-12	3-mercapturic acid conjugate of 3-thiomethyl-N-methyl-propionamide
MAK	Maximale Arbeitsplatzkonzentration (Maximum workplace concentration)
MIT	2-Methyl-2H-isothiazol-3-one
Mp	Melting Point
MW	Molecular weight/mass
n. r.	Not reported

NIK	Niedrigste Interessierende Konzentration (Lowest concentration of interest)
NMMA	N-methylmalonamic acid
NOAEC/L	No observed adverse effect concentration/level
OECD/OEZW	Organisation for Economic Co-operation and Development
OEL	Occupational Exposure Limit
OIT	Octylisothiazolinone
PND	Postnatal day
POD	Point of Departure
QSAR	Quantitative Structure-Activity Relationship
RAC	Committee for Risk Assessment
RD50	Concentration which elicits a respiratory rate decrease of 50 %
REACH	Registration, evaluation, authorization, and restriction of chemicals
SCOEL	Scientific Committee on Occupational Exposure Limits
SSCS	Scientific Committee on Consumer Safety
TG	Test Guideline
TLV	Threshold Limit Value

Summary

Substance profile and EU-LCI value for β-pinene

β-Pinene is an unsaturated monoterpene hydrocarbon. The compound is chiral, both forms and the racemate are widespread in nature, e. g. in essential oils and in turpentine oils. The content of β-pinene in turpentine oils varies considerably, depending on the origin of the turpentine. Compared to α-pinene and 3-carene, the concentration of β-pinene in turpentine oils is much lower; the ratio of α-pinene to β-pinene to 3-carene is reported to be approximately 10:1:5.

β-Pinene has a turpentine-like, woody green odour. An odour threshold of 0.033 ppm (0.185 mg/m^3) is reported (enantiomer not stated).

β-Pinene may be emitted from wooden construction materials or furniture, together with other monoterpenes. Concentrations of beta-pinene in indoor are mostly in the order of several µg/m^3. However, very high maximum levels exceeding 1000 µg/m^3 are also reported, presumably from complaint-related measurements.

Studies with controlled inhalation exposure of humans against turpentine containing β-pinene revealed that about two thirds of β-pinene were absorbed. Blood levels peaked 2 h after inhalation. Excretion is also rapid. Only small amounts taken-up are exhaled unchanged in the expired air, most is excreted as metabolites with urine. The metabolism of β-pinene differs from that of the isomeric α-pinene, since the presence of an exocyclic alkene function provides additional metabolic options.

The acute toxicity of β-pinene is low. Inhalation exposure of various animal species to saturated β-pinene vapour (approximately 19600 mg/m^3) was lethal to all animals, the shortest time to death was approximately 30 min. β-Pinene is a contact allergen, with the hydroperoxides formed during autoxidation in the air being held responsible for these effects.

β-Pinene, like structurally related terpenes (α-pinene, 3-carene, camphene), causes respiratory irritation. In humans, a 2-h exposure against a mixture of 54 % α-pinene, 11 % β-pinene, and 35 % 3-carene (total concentration: 450 mg/m^3) led to significant increase in airway resistance.

Studies with mice showed that the sensory irritation potency of (+)-β-pinene seems to be similar or slightly weaker than that of (+)-α-pinene. The (+)-enantiomers of β- and α-pinene are stronger irritants compared to the (-) enantiomers.

The data base regarding effects after repeated exposure to β-pinene is very limited. No data are available from exposure to β-pinene as individual substance. In a volunteer study, repeated inhalation exposure against a mixture of 280 mg/m^3 α-pinene, 30 mg/m^3 β-pinene and 140 mg/m^3 3-carene (overall terpene concentrations 450 mg/m^3) three hours/day on four days within two weeks caused an increase in alveolar macrophages and mast cells as signs of an acute alveolar reaction. In a subchronic inhalation study with rats exposed to aerosols of (-)-α-pinene, a NOAEC of 300 mg/m^3 was obtained for female rats, based on clinical effects at 900 mg/m^3. No further inhalation studies with mice with α-pinene or other terpenes structurally related to β-pinene were available.

There was no reliable evidence for genotoxicity of β-pinene *in vitro*, *in vivo* data were not available. No studies were identified relevant for the evaluation of the carcinogenicity of β-pinene. Similarly, no reproductive or developmental toxicity studies were available which were performed with exposure to β-pinene as individual substance.

Overall, the data base from studies with β-pinene is insufficient for the derivation of an EU-LCI value. Principally, read-across could be performed from α-pinene and the derived EU-LCI value

for α-pinene be applied to β-pinene. However, β-pinene shows a metabolic pattern different from α-pinene, so simple transfer or read-across from α-pinene to other bicyclic monoterpenes including β-pinene does not seem adequate. A similar concern holds for a read-across approach from 3-carene.

β-Pinene is a constituent of terpene hydrocarbon mixtures from natural sources which always contain α-pinene, β-pinene, and also 3-carene. Thus, β-pinene can be expected as emission product from buildings and in indoor air to always occur in combination with these bicyclic monoterpenes. The typical indoor air ratio between α-pinene, β-pinene and 3-carene is reported to be about 10:1:3. Therefore, α-pinene is the leading compound, and β-pinene emissions usually contribute only little (< 10 %) to the all-over bicyclic monoterpene emissions.

For the assessment of terpene hydrocarbon mixtures containing bicyclic monoterpenes, EU-LCI values for α-pinene and 3-carene are available. It is proposed not to derive a specific LCI value for β-pinene.

Substance profile and EU-LCI value for "other terpenes"

According to the specification of the project, only the group of "non-functionalised terpene hydrocarbons" is to be considered; specifically, these shall include "volatile compounds" which are mentioned in the literature in the context of building product emission and/or indoor air measurements. After examination of several reports, the following substances were selected to which the criteria apply:

- Acyclic terpenes: myrcene,
- Monocyclic monoterpenes: limonene, terpinene (without specification of isomers),
- Bicyclic monoterpenes: α-pinene, β-pinene, 3-carene, camphene
- Tricyclic terpenes: longifolene

Of these compounds, assessments and EU LCI values are already available for α-pinene, limonene and 3-carene, and β-pinene was assessed independently as part of this project.

"Non-functionalised terpene hydrocarbons" are emitted from wood, especially softwood (conifers), and wood-based materials. The main components of wood emissions or turpentine/turpentine oil have been reported as α-pinene (44 – 94 %), β-pinene (0.9 – 30 %), limonene (0.7 – 25 %), and camphene (1 – 15 %). The main constituents of an essential oil prepared from the oleoresin of the West European Pine species *Pinus pinaster* are reported to be α-pinene (about 60 – 68 %), β-pinene (about 19 – 23 %), longifolene (2.1 – 3.6 %), β-caryophyllene (1.8 – 3.2 %), camphene (1.0 – 1.2 %), and myrcene (0.9 – 9.3 %).

In addition to the compounds listed above, the cyclic sesquiterpenes longicyclene, and caryophyllene are also sometimes reported in indoor air measurements. Regarding the occurrence of hydrocarbon terpenes in indoor air measurement data confirm that the "standard terpenes" limonene, α-pinene, β-pinene, and 3-carene can be found in most indoor air situations, with detection frequencies in the order of 90 to 100 % for α-pinene, nearly as high for limonene (70 – 100 %), slightly less (60 – 90 %) for 3-carene, and over 50 % for β-pinene. The detection frequencies for the "other terpenes" are mostly much lower (below 1 to about 25 %). A similar trend can be seen regarding the concentrations of the individual terpenes. High (median and maximum) concentrations are especially found for limonene and α-pinene, followed by 3-carene and, mostly with markedly lower maximum values, β-pinene. The concentrations of the other

terpenes – the sesquiterpenes longifolene, longicyclene, and caryophyllene, the bicyclic monoterpene camphene, the monocyclic monoterpenes α- and γ-terpinene and the acyclic monoterpene myrcene – are much lower, especially when the maximum values are considered in comparison to those of the "common" terpene representatives limonene, α- and β-pinene, and 3-carene.

At room temperature, most of the "other terpenes" are colourless liquids (except for camphene, which is a solid) with a turpentine- or woody, resinous piney odour.

The data base regarding toxic effects on humans of the individual "other terpenes" considered here is insufficient for an evaluation. The data basis is also insufficient from animal studies for an evaluation of longicyclene and longifolene. Limited data are available for camphene and α-terpinene. At least subchronic toxicity studies are available for myrcene and caryophyllene and a combined repeated dose/reproductive developmental toxicity study according to OECD 422 is available for γ-terpinene.

However, inhalation toxicity studies are nearly completely lacking, so route-to route extrapolation would have to be performed using data from studies with oral exposure in order to derive LCI values. Data for myrcene from studies with oral exposure indicate that local effects (degeneration of the olfactory epithelium and necrosis of the respiratory epithelium, accompanied by chronic inflammatory change) seem to play a role in the assessment of the toxicity of this compound. Due to the insufficient data regarding inhalation toxicity, it cannot be decided whether local effects in the respiratory tract would have to be considered as critical for the derivation of an LCI value for inhalation exposure.

The "other terpenes" considered here may be emitted from wood, especially softwood, or wood-derived building products. In those cases, they will be emitted together with other terpenes. The composition of the terpene fractions which can be isolated from softwood or, respectively, the turpentines derived from these sources shows that the "other terpenes" are present at lower concentrations than the "lead terpenes" (especially α-pinene, but also limonene, 3-carene and β-pinene). Also, in indoor air, these "other terpenes" are present at lower concentrations and are detected less frequently than the "lead terpenes" (especially α-pinene, but also limonene, 3-carene and β-pinene).

EU-LCI values were derived and are published for the "lead terpenes" α-pinene, limonene, and 3-carene. Any emission of the "other terpenes" considered here may be expected to be accompanied by higher emissions of these "lead terpenes".

There is no evidence from the available (for some of the substances albeit rather limited) data that any of the "other terpenes" (myrcene, α- and γ-terpinene, camphene, caryophyllene, longicyclene, and longifolene) considered here is more toxic than the "lead substances" terpenes α-pinene, 3-carene, or limonene or shows effects markedly different from those compounds, possibly except for myrcene: There is some evidence for carcinogenicity of myrcene in rats and mice after oral exposure by gavage. However, the data do not indicate that myrcene is genotoxic, so that a non-genotoxic (threshold) mechanism may be considered.

As far as possible health risks are concerned, the emission of terpene hydrocarbon mixtures from construction products should be adequately characterised by the "lead substances" (mainly α-pinene, but also limonene and 3-carene) and assessed on the basis of the EU LCI values derived for these "lead substances" α-pinene, limonene and 3-carene.

It is concluded that the derivation of EU-LCI values for the "other terpenes" (myrcene, α- and γ-terpinene, camphene, caryophyllene, longicyclene, and longifolene) considered is not necessary and not recommended. No EU-LCI values are proposed.

Substance profile and EU-LCI value for pentanols

Pentanols represent a group of altogether eight alcohols: four primary alcohols, three secondary alcohols, and one tertiary alcohol. A mixture of 60 – 75 % pentan-1-ol, 25 – 40 % 2-methylbutan-1-ol and about 1 % 3-methylbutan-1-ol is produced and used industrially.

Most studies were performed with 3-methylbutan-1-ol and pentan-1-ol. Almost no toxicological data are available for 2,2-dimethylpropan-1-ol. However, this substance seems to be of very limited if any use in commercial products.

Pentanols are used as solvents and starting materials for the synthesis of esters (pentylacetates), which are used technically as solvents for various plastics, but also as flavourings. Pentan-1-ol, but also other isomers, are widely distributed in plants, including vegetables and fruits.

Pentan-1-ol is the most frequently detected isomer in indoor air. Overall, the concentrations are low in the order of a few µg/m^3, and even the maximum concentrations reported stay below 50 µg/m^3.

In a human experimental study with inhalation exposure to 3-methylbutan-1-ol, the mean respiratory absorption was 63 %, and a steady-state level was reached within a few minutes. Regarding the metabolism, the primary pentanols are largely oxidised to the corresponding aldehyde and further on to the corresponding acid. The secondary pentanols are mainly metabolised to the corresponding ketones. The proportion of metabolised dose decreases from primary to tertiary alcohol. Especially secondary pentanols and the tertiary pentanol 2-methyl-butan-2-ol are excreted unchanged as glucuronides in the urine.

Acute central nervous depression is associated with exposure to 3-methylbutan-1-ol for all exposure pathways. Oedema of the lung and cardiac arrhythmia are reported in case of exposure to 2-methylbutan-1-ol, and oral intake of 2-methylbutan-1-ol and 3-methylbutan-1-ol in combination with ethanol is reported to be correlated with neurological effects. Neurological effects with depression of central nervous reactions were also observed in inhalation studies with rodents after exposure against pentan-1-ol and 3-methylbutan-1-ol.

Sensory irritation was observed in studies with humans. Short-term exposure of volunteers with 100 ppm (363 mg/m^3) 3 methylbutan-1-ol for 3 to 5 minutes gave slight throat irritation to some subjects, whereas a majority estimated that this level would not be acceptable for an 8-hour exposure period. Exposure to 150 ppm (545 mg/m^3) evoked irritation of eyes and nose in most subjects and 200 ppm (726 mg/m^3) was objectionable to all. In a toxicokinetic study 25 ppm (91 mg/m^3) 3-methylbutan-1-ol, inhaled via mouth piece for 3–5 minutes, led to complaints about throat irritation. No irritation effects were observed in a volunteer study at 1 mg/m^3.

Animal experiments with alkanols of varying chain length indicate a decrease of the RD50 values, i. e. an increase in the potency for sensory irritation in the respiratory tract, with increasing chain length. The RD50 values for pentan-1-ol were similar or slightly higher than those for 3-methylbutan-1-ol. No data were available for other pentan-1-ols.

Animal studies with repeated exposure against pentan-1-ol, 3-methlybutan-1-ol, and 2-methylbutan-2-ol indicate that the toxicity of these pentanols is low. *In vitro* data provide no concern for genotoxic effects of pentanols, *in vivo* data were not available except for one micronucleus test with 3-methylbutan-1-ol of limited validity which provided no reliable evidence for a clastogenic effect. No reliable carcinogenicity studies were available. Reproductive or developmental toxicity was not or only observed at oral doses of various pentanols tested, which caused systemic toxicity.

Overall, the concentration of 25 ppm (91 mg/m^3) is regarded as a LOAEC for sensory irritation in humans and as a POD for the derivation of an EU-LCI value for pentanols.

The following assessment factors are used:

- LOAEC to NAEC: 10 (considering the uncertainty regarding the steepness of the concentration-response curve and the low number of exposed persons at the LOAEC)
- Adjusted study length factor: 1 (sensory irritation develops within minutes and is reversible)
- Interspecies extrapolation: 1 (study with humans)
- Intraspecies extrapolation (interindividual variability, general population): 10

The total assessment factor is 100, leading to a value of 91 mg/m^3 : 100 = for 910 µg/m^3 (rounded to 900 µg/m^3).

Animal data do not indicate substantial differences in the irritation potency (RD50 values) between different pentanol isomers. Therefore, it is proposed to adopt the value based on sensory irritation of 3-methylbutan-1-ol for (the sum of) all pentanol isomers.

An EU-LCI value of 900 µg/m^3 is proposed for all pentanol isomers.

The proposed LCI value of 900 µg/m^3 is far above the reported odour thresholds for 3-methyl-butan-1-ol of 0.0017 ppm or 6 µg/m^3 and for values reported for other pentanols. Thus, the odour of the compounds will be noticed at the proposed EU-LCI value.

Substance profile and EU-LCI value for 5-chloro-2-methyl-4-isothiazolin-3-one (CIT) and 2-methyl-2H-isothiazol-3-one (MIT)

5-Chloro-2-methyl-2H-isothiazol-3-one (CIT) and 2-methyl-2H-isothiazol-3-one (MIT) both belong to the 1,2-thiazoles. The individual substances and the CIT/MIT mixture (3:1) have antimicrobial and antifungal effects and are used as active substances in biocidal products mainly as preservatives in different products.

The inhalation absorption rate of a CIT/MIT mixture product with the brand name Nipaguard® CG is 100 %. The substances are mainly eliminated via urine and faeces.

While the toxicological effects of MIT have been partially investigated, no toxicological studies have been identified for CIT. Most of the reported studies were conducted with commercial products, which contain isothiazolinones (CIT and MIT) as active ingredients. The reported studies are based on information from secondary sources, as most of the studies have not been published.

Both the CIT/MIT mixture and MIT alone have a harmonised classification for acute toxicity with respect to all exposure routes (Acute Tox 2 or 3) as well as for Skin corrosion (1B or 1C). MIT is also classified for damage to the eyes (category 1). Available human data including epidemiological studies show that CIT, MIT, and the mixture of CIT/MIT are highly potent skin sensitisers, including case reports showing airborne contact dermatitis. Both, MIT and CIT/MIT mixture have a harmonised classification for Skin Sens. 1A.

The substances did neither show a genotoxic or carcinogenic potential nor is there a concern for reproductive or developmental toxicity.

There are only two studies available with the CIT/MIT mixture in which repeated inhalation exposure toxicity was investigated (a 90-day and a 14-day study in rats). For MIT as single

substance, only acute inhalation exposure studies are available. These studies demonstrate that local effects are predominant after inhalation exposure unless lethal concentrations were applied.

The derivation for EU-LCI values for CIT and MIT as well as for the mixture of CIT/MIT (3:1) are based on toxicological data for the CIT/MIT mixture obtained from the 90-day inhalation study in rats with a NOAEC of 0.34 mg/m³ and a LOAEC of 1.15 mg/m³ (product Kathon™ 886). Adjustment for continuous exposure (6 h/d, 5 d/week) was performed by using the factor of 5.6. Additionally, the study length was adjusted by the factor of 2. For interspecies extrapolation a factor of 2.5 (allometric scaling not performed since route of exposure is inhalation) was applied and for intraspecies extrapolation (interindividual variability, general population) a factor of 10 was used. The total assessment factor is 280.

- NOAEC CIT/MIT (3:1) mixture 0.34 mg/m³ : 280 = 0.0012 mg/m³ (1.2 µg/m³ for CIT/MIT, rounded to 1 µg/m³).

In a conservative approach and in analogy to the MIT Acceptable Exposure Concentration (AEC) derivation for biocidal products, the EU-LCI value for MIT is the same as the value for the mixture. Consequently, it is derived as follows:

- NOAEC CIT/MIT (3:1) mixture 0.34 mg/m³: 280 = 0.0012 mg/m³ (1.2 µg/m³ for MIT, rounded to 1 µg/m³).

However, for the EU-LCI value for CIT an adaption of the NOAEC of 0.34 mg/m³ by a factor of 0.75 is performed. This approach follows the procedure as suggested in the AEC derivation for biocidal products. In the absence of toxicological data for CIT and in a conservative approach, all toxicity caused by the CIT/MIT mixture (3:1), is attributed to CIT alone. This is reflected in the factor 0.75.

- NOAEC of 0.34 mg/m³ from CIT/MIT (3:1) mixture (product Kathon™ 886) x 0.75 = 0.26 mg/m³: 280 = 0.0009 mg/m³ (0.9 µg/m³ for CIT, rounded to 1 µg/m³).

The rounded EU-LCI values for the CIT/MIT (3:1) mixture, and the individual substances MIT and CIT all result in the same value: 1 µg/m³.

If both individual substances (MIT and CIT) are measured the total concentration of both substances should not exceed 1 µg/m³. Based on the cases of airborne contact dermatitis and considering the measured values indoors after application of wall paint, the occurrence of contact dermatitis cannot be ruled out at present at the proposed EU-LCI values of 1 µg/m³.

Zusammenfassung

Stoffprofil und EU-LCI-Wert für β-Pinen

β-Pinen ist ein ungesättigter Monoterpen-Kohlenwasserstoff. Die Verbindung ist chiral, beide Enantiomeren und das Racemat sind in der Natur weit verbreitet, z. B. in ätherischen Ölen und in Terpentin. Der Gehalt an β-Pinen in Terpentinölen ist je nach Herkunft des Terpentins sehr unterschiedlich. Im Vergleich zu α-Pinen und 3-Caren ist die Konzentration von β-Pinen in Terpentinölen viel geringer; das Verhältnis von α-Pinen zu β-Pinen zu 3-Caren wird mit etwa 10:1:5 angegeben.

β-Pinen hat einen terpentinartigen, holzigen, grünen Geruch. Es wird eine Geruchsschwelle von 0,033 ppm (0,185 mg/m^3) angegeben (Enantiomer nicht genannt).

β-Pinen kann zusammen mit anderen Monoterpenen aus Holzbaustoffen oder Möbeln emittiert werden. Die Konzentrationen von β-Pinen in Innenräumen liegen meist in der Größenordnung von einigen µg/m^3. Es wird jedoch auch über sehr hohe Höchstwerte von über 1000 µg/m^3 berichtet, die vermutlich aus anlassbezogenen Messungen stammen.

Studien mit kontrollierter Inhalationsexposition von Menschen gegenüber β-Pinen enthaltendem Terpentin ergaben, dass etwa zwei Drittel des β-Pinens absorbiert wurden. Die Blutspiegel erreichten 2 Stunden nach der Inhalation ihren Höhepunkt. Auch die Ausscheidung erfolgt schnell. Nur geringe aufgenommene Mengen werden unverändert mit der Ausatemluft ausgeatmet, der größte Teil wird als Metaboliten mit dem Urin ausgeschieden. Der Metabolismus von β-Pinen unterscheidet sich von dem des isomeren α-Pinens, da das Vorhandensein einer exozyklischen Doppelbindung zusätzliche metabolische Reaktionswege bietet.

Die akute orale Toxizität von β-Pinen ist gering. Die inhalative Exposition verschiedener Tierarten gegenüber gesättigtem β-Pinen-Dampf (19600 mg/m^3) war für alle Tiere tödlich, die kürzeste Zeit bis zum Tod betrug etwa 30 min. β-Pinen ist ein Kontaktallergen, wobei die bei der Autoxidation in der Luft gebildeten Hydroperoxide für diese Wirkungen verantwortlich gemacht werden.

β-Pinen verursacht, wie die strukturell verwandten Terpene (α-Pinen, 3-Caren, Camphen), Reizungen der Atemwege. Beim Menschen führte eine 2-stündige Exposition gegenüber einem Gemisch aus 54 % α-Pinen, 11 % β-Pinen und 35 % 3-Caren (Gesamtkonzentration: 450 mg/m^3) zu einer deutlichen Erhöhung des Atemwegswiderstands.

Studien mit Mäusen zeigten, dass die sensorische Reizwirkung von (+)-β-Pinen ähnlich oder etwas schwächer als die von (+)-α-Pinen zu sein scheint. Die (+)-Enantiomere von β- und α-Pinen sind im Vergleich zu den (-)-Enantiomeren stärkere Reizstoffe.

Die Datenbasis bezüglich der Wirkungen nach wiederholter Exposition gegenüber β-Pinen ist sehr begrenzt. Es liegen keine Daten über die Exposition gegenüber β-Pinen als Einzelsubstanz vor. In einer Studie an freiwilligen Probanden führte eine wiederholte Inhalationsexposition gegenüber einem Gemisch aus 280 mg/m^3 α-Pinen, 30 mg/m^3 β-Pinen und 140 mg/m^3 3-Caren (Gesamtterpenkonzentration 450 mg/m^3) drei Stunden/Tag an vier Tagen innerhalb von zwei Wochen zu einer Zunahme von Alveolarmakrophagen und Mastzellen als Zeichen einer akuten Alveolarreaktion. In einer subchronischen Inhalationsstudie mit Ratten, die Aerosolen von (-)-α-Pinen ausgesetzt waren, wurde für weibliche Ratten ein NOAEC-Wert von 300 mg/m^3 ermittelt, der auf klinischen Effekten bei 900 mg/m^3 beruht. Weitere Inhalationsstudien mit Mäusen mit α-Pinen oder anderen, mit β-Pinen strukturell verwandten Terpenen waren nicht verfügbar.

Aus *In-vitro*-Untersuchungen ergeben sich keine belastbaren Hinweise auf gentoxische Effekte von β-Pinen, *In-vivo*-Daten waren nicht verfügbar. Es wurden keine Studien ermittelt, die für die

Bewertung der Kanzerogenität von β-Pinen relevant wären. Ebenso lagen keine Studien zur Reproduktions- oder Entwicklungstoxizität vor, die mit einer Exposition gegenüber β-Pinen als Einzelsubstanz durchgeführt wurden.

Insgesamt ist die Datenbasis aus Studien mit β-Pinen für die Ableitung eines EU-LCI-Wertes nicht ausreichend. Grundsätzlich könnte ein Read-across von α-Pinen durchgeführt werden und der abgeleitete EU-LCI-Wert für α-Pinen auf β-Pinen übertragen werden. Allerdings zeigen β-Pinen und α-Pinen Unterschiede in der Metabolisierung, so dass eine einfache Übertragung oder ein Read-across von α-Pinen auf andere bicyclische Monoterpene einschließlich β-Pinen nicht angemessen erscheint. Ähnliches gilt für einen Read-across-Ansatz von 3-Caren.

β-Pinen ist ein Bestandteil von Terpenkohlenwasserstoffmischungen aus natürlichen Quellen, die immer α-Pinen, β-Pinen und auch 3-Caren enthalten. Es ist daher zu erwarten, dass β-Pinen als Emissionsprodukt aus Gebäuden und in der Innenraumluft immer in Kombination mit diesen bicyclischen Monoterpenen auftritt. Das typische Verhältnis zwischen α-Pinen, β-Pinen und 3-Caren in der Innenraumluft wird mit etwa 10:1:3 angegeben. Daher ist α-Pinen die führende Verbindung, und β-Pinen-Emissionen tragen in der Regel nur wenig (< 10 %) zu den Gesamtemissionen bicyclischer Monoterpene bei.

Für die Bewertung von Terpenkohlenwasserstoffgemischen, die bicyclische Monoterpene enthalten, liegen EU-LCI-Werte für α-Pinen und 3-Caren vor. Es wird vorgeschlagen, keinen spezifischen LCI-Wert für β-Pinen abzuleiten.

Stoffprofil und EU-LCI-Wert für „andere Terpene“

Nach der Spezifikation des Projekts soll nur die Gruppe der "nicht funktionalisierten Terpenkohlenwasserstoffe" betrachtet werden; konkret soll es sich um "flüchtige Verbindungen" handeln, die in der Literatur im Zusammenhang mit Bauproduktemissionen und/oder Innenraumluftmessungen genannt werden. Nach Prüfung mehrerer Berichte wurden die folgenden Stoffe ausgewählt, auf die die genannten Kriterien zutreffen:

- azyklische Terpene: Myrcen,
- monozyklische Monoterpene: Limonen, Terpinen (ohne Angabe der Isomere),
- bicyclische Monoterpene: α-Pinen, β-Pinen, 3-Caren, Camphen
- trizyklische Terpene: Longifolen.

Von diesen Verbindungen liegen für α-Pinen, Limonen und 3-Caren bereits Bewertungen und EU-LCI-Werte vor und β-Pinen wurde im Rahmen dieses Projekts eigenständig bewertet.

"Nicht funktionalisierte Terpenkohlenwasserstoffe" werden von Holz, insbesondere von Nadelholz (Koniferen), und Holzwerkstoffen emittiert. Als Hauptbestandteile von Holzemissionen oder Terpentin/Terpentinöl wurden α-Pinen (44 – 94 %), β-Pinen (0,9 – 30 %), Limonen (0,7 – 25 %) und Camphen (1 – 15 %) angegeben. Die Hauptbestandteile eines ätherischen Öls, das aus dem Oleoresin der westeuropäischen Kiefernart *Pinus pinaster* hergestellt wird, sind α-Pinen (etwa 60 – 68 %), β-Pinen (etwa 19 – 23 %), Longifolen (2,1 – 3,6 %), β-Caryophyllen (1,8 – 3,2 %), Camphen (1,0 – 1,2 %) und Myrcen (0,9 – 9,3 %).

Zusätzlich zu den oben genannten Verbindungen werden bei Raumluftmessungen gelegentlich auch die zyklischen Sesquiterpene Longicyclen und Caryophyllen erfasst. Hinsichtlich des Vorkommens von Terpen- Kohlenwasserstoffen in der Innenraumluft bestätigen die Messdaten, dass die "Standard-Terpene" Limonen, α-Pinen, β-Pinen und 3-Caren in den meisten Innenraum-

luftsituationen gefunden werden können, mit Nachweishäufigkeiten in der Größenordnung von 90 bis 100 % für α-Pinen, fast ebenso hoch für Limonen (70 - 100 %), etwas weniger (60 - 90 %) für 3-Caren und über 50 % für β-Pinen. Die Nachweishäufigkeiten für die "anderen Terpene" sind in der Regel viel niedriger (unter 1 bis etwa 25 %). Ein ähnlicher Trend lässt sich bei den Konzentrationen der einzelnen Terpene feststellen. Hohe (Median- und Maximal-) Konzentrationen finden sich vor allem für Limonen und α-Pinen, gefolgt von 3-Caren und, meist mit deutlich niedrigeren Maximalwerten, β-Pinen. Die Konzentrationen der anderen Terpene – die Sesquiterpene Longifolen, Longicyclen und Caryophyllen, das bizyklische Monoterpen Camphen, die monozyklischen Monoterpene α- und γ-Terpinen und das azyklische Monoterpen Myrcen – sind deutlich geringer, vor allem wenn man die Maximalwerte im Vergleich zu denen der "herkömmlichen" Terpenvertreter Limonen, α- und β-Pinen und 3-Caren betrachtet.

Bei Raumtemperatur sind die meisten der "anderen Terpene" farblose Flüssigkeiten (mit Ausnahme von Camphen, das ein Feststoff ist) mit einem terpentinartigen oder holzigen, harzigen, kiefernartigen Geruch.

Die Datenbasis bezüglich toxischer Wirkungen der einzelnen hier betrachteten "anderen Terpene" auf den Menschen ist für eine Bewertung nicht ausreichend. Für Longicyclen und Longifolen ist auch die Datengrundlage aus Tierversuchen für eine Bewertung nicht ausreichend. Für Camphen und α-Terpinen liegen nur begrenzte Daten vor. Für Myrcen und Caryophyllen liegen zumindest Studien zur subchronischen Toxizität vor, und für γ-Terpinen gibt es eine kombinierte Studie zur Toxizität bei wiederholter Verabreichung und zur Reproduktionsentwicklung gemäß OECD 422.

Studien zur Inhalationstoxizität fehlen jedoch praktisch vollständig, so dass eine Pfad-zu-Pfad-Übertragung unter Verwendung von Daten aus Studien mit oraler Exposition durchgeführt werden müsste, um LCI-Werte abzuleiten. Die Daten für Myrcen aus Studien mit oraler Exposition deuten indes darauf hin, dass lokale Effekte (Degeneration des Riechepithels und Nekrose des respiratorischen Epithels, begleitet von chronisch entzündlichen Veränderungen) bei der Bewertung der Toxizität dieser Verbindung eine Rolle zu spielen scheinen. Aufgrund der unzureichenden Daten zur Inhalationstoxizität kann nicht entschieden werden, ob lokale Wirkungen in den Atemwegen als kritisch für die Ableitung eines LCI-Wertes für die Inhalationsexposition zu betrachten sind.

Die hier betrachteten "anderen Terpene" können aus Holz, insbesondere aus Nadelholz, oder aus Bauprodukten aus Holz emittiert werden. In diesen Fällen werden sie zusammen mit anderen Terpenen emittiert. Die Zusammensetzung der Terpenfraktionen, die aus Nadelholz bzw. den daraus gewonnenen Terpentinen isoliert werden können, zeigt, dass die "anderen Terpene" in geringeren Konzentrationen vorhanden sind als die "Leitterpene" (insbesondere α-Pinen, aber auch Limonen, 3-Caren und β-Pinen). Auch in der Innenraumluft sind diese "anderen Terpene" in geringeren Konzentrationen vorhanden und werden seltener nachgewiesen als die "Leitterpene" (vor allem α-Pinen, aber auch Limonen, 3-Caren und β-Pinen).

Für die "Leitterpene" α-Pinen, Limonen und 3-Caren wurden EU-LCI-Werte abgeleitet und veröffentlicht. Es ist zu erwarten, dass jede Emission der hier betrachteten "anderen Terpene" mit einer höheren Emission dieser "Leitterpene" einhergeht.

Aus den verfügbaren (für einige der Stoffe allerdings recht begrenzten) Daten geht nicht hervor, dass eines der hier betrachteten "anderen Terpene" (Myrcen, α- und γ-Terpinen, Camphen, Caryophyllen, Longicyclen und Longifolen) toxischer ist als die "Leitterpene“ α-Pinen, 3-Caren oder Limonen oder deutlich andere Wirkungen als diese Verbindungen aufweist, möglicherweise mit Ausnahme von Myrcen: Es gibt Hinweise auf kanzerogene Wirkungen von Myrcen bei Ratten und Mäusen nach oraler Exposition per Schlundsonde. Die Daten deuten

jedoch nicht darauf hin, dass Myrcen genotoxisch ist, so dass ein nicht-genotoxischer Mechanismus (Schwellenwert) in Betracht gezogen werden kann.

Was mögliche Gesundheitsrisiken betrifft, so dürfte die Emission von Terpenkohlenwasserstoff-gemischen aus Bauprodukten durch die "Leitsubstanzen" (hauptsächlich α-Pinen, aber auch Limonen und 3-Caren) angemessen charakterisiert und anhand der für diese "Leitsubstanzen" α-Pinen, Limonen und 3-Caren abgeleiteten EU-LCI-Werte bewertet werden können.

Es wird der Schluss gezogen, dass die Ableitung von EU-LCI-Werten für die betrachteten "anderen Terpene" (Myrcen, α- und γ-Terpinen, Camphen, Caryophyllen, Longicyclen und Longifolen) nicht erforderlich und nicht empfehlenswert ist. Es werden keine EU-LCI-Werte vorgeschlagen.

Stoffprofil und EU-LCI-Wert für Pentanole

Pentanole stellen eine Gruppe von insgesamt acht Alkoholen dar: vier primäre Alkohole, drei sekundäre Alkohole und ein tertiärer Alkohol. Ein Gemisch aus 60 – 75 % Pentan-1-ol, 25 – 40 % 2-Methylbutan-1-ol und etwa 1 % 3-Methylbutan-1-ol wird industriell hergestellt und verwendet.

Die meisten Studien wurden mit 3-Methylbutan-1-ol und Pentan-1-ol durchgeführt. Für 2,2-Dimethylpropan-1-ol liegen fast keine toxikologischen Daten vor. Dieser Stoff scheint jedoch, wenn überhaupt, nur sehr begrenzt in kommerziellen Produkten eingesetzt zu werden.

Pentanole werden als Lösungsmittel und Ausgangsstoffe für die Synthese von Estern (Pentylacetaten) verwendet, die technisch als Lösungsmittel für verschiedene Kunststoffe, aber auch als Aromastoffe eingesetzt werden. Pentan-1-ol und andere Isomere sind in Pflanzen, einschließlich Gemüse und Obst, weit verbreitet.

Pentan-1-ol ist das am häufigsten nachgewiesenen Isomer in der Innenraumluft. Insgesamt sind die Konzentrationen niedrig und liegen in der Größenordnung von einigen µg/m^3, auch die angegebenen Höchstkonzentrationen bleiben unter 50 µg/m^3.

In einer experimentellen Studie am Menschen mit inhalativer Exposition gegenüber 3-Methylbutan-1-ol betrug die mittlere Absorption über die Atemwege 63 %, ein Gleichgewichtsniveau wurde innerhalb weniger Minuten erreicht. Was den Stoffwechsel betrifft, so werden die primären Pentanole größtenteils zu den entsprechenden Aldehyden und weiter zu den entsprechenden Säuren oxidiert. Die sekundären Pentanole werden hauptsächlich zu den entsprechenden Ketonen metabolisiert. Der Anteil der metabolisierten Dosis nimmt vom primären zum tertiären Alkohol ab. Vor allem sekundäre Pentanole und das tertiäre Pentanol 2-Methylbutan-2-ol werden als Glucuronide ohne anderweitige Metabolisierung mit dem Urin ausgeschieden.

Eine akute Exposition gegenüber 3-Methylbutan-1-ol geht bei allen Expositionswegen mit zentralnervösen Wirkungen einher. Ödeme der Lunge und Herzrhythmusstörungen werden bei Exposition gegenüber 2-Methylbutan-1-ol berichtet, und die orale Aufnahme von 2-Methylbutan-1-ol und 3-Methylbutan-1-ol in Kombination mit Ethanol wird mit neurologischen Wirkungen in Verbindung gebracht. Neurologische Wirkungen mit Depression zentralnervöser Reaktionen wurden auch in Inhalationsstudien mit Nagern nach Exposition gegenüber Pentan-1-ol und 3-Methylbutan-1-ol beschrieben.

In Studien am Menschen wurden sensorische Reizungen beobachtet. Die kurzzeitige Exposition von Freiwilligen mit 100 ppm (363 mg/m^3) 3-Methylbutan-1-ol für 3 bis 5 Minuten führte bei einigen Probanden zu einer leichten Reizung des Rachens, während die Mehrheit der Probanden

diesen Wert als für eine Expositionsdauer von 8 Stunden nicht akzeptabel bewertete. Eine Exposition mit 150 ppm (545 mg/m^3) führte bei den meisten Probanden zu einer Reizung von Augen und Nase, und 200 ppm (726 mg/m^3) wurde von allen als unangenehm empfunden. In einer toxikokinetischen Studie führte 25 ppm (91 mg/m^3) 3-Methylbutan-1-ol, über ein Mundstück 3-5 Minuten lang eingeatmet, zu Beschwerden über Halsreizungen. In einer Probandenstudie bei 1 mg/m^3 wurden keine Reizwirkungen beobachtet.

Tierversuche mit Alkanolen unterschiedlicher Kettenlänge deuten auf eine Abnahme der RD50-Werte, d. h. eine Zunahme der Potenz zur Reizung der Atemwege, mit zunehmender Kettenlänge hin. Die RD50-Werte für Pentan-1-ol waren ähnlich oder etwas höher als die für 3-Methylbutan-1-ol. Für andere Pentan-1-ole lagen keine Daten vor.

Tierstudien mit wiederholter Exposition gegenüber Pentan-1-ol, 3-Methylbutan-1-ol und 2-Methylbutan-2-ol deuten darauf hin, dass die Toxizität dieser Pentanole gering ist. *In-vitro*-Daten geben keinen Anlass zur Besorgnis hinsichtlich genotoxischer Wirkungen von Pentanolen; *In-vivo*-Daten waren nicht verfügbar, mit Ausnahme eines Mikronukleustests von begrenzter Validität mit 3-Methylbutan-1-ol, der keine zuverlässigen Hinweise auf eine klastogene Wirkung lieferte. Es lagen auch keine zuverlässigen Studien zur Kanzerogenität vor. Reproduktions- oder Entwicklungstoxizität wurde nicht oder nur bei oralen Dosen verschiedener getesteter Pentanole beobachtet, die systemische Toxizität verursachten.

Insgesamt wird die Konzentration von 25 ppm (91 mg/m^3) als LOAEC für sensorische Reizung beim Menschen und als POD für die Ableitung eines EU-LCI-Wertes für Pentanole angesehen.

Die folgenden Bewertungsfaktoren werden verwendet:

- LOAEC zu NAEC: 10 (unter Berücksichtigung der Unsicherheit bezüglich der Steilheit der Konzentrations-Wirkungs-Kurve und der geringen Anzahl der exponierten Personen bei der LOAEC)
- Zeitextralotion: 1 (sensorische Reizung entwickelt sich innerhalb von Minuten und ist reversibel)
- Interspezies-Extrapolation: 1 (Studie am Menschen)
- Intraspezies-Extrapolation (interindividuelle Variabilität, allgemeine Bevölkerung): 10

Der Gesamtfaktor ist 100, somit ergibt sich ein Wert von 91 mg/m^3 : 100 = für 910 µg/m^3 (gerundet: 900 µg/m^3).

Tierdaten deuten nicht auf wesentliche Unterschiede in der Reizwirkung (RD50-Werte) zwischen verschiedenen Pentanol-Isomeren hin. Daher wird vorgeschlagen, den auf der sensorischen Reizung von 3-Methylbutan-1-ol basierenden Wert für (die Summe) alle Pentanol-Isomere zu übernehmen.

Es wird ein EU-LCI-Wert von 900 µg/m^3 für alle Pentanol-Isomere vorgeschlagen.

Der vorgeschlagene LCI-Wert von 900 µg/m^3 liegt weit über den berichteten Geruchsschwellenwerten für 3-Methylbutan-1-ol von 0,0017 ppm (6 µg/m^3) und den für andere Pentanole berichteten Werten. Daher wird der Geruch der Verbindungen bei dem vorgeschlagenen EU-LCI-Wert wahrgenommen werden.

Stoffprofil und EU-LCI-Wert für 5-Chlor-2-methyl-4-isothiazolin-3-on (CIT) and 2-Methyl-2H-isothiazol-3-on (MIT)

5-Chlor-2-methyl-2H-isothiazol-3-on (CIT) und 2-Methyl-2H-isothiazol-3-on (MIT) gehören beide zu den 1,2-Thiazolen. Die Einzelsubstanzen und das CIT/MIT-Gemisch (3:1) haben antimikrobielle und antimykotische Effekte und werden als Wirkstoffe in Biozidprodukten hauptsächlich als Konservierungsmittel eingesetzt.

Die inhalative Absorptionsrate eines CIT/MIT-Gemischs mit dem Markennamen Nipaguard® CG beträgt 100 %. Die Substanzen werden hauptsächlich über Urin und Fäkalien ausgeschieden.

Während die toxikologischen Effekte von MIT teilweise untersucht wurden, liegen für CIT keine toxikologischen Studien vor. Die meisten der berichteten Studien wurden mit kommerziellen Produkten durchgeführt, die Isothiazolinone (CIT sowie MIT) als Wirkstoffe enthalten. Die vorliegenden Studien basieren auf Informationen aus Sekundärquellen, da die meisten Studien nicht veröffentlicht wurden.

Sowohl das CIT/MIT-Gemisch als auch MIT allein haben eine harmonisierte Einstufung für akute Toxizität bezüglich aller Expositionspfade (Akute Tox. 2 oder 3) und für Hautreizung (1B oder 1C). MIT ist außerdem als augenschädigend eingestuft (Kategorie 1). Verfügbare Humandaten, einschließlich epidemiologischer Studien, deuten darauf hin, dass CIT, MIT und das Gemisch aus CIT/MIT hochwirksame hautsensibilisierende Effekte haben. Zudem gibt es Fallberichte über das Auftreten von aerogener Kontaktdermatitis. Sowohl MIT als auch das CIT/MIT-Gemisch haben eine harmonisierte Einstufung für Hautsensibilisierung von 1A.

CIT, MIT und das CIT/MIT-Gemisch haben kein genotoxisches oder karzinogenes Potenzial und es gibt auch keine Bedenken hinsichtlich der Reproduktions- oder Entwicklungstoxizität.

Für das CIT/MIT-Gemisch liegen lediglich zwei Studien zur wiederholten inhalativen Exposition vor (eine 90-Tage und eine 14-Tage Studie an Ratten). Für MIT als Einzelstoff sind nur Studien zur akuten Inhalation verfügbar. Die Studien deuten darauf hin, dass nach einer inhalativer Exposition lokale Effekte überwiegen, es sei denn, es wurden letale Konzentrationen verwendet.

Die Ableitung der EU-LCI-Werte für CIT und MIT sowie für das Gemisch CIT/MIT (3:1) basiert auf toxikologischen Daten für das CIT/MIT-Gemisch aus der 90-Tage-Inhalationsstudie an Ratten mit einer NOAEC von 0,34 mg/m^3 und einer LOAEC von 1,15 mg/m^3 (Produkt Kathon™ 886). Die Anpassung an eine kontinuierliche Exposition (6 Stunden/Tag, 5 Tage/Woche) wurde mit dem Faktor 5,6 vorgenommen. Zusätzlich wurde die Studiendauer mit dem Faktor 2 angepasst. Für die Interspezies-Extrapolation wurde der Faktor 2,5 verwendet (allometrische Skalierung wurde nicht durchgeführt, da eine inhalative Exposition in der Studie vorlag) und für die Interspezies-Extrapolation wurde der Faktor 10 verwendet (interindividuelle Variabilität, allgemeine Bevölkerung). Daraus ergibt sich ein Gesamtbewertungsfaktor von 280.

- NOAEC CIT/MIT (3:1) Gemisch 0,34 mg/m^3 : 280 = 0,0012 mg/m^3 (1,2 µg/m^3 für CIT/MIT, gerundet auf 1 µg/m^3).

In einem konservativen Ansatz und in Analogie zur Ableitung der zulässigen Expositionskonzentration (AEC) für Biozidprodukte ist der EU LCI-Wert für MIT derselbe wie der Wert für das Gemisch. Daher wird er wie folgt abgeleitet:

- NOAEC CIT/MIT (3:1) Gemisch 0,34 mg/m^3: 280 = 0,0012 mg/m^3 (1,2 µg/m^3 für MIT, gerundet auf 1 µg/m^3).

Für den EU LCI-Wert für CIT wird die NOAEC von 0,34 mg/m^3 jedoch um den Faktor 0,75 angepasst. Dieser Ansatz folgt dem in der AEC-Ableitung für Biozidprodukte vorgeschlagenen

Verfahren. In Ermangelung toxikologischer Daten für CIT und in einem konservativen Ansatz wird die gesamte Toxizität, die durch das CIT/MIT-Gemisch (3:1) verursacht wird, dem CIT allein zugeschrieben. Dies spiegelt sich in dem Faktor 0,75 wider.

- NOAEC von 0,34 mg/m^3 aus CIT/MIT (3:1)-Gemisch (Kathon™ 886 Produkt) x 0,75 = 0,26 mg/m^3: 280 = 0,0009 mg/m^3 (0,9 µg/m^3 für CIT, gerundet auf 1 µg/m^3).

Die gerundeten EU LCI-Werte für das Gemisch CIT/MIT (3:1) und die Einzelstoffe MIT und CIT ergeben alle den gleichen Wert: 1 µg/m^3.

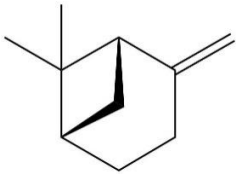
Im Falle, dass beide Einzelstoffe (MIT und CIT) gemessen werden, sollte die Gesamtkonzentration beider Stoffe 1 µg/m^3 nicht überschreiten. Ausgehend von den Fällen aerogener Kontaktdermatitis und unter Berücksichtigung der Messwerte für Innenräume nach dem Auftragen von Wandfarbe kann das Auftreten von Kontaktdermatitis bei den vorgeschlagenen EU-LCI-Werten von 1 µg/m^3 derzeit nicht ausgeschlossen werden.

1 Toxicological evaluation of β-pinene as basis for the derivation of an EU-LCI value

1.1 Substance identification

β-Pinene (6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane, pin-2(10)-ene, 2(10)-pinene) is an unsaturated bicyclic monoterpene. The molecule is chiral, both enantiomers, (+)- and (-)-β-pinene, occur naturally in essential oils and especially in turpentines, which are extracted from the resin of various coniferous trees. Both enantiomers are expected to have similar toxicological properties (AICIS, 2018). Conventional chemical analytical methods cannot distinguish between the two enantiomers. In this assessment, beta-pinene, if not otherwise stated, refers to the unspecified enantiomer.

Table 1: Substance identification of β-pinene*

CAS-No. EU-No. CLP-Index-No.	Systematic name, common name	Sum formula	Structural formula
CAS-No. 127-91-3 ((+)-pinene: 19902-08-0; (-)-pinene: 18172-67-3; mixture of (+,-)-α- and (+,-)-β-pinene: 1330-16-1) EU-No. 242-060-2 (for (-)-pinene) -	6,6-dimethyl-2-methylidene-bicyclo-[3.1.1]heptane; pin-2(10)-ene	$C_{10}H_{16}$	

* Source: PubChem (2023); (ECHA Dissemination, 2023a)

The content of β-pinene in turpentine oils varies considerably, depending on the origin of the turpentine (AICIS, 2018; Health Canada, 2020). Compared to α-pinene and 3-carene, the concentration of β-pinene in turpentine oils is much lower (the ratio of α-pinene to β-pinene to 3-carene is reported to be approximately 10:1:5 (HEAC, 2019)).

1.2 Substance properties and uses

Physicochemical properties of β-pinene are summarised in Table 2. At room temperature, β-pinene is a colourless, transparent liquid with a turpentine-like or dry woody, resinous piney odour. The liquid is almost insoluble in water but soluble in most organic solvents, e. g. ethanol, diethyl ether and oils (PubChem, 2023).

β-Pinene is a large scale industrial product (total tonnage band ≥ 10000 to < 100000 tonnes/a) (ECHA Dissemination, 2023b). Large amounts are produced by distillation of turpentine oils, most commonly available as the l-isomer ((-)-β-pinene). Besides turpentine, β-Pinene is also a constituent of many essential oils, e. g. of juniper berries, eucalyptus oil, and mandarin oil. The substances may be used as a flavouring agent. Used in household products include auto products and personal care products (PubChem, 2023).

Table 2: Physicochemical properties of β-pinene

Molar mass (g/mol)	Mp. (°C)	Boiling point (°C)	Vapour pressure (hPa) (at 25 °C)	Conversion 1 ppm = x mg/m³ (23 °C)	log pow	Solubility in water (g/L)
136.25	-61.5	166	3.9	5.61	4.16	0.00695

* Source: PubChem (2023); (ECHA Dissemination, 2023b)

1.3 Exposure

1.3.1 Indoor air

Data on the concentration of β-pinene in indoor air are summarised in Table 3. Due to the occurrence of β-pinene in softwood (pine, spruce, fir), this substance can frequently be detected in indoor air, especially in new buildings or after renovations involving the installation of wooden building materials (Health Canada, 2020). In rooms with solid-wood furniture, average levels of β-pinene (and also of α-pinene and 3-carene) were significantly higher than in rooms without such furniture (Schulz et al., 2010).

The reported median concentrations of β-pinene in indoor air are mostly in the order of several µg/m^3. Considerable variation is noted regarding maximum levels in indoor air, which may reach several hundred µg/m^3.

Table 3: Data on the occurrence of β-pinene, α-pine, and 3-carene in indoor air

Rooms	Terpene	N	LoQ (µg/m³)	N > LoD (%)	Median (µg/m³)	P95 (µg/m³)	Max. (µg/m³)	Ref.
Public buildings, evaluation of complaints	β-pinene	1897	0.5	861 (54)	2	16	630	(Petzold, 2015)
Homes with children 3 – 14 a, Germany	β-pinene	555	1.0	315 (57)	1.2	8.3	47.8	(Schulz et al., 2010)
Retirement and nursing homes, Germany	β-pinene	44	0.2 – 0.5	41 (93)	0.5	1.3	1.4	(Ostendorp and Heinzow, 2013)
Schools and kindergartens, Germany	β-pinene	285	0.5	260 (91)	6.0	71	200	(Ostendorp et al., 2009)
Offices, homes, (pre)-schools, Germany	β-pinene	2362	1.0	1460 (62)	1.0	22	370	(Hofmann and Plieninger, 2008)
Offices, homes, (pre)-schools, Germany	α-pinene	2395	1.0	2197 (92)	8.0	200	3200	(Hofmann and Plieninger, 2008)

Rooms	Terpene	N	LoQ (µg/m³)	N > LoD (%)	Median (µg/m³)	P95 (µg/m³)	Max. (µg/m³)	Ref.
Offices, homes, (pre)-schools, Germany	3-carene	2379	1.0	1713 (72)	2.5	65	1300	(Hofmann and Plieninger, 2008)

For comparison, data on the concentration of α-pinene and 3-carene reported by Hofmann and Plieninger (2008) have been included in Table 3. These two structurally related bicyclic monoterpenes are emitted from the same or similar sources as β-pinene. The data presented clearly indicate that all these three bicyclic monoterpenes can be very frequently detected in indoor air. However, among these three β-pinene is the compound least frequently detected and, moreover, is present at remarkably lower concentrations than 3-carene and, even more so, α-pinene. The typical indoor air ratio between α-pinene, β-pinene and 3-carene is reported to be about 10:1:3 (Sagunski and Heinzow, 2003).

1.3.2 Outdoor air

The concentrations of bicyclic monoterpenes including β-pinene in outdoor air are normally lower than in indoor air. In forest air, for which one can probably assume higher levels than for urban air, concentrations of bicyclic terpenes of < 0.05 to 1.6 µg/m^3 were measured above the treetops, with α- and β-pinene being the main components. In a clean air area (higher black forest), 0.1 – 3.2 µg/m^3 β-pinene, 0.3–4.7 µg/m^3 α-pinene, and 0.02 – 1.9 µg/m^3 3-carene were measured in summer (Sagunski and Heinzow, 2003).

1.4 Toxicokinetics

Beta-pinene is absorbed through the gastrointestinal and respiratory tract and skin. Elimination is mainly via urine as glucuronic acid conjugates of metabolites (NTP, 2002).

In a toxicokinetic study in humans, 8 male volunteers were exposed to 450 mg/m^3 (75 ppm) turpentine in an exposure chamber for 2 h during light physical exercise. The concentrations of the individual components were 242 mg/m^3 α-pinene, 49 mg/m^3 β-pinene and 157 mg/m^3 3-carene. Approximately two thirds of the inhaled β-pinene were absorbed. Blood levels peaked 2 h after inhalation. About 5 % of the substance taken up was detected in the expired air. The substance was rapidly eliminated after cessation of exposure. The elimination could be divided into three phases for all three compounds with half-times of 5.3, 41 and 25 min, respectively, for β-pinene (AICIS, 2018; DFG, 2000; Filipsson, 1996).

In general, the metabolic transformation of bicyclic terpene hydrocarbons including β-pinene in humans can be predicted to approximate that in other mammals (Adams et al., 2011). An overview of the metabolism of α- and β-pinene is presented in Figure 1.

In the urine of sawmill workers exposed to an atmosphere containing a-pinene, b-pinene, and 3-carene, cis and trans-verbenol were excreted as conjugates, probably with glucuronic acid. Analysis of urinary metabolites eliminated by human volunteers after exposure to a-pinene confirmed that the verbenols are metabolites of α-pinene. Another metabolic study on sawmill workers revealed that cis- and trans-4-hydroxymyrtenol, and trans-4-hydroxymyrtenal were also excreted in urine as probable metabolites of α-pinene (JECFA, 2006).

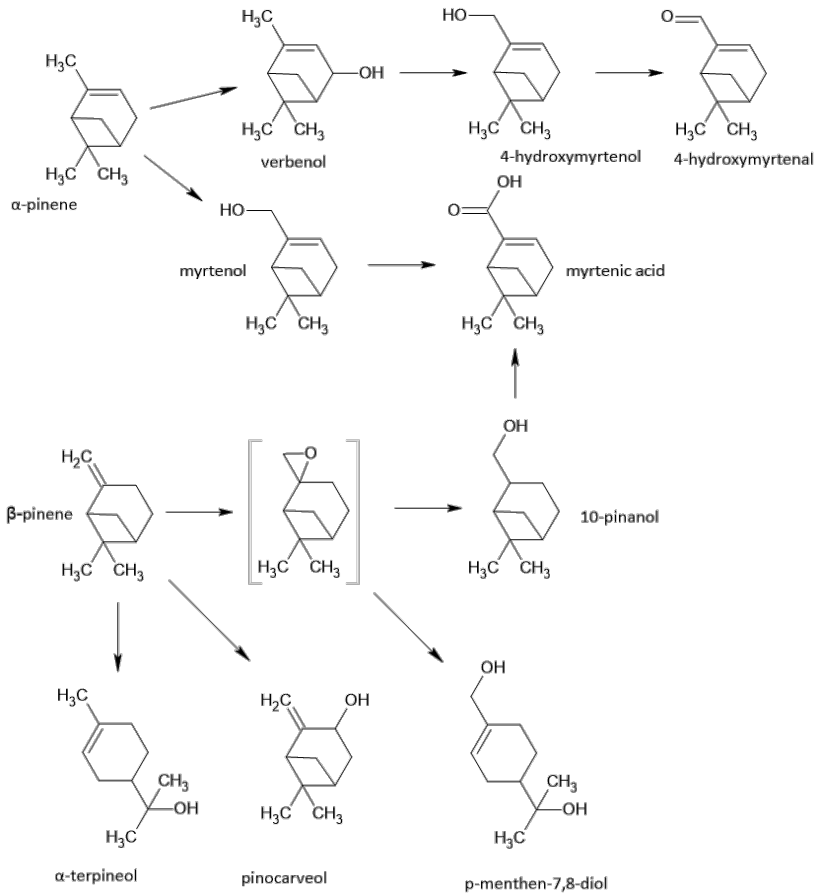
Studies in rabbits orally exposed to α- and β-pinene also showed that hydroxylated metabolites were excreted as (glucuronic acid) conjugates or as further oxidised metabolites, notably

carboxylic acids. The principal neutral metabolite formed by oxidation at the C4 position in the alicyclic ring of α-pinene was trans-verbenol. A minor pathway for α-pinene was allylic oxidation of the exocyclic methyl group to yield myrtenol and, by further oxidation, also myrtenic acid as minor metabolite (JECFA, 2006).

In β-pinene, the presence of an exocyclic alkene function provided additional metabolic options, and four neutral and one acidic metabolite were identified. Allylic oxidation of the C2 position yields trans-pinocarveol, while epoxidation of the exocyclic alkene (the epoxide could not be isolated) followed by hydration or rearrangement yields trans-10-pinanol and 1-p-menthene-7,8-diol, respectively. Ring cleavage yields α-terpineol. These metabolites comprised 11 % (trans-pinocarveol), 39 % (trans-pinanol), 30 % (1-p-menthene- 7,8-diol) or 5 % (α-terpineol) of the total urinary neutral metabolite fraction obtained from β-pinene, respectively. An acidic metabolite also found in urine was identical to the one identified for the α-pinene, i. e. myrtenic acid (Ishida et al., 1981; JECFA, 2006).

Similar metabolic reactions were also observed for 3-carene. In a metabolism study with oral intake of 10 mg 3-carene in humans, a number of urinary metabolites could be detected and part of them identified by GC/MS. Especially, carene-10-carboxylic acid was detected, the cumulative excretion within 24 h after exposure reached about 2 % of the applied dose (Schmidt, 2015; Schmidt et al., 2015). The formation of this compound corresponds to the formation of myrtenic acid from α- and β-pinene.

Figure 1: Metabolism of α- and β-pinene*



*: without speciation of enantiomers; redrawn from JECFA (2006) and Ishida et al. (1981)

In summary, α-pinene and β-pinene are both metabolised by hydroxylation. Both are oxidised to myrtenic acid as a minor metabolite, while the main metabolites formed are different between

α- and β-pinene. These differences in the metabolite profile should be kept in mind when considering read-across of data obtained for α-pinene in the toxicological evaluation of β-pinene.

1.5 Health effects

1.5.1 Acute toxicity, sensory irritation, and local effects

Acute toxicity

The data base for β-pinene is very limited.

Inhalation exposure of rats, guinea pigs and mice to saturated β-pinene vapour (19600 mg/m^3) was lethal to all animals after 5 h exposure. The shortest time to death was approximately 30 min in all species (AICIS, 2018).

The acute oral toxicity of β-pinene is low. In a study in rats the reported oral LD50 for β-pinene was 4700 mg/kg bw. Sub-lethal signs of toxicity included local irritation, CNS depression and respiratory distress (AICIS, 2018). In another study, one of 10 rats died after oral administration of 5000 mg/kg bw (LD50 > 5000 mg/kg bw) (ECHA Dissemination, 2023a).

Dermal exposure of rabbits led to local signs of irritation (redness of skin, moderate erythema formation) but not to systemic toxicity (LD50 > 5000 mg/kg bw) (ECHA Dissemination, 2023a).

Sensory irritation

β-Pinene, like structurally related terpenes (α-pinene, 3-carene, camphene), causes skin and respiratory irritation.

In humans, a 2-h exposure of 8 men against a mixture of 54 % α-pinene, 11 % β-pinene, and 35 % 3-carene (total concentration: 450 mg/m^3) led to significant increase in airway resistance compared to control (10 mg/m^3 3-carene) or exposure only against 450 mg 3-carene/m^3. Other lung function parameters were not affected (Filipsson, 1996).

The enantiomers (+)-β- and (-)-β-pinene differ in their respiratory irritant potency: The RD50 value as a measure of the inhalation irritant potential in mice was 7160 and 7950 mg/m^3 (1279 and 1419 ppm) for (+)-β-pinene and 26100 and 32500 mg/m^3 (4663 and 5881 ppm) for (-)-β-pinene, respectively. For the structurally similar bicyclic monoterpene (+)-α-pinene RD50 values of 5900 and 6200 mg/m^3 were determined; no RD50 value could be determined for the enantiomeric (-)-α-pinene because of its only very weak irritant effect. Thus, (+)-β-pinene seems to be a similar or slightly weaker respiratory irritant than (+)-α-pinene, and the (+)-enantiomers of β- and α-pinene are stronger irritants compared to the (-) enantiomers (Hartwig and MAK-Kommission, 2017; Sagunski and Heinzow, 2003).

An RD50 of 1345 ppm (7532 mg/m^3) was obtained for (+)-3-carene in a study with mice (Kasanen et al., 1999). This value for (+)-carene is similar to that for (+)-β-pinene. No RD50 was available for (-)-3-carene.

Sensitisation

β-Pinene is a contact allergen, with the hydroperoxides formed during autoxidation in the air being held responsible for these effects in particular. In a LLNA with (-)-β-pinene performed according to OECD 429 Guideline, an EC3 of 29 % was calculated from the experimental data. β-Pinene was considered a weak sensitiser (ECHA Dissemination, 2023b).

1.5.2 Repeated dose toxicity

No data are available from exposure to β-pinene as individual substance.

Human data

Eight adult volunteers were exposed against a mixture of 280 mg/m^3 α-pinene, 30 mg/m^3 β-pinene and 140 mg/m^3 3-carene (overall terpene concentrations 450 mg/m^3) three hours/day on four days within two weeks. Twenty hours after the last exposure, the bronchoalveolar lavage revealed a two-fold increase in alveolar macrophages and a five-fold increase in mast cells as signs of an acute alveolar reaction. Albumin, fibronectin, hyaluronic acid, and tryptase were not elevated. FEV1 (Forced Expiratory Volume during the first second) showed a non-significant decrease up to 20 % (Johard et al., 1993; Voss et al., 2023).

A cross-sectional study of 38 workers was carried out in four Swedish joinery shops. Exposure to monoterpenes (α-pinene, β-pinene and 3-carene) in joinery shops was studied during the processing of Scot's pine, and the acute respiratory effects among the employees were evaluated. The personal exposure to monoterpenes in the joinery shops was 10 - 214 mg/m^3 (no data on individual compounds). There were no acute effects on forced vital capacity or forced expiratory volume during 1 s. However, the workers had significantly reduced pre-shift lung function values when compared with the values of a local reference group, even when smokers and ex-smokers were excluded. The results from the lung function tests may indicate chronic rather than acute reactions in the airways (Eriksson et al., 1997; Voss et al., 2023).

Animal data

Sprague-Dawley rats (5 M + 5 F/group) were fed diets containing "**Galbelica**", a solution composed of 80 % β-pinene and 20 % 1,3,5-undecatriene for 14 days. The diet was calculated to provide a dose of 10 mg Galbelica/(kg bw x d) (8 mg β-pinene/(kg bw x d)). Galbelica was administered in a vehicle called „Pinene Beta Supra" (no further data), in a ratio of 1 (Galbelica) : 4 (vehicle). Two additional groups served as controls and were fed a basal diet with or without the vehicle. All the animals survived and appeared to be active and healthy throughout the study without signs of gross toxicity, adverse pharmacologic effects, or abnormal behaviour in any of the Galbelica-treated rats. Food consumption and body weights were not significantly different between groups. Absolute liver and kidney weights of treated rats were similar to controls, and no signs of gross toxicity were observed at necropsy (Adams et al., 2011; JECFA, 2006).

Several studies were carried out with other bicyclic monoterpene hydrocarbons structurally related to β-pinene.

Read-across: In the subacute inhalation NTP-study with **α-pinene** conducted prior to the subchronic study (see below), exposure of Sprague-Dawley rats and B6C3F1 mice (5 M + 5 F each/group) to the two highest exposure concentrations (800 and 1600 ppm) for 6 h/d, 5 d/week for 16 days led to clinical signs (ataxia, tremors, abnormal breathing, nasal/eye discharge) and death. In the remaining groups exposed to 100, 200, and 400 ppm, an increase in absolute and/or relative liver weights compared to chamber control rats was observed in both sexes (about 17 % increases in relative liver weight at 400 ppm males). Relative kidney weights were increased in an exposure concentration-dependent manner in male and female rats (ECHA Dissemination, 2023b).

In the subchronic inhalation study of the NTP with **α-pinene** (69 % (+)-α-pinene and 31 % (−)-α-pinene, 1.73 % β-pinene as impurity), groups of F344/N rats and B6C3F1 mice (each 10 M + 10 F/group) were exposed to α-pinene by whole body inhalation at concentrations of 0, 25, 50, 100, 200, or 400 ppm, 6 h/d, 5 d/week for 14 weeks. The major targets for α-pinene toxicity were the liver, urinary system, and male reproductive system. The absolute liver weights were significantly increased in high-dose male rats (13 %), male mice (21 %), and female mice (18 %), and in female rats at ≥ 50 ppm (14 – 17 %). However, there were no treatment-related histopathologic lesions in the liver. Changes in the kidneys of male rats (increased organ weight,

hyaline droplet accumulation, granular casts) were observed in male rats indicating sex- and species-specific effects (α-2u-globulin nephropathy) (NTP, 2016; Voss et al., 2023).

In the corresponding NTP-study with male and female mice, increased incidences of transitional epithelium hyperplasia of the urinary bladder were observed at ≥ 100 ppm **α-pinene**. Transitional epithelium hyperplasia in the urinary bladder can be either reparative (e.g., regenerative or reactive) or preneoplastic. Specific histopathologic indicators of either type of hyperplasia (e.g., calculi for reparative, cellular atypia for preneoplastic) were not evident in male or female mice from the current study; therefore, the neoplastic potential of the transitional epithelium hyperplasia of the urinary bladder that did occur is uncertain. There were also significantly lower numbers of sperm per cauda compared to controls in male rats at ≥ 200 ppm (NOAEC 100 ppm) and in male mice at ≥ 100 ppm (not evaluated at lower concentrations). Based on minor to moderate hyperplasia of the bladder urothel in mice, the NOAEC is 50 ppm (281 mg/m^3) (NTP, 2016; Voss et al., 2023).

The relevance of the decreased numbers of sperm/mg cauda in 200 and 400 ppm male mice and of sperm number/cauda in 100, 200, and 400 ppm male mice was discussed and questioned because, firstly, the fixation of caudae samples at 65 °C for sperm counts may have altered the integrity of the samples and, secondly, these changes in sperm levels were not corroborated by other findings such as histopathological changes in other reproductive organs/tissues or other sperm parameters (motility, spermatid counts). Also, these decreases could be secondary to stress induced by hyperplasia of bladder epithelium at ≥ 100 ppm. However, in a conservative approach, the LOAEC for males was considered to be 100 ppm, based on significantly decreased sperm counts/cauda at ≥ 100 ppm, and this effect was selected as the critical effect to calculate DNELs (ECHA Dissemination, 2023b).

In a 2-week dose range finding inhalation study, Sprague-Dawley rats (3 M + 3 F/group) were exposed "whole body" to aerosols of **(-)-α-pinene** (3 rats/sex/ group) at nominal concentrations of 0, 200, 600, 1200, and 2400 mg/m^3 (analytical concentrations reported: 0, 187, 621, 1610, 2750 mg/m^3) on 6 h/d, 5 d/week for two weeks (ECHA Dissemination, 2023a). Following the second exposure, all females and one male exposed to the highest concentration and one female exposed to 1610 mg/m^3 were sacrificed due to clinical signs of severe toxicity (decreased activity, breathing irregularities, tremor/spasms/repetitive movements, gait abnormalities, flattened posture). No pathological findings were attributable to the early termination of any decedent animal. There were no test item related effects in animals at 187 or 621 mg/m^3 on clinical observations, body weight, food consumption or organ weights (ECHA Dissemination, 2023a).

In the subsequent subchronic inhalation study according to OECD guideline 413, Sprague-Dawley rats (10 m + 10 F/group, + 5 M + 5 F/group for recovery) were exposed to **(-)-α-pinene** aerosols at nominal concentrations of 0, 150, 300, and 900 mg/m^3 on 6 h/d, 5 d/week for 90 days. Analytical concentrations were not reported. No treatment related effects were observed regarding clinical signs, effects on food consumption, blood chemistry, ophthalmoscopy, urinalysis, organ weights or broncho-alveolar lavage examinations. At the highest concentration, two females died during the exposure phase of the study. No histopathological cause for either death was established at microscopical examinations. In the lungs and bronchi of exposure phase males, foamy alveolar macrophages and alveolar eosinophilic crystals with associated inflammatory cell infiltrate were seen at a higher incidence at 900 mg/m^3 than in control males. The relationship of these findings to the test item was considered as uncertain. In the kidneys of males, accumulation of hyaline droplets in the cortical tubular epithelium and basophilia of the cortical tubular epithelium was seen in a concentration-dependent manner. Immunohistochemical staining for alpha-2u globulin confirmed the presence of this protein in

the epithelial hyaline droplets. Tubular granular casts in the outer medulla were seen in most males exposed to 300 or 900 mg/m^3 and a few males exposed to 150 mg/m^3. There were no pathological correlates seen in the liver to account for the slightly higher weight in animals exposed to 900 mg/m^3; hence this change was considered not adverse and of no toxicological significance. The NOAEC was considered to be 300 mg/m^3, based on the two females decedents at the concentration level of 900 mg/m^3 due to the general poor clinical condition (ECHA Dissemination, 2023a).

In a dose-finding pre-study for an oral screening study (Reproduction/Developmental Toxicity Screening Test following OECD guideline 421), rats (4 M + 4 F/group) received food with 0, 3000, 6000, or 12000 ppm **α-pinene** for 21 days. The mean achieved doses were 0, 196, 410 and 788 mg/(kg bw x d) in males and 0, 201, 411 and 827 mg/(kg bw x d) in females. Up to the highest dose. There were no premature deaths and no test item-related changes in clinical condition, oestrous cycles, blood chemistry and macropathology. Initially, food consumption was reduced. There was also an initial body weight loss followed by a reduced weight gain, especially in males. Males also showed a dose-dependent increase in thymus weight (ECHA Dissemination, 2023b).

In a dose-finding pre-study for an oral developmental toxicity study following OECD guideline 414 (see chapter 1.5.4), non-pregnant rabbits (3 f/group) received 0, 100, 200, 300, and 400 mg/(kg bw x d) **α-pinene** in corn oil for 14 days. Administration of 400 mg/(kg bw x d) was considered to exceed the maximum tolerated dose (MTD) and was associated with persistent marked body weight loss and reduced food intake, signs of decreased faecal output/faecal pellet size, reduced urine output and abnormal pale coloured faeces. Macroscopic examination did not reveal any abnormalities. Slight overall body weight loss and reduced food intake, but no clinical signs were noted at 300 mg/(kg bw x d). No evidence of adverse treatment-related effects and no macroscopic abnormalities were noted at 100 and 200 mg/(kg bw x d) (ECHA Dissemination, 2023b).

In a subchronic oral toxicity study following OECD guideline 408, Sprague-Dawley rats (10 M + 10 F/group) were exposed to **(+)-carene** (purity 82.2 %, impurities other mono- and bicyclic monoterpenes) at concentrations of 0, 2000, 4500, or 12000 ppm in food for a total of 13 weeks. Reduced grip strength was observed in female rats. The effects on grip strength were considered non-adverse in the registration dossier and the NOAEC considered to be 12000 ppm (752 mg/(kg bw x d)), the highest concentration (ECHA Dissemination, 2021; Voss et al., 2023). However, since the effects were outside the background control range and were only partially reversible within the four-week recovery period, adversity cannot be excluded. Thus, within the context of the proposal for an EU-LCI value for 3-carene, the highest concentration in the study was regarded as a LOAEL (NOAEL: 4500 ppm, by linear extrapolation: about 282 mg/(kg bw x d)) (Voss et al., 2023).

In a subacute oral toxicity study following OECD guideline 407, Wistar rats (5 M + 5 F/group) were exposed by gavage to 0, 62.5, 250, or 1000 mg **camphene**/(kg bw x d) for 28 days. An increased salivation was observed at the highest dose. Absolute and relative liver weight were also increased in males and females. No other effects were noted in females, but males showed histological changes in the kidney and clinical chemistry tests revealed an increase in urea nitrogen levels and a decrease in phosphorus level. A NOEL of 250 mg/(kg bw x d) was obtained for females (ECHA Dissemination, 2022a).

1.5.3 Genotoxicity and carcinogenicity

Genotoxicity

There was no evidence of mutagenicity in bacteria when β-pinene was tested (in studies according to OECD guideline 471 and non-guideline studies) with various strains of Salmonella typhimurium with and without S9 metabolic activation (Adams et al., 2011).

No conclusive results were obtained with (-)-β-pinene in an *in vitro* mammalian cell gene mutation test (performed similarly to OECD Guideline 476) in mouse lymphoma L5178Y TK+/- cells. In the absence of metabolic activation, one evaluation showed a positive result, while a second evaluation was negative for genotoxicity. In the presence of metabolic activation, one result was negative, the other was inconclusive (ECHA Dissemination, 2023a; ECHA Dissemination, 2023b).

β-Pinene did not induce sister chromatid exchanges (SCE) in Chinese Hamster ovary (CHO) cells *in vitro* (no information on metabolic activation) (ECHA Dissemination, 2023a; ECHA Dissemination, 2023b).

Data from *in vivo* studies are not available.

Read-across: No increase in micronucleate erythrocytes was seen in male or female mice after subchronic (three month) inhalation with **α-pinene** at concentrations up to 400 ppm (2240 mg/m^3) (NTP, 2016).

In a micronucleus test following OECD guideline 474, gavage administration of a single dose of 4000 mg **camphene**/kg bw to NMRI mice (5 M + 5 F/group) did not increase the number or the ratio of polychromatic and normochromatic cells in the bone marrow. A preliminary study had shown that the applied dose was the maximum applicable dose. The results indicate that camphene is not genotoxic in the micronucleus test (ECHA Dissemination, 2022a).

Carcinogenicity

No studies were identified relevant for the evaluation of β-pinene.

1.5.4 Toxicity to reproduction

No data are available from exposure to β-pinene as individual substance. Several studies were carried out with other, structurally related bicyclic monoterpene hydrocarbons.

Read-across:

Reproductive toxicity

In an OECD 421 screening study (Reproduction/Developmental Toxicity Screening Test), Sprague-Dawley rats (10 m + 4 f/group) received **(-)-α-pinene** at dietary concentrations of 3000, 6000 and 12000 ppm (control group: corn oil) (ECHA Dissemination, 2023a). The achieved doses for animals during treatment and for reproductive phase females before pairing (F0) were 181, 355 and 728 mg/(kg bw x d) for males and 180, 348 and 690 mg/(kg bw x d) for females. During the reproductive phase females received 186, 377 and 711 mg/(kg bw x d), and during lactation the doses were 418, 807 and 1494 mg/(kg bw x d). Males were exposed for three weeks before pairing and up to necropsy after a minimum of four consecutive weeks; females were exposed for three weeks before pairing, throughout pairing, gestation and until Day 12 of lactation. The F1 generation received no direct administration of the test item; any exposure was in utero or via the milk.

Male rats showed histopathological changes in the kidneys in a dose dependent manner for all treated groups, indicative of an accumulation of alpha-2 urinary globulin considered as a

phenomenon specific to male rats and of no significance to humans. Otherwise, females were more affected by treatment than males: all groups of treated females but not males showed a dose-related initial mean body weight loss on Days 1-4 of study. This initial bodyweight loss associated with reduced food consumption is probably the consequence of the low palatability of the diet, especially at high test item concentrations. This lower food intake was confirmed during gestation and lactation periods in the females exposed to 12000 ppm, and mean bodyweights of females at 12000 ppm were statistically significantly lower than controls from GD7 to day 13 of lactation.

There was no effect of treatment on oestrus cycles, mating performance, fertility, gestation length or index, litter size or offspring survival. At 12000 ppm, offspring body weights on day 1 of age were slightly low and subsequent weight gain was about 30 % lower than in controls, this was considered to reflect the smaller size of the dams and the lower food consumption during lactation and not any specific or intrinsic property of (-)-α-pinene. The slightly reduced offspring body weight gain (< 15 % lower) at the other doses was not dose-related and was not considered adverse. There was no effect of treatment on serum T4 levels in F0 males (not measured in females) and in male and female offspring at day 13 of age, offspring anogenital distance, nipple counts or external genitalia, or microscopic changes in male and female reproductive organs and thyroids. There is thus no evidence that (-)-α-pinene is an endocrine disruptor. The NOAEL for reproductive performance and survival of the offspring is 12000 ppm and the NOAEL for parental toxicity is 12000 ppm for males (728 mg/kg bw/day) and 6000 ppm for females (690 mg/kg bw/day) due to the lower food consumption and lower mean bodyweights and bodyweight gains observed in females at the highest dose, especially during gestation and lactation. Due to the about 30 % reduction in offspring body weight gain in offspring from mothers treated with 12000 ppm, the NOAEL for offspring growth and development is 6000 ppm (807 mg/kg bw/day during lactation) (ECHA Dissemination, 2023a).

In the corresponding OECD 421 screening study (Reproduction/Developmental Toxicity Screening Test) on rats (strain, number of animals and concentration in food as described above) with **α-pinene,** the achieved doses for animals during treatment were 186, 362 and 749 mg/(kg bw x d) for males. Doses for females were 179, 358, and 677 mg/(kg bw x d) during pore-pairing, 192, 381 and 740 mg/(kg bw x d) during gestation, and 432, 894, and 1613 mg/(kg bw x d) during lactation. Two additional groups of 10 M/group received the high-dose diet with 12000 ppm α-pinene (638 mg/(kg bw x d) or diet with vehicle (control) for 13 consecutive weeks. These animals constituted the toxicity phase of the study and were used for sperm analysis and histopathology of testes and epididymides at the end of the treatment period.

No changes related to treatment with α-pinene were seen in the reproductive tissues and on sperm parameters examined in reproductive and toxicity males (i.e. up to 90 days of exposure at 12000 ppm). There was no evidence for an endocrine disruptive effect of α-pinene in the parental generation or offsprings. The only histopathological changes related to treatment were seen in the kidneys of males manifesting as multifocal tubular basophilia in all treated groups and granular casts at 6000 or 12000 ppm, indicative of the species- and sex-specific alpha-2μ globulin nephropathy. When excluding the findings observed in male kidneys, the NOAEL for parental systemic toxicity and reproductive/developmental toxicity is 12000 ppm (males: 749 mg/(kg bw x d); females before pairing: 677 mg/(kg bw x d); during gestation: 740 mg/(kg bw x d); after 13 days of lactation: 1613 mg/(kg bw x d)) (ECHA Dissemination, 2023b).

Additionally, an Extended One-Generation Reproductive Toxicity Study (EOGRT study following OECD guideline 443) was conducted with Sprague-Dawley rats (25 M + 25 F/group). The F0-generation was fed diets containing, 0, 2000, 4300 or 6900 ppm **α-pinene** (with the F1-

generation exposed pre-weaning via lactation), the F1-generation after weaning received diets with 0, 2100, 4600 or 7300 ppm (in corn oil as vehicle). Males were treated for ten weeks before pairing and up to scheduled termination, after litters had weaned. Females were treated for ten weeks before pairing, throughout pairing and up to scheduled termination on day 28 of lactation. In the F1 generation, 20 males and 20 females were treated from weaning to their scheduled termination (relevant to each cohort).

In males, no NOAEL could be established for kidney changes in the F0- and F1-generation, consistent with the accumulation of alpha-2u-globulin, which is generally considered not to be relevant to human health. The observation reductions in the growth of both generations of offspring was likely caused by reduced palatability of the diet, and the consequent reduced food consumption and bodyweight and/or body weight gain of the females during gestation and lactation, and was therefore considered not relevant with respect to human health. Otherwise, no adverse treatment-related effects were observed up to the highest concentration of α-pinene in food tested (6900 or 7300 ppm, respectively). Presented as delivered body doses, the NOAEL for systemic toxicity in the F0 parent generation and in the F1 Cohort 1A and 1B adults corresponded to 469 mg/(kg bw x d) for F0 males, 567 mg/(kg bw x d) for F0 females before pairing, 648 mg/(kg bw x d) for F1 males and 659 mg/(kg bw x d) for F1 females before pairing. Accordingly, the NOAEL for reproductive performance of the F0 and F1B parent animals and survival of the F1 and F2 offspring was 466 mg/(kg bw x d) and 1047 mg/(kg bw x d) during F0 gestation and lactation and 494 mg/(kg bw x d) and 1044 mg/(kg bw x d) during F1B gestation and lactation. The NOAEL for the growth of the F1 offspring (days 1-21 of age) was 466 mg/(kg bw x d) and 1047 mg/(kg bw x d) in F1 offspring and 494 mg/(kg bw x d) and 1044 mg/(kg bw x d) in F2 offspring (ECHA Dissemination, 2023b).

In dose-finding studies for an EOGRT, Sprague-Dawley rats (6 F/group) received 0, 3000, 6000, or 12000 ppm **(+)-α-pinene** orally via the diet for three weeks before pairing and until termination on Day 21 of lactation. The F1 generation (6 M + 6 F/group) receive the diet with the mentioned concentrations from day 21 to week 7 of age. In the F0-generation, the overall body weight gain and food intake were significantly lower (75 % and 83 % of control, respectively) during gestation and food intake was low (83 % of control) during lactation. Fertility and prenatal foetal developmental parameters were unaffected by treatment. In the F1-generation, the absolute body weight on day 1 of age and the body weight gain to day 28 of age were statistically significantly lowered, and weight gain in males did not improve to week 7 of age, leading to final mean body weight 22 % lower than control. Postnatal development was delayed in males and females. It was concluded that 12000 ppm exceeded the maximum tolerated dose for an EOGRT study (ECHA Dissemination, 2023b).

In a dose-finding study for an EOGRT study in rats (the main study following OECD guideline 443 is ongoing), the F0 generation of Sprague Dawley rats (8 M + 8 F/Group) were fed diets containing 0, 3000, 6000 or 12000 ppm **(+)-3-carene.** Males were treated for three weeks before pairing, throughout pairing and up to necropsy after litters were weaned. Females were additionally treated up to Day 20 of lactation. The F1 generation was treated from weaning for up to week 7 of age at the same dietary concentrations as the F0 generation. Treatment with (+)-3-carene up to the highest dose had no effect on reproductive performance. Food intake was lower at ≥ 6000 ppm, probably due to reduced palatability, and body weight gain at these carene doses in food was also lowered. Lower body weight gain and food consumption, delayed female sexual maturation and increased liver and kidney weights in F1 animals were also observed at 12000 ppm. Depending on the age, sex and life stage of the animals, 12000 ppm in food corresponded to doses between 639 and 1622 mg/(kg bw x d), and 6000 ppm corresponded to 314 to 841 mg/(kg bw x d) (ECHA Dissemination, 2021; Voss et al., 2023).

Developmental toxicity

An **essential oil** consisting predominantly (80 – 90 %) of α-pinene (20 – 25 %), **β-pinene** (15 – 18 %), and sabinene (38 – 42 %) was evaluated in pregnant Wistar rats, CD-1 mice, and golden hamsters. Wistar rats (22 – 23 F/group) received 0, 6, 26, 150 or 560 mg/(kg bw x d) of the test material in corn oil on GD6 – 15 by gavage. No effects were observed on maternal survival, implantation, or any measured foetal parameter. Similar results were reported for hamsters receiving up to 600 mg/(kg bw x d) and mice receiving up to 560 mg/(kg bw x d) (FFHPVC, 2006).

In a developmental toxicity study, pregnant Sprague-Dawley rats (12 – 17/group) was given 0.16, 0.80, or 1.60 ml **Rowachol**/(kg bw x d) orally once daily on days 9 – 14 of gestation. Rowachol is a mixture of L-menthol (32 %), α- and **β-pinene** (not further specified, 17 % in sum of both), menthone (6 %), borneol (5 %), D-camphene (5 %), eucalyptol (2 %), and olive oil (33 %). The control group received olive oil. Autopsies were performed on day 20 of pregnancy. At the highest dose, significant reductions in maternal, placental, foetal and newborn body weight were noted compared to controls (no further data available). Newborn body weights recovered within one week. There were no gross, visceral or skeletal anomalies or any significant differences in the incidence of foetal malformations or retarded ossifications between Rowachol -treated and control rats. In this study, the maternal and foetal NOAEL for the mixture was determined to be 0.80 ml/kg bw (Adams et al., 2011; EFSA CEF Panel, 2013).

In a prenatal developmental toxicity study according to OECD Guideline 414, pregnant Sprague-Dawley rats (22/group) received 0, 30, 50 or 100 mg/(kg bw x d) of **(-)-α-pinene** by gavage on GD 6 – 20. There were two test item related early deaths in the study at the highest dose. Two females that received the highest dose showed marked clinical signs (e. g. abnormally cold to touch, piloerection and hunched posture, weight loss) and were euthanised on GD 12. At the highest dose, the group mean body weight, body weight gain and food consumption were significantly lower than controls. There were no statistically significant differences in serum TSH levels at any pinene dose when compared to the control group. T3 and T4 concentrations in serum was statistically significant decreased at 100 mg/(kg bw x d) but was within the laboratory historical control range. There were no effects on weight or histology of the thyroid. There was no effect of treatment on embryo foetal survival or sex ratio. The overall litter weight was significantly but marginally lower (93 % of control) at the highest dose, and there was an increase in foetal incidence of delayed/incomplete ossification/unossified sternebrae. As incomplete ossification is a transient stage in foetal development, indicative of foetal immaturity and may be associated with the statistically significant decrease in mean foetal weight seen at this dose level, it was not considered adverse. No maternal or developmental effects were observed at 30 or 50 mg/(kg bw x d). Based on the treatment related body weight loss and low food consumption of females at 100 mg/(kg bw x d), the maternal NOAEL is 50 mg/(kg bw x d) and the NOAEL for embryo-foetal survival, growth and development is 100 mg/(kg bw x d) (ECHA Dissemination, 2023a).

In a corresponding developmental toxicity study with **α-pinene,** pregnant Sprague-Dawley rats /20 F/group) received 0, 30, 60, or 110 mg/(kg bw x d) on GD 6 – 19 (In pre-studies, a steep dose-response was observed with mortality of dams at 150 mg/(kg bw x d)). One dam in the high-dose group was euthanised on day 14 because of marked weight loss and poor condition. At the highest dose, reduced bodyweight gain was observed which was correlated with the reduced food consumption at this dose. Reproductive parameters and postnatal development were not affected by treatment at any dose. The maternal NOAEL was 60 mg/(kg bw x d), based on lower food intake and reduced bodyweight gain, and the NOAEL for development was 110 mg/(kg bw x d) (ECHA Dissemination, 2023a).

In pregnant New Zealand White rabbits (24 F/group), oral gavage administration of 0, 75, 150, or 300 mg/(kg bw x d) **α-pinene** on GD 6 – 28 was associated with slight reductions in body weight gain and in mean food consumption in high-dose females, which were judged as non-adverse. At ≥150 mg/(kg bw x d), mean foetal weights were slightly lower than control (≤ 10 %). Differences were within historical control data and not associated with any structural foetal abnormalities, therefore they were not considered adverse. There were no treatment-related major foetal abnormalities or minor skeletal or visceral structural abnormalities. An increased incidence of incomplete ossification at ≥ 150 mg/(kg bw x d) was also within historical control data range. The NOAEL for maternal toxicity, embryo-foetal survival and development was 300 mg/(kg bw x d), the highest dose tested (ECHA Dissemination, 2023a).

In a prenatal developmental toxicity study according to OECD Guideline 414, pregnant Sprague-Dawley rats (20/group) received 0, 90, 175 or 350 mg/(kg bw x d) of **(+)-3-carene** by gavage on GD 6 – 19. The treatment led to a small body weight loss after the first dose at all concentrations and subsequent to a low weight gain (-24 %) and food intake at 350 mg/(kg bw x d). No data have yet been presented regarding effects of treatment on offsprings. In a pre-study with six dams/group, mean pre-implantation loss (%) appeared high at 600 mg/(kg bw x d) and resulted in a slightly low litter size. No developmental toxicity was observed at 300 and 450 mg/(kg bw x d); however, food intake and weight gain of dams were reduced at all doses (ECHA Dissemination, 2021; Voss et al., 2023).

In rabbits, local and systemic effects were observed at ≥ 500 mg **(+)-3-carene**/(kg bw x d) after one or two weeks of gavage exposure (decreased faecal output, persistent weight loss, reduced food consumption, dark areas on the glandular mucosa of the stomach) in a dose-finding study of an ongoing developmental toxicity study (ECHA Dissemination, 2021; Voss et al., 2023).

In a developmental toxicity study according to OECD Guideline 414, pregnant Sprague-Dawley rats (20/group) received 0, 250, or 1000 mg/(kg bw x d) of **camphene** by gavage on GD 6 – 15, using sesame oil as vehicle. The animals were examined on day 20 of pregnancy. In most of the high-dose dams, temporary salivation and reduced activity was observed after gavage administration of the compound. No clinical signs were observed in the remaining high-dosed and the low-dosed dams. No effects on body weight, weight gain, and drinking-water consumption were observed, and there were no substance-related pathological changes at autopsy. The highest dose caused a slight but not significant increase in the resorption rate and in post-implantation losses (substance-treated group: 11.5 %, control group: 5.2 %). No substance-related variations or retardations were observed regarding external macroscopic signs or soft tissue and skeletal examination. The occurrence of one malformed foetus at the highest dose (shifted and fused dorsal, lumbar and coccygeal vertebrae, bilateral crossed legs, stump tail, omphalocele) was regarded as spontaneous, not substance-related. The dose of 250 mg/(kg bw x d) was regarded a NOEL (ECHA Dissemination, 2022a).

1.5.5 Odour perception

As an isolated substance β-pinene has a turpentine-like, woody-green odour. Measurements using the triangle odour bag method revealed an odour threshold of 0.033 ppm (0.185 mg/m^3) for β-pinene (enantiomer not stated) and (for comparison) of 0.018 ppm (0.101 mg/m^3) for α-pinene (enantiomer not stated) (Nagata, 2003).

Comparative investigations of the two enantiomers, (+)- and (-)- β-pinene, dissolved in water, yielded only minor differences regarding the odour detection threshold ((+)-β-pinene: 2.54 ppm, (-)-β-pinene: 4.16 ppm). Both thresholds were much higher than those for the corresponding α-pinenes (0.02/0.10 ppm) (Padrayuttawat et al., 1997).

1.6 Evaluation

1.6.1 Existing regulations and classifications

There is no harmonised classification for β-pinene (ECHA C&L Inventory, 2023), and the substance has not yet been evaluated by IARC regarding carcinogenicity (IARC).

Existing guide values for β-pinene and for bicyclic terpenes using α-pinene as indicator substance are summarised in Table 4.

No registration dossier according to REACH and no DNEL are available for racemic β-pinene or (+)-β-pinene, respectively.

In the registration dossier for (-)-β-pinene, a DNEL of 1.0 mg/m^3 is derived for the protection of the general population via inhalation. This DNEL is based on read-across using data for the structurally similar isomer α-pinene. In a subchronic inhalation toxicity study with α-pinene a NOAEC of 50 ppm (283 mg/m^3) was derived in male and female mice based on minimal to moderate hyperplasia in the transitional epithelium of the urinary bladder from 100 ppm. Although the relevance of this effect for humans was considered uncertain, this study was selected for calculating the systemic long-term DNEL for β-pinene. The exposure was recalculated for continuous exposure conditions (6 h/25, 5 d/7 d) and the DNEL was derived using standard factors for time (2), toxicodynamic interspecies (2.5) and intraspecies extrapolation (10). A DNEL of 5.69 mg/m^3 for workers was also derived, based on the same NOAEC and corresponding standard factors for workers (ECHA Dissemination, 2022b).

It must be noted that although data for read-across were used from a study with α-pinene, the derived DNEL and the POD differs from those used in the DNEL derivation for α-pinene and (-)-α pinene in the corresponding registration dossiers as shown in Table 5. In case of α-pinene, the results from the NTP-study with mice were also used (as for read-across) but a different POD (decreased sperm count) with a lower estimated NAEC (obtained by extrapolating from a LOAEC) and finally leading to a lower DNEL. In contrast, the DNEL derived for (-) α pinene is based on a NOAEC from a subchronic inhalation toxicity study with rats (Table 5).

The EU-LCI for α-pinene is based on the same study and POD as the DNEL but omits the interspecies extrapolation factor of 2.5, stating that mice are considered more susceptible than humans to the critical effect on the bladder epithelium (EC, 2013). Consequently, the EU-LCI value for α-pinene is 2.5-fold higher than the DNEL reported above.

Table 4: Guide values for β-pinene and bicyclic terpenes, part I (for explanation, see text)

Guide value Parameter/ Organisation	ECHA Dissemination (2022b)	Sagunski and Heinzow (2003)
Substance	(-)-β-pinene	Bicyclic terpenes (indicator substance: α-pinene)
Name (reference period)	DNEL (chronic), general population	Hazard guide value (RW II) (chronic) Precautionary guide value (RW I) (chronic)
Value (mg/m³)	1.0 mg/m³	RW II: 2 mg/m³ RW I: 0.2 mg/m³
Organ/critical effect	Urinary bladder: hyperplasia in the transitional epithelium	Irritation / inflammation reactions in respiratory tract
Species	Mouse	Human
Basis	NOAEC: 50 ppm (283 mg/m³)	LOAEC: 450 mg/m³
Adjusted for continuous exposure	281 mg/m³ x 6/24 x 5/7 = 50.6 mg/m³	
Extrapolation factors Time LOAEC to NOAEC Interspecies Intraspecies Other Total	 2 - 2.5 10 - 50	 12 RW II to RW I: 10 - 10 2 (children) 240 (RW I: 2400)
Remark	Read-across from NTP-study with α-pinene	Values for substance group of bicyclic monoterpenes

Finally, the Guidance value II ("Richtwert II") of the Ad-hoc Working Group of the German Indoor Air Commission (Sagunski and Heinzow, 2003) for bicyclic monoterpenes is based on a LOAEC of 450 mg/m^3 for inflammation reactions observed in studies in which volunteers were exposed intermittently to a (about 10:1:5) mixture of 280 mg α-pinene, 30 mg β-pinene und 140 mg 3-carene/m^3 for two weeks (Johard et al., 1993). Support comes from another study with humans in which eye and nasal irritation were reported by the participants at 450 mg/m^3 3-carene or (+)-α-pinene, respectively (Falk et al., 1991; Falk et al., 1990). A factor of 12 was used to extrapolate to chronic extrapolation, a standard factor of 10 for intraspecies extrapolation and an additional factor of two for a possibly higher susceptibility of children were considered to derive a guidance value II of 2 mg/m^3. One tenth of this concentration was set as guidance value I ("Richtwert I") (Sagunski and Heinzow, 2003).

Table 5: Guide values for α-pinene and bicyclic terpenes, part II (for explanation, see text)

Guide value Parameter/ Organisation	ECHA Dissemination (2023b)	ECHA Dissemination (2023a)	EC (2013)
Substance	α-pinene	(-)-α-pinene	α-pinene
Name (reference period)	DNEL (chronic), general population	DNEL (chronic), general population	EU-LCI, general population
Value (mg/m³)	0.674 mg/m³	1.07 mg/m³	2.5 mg/m³
Organ/critical effect	Fertility (decreased sperm count)	General systemic toxicity (clinical effects)	Bladder epithelial changes
Species	Mouse	Rat	Mouse
Basis	LOAEC: 100 ppm (566 mg/m³)	NOAEC: 300 mg/m³	NOAEC: 50 ppm (283 mg/m³)
Adjusted for continuous exposure	566 mg/m³ x 6/24 x 5/7 = 101 mg/m³	300 mg/m³ x 6/24 X 5/7 = 53.6 mg/m³	281 mg/m³ x 6/24 x 5/7 = 50.6 mg/m³
Extrapolation factors Time LOAEC to NOAEC Interspecies Intraspecies Other Total	 2 3 2.5 10 - 150	 2 - 2.5 10 - 50	 2 - - 10 - 20
Remark	Subchronic NTP inhalation study with α-pinene	Subchronic inhalation study with (-)-α-pinene	Subchronic NTP inhalation study with α-pinene

1.6.2 Derivation of an EU-LCI value

Bicyclic terpenes in general are rapidly absorbed upon inhalation and distributed. In a toxicokinetic study in humans, approximately two thirds of the inhaled β-pinene were absorbed. Blood levels peaked 2 h after inhalation. Excretion is also rapid. Only small amounts taken-up are exhaled unchanged in the expired air, most is excreted as metabolites with urine (AICIS, 2018; DFG, 2000; Filipsson, 1996).

In general, the metabolic transformation of bicyclic terpene hydrocarbons including β-pinene in humans can be predicted to approximate that in other mammals (Adams et al., 2011). The metabolism of β-pinene differs from that of the isomeric α-pinene, since the presence of an exocyclic alkene function provides additional metabolic options (Ishida et al., 1981; JECFA, 2006).

The acute oral toxicity of β-pinene is low (oral LD50, rats: ≥ 4700 mg/kg bw (AICIS, 2018; ECHA Dissemination, 2023a). Exposure of various animal species to saturated β-pinene vapour (19600 mg/m^3) was lethal to all animals, the shortest time to death was approximately 30 min (AICIS, 2018). β-Pinene is a contact allergen, with the hydroperoxides formed during autoxidation in the air being held responsible for these effects in particular (ECHA Dissemination, 2023b).

β-Pinene, like structurally related terpenes (α-pinene, 3-carene, camphene), causes respiratory irritation. In humans, a 2-h exposure against a mixture of 54 % α-pinene, 11 % β-pinene, and

35 % 3-carene (total concentration: 450 mg/m³) led to significant increase in airway resistance (Filipsson, 1996).

Studies with mice showed that the enantiomers (+)-β- and (-)-β-pinene differ in their respiratory irritant potency: The RD50 value as a measure of the inhalation irritant potential was 7160 and 7950 mg/m³ (1279 and 1419 ppm) for (+)-β-pinene and 26100 and 32500 mg/m³ (4663 and 5881 ppm) for (-)-β-pinene, respectively. The structurally similar bicyclic monoterpene (+)-α-pinene showed approximately the same potency (RD50 5900 and 6200 mg/m³ in two studies) as (+)-β-pinene, whereas (-)-α-pinene is only a very weak irritant. Thus, (+)-β-pinene seems to be a similar or slightly weaker respiratory irritant than (+)-α-pinene, and the (+)-enantiomers of β- and α-pinene are stronger irritants compared to the (-) enantiomers (Hartwig and MAK-Kommission, 2017; Sagunski and Heinzow, 2003).

The data base regarding effects after repeated exposure to β-pinene is very limited. No data are available from exposure to β-pinene as individual substance.

In a volunteer study, repeated inhalation exposure against a mixture of 280 mg/m³ α-pinene, 30 mg/m³ β-pinene and 140 mg/m³ 3-carene (overall terpene concentrations 450 mg/m³) three hours/day on four days within two weeks caused an increase in alveolar macrophages and mast cells as signs of an acute alveolar reaction (Johard et al., 1993; Voss et al., 2023).

Several studies were carried out with other bicyclic monoterpene hydrocarbons structurally related to β-pinene.

A subchronic inhalation study in rats with **α-pinene** (69 % (+)-α-pinene and 31 % (−)-α-pinene, 1.73 % β-pinene as impurity) observed that absolute liver weights were significantly increased (by no more than 20 %) at concentrations ≥ 50 ppm (280 mg/m³). However, there were no treatment-related histopathologic lesions in the liver. In mice, hyperplasia of the transitional epithelium of the urinary bladder were observed at ≥ 100 ppm (NOAEC: 50 ppm). There were also possible effects on sperm counts at ≥ 100 ppm (not studied at 50 ppm) . These decreases could be secondary to stress induced by hyperplasia of the bladder epithelium at ≥ 100 ppm, but, in a conservative approach, the LOAEC for male mice was considered to be 100 ppm in the registration dossier (ECHA Dissemination, 2023b).

In a subchronic inhalation study with rats exposed to aerosols of the **(-)-α-pinene** enantiomer, a NOAEC of 300 mg/m³ was obtained for female rats, based on clinical effects at 900 mg/m³. In males, no NOAEC was derived, since effects on the kidney consistent with the well-known male rat specific alpha-2u globulin nephropathy were observed at all concentrations (ECHA Dissemination, 2023a).

No further inhalation studies with mice with α-pinene or other terpenes structurally related to β-pinene were available.

A subchronic oral toxicity study with rats fed diets containing **(+)-carene** showed a reduced grip strength in females. Within the context of the proposal for an EU-LCI value for 3-carene, the highest concentration of 12000 ppm was regarded as a LOAEL (NOAEL: 4500 ppm, about 282 mg/(kg bw x d)) (Voss et al., 2023). A similar NOEL of 250 mg/(kg bw x d) was reported for rats in a subacute toxicity study with **camphene**, based on an increased liver weight at 1000 mg/(kg bw x d) (ECHA Dissemination, 2022a).

There was no reliable evidence for genotoxicity of **β-pinene** *in vitro* (no data *in vivo*). No clastogenicity was observed *in vivo* in studies with **α-pinene** and **camphene** in mice (ECHA Dissemination, 2022a; NTP, 2016). No studies were identified relevant for the evaluation of the carcinogenicity of β-pinene.

No reproductive or developmental toxicity studies were available which were performed with exposure to β-pinene as individual substance. Several studies are available with oral exposure of animals against α-pinene or 3-carene. Briefly, these studies provided no evidence of specific effects on reproduction or development in the absence of general systemic toxicity.

Overall, the data base from studies with β-pinene is insufficient for the derivation of an EU-LCI value. Principally, read-across could be performed from α-pinene and the derived EU-LCI value of 2500 µg/m³ for α-pinene be applied to β-pinene. However, in the fact sheet for α-pinene it is stated that *"beta pinene and other terpenes showed a different metabolic pattern and may differently affect bladder epithelium, so simple transfer or read-across to other bicyclic monoterpenes seem to be inadequate"* (EC, 2013) (see also chapter 1.4 for further data on the metabolism of α- and β-pinene). These differences raise additional concerns when it comes to justifying a read-across of α-pinene.

Read-across could also be performed from 3-carene, and applying the derived EU-LCI value for 3-carene (EU-LCI Working Group, 2022) to β-pinene would also lead to a value of 2500 µg/m³. However, similar concern holds for this read-across approach.

It must be remembered that β-pinene is a constituent of terpene hydrocarbon mixtures from natural sources which always contain α-pinene, β-pinene, and also 3-carene (see chapter 1.1.). Thus, β-pinene can be expected as emission product from buildings and in indoor air to always occur in combination with these bicyclic monoterpenes. According to Sagunski and Heinzow (2003), the typical indoor air ratio between α-pinene, β-pinene and 3-carene is about 10:1:3. Therefore, α-pinene is the leading compound, and β-pinene emissions usually contribute only little (< 10 %) to the all-over bicyclic monoterpene emissions (see also chapter 1.3.1).

For the assessment of terpene hydrocarbon mixtures containing bicyclic monoterpenes, EU-LCI values for α-pinene and 3-carene are available. It is proposed not to derive a specific LCI value for β-pinene.

1.7 List of references

The links listed in the bibliography were last checked and confirmed on 3 November 2023.

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A Appendix

A.1 Fact and Data collection sheet for β-pinene

Table 6: Data collection sheet for β-pinene

Compound	β-Pinene
Nº CAS: 127-91-3 1 ppm = 5.61 mg/m³ (23 °C)	EU-Classification: no CLP, harmonised classification: -
Organisation name	**REACH Registrants**
Risk value name	DNEL
Risk value (mg/m³)	1.0
Reference period	Chronic (general population)
Risk value (mg/m³) **Short term (15 min)**	not derived (no hazard identified)
Year	2022
Key study	NTP (2016)
Study type	Subchronic inhalation study with α-pinene
Species	Mouse
Duration of exposure in key study	6 h/d, 5 d/week, 90 d
Critical effect	Systemic toxicity (hyperplasia of the transitional epithelium of the urinary bladder)
Critical dose value	NOAEC: 50.6 mg/m³
Adjusted critical dose	Adjusted to continuous exposure (see remarks)
Single assessment factors	UFs 2 x U_{FI} 1 x UF_A 2.5 x UF_H 10 = 50
Other effects	-
Remarks	*Read-across: NOAEC mouse (mg/m³) = (NOAEC (ppm) x MW) / Vmol = (50 x 136.24) / 24.05 = 283.24 mg/m³; Modification for exposure (experiment to human): (6/24)*(5/7) (Mouse Exposure condition (6h – 5/7 days) / General population Exposure condition (24 h – 7/7 days). Thus, the corrected starting point for inhalation is: 283.24 mg/(kg bw x d) x (6 h/24 h) x (5 d / 7 d) = 50.6 mg/m³*

*: remaining differences interspecies toxicodynamics factor UF_L Used LOAEL; UF_H Intraspecies variability; UF_A interspecies variability; UF_S Used subchronic study

Note: The same study was used by the LCI-Working group to derive an LCI value of 2500 µg/m³ for α-pinene. However, "It was decided not to use an additional factor for interspecies kinetic and dynamic differences, because it is reasonable to assume that mice are more sensitive than

humans because of faster metabolism, leading to higher exposition of bladder epithelium cells (in the NTP-study the effect was only found in mice, not in rats)".

Table 7: Fact sheet for β-pinene

Compound	**β-Pinene**		**Fact sheet**
Parameter	**Note**	**Comments**	**Value / descriptor**
EU-LCI value and status			
EU-LCI value	1	[µg/m³]	-
EU-LCI status	2	Draft/Final	Draft
EU-LCI year of issue	3	Year when EU-LCI value has been issued	2023
General information			
CLP-Index No.	4	INDEX	-
EC-No.	5	EINECS	242-060-2
CAS-No.	6	Chemical Abstract Service number	β-Pinene: 127-91-3 (+)-β-Pinene: 19902-08-0 (-)-β-Pinene: 18172-67-3 (+,-)-α- and (+,-)-β-pinene: 1330-16-1
Harmonised CLP classification	7	Human health risk related classification	-
Molar mass and conversion factor	8	[g/mol] and [ppm – mg/m³]	136.25 1 ppm = 5.61 mg/m³
Key data / database			
Key study, authors, year	9	Critical study with lowest relevant effect level	
Read across compound	10	Where applicable	
Species	11	Rat, human, etc.	
Route / type of study	12	Inhalation, oral feed, etc.	
Study length	13	Days, subchronic, chronic, etc.	
Exposure duration	14	h/d, d/w	
Critical endpoint	15	Effect (s), site of	
Point of departure (POD)	16	LOAEC, NOAEC, BMD, etc.	
POD value	17	[mg/m³] or ppm or [mg/kg$_{BW}$×d]	
Assessment factors (AF)			
Adjustment for exposure duration	19	Study exposure h/d, d/w	
Study length	20	sa→sc→c	

Compound	β-Pinene		Fact sheet
Route-to-route extrapolation factor	21	-	
Dose-response	22a	Reliability of dose-response, LOAEL to NOAEL	
	22b	Severity of effect (R8 6d)	
Interspecies differences	23a	Allometric Metabolic rate (R8-3)	
	23b	Kinetic + dynamic	
Intraspecies differences	24	Kinetic + dynamic General population	
AF (sensitive population)	25		
Other adjustment factors Quality of database	26	Route-to-route-extrapolation	
Results			
Summary of assessment factors	27	Total Assessment Factor	
POD/TAF	28	Calculated value [µg/m³ and ppb]	
Molar adjustment factor	29		
Rounded value	30	[µg/m³]	-
Additional comments	31		
Rationale selection	32		

Data compilation and evaluation is based on a project funded by the German Environment Agency (Voss et al., 2024).

Rationale for critical effects

The data basis for a derivation of an EU-LCI value for β-pinene is considered insufficient.

For the structurally related substance α-pinene, the critical effect for the derivation of the EU-LCI value is the hyperplasia observed in the transitional epithelium of the urinary bladder of mice exposed to α-pinene by inhalation (EC, 2013).

No corresponding study is available with β-pinene (inhalation or oral) exposure of mice. Therefore, it cannot be evaluated whether a similar effect would be critical for the evaluation of β-pinene.

Read-across could principally be performed using data from studies with other bicyclic monoterpenes, i. e. α-pinene or 3-carene, respectively. However, as stated in the fact sheet for α-pinene, *"beta pinene and other terpenes showed a different metabolic pattern and may differently*

affect bladder epithelium, so simple transfer or read-across to other bicyclic monoterpenes seem to be inadequate." (EC, 2013)

Read-across could also be performed from 3-carene, and applying the derived EU-LCI value for 3-carene (EU-LCI Working Group, 2022) to β-pinene would also lead to a value of 2500 µg/m³. However, similar concern holds for this read-across approach.

It must be remembered that β-pinene is a constituent of terpene hydrocarbon mixtures from natural sources which always contain α-pinene, β-pinene, and 3-carene. Thus, β-pinene can be expected as emission product from buildings and in indoor air to always occur in combination with these bicyclic monoterpenes. According to Sagunski and Heinzow (2003), the typical indoor air ratio between α-pinene, β-pinene and 3 carene is about 10:1:3. Therefore, α-pinene is the leading compound, and β-pinene emissions usually contribute only little (< 10 %) to the all-over bicyclic monoterpene emissions.

For the assessment of terpene hydrocarbon mixtures containing bicyclic monoterpenes, EU-LCI values for α-pinene and 3-carene are available. It is concluded not to derive a specific LCI value for β-pinene.

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2 Toxicological evaluation of “other terpenes” as basis for the derivation of an EU-LCI value

2.1 Substance identification

According to the specification of the project, only the group of "non-functionalised terpene hydrocarbons" is to be considered; specifically, these shall include "volatile compounds" which are mentioned in the literature in the context of building product emission and/or indoor air measurements. Reference is made to five reports (Horn et al., 2007; Müller et al., 2016; Müller et al., 2011; Schieweck, 2018; Wilke et al., 2012). After examination of these reports, the following substances are selected to which the criteria apply:

- Acyclic terpenes: myrcene,
- Monocyclic monoterpenes: limonene, terpinene (without specification of isomers),
- Bicyclic monoterpenes: α-pinene, β-pinene, 3-carene, camphene
- Tricyclic terpenes: longifolene.

"Non-functionalised terpene hydrocarbons" are emitted from wood, especially softwood (conifers), and wood-based materials (Schieweck, 2018). The terpenes present in the resins from softwood are also present in turpentine which is obtained by collecting and distillation of the resin harvested from living trees, mainly pines. Turpentine (CAS-No. 9005-90-7) and turpentine oil (CAS-No. 8006-64-2) are often used interchangeably and the definitions in the literature are inconsistent; however, their compositions are practically identical. The main components of turpentine/turpentine oil have been reported as α-pinene (44 – 94 %), β-pinene (0.9 – 30 %), limonene (0.7 – 25 %), and camphene (1 – 15 %). In some samples, the main components of the volatile fraction were also limonene, terpinolene (δ-Terpinene), and α- and γ-terpinene (Health Canada, 2020). Turpentine produced in the United States is made up primarily of amounts of α-pinene (75 – 85 %), β-pinene (up to 3 %), camphene (4 – 15 %), limonene (5 – 15 %), 3-carene, and terpinolene (percentages not provided) (NTP, 2002). The registration dossiers according to REACH for turpentine oils also state α-pinene, β-pinene, limonene, 3-carene, and camphene as the main constituents and other acyclic, monocyclic, or bicyclic terpenes (and oxygenated terpenes) as minor constituents, the exact composition varying with refining methods and age, location, and species of the softwood source (ECHA Dissemination, 2021b; ECHA Dissemination, 2022d). The main constituents of an essential oil prepared from the oleoresin of the West European Pine species *Pinus pinaster* are reported to be α-pinene (about 60 – 68 %), β-pinene (about 19 – 23 %), longifolene (2.1 – 3.6 %), β-caryophyllene (1.8 – 3.2 %), camphene (1.0 – 1.2 %), and myrcene (0.9 – 9.3 %). In total, up to 88 constituents were detected, 40 of which were identified, accounting on average for 99.6 % of the oil. Besides the six compounds indicated noted above, 15 other compounds[1] were detected at individual levels > 0.1 %, and these 21 compounds > 0.1 % together accounted for 98.8 % of the peaks in the gas chromatographical analysis (EFSA FEEDAP et al., 2023).

Toxicological evaluations are already available and EU-LCI values were derived for α-pinene (EC, 2013; EU-LCI Working Group, 2021), 3-carene (EU-LCI Working Group, 2021; Voss et al., 2023), and limonene (EU-LCI Working Group, 2021). The bicyclic monoterpene β-pinene is evaluated

1 Including the hydrocarbon terpenes limonene, β-phellandrene, terpinolene, α-longipinene, α-cubebene, α-copaene, 3,7,10-humulatriene, 1-isopropyl-4-methylbenzene (p-cymene, EU-LCI value derived (Voss et al., 2023)), longicyclene, and sativene.

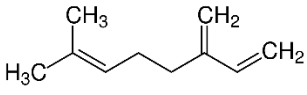
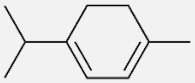
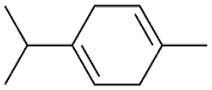
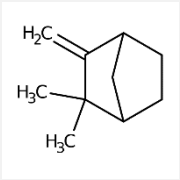
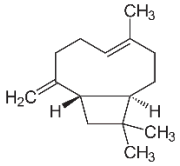
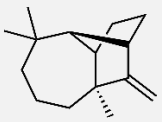
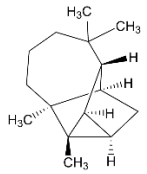
within the scope of this project (see chapter 1). These terpenes represent the most common terpenoid indoor air components (Ad-hoc AG, 2010).

Data on the concentration of several "other terpenes" in indoor air are summarised in Table 10 to Table 14. In addition to the compounds listed above, the cyclic sesquiterpenes longicyclene, and caryophyllene were included since some measurement data for these compounds were also reported. Data for the monocyclic monoterpene limonene and the bicyclic monoterpenes α-pinene, β-pinene, and 3-carene are included in each of these tables to allow for a comparison of the frequency of detection and of the measured concentrations of these "standard terpenes" with "other terpenes". This compilation confirms that limonene, α-pinene, β-pinene, and 3-carene can be found in most indoor air situations, with detection frequencies in the order of 90 to 100 % for α-pinene, nearly as high for limonene (70 – 100 %), slightly less (60 – 90 %) for 3-carene, and over 50 % for β-pinene. The detection frequencies for the other terpenes listed in Table 10 to Table 14 are mostly much lower (below 1 to about 25 %, except for longifolene in one study).

A similar trend can be seen regarding the concentrations of the individual terpenes. High (median and maximum) concentrations are especially found for limonene and α-pinene, followed by 3-carene and, mostly with markedly lower maximum values, β-pinene. The concentrations of the other terpenes – the sesquiterpenes longifolene, longicyclene, and caryophyllene, the bicyclic monoterpene camphene, the monocyclic monoterpenes α- and γ-terpinene and the acyclic monoterpene myrcene – are much lower, especially when the maximum values are considered in comparison to those of the "common" terpen representatives limonene, α- and β-pinene, and 3-carene.

Data on the substance identification of the "other terpenes" are summarised in Table 8.

Table 8: Substance identification of various "other terpenes"

Substance name* CAS-No. EU-No. CLP-Index-No.	Systematic name#	Sum formula	Structural formula
Myrcene 123-35-3 204-622-5 -	7-Methyl-3-methyleneocta-1,6-diene	$C_{10}H_{16}$	
α-Terpinene 99-86-5 202-795-1 601-095-00-7	1-methyl-4-(propan-2-yl)cyclohexa-1,3-diene; 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	$C_{10}H_{16}$	
γ-Terpinene 99-85-4 202-794-6 -	1-methyl-4-(propan-2-yl)cyclohexa-1,4-diene; 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	$C_{10}H_{16}$	
Camphene 79-92-5 201-234-8	2,2-Dimethyl-3-methylidene-bicyclo[2.2.1]heptane	$C_{10}H_{16}$	
β-Caryophyllene 87-44-5 201-746-1 -	(1R,4E,9S)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	$C_{15}H_{24}$	
Longifolen 475-20-7 207-491-2 -	(1R,2S,7S,9S)-3,3,7-trimethyl-8-methylidenetricyclo[5.4.0.0^{2,9}]undecane; Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene	$C_{15}H_{24}$	
Longicyclen 1137-12-8 214-504-5 -	[1S-(1α,2α,3aβ,4α,8aβ,9R*)]-decahydro-1,5,5,8a-tetramethyl-1,2,4-methenoazulene; 1,2,6,6-Tetramethyl-tetracylo[8.1.0.02.8.07.11]-undecane	$C_{15}H_{24}$	

*: without speciation of stereoisomers; #: referring to the name given in the Brief Profile of the corresponding substance at echa.europa.eu

2.2 Substance properties and use

Physicochemical properties of "other terpenes" as selected by the criteria described above are summarised in Table 9. At room temperature, most of these compounds are colourless liquids (except for camphene, which is a solid) with a turpentine- or woody, resinous piney odour. Their boiling point is in the range between 116 and 182 °C for the monoterpenes and around 250 °C for the sesquiterpenes. All of these compounds are very lipophilic and almost insoluble in water.

The tonnage bands (production and use in the EU) as reported in the registration dossiers are ≥ 10 to < 100 tonnes/a for myrcene and ≥ 100 to < 1 000 tonnes/a for α-terpinene, γ-terpinene,

camphene and caryophyllene (ECHA Dissemination, 2021a; ECHA Dissemination, 2022b; ECHA Dissemination, 2022c; ECHA Dissemination, 2023a; ECHA Dissemination, 2023b). For longifolene, a tonnage band of ≥ 1 to < 10 tonnes/year is reported (ECHA Dissemination, 2022a). No data are available for longicyclene.

Table 9: Physicochemical properties of various „other terpenes“*

	Molar mass (g/mol)	Mp. (°C)	Boiling point (°C)	Vapour pressure	Conversion 1 ppm = x mg/m³ (23 °C)	log pow	Solubility in water (g/L)
Myrcene	136.24	-80	116	2.51 hPa (25 °C)	5.61	4.82	0.00506
α-Terpinene	136.24	-20	173	2.22 hPa (25 °C)	5.61	5.3	0.0091
γ-Terpinene	136.24	-70	181.8	86 hPa (20 °C)	5.61	5.4	0.00744
Camphene	136.24	43 – 46	156 – 160	3.8 hPa (20 °C)	5.61	4.22	0.0042
β-Caryophyllene	204.35	-100	262	6 Pa (20 °C)	8.41	6.23	0.000088
Longifolen	204.35	-20	257.8	5.3 Pa (24 °C)	8.41	5.0	0.0079
Longicyclen	204.35	No data	252 – 254	8.1 Pa (25 °C)⁺	8.41	5.9⁺	0.000197⁺

*: Data from the registration dossier and Brief Profile of the corresponding substance at echa.europa.eu; +: The Good Scent's Company TGSC Information System, https://www.thegoodscentscompany.com/data/rw1465881.html (accessed on 3.11.2023)

2.3 Exposure

2.3.1 Indoor air

Data on the concentration of several "other terpenes" in indoor air are summarised in Table 10 to Table 14. Data for the monocyclic monoterpene limonene and the bicyclic monoterpenes α-pinene, β-pinene, and 3-carene are included in each of these tables to allow for a comparison (see chapter 2.1) of the frequency of detection and of the measured concentrations with "other terpenes".

Table 10: Data on the occurrence of terpenes in indoor air: Public buildings, evaluation of complaints (Petzold, 2015)

Terpene	N	LoQ (µg/m³)	N > LoQ (%)	Median (µg/m³)	P95 (µg/m³)	Max. (µg/m³)
Myrcene*	1897	0.5	11 (0.6)	2	10	10
α-Terpinene*	1897	0.5	28 (1.5)	3	20	20
Camphene*	1897	0.5	28 (1.5)	4	38	41
β-Caryo-phyllene	1897	0.5	11 (0.6)	1	16	16
Longifolen	1897	0.5	471 (25)	1	4	40
Limonene	1897	0.5	1505 (73)	5	47	544
α-Pinene	1897	0.5	1654 (87)	7	94	2400
β-Pinene	1897	0.5	861 (54)	2	16	630
3-Carene	1897	0.5	1138 (60)	3	36	546

*: calculated as toluene equivalent

The reported median concentrations of the "other terpenes" (myrcene, α- und γ-terpinene, camphene, longifolene, longicyclene, and β-caryophyllene) are mostly at or only slightly above the limit of quantification. The highest concentration within this group is recorded for camphene (maximum 106 µg/m^3, in the compilation of Hofmann and Plieninger (2008)). The 95th percentiles of these "other terpenes" typically range in the order of 10 µg/m^3 or below, but higher values for these and the other (predominant terpenes) were measured in public buildings where measurements were carried out after complaints about the indoor air quality (Petzold, 2015).

Table 11: Data on the occurrence of terpenes in indoor air: Homes with children 3 – 14 a, Germany (Schulz et al., 2010)

Terpene	N	LoQ (µg/m³)	N > LoQ (%)	Median (µg/m³)	P95 (µg/m³)	Max. (µg/m³)
Longifolen	555	1.0	90 (16)	< 1.0	1.8	8.0
Limonene	555	1.0	517 (93)	11.5	103	400
α-Pinene	555	1.0	547 (99)	9.8	67.6	800
β-Pinene	555	1.0	315 (57)	1.2	8.3	47.8
3-Carene	555	1.0	414 (75)	2.6	22.7	336

Table 12: Data on the occurrence of terpenes in indoor air: Retirement and nursing homes, Germany (Ostendorp and Heinzow, 2013)

Terpene	N	LoQ (µg/m³)	N > LoQ (%)	Median (µg/m³)	P95 (µg/m³)	Max. (µg/m³)
Longifolen	44	0.2	37 (84)	0.3	0.5	0.8
Limonene	44	0.2	44 (100)	4.1	28	85

Terpene	N	LoQ (µg/m³)	N > LoQ (%)	Median (µg/m³)	P95 (µg/m³)	Max. (µg/m³)
α-Pinene	44	0.2	44 (100)	1.1	5.0	6.7
β-Pinene	44	0.2	41 (93)	0.5	1.3	1.4
3-Carene	44	0.2	38 (86)	0.5	3.0	3.8

Table 13: Data on the occurrence of terpenes in indoor air: Schools and kindergartens, Germany (Ostendorp et al., 2009)

Terpene	N	LoQ (µg/m³)	N > LoQ (%)	Median (µg/m³)	P95 (µg/m³)	Max. (µg/m³)
Myrcene	285	0.5	4 (1.4)	< 2.0	< 2.0	13
α-Terpinene	285	0.5	9 (3.2)	< 2.0	< 2.0	5.0
γ-Terpinene	285	0.5	2 (0.7)	< 2.0	< 2.0	7.0
Camphene*	285	0.5	6 (2.1)	< 2.0	< 2.0	3.0
Longifolene	285	0.5	59 (21)	< 1.0	1.7	10
Limonene	285	0.5	234 (82)	3.0	51	880
α-Pinene	285	0.5	260 (91)	6.0	71	200
β-Pinene	285	0.5	168 (59)	1.0	8.0	24
3-Carene	285	0.5	218 (76)	2.0	23	130

*: calculated as toluene equivalent

Table 14: Data on the occurrence of terpenes in indoor air: Schools and kindergartens, Germany (Hofmann and Plieninger, 2008)

Terpene	N	LoQ (µg/m³)	N > LoQ (%)	Median (µg/m³)	P95 (µg/m³)	Max. (µg/m³)
α-Terpinene	999	1.0	36 (4)	0.5	1.0	29
γ-Terpinene	718	1.0	1 (< 1)	0.7	0.9	9.0
Camphene	1455	1.0	375 (26)	0.7	5.1	106
β-Caryo-phyllen	1190	1.0	59 (5)	0.9	1.1	11
Longifolene	2047	1.0	575 (28)	0.9	4.0	30
Longicyclene	369	1.0	3 (< 1)	0.7	0.7	5.0
Isolongifolene/ isolongicyclen	1227	1.0	21 (2)	0.9	1.0	13
Limonene	2394	1.0	2167 (91)	6.0	56	2500
α-Pinene	2395	1.0	2197 (92)	8.0	200	3200
β-Pinene	2362	1.0	1460 (62)	1.0	22	370
3-Carene	2379	1.0	1713 (72)	2.5	65	1300

2.3.2 Other sources

Terpenoids are main constituents of plant-derived essential oils. Because of their pleasant odour, they are widely used in the food, fragrance, and pharmaceutical industry (Buchbauer and Bohusch, 2015). The terpenes listed in Table 8 are widely distributed in plants, including vegetables and fruits. A comprehensive list of plants, plant products, essential oils, beverages and stimulants (e. g., hemp) where these compounds have been detected can be found in Duke's Phytochemical and Ethnobotanical Databases, USDA (2016). E. g., myrcene is the major component of hop and bay oils and lemongrass tea (NTP, 2010) and also is the major constituent of the essential oils of *Humulus lupulus* and *Levisticum officinale* (Buchbauer and Bohusch, 2015). Longifolene is primarily found in Indian turpentine oil, which is commercially extracted from *Pinus roxburghii* (Buchbauer and Bohusch, 2015). As a consequence, longifolen is one of the main flavour components of Lapsang Souchong Tea, which is produced by smoking tea leaves over wood from this pine (Yao et al., 2005). (−)-β-Caryophyllene is a common sesquiterpene with a clove- or turpentine-like odour. It can be found in the essential oils of *Syzygium aromaticum, Piper nigrum,* and *Humulus lupulus*. It is used as flavouring substance, for example, for chewing gums (Buchbauer and Bohusch, 2015). Longicyclene was identified as one of the components (9.1 %) of the essential oil of *Eucalyptus gunnii* (Caputo et al., 2020).

Because of the widespread occurrence of these terpenes in plant-based materials including food, a substantial or the predominant amount of the total human exposure to these terpenes can be expected to occur via food. E. g., the potential human daily intake of β-caryophyllene from food has been estimated to be 10.24 mg (Schmitt et al., 2016).

A “Maximised Survey-derived Daily Intake” (MSDI) approach to estimate the per capita intakes of flavouring substances in Europe (based on the information provided by the European Flavour Industry on the use levels of flavouring agents in various foods) reported that the approximate intake of α-pinene, β-pinene, and β-caryophyllene together amounts to 3400 µg/person x day (EFSA CEF, 2015a). For β-caryophyllene as single substance a MSDI of 330 µg/person x d was reported (EFSA CEF, 2015b).

The estimated intake of myrcene from its use as a flavouring agent in Europe was reported to be around 140 µg/(kg bw x d) (JECFA, 2005). However, exposure to myrcene from natural food sources was estimated in the U.S.A. to be 16,500 times more than from its synthetic use as a flavour substance (Surendran et al., 2021).

Other uses of these compounds include cosmetic products such as soaps and detergents (AICIS, 2018; AICIS, 2022; NTP, 2010).

2.4 Toxicokinetics

No data on the absorption, metabolism, distribution, or excretion of β-myrcene in humans were available (IARC, 2019). Metabolism studies conducted with rats and rabbits showed that **myrcene** as an acyclic alkene is metabolised via epoxidation of the double bonds, ultimately leading to diols, which can be further conjugated before excretion with urine. Epoxidation is selective, the 3,10-double bond being favoured over the 1,2-double bond. The studies indicate that the formation of diols from the epoxides is very efficient. Myrcene-3,10-glycol, formed from the hydration of the epoxide intermediate, was the principal urinary metabolite of myrcene in both species (EFSA FeedAP, 2016).

Toxicokinetic data on **terpinenes** are limited. It is expected that the kinetics and metabolism of these compounds will be very similar to that of limonene, a structural isomer of α- and γ-terpinene. Limonene is rapidly absorbed orally and via inhalation. Various hydroxylated

metabolites and a carboxy acid were identified in urine of rats after oral exposure with limonene. Urinary excretion of metabolites was rapid (77 – 96 % within three days) in rats, guinea pigs, hamsters, and dogs (AgraQuest, 2011; EFSA, 2014).

For the substance group of bi- and tricyclic in general, the few available data show that these substances will be orally absorbed to some extent. For the supporting substances α- and β-pinene and 3-carene kinetic data including those from humans show that these substances can be absorbed after inhalation exposure and that metabolites will be excreted into the urine, e. g. as glucuronide conjugates. The elimination follows a triphasic pattern with rather long terminal half-lives. The absorbed amount will be eliminated within several days. Based on the lipophilic character of these substances it may be anticipated that they will preferentially distribute in the adipose tissues, which is supported by the slow terminal elimination rates (EFSA CEF, 2010).

Metabolism data of various bicyclic monoterpenes (pinenes, camphene, caryophyllene, 3-carene, pinane and carane) and longifolene indicate that in general the metabolic options for these substances include oxidation of methyl substituents to the corresponding alcohols which are further oxidised. Epoxidation of carbon double bonds has also been demonstrated. Ring cleavage may lead to the formation of monocyclic terpenoid derivatives (e. g. for β-pinene, forming α-terpineol). Hydroxylated metabolites may be excreted as glucuronides (EFSA CEF, 2015a).

Camphene was shown to be eliminated from the human body by exhalation, and a study in a young pig indicated that the substance was eliminated via bile as unchanged substance or as glucuronide conjugate via the urine (EFSA CEF, 2010).

The metabolism of **(+)-longifolene** and **caryophyllene** was studied in rabbits after gavage exposure. Two days after the administration, metabolites of these substances could be detected in the urine, from which it can be concluded that these substances are absorbed. No mass balance data were given; therefore, the extent of absorption cannot be assessed (EFSA CEF, 2010).

In a study with **(+)-longifolene,** a number of peaks were observed in the neutral fraction isolated from the urine of male rabbits, but only one was further characterised to be (2S, 7S)-(+)-14-hydroxyisolongifolaldehyde (35 % of the neutral metabolite fraction) (EFSA CEF, 2015a). Then rapid CYP-catalyzed hydroxylation of this endo-aldehyde occurs (Buchbauer and Bohusch, 2015). It was concluded that (+)-longifolene is metabolised at two sites in two subsequent steps: The first step involves the oxidation of the exo-methylene group to an epoxide with subsequent isomerisation to an aldehyde, and the second step is a hydroxylation of the gem-dimethyl group to form a primary alcohol (EFSA CEF, 2015a).

Epoxidation of the endocylic 5,6-double bond to yield a stable epoxide metabolite and hydroxylation at the gem-dimethyl group of **β-caryophyllene** was reported in a metabolism study in rabbits. The resulting metabolite 14-hydroxycaryophyllene-5,6-epoxide and its C14-acetylated conjugate could be detected in the urine. A second epoxidation of the 5,6-epoxide's exocyclic 2,12-double bond, ultimately resulting in the 14-hydroxycaryophyllene-5,6-epoxide-2,12-diol, was also reported (Buchbauer and Bohusch, 2015; EFSA CEF, 2010).

No data were available for **longicyclene**. The EFSA FEEDAP noted that for the sesquiterpene longicyclene a metabolic profile similar to other cyclic terpenes can be expected (oxidation of the highly lipophilic compound to polar oxygenated metabolites which are conjugated and excreted in urine) (EFSA FEEDAP et al., 2023).

2.5 Health effects

The overview presented in chapter 2.1 to chapter 2.3 indicates that the "other terpenes", especially compared to limonene and α-pinene (which are used as "active lead substance"), are much less often measured and, if found, in much lower concentrations. Also, the toxicological data base for these substances (as individual compounds) is rather limited. A brief compilation of the toxicological data of the selected "other terpenes" is given as follows, largely based on the available REACH registration dossiers (no such dossier is available for longicyclene up to now) and scientific opinions of the EFSA. In the registration dossier, only studies regarding acute toxicity, skin and eye irritation, sensitisation, and genotoxicity in vitro are reported (ECHA Dissemination, 2022a).

2.5.1 Acute toxicity, sensory irritation, and local effects

Acute toxicity

The acute oral and dermal toxicity of the compounds is low. Data regarding acute inhalation toxicity are not available.

An approximate lethal oral dose (LDlo) for myrcene of > 5060 mg/kg bw was reported for mice and of 11390 mg/kg bw for rats, respectively. The LD50 were reported to exceed 5000 mg/kg bw for oral exposure of rats or dermal exposure of rabbits (Surendran et al., 2021).

The oral LD50 of γ-terpinene for rats is > 2000 mg/kg bw. No deaths or signs of local or systemic effects were observed in rabbits after dermal exposure with 2000 mg/kg bw (ECHA Dissemination, 2023b).

An oral LD50 value of 1680 mg/kg bw was obtained for rats. No clinical signs were noted at 1310 mg/kg bw, 1640 mg/kg bw caused lethargy. Lethargy, loss of righting reflex, and piloerection were observed at 2050 and 5000 mg/kg bw. Dermal exposure of rabbits with 2000 mg/kg bw caused local reversible erythema at the application site but no other signs or death (LD50 > 2000 mg/kg bw) (ECHA Dissemination, 2023a).

For camphene, oral LD50 values > 5000 mg/kg bw were reported for rats and mice. In mice, this dose caused Stilted gait, increased tonus of the abdominal position, diarrhoea, narrowed palpebral fissures back-arched position, reduced spontaneous activity, piloerection. The dermal LD50 for rabbits is reported to be > 2500 mg/kg bw (ECHA Dissemination, 2022c).

No mortality was observed in mice after oral exposure with up to 5000 mg/kg bw caryophyllene. Further acute toxicity data are not reported (ECHA Dissemination, 2021a).

The LD50 for longicyclene is reported to be > 5000 mg/kg bw. No further acute toxicity data were reported(ECHA Dissemination, 2022a). No data are available for longifolene.

Sensory irritation

In a study with a group of three anosmic volunteers, a nasal pungency threshold could only be determined for α-terpinene (at about 2000 ppm as read from a figure), but not for γ-terpinene, (-)-α-pinene or (-)-β-pinene or limonene (both enantiomers) (Cometto-Muñiz et al., 1998).

RD50 values for sensory irritation derived in animal studies for not available.

Sensitisation

In a European multicentre study, only one patient out of 1,511 consecutive dermatitis patients reacted adversely to 3 % oxidised **myrcene** (containing 30 % of myrcene). Myrcene (5 %) was sensitising to two of eleven patients sensitive to tea tree oil (Surendran et al., 2021). Myrcene

did not show a sensitising potential in a LLNA (Local Lymph Node Assay) according to OECD Guideline 429 in mice (ECHA Dissemination, 2022b).

α-Terpinene did show a sensitising potential in a LLNA (Local Lymph Node Assay) according to OECD Guideline 429 in mice. Based on the results, α-terpinene was shown to be a sensitizer of moderate potency with an EC3 value of 0.65 M (8.9 % w/v). (ECHA Dissemination, 2023a).

γ-Terpinene did not show a sensitising potential in an OECD Guideline 442D Study (In Vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method) and in an OECD Guideline 442C Assay (In Chemico Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA)) (ECHA Dissemination, 2023b).

QSAR predictions lead to the conclusion that **camphene** is not a skin sensitiser. A maximization test carried out on 25 volunteers with camphene at a concentration of 4 % in petrolatum produced no sensitization reactions (ECHA Dissemination, 2022c).

In Patch tests performed in six European dermatology centres, 10 out of 1606 patients (0.6 %) exhibited reactions to 5 % **caryophyllene** in petrolatum. About 0.5 % of 1511 patients reacted to a 3.0 % oxidation mixture of caryophyllene in non-stabilised white petrolatum, containing 25 % β-caryophyllene and 35 % caryophyllene oxide. Only 0.1 % (n = 1511) of patients reacted to caryophyllene oxide in non-stabilised white petrolatum (ECHA Dissemination, 2021a).

In a modified Freund's Complete Adjuvant Test (FCAT) similar to OECD 406 Guideline, β-caryophyllene did not induce positive reactions in exposed guinea pigs and was not considered to be a contact allergen. Caryophyllene oxide did show a weak sensitising potential in a LLNA (Local Lymph Node Assay) according to OECD Guideline 429 in mice. An EC3 value of 26.2 % w/v was calculated (ECHA Dissemination, 2021a).

Longifolene did show a sensitising potential in a LLNA (Local Lymph Node Assay) according to OECD Guideline 429 in mice. An EC3 value of 31.4 % w/v was calculated (ECHA Dissemination, 2022a).

No data were available for longicyclene.

2.5.2 Repeated dose toxicity

Human data

No data were available from studies conducted with the compounds under assessment as individual substances.

Animal data

Three subchronic oral toxicity studies 90-day studies are available with **myrcene:** a feeding study with rats and one gavage study each with rats and mice. Furthermore, a two-year carcinogenicity study with oral (gavage) **myrcene** exposure of rats and mice was conducted; the results are described in chapter 2.5.3.

In the feeding study (compliant with OECD guideline 408), Sprague-Dawley rats (10 M + 10 F/ group) received diets with 0, 700, 2100, or 4200 ppm **myrcene** for 90 days. Since the myrcene content of the diet decreased considerably over 7 days, every week a new charge of the diet was prepared. Based on the concentration on the last day of the week, an adjusted mean daily intake was calculated of 0, 8.0, 40, and 44 mg/(kg bw x d) for males and of 0, 9.6, 48 and 53 mg/(kg bw x d) for females over the whole exposure period. There were no mortalities, clinical, or ophthalmological changes and no statistically significant, concentration-dependent changes in body weight, weight gain, or food consumption during the study. Also, there were no substance-

related histopathological or organ weight changes. Some incidental changes in clinical chemistry and haematology parameters were within approximate historical control values, did not correlate with macroscopic or histopathological findings, were without biologic impact, and were considered not toxicologically relevant. The NOAEL was determined to be the highest calculated dose (44 mg/(kg bw x d) for males and 53 mg/(kg bw x d) for females, respectively) (EFSA CEF, 2015b).

Male and female F344N Fisher rats (10/sex/group) were administered 0, 250, 500, 1000, 2000 or 4000 mg/kg bw of **myrcene** by gavage 5 days a week for 14 weeks. All animals in the high-dose group died within the first 11 days of the study. Also, some animals (up to four) died in the groups receiving ≥ 500 mg/(kg bw x d) before the end of the study. Final mean body weight and weight gains of males and females administered ≥ 500 mg/(kg bw x d) were significantly lower compared to controls. At study termination, a dose-related decrease in plasma creatinine concentration in both sexes were observed. These decreases were suggested by the authors to be associated with the decreased body weight gains observed in treated rats. No other consistent changes in clinical chemistry parameters were found. A dose-related effect of myrcene in the nose was observed in both sexes as degeneration of the olfactory epithelium and necrosis of the respiratory epithelium (significant only at 2000 mg/(kg bw x d)) accompanied by chronic inflammatory change (significant at 1000 and 2000 mg/(kg bw x d)). Absolute kidney and liver weights were significantly increased in both male and female rats receiving myrcene. Also, a dose-dependent increase in the relative liver and kidney weights were observed for males (25 – 150 % in kidney, 13 – 46 % in liver) and females (27 – 100 % in kidney, 13 – 67 % in liver). The incidence of renal tubular necrosis was significantly increased in all dosed groups of males and females, with increasing severity from minimal to moderate related to dose. Both control and treated rats showed development of chronic progressive nephropathy (CPN), but the incidence was higher in treated rats. Treatment-related increases in the incidences and severity of hyaline droplet accumulation were found in 250, 500 and 1000 mg/kg bw males, accompanied by granular casts in the outer medulla of the kidney. Hyaline droplet formation was not observed in the 2000 mg/(kg bw x d) males, although the animals showed a high incidence of renal tubular necrosis, nephrosis and CPN. No evidence of hyaline droplet accumulation was found in female rats, however treated females showed both nephrosis and CPN. A significant increase in nephrosis was observed in the 1000 and 2000 mg/(kg bw x d) dose groups of both males and females, with a dose-related increase in severity from minimal to moderate. The incidence of splenic atrophy was significantly increased in both sexes receiving 2000 mg/kg bw, accompanied by thymic necrosis in one male and three females. In the mesenteric lymph node, the incidence of atrophy was increased in males receiving 2000 mg/kg bw and females receiving 1000 or 2000 mg/kg bw. Observed lymphoid changes (splenic and mesenteric lymph node atrophy, thymic necrosis) at 1000 or 2000 mg/(kg bw x d) were considered by the authors to be secondary to morbidity rather than a direct toxic effect of myrcene (NTP, 2010). Increased hyaline droplet accumulation in male rats is characteristic of α2u-globulin nephropathy, which is a male rat specific effect with little relevance for humans. However, in the present study myrcene exposure also led to renal toxicity in the female animals. Therefore, based on the presence of renal tubular necrosis in all test groups, a NOAEL could not be assigned (EFSA CEF, 2015a).

In the corresponding gavage study with B6C3F1 mice (10 M + 10 F/ group) 0, 250, 500, 1000, 2000 or 4000 mg **myrcene**/(kg bw x d) were administered by gavage 5 d/week for 14 weeks (NTP, 2010). All animals at the highest dose died within the first three days; and most males and females in the 2000 mg/(kg bw x d) group died prior to week 5. In animals that died prior to study termination, clinical signs included lethargy, abnormal breathing and/or thin appearance.

Because of the low survival in the two top doses, further results are not reported for these doses. The final mean body weights and body weight gain of males treated with 1000 mg/(kg bw x d) and females treated with 500 mg/(kg bw x d) were significantly less than those of vehicle controls. Haematological effects (15 – 20 % decrease in haematocrit, haemoglobin and erythrocyte counts) were observed in the 1000 mg/kg bw dose males and females. A dose-dependent, but modest increase in the relative liver weight was observed for all doses in male (approximately 7 %, 6 % and 17 %) and females (approximately 8 %, 17 % and 26 %). Female mice also showed a dose-dependent significant increase in relative kidney weight at all doses (approximately 14 %, 12 % and 22 %). No significant histopathological changes in other organs examined, including kidney, were observed in mice receiving up to 1000 mg/kg bw myrcene for 14 weeks. The EFSA CEF Panel concluded in its evaluation that no NOAEL could be allocated for this study, because a significant dose-dependent increase in the relative kidney weight was observed for female mice at all treatment doses (EFSA CEF, 2015a).

In the registration dossier for **α-terpinene,** only oral toxicity studies with limonene are reported (ECHA Dissemination, 2023a).

The results of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test conducted with oral exposure of rats against **γ-terpinene** are described in chapter 2.5.4.

In a subacute oral toxicity study following OECD guideline 407, Wistar rats (5 M + 5 F/group) were exposed by gavage to 0, 62.5, 250, or 1000 mg **camphene**/(kg bw x d) for 28 days. An increased salivation was observed at the highest dose. Absolute and relative liver weight were also increased in males and females. No other effects were noted in females, but males showed histological changes in the kidney and clinical chemistry tests revealed an increase in urea nitrogen levels and a decrease in phosphorus level. A NOEL of 250 mg/(kg bw x d) was obtained for females (ECHA Dissemination, 2022c).

A subchronic oral toxicity study (similar to OECD guideline 408) with **β-caryophyllene** was conducted with Sprague–Dawley rats (10 M + 10 F/group). The animals were fed diets with 0, 3500, 7000, and 21000 ppm β-caryophyllene for 90 d, providing daily intakes of 0, 222, 456 and 1367 mg/(kg bw x d) of β-caryophyllene for males and 0, 263, 1033 and 4278 mg/(kg bw x d) for females, respectively. Effects were observed at the middle and high dose. A dose-dependent increase in white blood cells and several changes in other blood cells were observed in males. Hepatocellular hypertrophy with increased absolute and relative liver weight was noted in males and females. Erythrocytes in mesenteric lymph nodes in both sexes and an increase in relative kidney weight in females (without histological effects) were also observed. The EFSA CEF Panels concluded that only the lowest dose in male rats (222 mg/(kg bw x d)) provides a NOAEL for β-caryophyllene (EFSA CEF, 2015a; EFSA FEEDAP et al., 2023).

In the 14-day range-finding study conducted prior to the subchronic study, Sprague-Dawley rats (3 M + 3 F/group) received diets with 0, 6000, 18000, and 48000 ppm **β-caryophyllene,** corresponding to intakes of 0, 516, 1547 and 3569 mg/(kg bw x d) for males and 0, 528, 1582 and 4438 mg/(kg bw x d) for females, respectively. No mortality was observed. At the highest dose, hyperactivity observed in 33 % of males and females, and necropsy at the end of the study revealed signs of gastrointestinal irritation (EFSA CEF, 2015a).

In another subchronic toxicity study, Wistar rats (10 M + 10 F/group in main study, additional 5 M + 5 F/group for recovery groups) received **β-caryophyllene** (77 % pure, remainder: other essential oils) by gavage at dosages of 0, 150, 450, or 700 mg/(kg bw x d) for 90 days, including a 21-day recovery period. No significant toxicologic manifestations were observed. No “major treatment-related changes” were observed in the general condition and appearance,

neurobehavioral end points, growth, feed and water intake, ophthalmoscopic examinations, routine haematology and clinical chemistry parameters, urinalysis, and necropsy findings. The NOAEL was 700 mg/(kg bw x d), the highest dose tested (Schmitt et al., 2016). No additional relevant data for caryophyllene were available in the registration dossier (ECHA Dissemination, 2021a).

No repeated dose toxicity studies are available with longifolene or longicyclene.

2.5.3 Genotoxicity and carcinogenicity

Genotoxicity

Myrcene was not mutagenic *in vitro* in Ames tests with several strains of S. typhimurium in the presence or absence of metabolic activation. **Myrcene** also was not mutagenic in an HPRT (Hypoxanthine guanine phosphoribosyl transferase) assay in Chinese hamster V79 cells, did not induce sister-chromatid exchanges (SCE) in V79 cells, human hepatic tumour cell or cultured human lymphocytes, or chromosomal aberrations in cultured human lymphocytes *in vitro* (EFSA CEF, 2015a; 2015b). With respect to the key characteristics of carcinogens, it was consistently demonstrated in bacterial and mammalian assays, including tests *in vivo* and *in vitro,* that β-myrcene is not genotoxic (IARC, 2019).

In a cytogenetic assay *in vivo,* Wistar rats received oral doses of 100, 500, or 1000 mg **myrcene**/kg bw by gavage. Myrcene caused a dose-dependent increase in the mitotic index in bone-marrow cells, indicating that it was present at a sufficient dose in the target tissue. Compared with the negative control group, treatment with myrcene did not result in an increase of metaphase cells with chromosomal aberrations upon examination at 24 or 48 h. No increase in micronucleus formation in peripheral blood erythrocytes of mice was observed within 24 h of the final exposure in a 13-week gavage study at a dose of up to 2000 mg myrcene/(kg bw x d) (EFSA CEF, 2015b).

γ-Terpinene (p-mentha-1,4-diene) was not mutagenic *in vitro* in Ames tests with several strains of S. typhimurium in the presence or absence of metabolic activation. **γ-Terpinene** did not induce unscheduled DNA synthesis (UDS) in isolated rat hepatocytes *in vitro* (EFSA CEF, 2015a; 2015b).

Camphene was not mutagenic *in vitro* in Ames tests with several strains of S. typhimurium in the presence or absence of metabolic activation. **Camphene** also did not induce SCE in Chinese Hamster Ovary (CHO) cells in the absence of metabolic activation (EFSA CEF, 2015a; 2015b).

Camphene metabolites in rat urine were tested for mutagenicity in bacteria. After treating rats with a single oral dose of 1684 mg/kg bw, the urine was collected for 24 h. Three types of urine sample were tested in the Ames assay with S. typhimurium strains TA98 and TA100 with metabolic activation: a direct urine sample, a urine-ether extract, and the aqueous fraction of the urine-ether extract. Only the urine-ether extract showed a weak mutagenic response, and only in the strain TA100, not in TA98 (EFSA CEF, 2015b).

In a micronucleus test *in vivo* following OECD guideline 474, gavage administration of a single dose of 4000 mg **camphene**/kg bw to NMRI mice (5 M + 5 F/group) did not increase the number or the ratio of polychromatic and normochromatic cells in the bone marrow. A preliminary study had shown that the applied dose was the maximum applicable dose. The results indicate that camphene is not genotoxic in the micronucleus test (ECHA Dissemination, 2022c).

No evidence of genotoxic potential of **β-caryophyllene** was observed in several *in vitro* tests with bacteria (various strains of S. typhimurium and E. coli WP2uvrA) in the presence or

absence of an exogenous metabolic activation system. **β-Caryophyllene** also did not induce SCE in Chinese Hamster Ovary (CHO) cells in the absence of metabolic activation (EFSA CEF, 2015a; 2015b).

β-caryophyllene also did not induce UDS in rat hepatocytes (EFSA CEF, 2015a; 2015b).

A sister-chromatid-exchange (SCE) assay *in vivo* was conducted with **β-caryophyllene** in male mice (6/group). The animals were orally administered 20, 200 and 2000 mg of the test substance/kg bw or vehicle (10 % w/v corn oil). Positive controls received 200 mg/kg bw of benzo[a]pyrene (BaP). No genotoxic effects were observed up to highest dose (no significant increase in numbers of SCE, no significant differences in the average generation time, numbers/types of chromosome aberrations or mitotic index between the test substance and control). In a second experiment, mice orally treated with β-caryophyllene (20, 200 and 2000 mg/kg bw) were intraperitoneally injected with 200 mg BaP/kg 30 min later and sacrificed 25 hours after administration. Treatment with β-caryophyllene ameliorated the genotoxicity of BaP at the high dose caryophyllene (ECHA Dissemination, 2021a).

Longifolene was not mutagenic *in vitro* in a bacterial mutagenicity assay (according to OECD guideline 471) in all tested strains of S. typhimurium (TA 1535, TA 1537, TA 98 and TA 100) and in Escherichia coli WP2 uvr A in the presence or absence of exogenic metabolic activation (ECHA Dissemination, 2022a).

No data were available for longicyclene.

Carcinogenicity

No carcinogenicity studies are available except for myrcene.

In a NTP-carcinogenicity study, F344/N rats (50 M + 50 f/group) received 0, 250, 500, or 1000 mg **myrcene**/(kg bw x d) in corn oil by gavage, 5 d/week, for 105 weeks (purity of myrcene: 93 %, purity of > 93 %, the major contaminant was psi-limonene). All high-dose males died before the end of the study due to renal toxicity and were not included in the evaluation of carcinogenic effects. Compared to vehicle controls, the mean body weight of high-dose females was at least 8 % less than those of vehicle controls after 11 weeks and 13 weeks, respectively. Signs of irritation were observed in male rats (chronic active inflammation of the nose and the forestomach at 500 mg/(kg bw x d)). Systemic effects were apparent in the kidney. The incidences of renal tubule adenoma (0/50, 12/50, 13/50 with increasing doses) and the combined incidences of renal tubule adenoma or carcinoma (0/50, 13/50, 14/50) were significantly increased in the remaining groups of myrcene-treated males. The incidences of renal adenoma in females were 0/50, 2/50, 1/50 and 3/50). The incidences of renal tubule nephrosis were markedly increased in all dosed groups of both sexes except in low-dose females. The incidences of papillary mineralisation in males were also significantly increased. The incidences of hyperplasia of the transitional epithelium lining the pelvis and overlying the renal papilla were significantly increased in all dosed groups of males and females. There was *clear evidence of carcinogenic activity* of myrcene in male rats based on increased incidences of renal tubule neoplasms. There was *equivocal evidence of carcinogenic activity* of myrcene in female rats based on increased incidences of renal tubule adenoma (NTP, 2010).

In the corresponding NTP-study with B6C3F1 mice, 50 M + 50 F/group received 0, 250, 500, or 1000 mg **myrcene**/(kg bw x d) in corn oil by gavage, 5 d/week, for 104 or 105 weeks. Survival was reduced at the highest dose in males and females. High-dose males and females were excluded from the evaluation of carcinogenic effects because of the high number of early deaths. The cause of the early deaths was not determined. The mean body weights of high-dose males and mid- and high-dose females was at least 7 % less than those of the vehicle controls during

some phase of the study. Signs of irritation were observed in the forestomach (inflammation and epithelial hyperplasia) of 500 mg/(kg bw x d) females. In the liver of mid-dose males and females, the incidences of hepatocellular hypertrophy were significantly increased, as was the incidence of mixed cell foci in females. The incidences of bone marrow atrophy and lymph node follicle atrophy in the spleen were also higher at this dose. Regarding neoplasia, the incidences of liver neoplasms (hepatocellular adenoma and hepatocellular carcinoma and hepatoblastoma: 34/50, 45/50, 48/50) were significantly increased at 250 and 500 mg/(kg bw x d) in males and at 250 mg/(kg bw x d) in females (hepatocellular adenoma and carcinoma: 7/50, 18/50, 8/50). There was *clear evidence of carcinogenic activity* of myrcene in male mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma. There was *equivocal evidence of carcinogenic activity* in female mice based on marginally increased incidences of hepatocellular adenoma and carcinoma (NTP, 2010).

No carcinogenicity studies conducted with α- or γ-terpinene, camphene, caryophyllene, longifolene, or longicyclene were available.

2.5.4 Toxicity to reproduction

In the subchronic toxicity study of the NTP with oral (gavage) **myrcene** exposure of rats and mice, respectively (see 2.5.2), no significant changes were seen in the weights of the reproductive organs nor in the sperm parameters of males or oestrous cyclicity of female rats and mice at any dose level (NTP, 2010). No such evaluation was performed in the two-year NTP-carcinogenicity study.

Female Wistar rats (28, 15, 20, 26 and 27/group) received 0, 30, 60, 125 or 250 mg **α-terpinene**/(kg bw x d) by gavage from GD 6 – 15. The study was not performed according to OECD TG 414; no individual data were available, and it is not known whether mated (sperm-positive females) were assigned in an unbiased manner to the control and treatment groups. In addition, no historical control data were provided to assess the variability of the examined parameters. Maternal toxicity was observed at 250 mg/(kg bw x d) by initial reductions in maternal body weight and reduced maternal body weight gain. Body weight gain minus uterus weight was reduced during GD 0 – 21 by 58 % at the highest and by 23.7 % at the second highest dose. No clinical signs of maternal toxicity were reported, and at caesarean section no gross pathological alterations were found in maternal organs of any group. The only parameter related to fertility which was affected was an increase to 44 % of sperm positive females without any implantation site in the highest dose. It is unclear how this could be related to treatment. All the other parameters, which could indicate alterations of fertility, were not altered by treatment at any dose. Regarding developmental effects, neither the number of live foetuses nor the number of resorptions per pregnant female were affected at any dose. The foetal weight was significantly reduced at 250 mg/(kg bw x d). The number and percentage of foetuses with delayed ossification was increased at ≥ 60 mg/(kg bw x d). This retardation of ossification and a small reduction in foetal body weight at the highest dose were not considered as significant adverse effects (RAC, 2019).

This study is also reported in the registration dossier for **α-terpinene** where it was concluded that *"α-terpinene can adversely affect embryofoetal development in the rat at oral doses higher than 60 mg/kg body weight and is toxic to the mother at oral doses higher than 125 mg/kg body weight. Thus, the NOAEL of alpha terpinene for embryofoetal toxicity was determined to be 30 mg/kg body weight."* The dose of 125 mg/(kg bw x d) was described as a LOEL, based on reduced body weight and weight gain, and the dose of 250 mg/(kg bw x d) as a LOEL for changes in the number of pregnant animals (ECHA Dissemination, 2023a).

A combined repeated dose/reproductive developmental toxicity study according to OECD 422 was conducted in Wistar rats orally exposed to a **γ-terpinene.** The animals (10 M + 10 F/group) received 0, 25, 100, or 250 mg/(kg bw x d) by gavage. Males were treated during 14 days pre-mating and 14 days mating (maximum). Females were treated during 14 days pre-mating, 14-days of mating (maximum) and during gestation (22-days maximum) until day 13 of lactation. The animals designated for post-treatment observation (5 M + 5 F in control and high dose, respectively) remained untreated for subsequent 14 days. There were no test item-related deaths and no effects on body weight gain. There were no substance-related effects on functional behaviour tests, haematology, clinical chemistry, urine analysis or organ weights. In F1 males, the relative weights of the liver were significantly slightly increased compared to control at all doses. Significant increases of kidney and spleen relative weight and decreases of relative weight of thymus in high dose males were also observed. High dose females showed a significant decrease in pregnancy achievement and decreased number of implantations (no details reported). A NOAEL of 250 mg/(kg bw x d) is reported for systemic toxicity in the P0- and F1-generation and for reproductive performance in males; for females the NOAEL for reproductive toxicity was 100 mg/(kg bw x d) (ECHA Dissemination, 2023b).

In a prenatal developmental toxicity study (following OECD Guideline 414, but only with two doses of the test substance) pregnant Sprague-Dawley rats (20/group) received 0, 250 or 1000 mg/(kg bw x d) of **camphene** (purity 78 %, no further data) by gavage on GD 6 – 15. No substance-related mortality was observed in the dams. At the higher dose, 6/20 dams showed reduced motor activity and salivation after first dosing, and 2/20 salivation after second dosing. A transient decrease of the food consumption was observed at the highest dose. Otherwise, no clinical signs were observed at any dose. Body weight and weight gain were reported to be unaffected by treatment. No substance-related pathological changes were detected at autopsy of the dams. A non-significant increase of the resorption rate occurred at 1000 mg/(kg bw x d) (resorption rate and post-implantation loss: 11.5 %, control group: 5.2 %). Otherwise, no effects on the prenatal development were detected, and all other foetal parameters were within the normal range of the control group. No dead foetuses occurred in any group. External macroscopic inspection of soft tissue and skeleton revealed no substance-related variations and/or retardations. One malformed foetus at 1000 mg/(kg bw x d) (shifted and fused vertebrae, crossed legs, stump tail, omphalocele) was considered as spontaneous and not treatment-related (control: one foetus with stump tail). The dose of 250 mg/(kg bw x d) was identified as a NOEL for the dams (ECHA Dissemination, 2022c).

No studies were available with caryophyllene, longifolene, and longicyclene.

2.5.5 Odour perception

Without further details, an odour threshold for caryophyllene of 0.535 mg/m^3 and for myrcene of 0.0723 mg/m^3 are reported (Ruth, 1986).

In a study with a group of four normosmic volunteers, an odour threshold of 1.4 ppm was reported for α-terpinene. An odour threshold of about 10 ppm can be estimated for γ-terpinene from a figure presented in the same study. The reported odour threshold determined in the same study for (-)-α-pinene was 19 ppm (Cometto-Muñiz et al., 1998). Though the absolute values reported seem rather high, it may be concluded that the two terpinenes have a considerably lower odour threshold than (-)-pinene (see also chapter 1.5.5 for further data on pinenes).

The odour of myrcene was described as “green mango, fresh green grass-like, warm, balsamic”, that of γ-terpinene as “refreshing citrus-like, gasoline”, and that of β-caryophyllene “sweet floral,

dry woody, clove leaf oil-like". In the same study, odour detection thresholds from aqueous solutions of these compounds were reported of 0.10 ppm for myrcene, 0.26 ppm for γ-terpinene, 0.15 ppm for β-caryophyllene, and 0.19 ppm for α-pinene (isomer not reported) (Tamura et al., 2001). A tenfold lower odour threshold of 0.018 ppm (0.101 mg/m^3) α-pinene (enantiomer not stated) in air was determined using the triangle bag method (Nagata, 2003). Thus, while the absolute values may not be relevant for the odour threshold of the pure compounds in air, the values relative to that for α-pinene suggests that all of these terpenes have odour thresholds in a similar concentration range.

2.6 Evaluation

2.6.1 Existing regulations and classifications

There is no harmonised classification according to Annex VI of Regulation (EC) No. 1272/2008 for myrcene, γ-terpinene (p-mentha-1,4-diene), camphene, longifolene, longifolene, and caryophyllene (ECHA C&L Inventory, 2023; ECHA Dissemination, 2021a; ECHA Dissemination, 2022a; ECHA Dissemination, 2022b; ECHA Dissemination, 2022c; ECHA Dissemination, 2023b).

α-Terpinene is classified with respect to toxicity as aspiration hazard (Asp. Tox. 1, H304) and as skin sensitising (Skin Sens. 1, H317) (ECHA C&L Inventory, 2023). This classification follows the opinion of the Committee for Risk Assessment (RAC). The RAC also concluded that a classification for adverse effects on development is not deemed necessary (RAC, 2019).

IARC has evaluated myrcene with respect to carcinogenicity. Based on *inadequate evidence* in humans and *sufficient evidence* in experimental animals for the carcinogenicity of β-myrcene, it was concluded that β-myrcene is possibly carcinogenic to humans (Group 2B) (IARC, 2019).

No DNEL was derived or reported in the toxicological summary of the registration dossier for **myrcene** (ECHA Dissemination, 2022b), β-caryophyllene (ECHA Dissemination, 2021a), **longifolene** (no repeated dose toxicity data in the dossier) (ECHA Dissemination, 2022a), and **longicyclene** (no registration dossier available).

Existing DNEL values for terpinenes and camphene are summarised in Table 15.

In the registration dossier for **α-terpinene,** a DNEL of 0.725 mg/m^3 is derived for the protection of the general population via inhalation. This DNEL is based on a NOAEL stated to be 250 mg/(kg bw x d) obtained in a developmental toxicity/ teratogenicity study. Standard factors were used for route-to-route extrapolation (1.15 kg bw x d/m^3), to account for differences between inhalation and oral absorption (2), for time extrapolation (6 for subacute to chronic), for toxicodynamic interspecies (2.5) and for intraspecies extrapolation (10). A DNEL of 2.939 mg/m^3 for workers was also derived, based on the same NOAEL and corresponding standard factors for workers (ECHA Dissemination, 2023a). However, the NOAEL of 250 mg/(kg bw x d) used as POD is not in line with the data presented in the registration dossier. In the developmental toxicity study described in the registration dossier, it is reported that *"a reduction in body weight minus uterine weight at term indicated that the two highest doses tested (125 and 250mg/kg bw) were maternally toxic*" and *"the NOAEL for alpha terpinene-induced embryofoetotoxicity can be set at 30 mg/kg bw by the oral route"* (ECHA Dissemination, 2023a). The discrepancy between the described effects levels and the DNEL derivation cannot be clarified on the basis of the available data, and the validity of the presented DNEL must be questioned.

A DNEL of 0.725 mg/m^3 is presented for **γ-terpinene.** The derivation is based on a NOAEL of 250 mg/(kg bw x d) obtained in an oral developmental toxicity study with rats, considering a

total extrapolation factor of 150 (ECHA Dissemination, 2023b). However, the study and the individual extrapolation factors are not specified. It is likely that the NOAEL is derived from a combined repeated dose toxicity study with the reproduction / developmental toxicity screening test (OECD Guideline 422) in rats (see chapter 2.5.4). Applying standard factors for route-to-route extrapolation (1.15 kg bw x d/m^3), to account for differences between inhalation and oral absorption (2), for time extrapolation (6 for subacute to chronic), for toxicodynamic interspecies (2.5) and for intraspecies extrapolation (10) leads to the reported value of 0.725 mg/m^3. A DNEL of 2.939 mg/m^3 for workers was also derived, applying a total extrapolation factor of 75 (no details presented).

A DNEL of 54.3 mg/m^3, based on "repeated dose toxicity", is available for **camphene**. "ECHA REACH Guidance" is stated in the dossier as DNEL derivation method, but no details are presented except for an overall assessment factor of 600. The same value of 54.3 mg/m^3 is also presented as DNEL for acute/short-term exposure, stating that an overall assessment factor of 100 was applied (ECHA Dissemination, 2022c). A DNEL of 54.3 mg/m^3 derived with an assessment factor of 600 would imply a concentration of 32580 mg/m^3 as a POD obtained in a study with repeated inhalation exposure (or a dose of at least 37500 mg/(kg bw x d) if route-to-route extrapolation would have been performed). No such study is presented in the dossier (or could otherwise be identified from a study with camphene), and such high values are not realistic. A similar objection holds true regarding the DNEL for acute/ short-term exposure. It is concluded that the derivations cannot be reproduced and are to be doubted.

Table 15: Guide values for terpinenes and camphene (for explanation, see text)

Guide value Parameter/ Organisation	ECHA Dissemination (2023b)	ECHA Dissemination (2023a)	(ECHA Dissemination, 2022c)
Substance (CAS No.)	α-Terpinene, p-mentha-1,3-diene (99-86-5)	γ-Terpinene, p-mentha-1,4-diene (99-85-4)	Camphene (79-92-5)
Name (reference period)	DNEL (chronic)	DNEL (chronic)	DNEL (chronic)
Value (mg/m³)	0.725 mg/m³	0.725 mg/m³	54.3 mg/m³
Organ/critical effect	Not specified	Not specified	Not specified
Species	Rat	Not specified	Not specified
Basis	NOAEL: 250 mg/(kg bw x d), route-to-route extrapolation	NOAEL: not specified, route-to-route extrapolation	Not specified
Adjusted for continuous exposure	-	-	Not specified
Extrapolation factors Time LOAEC to NOAEC Interspecies Intraspecies Other Total	 6 - 2.5 10 0.5, 1.15 150	 Not specified - Not specified Not specified Not specified 150	 Not specified Not specified Not specified Not specified Not specified 600
Critical study, remarks	Oral developmental toxicity study; Derivation not in line with data presented in registration dossier	Oral toxicity study (not further specified)	No details of the derivation available

2.6.2 Derivation of an EU-LCI value

The data base regarding toxic effects on humans of the individual compounds considered here is insufficient for an evaluation.

The data basis from animal studies is insufficient for an evaluation of longicyclene and longifolene. Limited data are available for camphene and α-terpinene. At least subchronic toxicity studies are available for myrcene and caryophyllene and a combined repeated dose/reproductive developmental toxicity study according to OECD 422 is available for γ-terpinene.

However, inhalation toxicity studies are nearly completely lacking, so route-to route extrapolation would have to be performed using data from studies with oral exposure in order to derive LCI values. Data for myrcene from studies with oral exposure indicate that local effects (degeneration of the olfactory epithelium and necrosis of the respiratory epithelium, accompanied by chronic inflammatory change) seem to play a role in the assessment of the toxicity of this compound. Due to the insufficient data regarding inhalation toxicity, it cannot be decided whether local effects in the respiratory tract would have to be considered as critical for the derivation of an LCI value for inhalation exposure.

The “other terpenes” considered here may be emitted from wood, especially softwood, or wood-derived building products. In those cases, they will be emitted together with other terpenes. The composition of the terpene fractions which can be isolated from softwood or, respectively, the turpentines derived from these sources shows that the “other terpenes” are present at lower concentrations than the “lead terpenes” (especially α-pinene, but also limonene, 3-carene and β-pinene).

In indoor air, these “other terpenes” will also be derived from other sources, because they are widely occurring in essential oils of plants[2]. However, the data presented in the Table 10 to Table 14 in chapter 2.3.1 show that these “other terpenes” are present in indoor air at lower concentrations and are detected less frequently than the “lead terpenes” (especially α-pinene, but also limonene, 3-carene and β-pinene).

EU-LCI values were derived and are published for the “lead terpenes” α-pinene, limonene, and 3-carene. Any emission of the “other terpenes” considered here may be expected to be accompanied by higher emissions of these “lead terpenes”.

There is no evidence from the available (for some of the substances albeit rather limited) data that any of the “other terpenes” (myrcene, α- and γ-terpinene, camphene, caryophyllene, longicyclene, and longifolene) considered here is more toxic than the “lead substances” terpenes α-pinene, 3-carene, or limonene or shows effects markedly different from those compounds, possibly except for myrcene: There is some evidence for carcinogenicity of myrcene in rats and mice after oral exposure by gavage. However, the data do not indicate that myrcene is genotoxic, so that a non-genotoxic (threshold) mechanism may be considered. Myrcene is not classified as carcinogenic or mutagenic in Annex VI of Regulation (EC) No. 1272/2008 (ECHA C&L Inventory, 2023).

Regarding possible health risks, the emission of terpene hydrocarbon mixtures from building products appears to be adequately characterised by the “lead substances” (mostly α-pinene and also limonene, and 3-carene) and can be assessed via the EU-LCI values derived for these “lead substances” α-pinene, limonene, and 3-carene.

It is concluded that the derivation of EU-LCI values for the “other terpenes” (myrcene, α- and γ-terpinene, camphene, caryophyllene, longicyclene, and longifolene) considered is not necessary and not recommended. No EU-LCI values are proposed.

[2] It should be noted that, all over, exposure against these “other terpenes” will occur largely via food and only to a smaller extent by inhalation. Any evaluation would therefore have to consider the oral exposure pathway.

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B Appendix

B.1 Fact and data collection sheet for "other terpenes"

No data collection sheets are presented for myrcene, β-caryophyllene, longicyclene and longifolene because no DNELS or other inhalation exposure guide values are available.

Table 16: Data collection sheet for "other terpenes": α-terpinene

Compound	**α-Terpinene**
Nº CAS: 99-86-5 1 ppm = 5.61 mg/m³ (23 °C)	EU-Classification: yes CLP: Acute Tox 4 (H302), Asp. Tox. 1 (H304), Skin Sens. 1 (H317)
Organisation name	**REACH Registrants**
Risk value name	DNEL
Risk value (mg/m³)	0.725
Reference period	Chronic (general population)
Risk value (mg/m³) **Short term (15 min)**	
Year	2023
Key study	Not further specified
Study type	Developmental toxicity / teratogenicity
Species	Rat
Duration of exposure in key study	Subacute
Critical effect	Not further specified
Critical dose value	NOAEL: 250 mg/(kg bw x d)
Adjusted critical dose	250 mg/(kg bw x d), corrected for 50 % oral absorption: 125 mg/(kg bw x d) : 1.15 m³/(kg bw x d) = 108.7 mg/m³
Single assessment factors	UFs 6 x UF_A 2.5 x UF_H 10 = 150
Other effects	-

UF_L Used LOAEL; UF_H Intraspecies variability; UF_A interspecies variability; UF_S Used subacute study

Table 17: Data collection sheet for "other terpenes": γ-Terpinene

Compound	**γ-Terpinene**
Nº CAS: 99-85-4 1 ppm = 5.61 mg/m³ (23 °C)	EU-Classification: no CLP: -
Organisation name	**REACH Registrants**
Risk value name	DNEL
Risk value (mg/m³)	0.725
Reference period	Chronic (general population)
Risk value (mg/m³) **Short term (15 min)**	
Year	2023
Key study	Not further specified
Study type	Oral toxicity study, not further specified
Species	Not specified
Duration of exposure in key study	Not specified
Critical effect	Not further specified
Critical dose value	NOAEL: not specified
Adjusted critical dose	Not specified
Single assessment factors	Single factors not specified, total factor: 150
Other effects	-
Remarks	DNEL derivation method: ECHA REACH Guidance (not further specified)

Table 18: Data collection sheet for "other terpenes": Camphene

Compound	**Camphene**
Nº CAS: 79-92-5 1 ppm = 5.61 mg/m³ (23 °C)	EU-Classification: no CLP: -
Organisation name	**REACH Registrants**
Risk value name	DNEL
Risk value (mg/m³)	54.3
Reference period	Chronic (general population)
Risk value (mg/m³) **Short term (15 min)**	
Year	2022
Key study	Not specified
Study type	Not specified
Species	Not specified
Duration of exposure in key study	Not specified
Critical effect	Not specified
Critical dose value	Not specified
Adjusted critical dose	Not specified
Single assessment factors	Single factors not specified, total factor: 600 (also reported: 100)
Other effects	-
Remarks	DNEL derivation method: ECHA REACH Guidance (not further specified)

Table 19: Fact sheet for "other terpenes": Myrcene, α-terpinene, gamma-terpinene, camphene, β-caryophyllene, longicyclene, longifolene

Compound			**Fact sheet**
Parameter	**Note**	**Comments**	**Value / descriptor**
EU-LCI value and status			
EU-LCI value	1	[µg/m³]	not proposed
EU-LCI status	2	Draft/Final	
EU-LCI year of issue	3	Year when EU-LCI value has been issued	
General information			
CLP-Index No.	4	INDEX	
EC-No.	5	EINECS	
CAS-No.	6	Chemical Abstract Service number	
Harmonised CLP classification	7	Human health risk related classification	
Molar mass and conversion factor	8	[g/mol] and [ppm – mg/m³]	
Key data / database			
Key study, authors, year	9	Critical study with lowest relevant effect level	
Read across compound	10	Where applicable	
Species	11	Rat, human, etc.	
Route / type of study	12	Inhalation, oral feed, etc.	
Study length	13	Days, subchronic, chronic, etc.	
Exposure duration	14	h/d, d/w	
Critical endpoint	15	Effect (s), site of	
Point of departure (POD)	16	LOAEC, NOAEC, BMD, etc.	
POD value	17	[mg/m³] or ppm or [mg/kg_{BW}×d]	
Assessment factors (AF)			
Adjustment for exposure duration	19	Study exposure h/d, d/w	
Study length	20	sa→sc→c	

Compound			Fact sheet
Route-to-route extrapolation factor	21	-	
Dose-response	22a	Reliability of dose-response, LOAEL to NOAEL	
	22b	Severity of effect (R8 6d)	
Interspecies differences	23a	Allometric Metabolic rate (R8-3)	
	23b	Kinetic + dynamic	
Intraspecies differences	24	Kinetic + dynamic General population	
AF (sensitive population)	25		
Other adjustment factors Quality of database	26	Route-to-route-extrapolation	
Results			
Summary of assessment factors	27	Total Assessment Factor	
POD/TAF	28	Calculated value [µg/m³ and ppb]	
Molar adjustment factor	29		
Rounded value	30	[µg/m³]	
Additional comments	31		
Rationale selection	32		

Data compilation and evaluation is based on a project funded by the German Environment Agency (Voss et al., 2024).

Rationale

The data base regarding toxic effects on humans of the individual compounds considered here is insufficient for an evaluation.

The data basis from animal studies is insufficient for an evaluation of longicyclene and longifolene. Limited data are available for camphene and α-terpinene. At least subchronic toxicity studies are available for myrcene and caryophyllene and a combined repeated dose/reproductive developmental toxicity study according to OECD 422 is available for γ-terpinene (ECHA Dissemination, 2021; ECHA Dissemination, 2022a; ECHA Dissemination, 2022b; ECHA Dissemination, 2022c; ECHA Dissemination, 2023a; ECHA Dissemination, 2023b).

However, inhalation toxicity studies are nearly completely lacking, so route-to route extrapolation would have to be performed using data from studies with oral exposure. Data for myrcene from studies with oral exposure indicate that local effects (degeneration of the

olfactory epithelium and necrosis of the respiratory epithelium, accompanied by chronic inflammatory change) seem to play a role in the assessment of the toxicity of this compound. Due to the insufficient data regarding inhalation toxicity, it cannot be decided whether local effects in the respiratory tract would have to be considered as critical for the derivation of an LCI value for inhalation exposure.

The "other terpenes" considered here may be emitted from wood, especially softwood, or wood-based building products. In those cases, they will be emitted together with other terpenes. The composition of the terpene fractions which can be isolated from softwood or, respectively, the turpentines derived from these sources shows that the "other terpenes" are present at lower concentrations than the "lead terpenes" (especially α-pinene, but also limonene, 3-carene and β-pinene).

In indoor air, these "other terpenes" will also be derived from other sources, because they are widely occurring in essential oils of plants. However, these "other terpenes" are present in indoor air at lower concentrations and are detected less frequently than the "lead terpenes" (especially α-pinene, but also limonene, 3-carene and β-pinene).

EU-LCI values were derived and are published for the "lead terpenes" α-pinene, limonene, and 3-carene (EU-LCI Working Group, 2022; EC, 2013). Any emission of the "other terpenes" considered here may be expected to be accompanied by higher emissions of these "lead terpenes".

There is no evidence from the available (for some of the substances albeit rather limited) data that any of these "other terpenes" (myrcene, α- and γ-terpinene, camphene, caryophyllene, longicyclene, and longifolene) considered here is more toxic than the "lead substances" terpenes α-pinene, 3-carene, or limonene or shows effects markedly different from those compounds, possibly except for myrcene: There is some evidence for carcinogenicity of myrcene in rats and mice after oral exposure by gavage. However, the data do not indicate that myrcene is genotoxic, so that a non-genotoxic (threshold) mechanism may be considered. Myrcene is not classified as carcinogenic or mutagenic in Annex VI of Regulation (EC) No. 1272/2008 (ECHA C&L Inventory, 2023).

Regarding possible health risks, the emission of terpene hydrocarbon mixtures from building products appears to be adequately characterised by the "lead substances" (mostly α-pinene and also limonene, and 3-carene) and can be assessed via the EU-LCI values derived for these "lead substances" α-pinene, limonene, and 3-carene.

It is concluded that the derivation of EU-LCI values for the "other terpenes" (myrcene, α- and γ-terpinene, camphene, caryophyllene, longicyclene, and longifolene) considered is not necessary and not recommended. No EU-LCI values are proposed.

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ECHA Dissemination (2023a) p-mentha-1,3-diene (alpha-terpinene). In: European Chemicals Agency (ECHA), Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland. https://echa.europa.eu/registration-dossier/-/registered-dossier/24217. Accessed on 3.11.2023.

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3 Toxicological evaluation of pentanols as basis for the derivation of an EU-LCI value

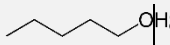
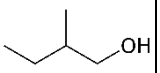
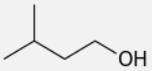
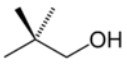
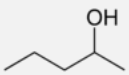
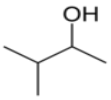
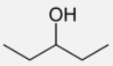
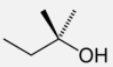
3.1 Substance identification

Pentanols represent a group of altogether eight alcohols: four primary alcohols, three secondary alcohols, and one tertiary alcohol (Table 20). 2-Methylbutan-1-ol, pentan-2-ol und 3-methyl-butan-2-ol each contain a chiral centre; the commercially available formulations usually are a 1:1 mixture of both enantiomers, and if not stated otherwise, the toxicological studies were conducted with such mixtures.

The toxicology of pentanols is summarised and evaluated in several dossiers and reviews, e.g.: (Api et al., 2019; Api et al., 2017; ECHA Dissemination, 2008; ECHA Dissemination, 2022a; ECHA Dissemination, 2022b; ECHA Dissemination, 2022c; ECHA Dissemination, 2022d; ECHA Dissemination, 2023; Hartwig and MAK Commission, 2016; Johanson et al., 2017; MAK Commission, 2008; SCOEL, 2013; SCOEL, 2016). No registration dossier according to REACH is available for 2,2-dimethylpropan-1-ol (neopentyl alcohol), pentan-2-ol (sec-amyl alcohol), 3-methylbutan-2-ol (sec isoamyl alcohol) and pentan-3-ol.

Most studies were performed with 3-methylbutan-1-ol and pentan-1-ol. Almost no toxicological data are available for 2,2-dimethylpropan-1-ol. However, this substance seems to be of very limited if any use in commercial products.

Table 20: Substance identification of pentanols

CAS-No. EU-No. CLP-Index-No.	Systematic name, common name	Sum formula	Structural formula	Molar mass (g/mol)
Primary pentanols				
71-41-0 200-752-1 603-200-00-1	Pentan-1-ol, n-amyl alcohol	$C_5H_{11}OH$		88.15
137-32-6 205-289-9 -	2-Methylbutan-1-ol*	$C_5H_{11}OH$		88.15
123-51-3 204-633-5 -	3-Methylbutan-1-ol, isoamyl-alcohol	$C_5H_{11}OH$		88.15
75-84-3 200-907-3 -	2,2-Dimethylpropan-1-ol, neopentyl alcohol	$C_5H_{11}OH$		88.15
94624-12-1 305-536-1 (903-139-3) -	Pentanol, branched and linear (reaction mass of 2-methylbutan-1-ol and pentan-1-ol)	$C_5H_{11}OH$	See individual compounds	88.15
Secondary pentanols				
6032-29-7 227-907-6 -	Pentan-2-ol, sec-amyl alcohol*	$C_5H_{11}OH$		88.15
598-75-4 209-950-2 -	3-Methylbutan-2-ol, sec-isoamyl-alcohol*	$C_5H_{11}OH$		88.15
584-02-1 209-526-7 -	Pentan-3-ol	$C_5H_{11}OH$		88.15
Tertiary pentanols				
75-85-4 200-908-9 603-007-00-2	2-Methylbutan-2-ol, tert-amyl alcohol	$C_5H_{11}OH$		88.15

*: contains a chiral centre

3.2 Substance properties and uses

Pentanols are used as solvents and starting materials for the synthesis of esters (pentylacetates), which are used technically as solvents for various plastics, but also as flavourings. The tonnage bands (production and use in the EU) of pentan-1-ol, 2-methylbutan-1-ol, and 2-methylbutan-2-ol each are ≥ 100 tonnes/a. The tonnage bands of 3-methylbutan-1-ol and the reaction mass of pentan-1-ol and 2-methylbutan-1-ol each are ≥ 1000 tonnes/a.

The physicochemical properties of the pentanols are summarised in Table 21. At room temperature all pentanols are colourless liquids, except for 2,2-dimethylpropan-1-ol, which is a

white powder (MAK Commission, 2008). All pentanols are somewhat soluble in water and soluble or miscible with most other alcohols and many organic solvents.

A mixture of 60 – 75 % pentan-1-ol, 25 – 40 % 2-methylbutan-1-ol and about 1 % 3-methyl-butan-1-ol (CAS No. 94624-12-1), also known as amyl alcohol, is also used industrially (MAK Commission, 2008).

Table 21: Physicochemical properties of pentanols

Substance name	Melting point (°C)#	Boiling point (°C)#	Vapour pressure (hPa) (at 25 °C)*	Conversion 1 ppm = x mg/m³ (23 °C)	log pow*	Solubility in water (g/L) @ 20°C*
Pentan-1-ol	-78	138	2.93	3.63	1.51	22
2-Methylbutan-1-ol	-70	129	4.15	3.63	1.29	29,7
3-Methylbutan-1-ol	-117	131	3.15	3.63	1.16	26,7
2,2-Dimethyl-propan-1-ol	53	113	21.28 (20 °C)	3.63	1.31	35
Pentan-2-ol	-50	119	8.13	3.63	1.19	44,6
3-Methylbutan-2-ol	-117	112	12.17	3.63	1.28	56
Pentan-3-ol	-8	116	11.7	3.63	1.21	51,5
2-Methylbutan-2-ol	-8	102	19.0	3.63	0.89	110

#: Data from (DGUV, 2023); *: Data from (Hartwig and MAK Commission, 2016)

3.3 Exposure

3.3.1 Indoor air

Data on the concentration of pentanols in indoor air are summarised in Table 22.

Among the isomers listed, most data refer to pentan-1-ol which is also the most frequently detected isomer in indoor air. Overall, the concentrations are low in the order of a few µg/m³, and even the maximum concentrations reported stay below 50 µg/m³.

Table 22: Data on the occurrence of pentanols in indoor air

Rooms	Substance	No.	LoD# (µg/m³)	N > LoD	Median (µg/m³)	P95 (µg/m³)	Max. (µg/m³)	Ref.
Indoor, Germany	Pentan-1-ol	462	1.0	280	1.8	2.0	39	(Hofmann and Plieninger, 2008)
Indoor, Germany	Pentan-2-ol	127	1.0	17	5	2.5	5	(Hofmann and Plieninger, 2008)
Indoor, Germany	2-methyl-butan-1-ol	89	1.0	15	0.1	0.5	3.0	(Hofmann and Plieninger, 2008)

Rooms	Substance	No.	LoD# (µg/m³)	N > LoD	Median (µg/m³)	P95 (µg/m³)	Max. (µg/m³)	Ref.
Indoor, Germany	3-Methyl-butan-1-ol, isoamyl alcohol	729	1.0	34	0.3	0.7	3	(Hofmann and Plieninger, 2008)
Indoor, Germany	3-methyl-butan-2-ol	57	1.0	6	0.1	0.7	3	(Hofmann and Plieninger, 2008)

#: Median of LoD in all analytical determinations

A guidance value (corresponding to the 90th percentile of measured concentrations) of 5.4 µg pentan-1-ol/m³ was published by AGÖF (2013). AGÖF did not publish corresponding values for other pentanols.

In a study by Horn et al. (2007), 50 construction products were tested in emission test chambers according to the requirements of the AgBB-scheme, including acrylic and silicone sealants, paste-like synthetic resin plasters, wood based products, adhesives, lacquers, and wall paints. VOC and odour emissions were measured on day 1, 3, 10, and 28. Pentanol was within the 10 most often found VOC from wood and wood-based materials. The reported data are presented in Table 23. In OSB plates, the concentrations were in the range of several tens of µg/m³ and declined within 28 days. Other building materials such as glued pine board or wooden chip-board caused low concentrations of pentan-1-ol which remained at the initial level or only showed a minute decline.

Table 23: Pentanols concentrations from construction products in emission chamber measurements (Horn et al., 2007)

Substance/source	No. of determinations/day of measurement	Median (µg/m³)	Max. (µg/m³)
Pentanol (no specification)/all products	7, day 28	19	130
Pentanol (no specification)/wood and wood-based materials	6, day 28	18	29
Pentan-1-ol/OSB plate	n. r.*/ day 1 day 3 day 10 day 28 day 81		33/30/55/98/19/51/42# 48/21/46/62/10/31/31 42/17/44/42/14/20/21 28/17/21/29/23/9/19 -/-/14/-/-/-/21
Pentan-1-ol/glued pine board	n. r./ day 1 day 3 day 10 day 28		11 11 11 10
Pentan-1-ol/wooden chip-board panel	n. r./ day 1 day 3 day 10 day 28		2 2 3 n. d.

Substance/source	No. of determinations/day of measurement	Median (µg/m³)	Max. (µg/m³)
Pentan-1-ol/floor paint	n. r./ day 1 day 3 day 10 day 28		10 9 3 2
Pentan-1-ol/gypsum plaster board	n. r./ day 1 day 3 day 10 day 28		15 8 1 n. d.

#: measurements for different plates; *: not reported

3.3.2 Other sources

Pentan-1-ol, but also other isomers, are widely distributed in plants, including vegetables and fruits. A comprehensive list of plants, plant products, and essential oils where pentanols have been detected can be found in Duke's Phytochemical and Ethnobotanical Databases, USDA (2016). E.g., according to this data base, pentan-1-ol was detected in apples, isoamyl alcohol in celery and eucalyptus leaves, lemongrass, pears and blackberries, and pentan-2-ol in rosemary leaves and ginger rhizome.

3.4 Toxicokinetics

Based on PBPK modelling, the nasal uptake of **3-methylbutan-1-ol** (isoamyl alcohol) in rats was estimated to be 80 % (Johanson et al., 2017).

In a human experimental study, 3 healthy volunteers were exposed at rest through a mouthpiece to 25 ppm (91 mg/m^3) **3-methylbutan-1-ol** for 10 min. The mean respiratory absorption was 63 % during the last 5 min of exposure, and a steady-state level was reached within a few minutes (Johanson et al., 2017; Kumagai et al., 1999).

3-Methylbutanal and 3-methylbutanoic acid were identified as metabolites in the blood of humans. Some individuals show a deficiency of a specific aldehyde dehydrogenase isoenzyme and may therefore have elevated 3-methylbutanal blood levels after exposure to **3-methyl-butan-1-ol** (Johanson et al., 2017).

An overview of the metabolism of pentanols is presented by the MAK Commission (2008):

The primary isomers pentan-1-ol, 2-methylbutan-1-ol and 3-methylbutan-1-ol were both oxidised and glucuronidated, when incubated with liver microsomes from rats. The secondary alcohols pentan-2-ol and 3-methylbutan-2-ol were not oxidised, but glucuronidated.

Studies with rabbits indicated that approx. 9 % of an orally administered dose of 733 mg 3-methylbutan-1-ol/kg bw was excreted as glucuronic acid conjugate. *In vitro* studies with class I to III alcohol dehydrogenase from human liver revealed that 2-methyl-butan-1-ol and 3-methylbutan-1-ol are mainly (about 99 %) oxidised to the corresponding methyl-butyraldehyde by class I alcohol dehydrogenase.

In the case of the tertiary alcohol 2-methylbutan-2-ol no volatile metabolite was detected after the application of 1000 mg/kg bw in rats.

In summary, it was concluded that the primary alcohols pentan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol and 2,2-dimethylpropan-1-ol are largely oxidised to the corresponding

aldehyde and further on to the corresponding acid (MAK Commission, 2008). Pentanoic acid (valeric acid) and 3-methylbutanoic acid (isovaleric acid) are known to enter physiological metabolic pathways (Api et al., 2019; DGUV (Gestis), 2019b).

The secondary alcohols pentan-2-ol, pentan-3-ol and 3-methylbutan-2-ol are mainly metabolised to the corresponding ketones (methyl n-propyl ketone, diethyl ketone or methyl isopropyl ketone). The proportion of metabolised dose decreases from primary to tertiary alcohol. Especially secondary pentanols and the tertiary pentanol 2-methylbutan-2-ol are excreted unchanged as glucuronides in the urine (MAK Commission, 2008).

3.5 Health effects

3.5.1 Acute toxicity, sensory irritation, and local effects

Acute toxicity

Acute central nervous depression is associated with exposure to **3-methylbutan-1-ol** for all exposure pathways.

Oral intake of 50 – 100 ml of this compound led to central nervous system depression, weakness, pain, nausea, headache, sleep within 10–15 min, terminal coma and death within 1 hour to 6 days (Johanson et al., 2017). In case of **2-methylbutan-1-ol**, oedema of the lung and cardiac arrhythmia are reported (no further details). Oral intake of **2-methylbutan-1-ol** and **3-methylbutan-1-ol** in combination with ethanol is reported to be correlated with neurological effects and hangover during the late phase nine hours after intake (MAK Commission, 2008).

For (S)-pentan-2-ol, an LD50 > 2000 mg/kg bw is reported for rats based on an acute toxicity test according to the acute toxic class method (OECD 423) (ECHA Dissemination, 2008).

No quantitative data are provided for inhalation exposure (Johanson et al., 2017).

In studies with rats, no deaths occurred during or after an 8-h exposure to **pentan-1-ol** (saturated atmosphere at 20 °C, calculated concentration 7370 mg/m^3). Marked (in some cases, bloody) irritation of mucous membranes was observed (MAK Commission, 2008). Similar exposure of rats and mice to **2-methylbutan-1-ol** and **3-methylbutan-1-ol** at concentrations around vapour saturation also led to signs of irritation (laboured breath, lacrimation, nasal discharge, in mice: lung oedema) and CNS depression. Deaths were noted after 6-h exposure of rats and mice against an aerosol exposure concentration of about 14000 mg/m^3 of a technical mixture of pentan-1-ol (74 %), 2-methylbutan-1-ol (25 %) and 3-methylbutan-1-ol (MAK Commission, 2008; Scala and Burtis, 1973).

Four male Wistar rats or eight female strain H mice were exposed to different concentrations of **pentan-1-ol** for 4 h (rats) or 2 h (mice). The duration and latency of seizures (tonic extensions) in the hind limbs following electric stimulation of the ears were reported, as these endpoints were considered the most sensitive and reproducible. The concentration of pentan-1-ol that evoked a 30 % depression (EC30, shortened duration and increased latency of seizures) in recorded activity immediately after the exposure was determined to be 1600 ppm (5800 mg/m^3) in rats and 2600 ppm (9438 mg/m^3) in mice (Frantik et al., 1994).

The same approach provided EC30 values for **3-methylbutan-1-ol** of 1700 ppm (6171 mg/m^3) in rats and 950 ppm (3449 mg/m^3) in mice (Frantik et al., 1994).

A comparative study of the pentanol isomers involving intraperitoneal application of the substance to rodents indicated that all pentanols triggered stronger toxic effects than ethanol.

2,2-Dimethylpropan-1-ol appeared to belong to the less effective pentanol isomers (DGUV (Gestis), 2019a).

Sensory irritation

Human data

Experiments with very brief exposure of humans for a few seconds to **pentan-1-ol** indicated a threshold for nasal irritation of about 2000 ppm (7260 mg/m^3). Similar studies with hexan-1-ol indicated a threshold for eye irritation in the order of 400 ppm (1680 mg/m^3) and of about 1000 ppm (4200 mg/m^3) for nasal irritation and of 200 ppm (960 mg/m^3) as a threshold for nasal irritation for heptan-1-ol. Other studies with short-term exposure to octan-1-ol revealed thresholds for eye irritation of 40 ppm (214 mg/m^3) and for nasal irritation of 56.2 ppm (300 mg/m^3) and 100 ppm (536 mg/m^3) (the nasal threshold concentration was too high to be determined in a third study). For 2-ethylhexan-1-ol a threshold for nasal irritation of 66.6 ppm was reported (357 mg/m^3) (AGS, 2019).

30 healthy volunteers (16 men, 14 women) were exposed in random order to 1 mg/m^3 (0.275 ppm) **3-methylbutan-1-ol** (isoamyl alcohol) or clean air for 2 h at controlled conditions. Ratings with visual analogue scales revealed slightly increased perceptions of eye irritation and smell compared with control exposure: The median rating of eye irritation during exposure to isoamyl alcohol reached 5 mm at 1 hour of exposure, versus 3 mm for clean air (Zero - 0 - mm on the visual analogue scale corresponds to “Not at all” and 6 mm to “Hardly at all”). The other ratings (irritation in nose and throat, dyspnoea, headache, fatigue, dizziness, nausea, and intoxication) were not significantly affected. No significant exposure-related effects were found in blinking frequency, tear film break-up time, vital staining of the eye, nasal lavage biomarkers, lung function, and nasal swelling. Thus, the study revealed no irritation effects at 1 mg/m^3 (Ernstgard et al., 2013). The SCOEL also considered that 1 mg/m^3 can be regarded as the NOAEC for eye irritation in this study as the effect was minimal (below “hardly at all”) (Johanson et al., 2017).

In a toxicokinetic study (Kumagai et al., 1999), three volunteers inhaling 25 ppm (91 mg/m^3, concentration analytically monitored) **3-methylbutan-1-ol** via a mouth piece for 3–5 minutes complained about throat irritation. The mean respiratory rate was slightly increased (15.3/min, as compared to 12.1 – 14.0/min for the other compounds). 3-Methylbutan-1-ol was considered to be the causative agent, as no irritation and no effect on the respiratory rate were reported during exposure to nine other polar oxygen-containing solvent vapours (50 ppm methyl isobutyl ketone, up to 100 ppm methanol, methyl acetate, methyl propyl ketone, ethylene glycol monobutyl ether, and propylene glycol monomethyl ether, and up to 200 ppm acetone, ethyl acetate and isopropanol) using the same protocol (Johanson et al., 2017; Kumagai et al., 1999).

Short-term exposure for 3 to 5 minutes of volunteers (about ten subjects, males and female) with 100 ppm (363 mg/m^3) **3-methylbutan-1-ol** gave slight throat irritation to some subjects, whereas a majority estimated that this level would not be acceptable for an 8-hour exposure period. Exposure to 150 ppm (545 mg/m^3) evoked irritation of eyes and nose in the majority of subjects and 200 ppm (726 mg/m^3) was objectionable to all (Nelson et al., 1943). It must be noted that only nominal but no analytically confirmed concentrations were reported in this study.

The SCOEL (2016) briefly reported data obtained in studies with other alkanols: In a study with 24 male volunteers, 4-hour exposure to 6.4 ppm octan-1-ol caused increased ratings of sensory irritation and annoyance, whereas up to 190 ppm isopropanol produced no such effects (van Thriel et al., 2003). Similar to octan-1-ol, increased blink frequency was observed in men

exposed to 10 ppm or 20 ppm 2-ethylhexan-1-ol, using 1.5 ppm as control condition (Kiesswetter et al., 2005).

The SCOEL (2016) also reported work history and records of physical examination of men occupationally exposed to n-butanol as solvent for 10 years. Signs of marked eye irritation (blurred vision, lacrimation, photophobia, burning, moderate corneal oedema, oedematous conjunctiva) were commonly recorded at 200 ppm (measured at the breathing zone). Reduction of the exposure levels to an average of ≤ 100 ppm greatly reduced the number of complaints, and these complaints "were associated with short runs where the concentration frequently exceeded 100 ppm" (Sterner et al., 1949).

Based on these data, the SCOEL (2016) concluded that *"there is a clear trend of decreasing effect levels* [i. e. increased potency of the compound] *with increasing carbon chain length".*

Animal data

Animal data regarding sensory irritation as expressed by the RD50 obtained in inhalation studies with mice are presented in Table 24. These data also indicate a clear trend for a decrease of the RD50 values, i. e. an increase in the potency for sensory irritation in the respiratory tract, with increasing chain length. The RD50 values for pentan-1-ol are similar or maybe slightly higher than those for 3-methylbutan-1-ol. No data are available for other pentan-1-ols.

Table 24: Vapour pressure and RD values for C4 – C8 alkanols#

Substance or mixture	Vapour pressure (hPa)at ambient temperature	RD50 (ppm)	RD0 (ppm)
n-Butanol	10 (20 °C)*	3870 – 35670 (1268 – 11696)	233
2-Methypropan-1-ol (isobutanol)	11.8 (20 °C)	1818	
Pentan-1-ol	2.04 (20 °C)*	610 (810) 3000 4039	120
3-Methylbutan-1-ol (isoamyl alcohol, isopentanol)	3 (20 °C)*	729 2583 4452 6160	
Pentan-2-ol		2744	
3-Methylbutan-2-ol		2672	
Hexan-1-ol	1.3 (20 °C)	(200) 220 239	
4-Methylpentan-2-ol	3.7 (20 °C)	425	
Heptan-1-ol	0.7 (20 °C)	98 770	28
Octan-1-ol	0.1 (25 °C)	48 47	
Octan-3-ol		255	

Substance or mixture	Vapour pressure (hPa)at ambient temperature	RD50 (ppm)	RD0 (ppm)
2-Ethylhexan-1-ol	0.93 (20 °C)* 0.3 (20 °C)+	45 44	

#: Data from (AGS, 2019; Alarie, 2015; Bos et al., 1992; Kane et al., 1980; Korpi et al., 2009; MAK Commission, 2008; Muller and Greff, 1984; Nielsen and Wolkoff, 2017); * from ECHA Disseminations (ECHA, 2022) or AGS (2019); + (Ad-hoc AG, 2013)

Sensitisation

There are no clear indications of a contact sensitising effect. Individual cases of positive reactions to pentan-1-ol, 2-methylbutan-1-ol and 3-methylbutan-1-ol were described in epicutaneous tests (MAK Commission, 2008).

No sensitisation was observed with 8 % isoamyl alcohol in petrolatum in a Kligman-maximisation test with 25 volunteers. In a patch test, three patients reacted positively to isoamyl alcohol as well as to other substances (SCOEL, 2016).

2-Methyl-butan-2-ol and (S)-pentan-2-ol did not show a sensitising potential in a LLNA (Local Lymph Node Assay) according to OECD Guideline 429 in mice (ECHA Dissemination, 2008; ECHA Dissemination, 2022b).

No data are available on respiratory sensitising effects.

3.5.2 Repeated dose toxicity

Human data

No relevant toxicity studies were available regarding health effects of repeated exposure to pentanols in humans.

Animal data

Wistar rats /5 m/group) were exposed via inhalation to 0, 100, 300 or 600 ppm **pentan-1-ol** (0, 363, 1089, 2178 mg/m^3) 6 h/d, 5 d/week for up to 14 weeks. The exposure had no effect on the body weight of the animals. The activity of the pentan-1-ol dehydrogenase in liver and kidneys and the activities of CYP450 monooxygenase and 7-ethoxycoumarin-O-deethylase (EOD) in the liver showed no changes. In the kidneys, a dose-dependent increase in EOD activity was observed after seven weeks, which was slightly lower after 14 weeks. Brain and muscle acetylcholinesterase activities showed a dose-dependent increase after 7 weeks although this effect ameliorated after 14 weeks. Other parameters were not investigated. The study showed rapid absorption and metabolism of pentan-1-ol to valeraldehyde, which in turn was rapidly further metabolised (Savolainen et al., 1985).

In subchronic oral toxicity study, the exposure of ASH/CSE rats (5 M + 5 F/group) by gavage with 0, 150, or 1000 mg/(kg bw x d) of **pentan-1-ol** on 7 d/week for 6 weeks had no treatment-related effect. Similarly, gavage exposure of ASH/CSE rats with 0, 50, 150, or 1000 mg pentan-1-ol/(kg bw x d) on 7 d/week for 2 weeks (5 M + 5 F/group) or 13 weeks (15 M + 15 F/group and 5 M + 5 F/satellite group) had no treatment-related adverse effect (MAK Commission, 2008). The initially increased stomach weights found at week 2 in some rats at the highest dose could indicate a mild local irritation. However, the effect did not persist throughout the study (ECHA Dissemination, 2022c).

The results of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test conducted with oral exposure of rats against **pentan-1-ol** are described in chapter 3.5.4.

The oral toxicity of **3-methylbutan-1-ol** was studied in Wistar rats in a 90-day study (according to OECD guideline 408). The animals (10 M + 10 F/group) received 0, 1000, 4000 and 16000 ppm of the test substance in drinking water, corresponding to approximately 0, 80, 340, 1250 mg/(kg bw x d) (for males: 0, 73, 295 and 1068 mg/(kg bw x d), for females: 0, 90, 385 and 1431 mg/(kg bw x d)) . No substance-related clinical signs were identified at any dose. Haematological alterations (increase in the red blood cell count and a decrease in the mean corpuscular volume and the mean corpuscular haemoglobin content) were observed in high-dose males and, to a slight extent, also at the mid dose. The alterations were reported to be within the range of normal biological variation and thus not characteristic for a specific toxic effect. It was concluded that dose level which cause clear signs of toxicity would be above 16000 ppm and the highest dose of 16000 ppm (about 1250 mg/(kg bw x d)) represented a NOAEL (ECHA Dissemination, 2022c; 2023).

In a further subchronic oral toxicity study, ASH/CSE rats (15 M + 15 F/group) were exposed to 0, 150, 500 or 1000 mg/(kg bw x d) **3-methylbutan-1-ol** for 17 weeks. Two rats at the high dose died, but these deaths were due to dosing into the lungs. The body-weight gain was slightly reduced at the highest dose level in males due to a reduced food intake. Otherwise, no substance-related effects were noted regarding haematology, serum analyses, urinary cell counts, renal concentration tests, or organ weights (NOAEL: 1000 mg/(kg bw x d), NOEL 500 mg/(kg bw x d)) (ECHA Dissemination, 2022c; 2023).

The results of combined repeated dose toxicity studies with the reproduction/developmental toxicity screening test conducted with oral exposure of rats against **3-methylbutan-1-ol** are described in chapter 3.5.4.

Rats (8/group, sex and strain not specified) were exposed against 0, 150, 500, or 1500 ppm (0, 540, 1800, or 5400 mg/m^3) **2-methylbutan-2-ol** for 6 h/d on seven consecutive days. Motor incoordination and lethargic appearance were observed over 3 to 4 hours after the first two exposures but not later on, suggesting the development of some tolerance. Absolute and relative liver and kidney weights were increased and blood glucose level decreased; gross pathologic examination did not reveal any consistent changes attributable to exposure (ECHA Dissemination, 2022b).

The results of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test conducted with inhalation exposure of rats against **2-methylbutan-2-ol** (ECHA Dissemination, 2022b) are described in chapter 3.5.4.

In a subchronic inhalation study, CD1-mice (10 M + 10 f/group), F344 rats (10 m + 10 F/group), and Beagle dogs (4 M/group) were exposed to 0, 50, 225, or 1000 ppm **2-methylbutan-2-ol** (0, 182, 817, 3630 mg/m^3) 6 h/d, 5 d/week for 87 days. No effects on body weight, clinical-chemical parameters or histopathology of the organs were observed in mice. In rats and dogs, effects on the central nervous system and the liver were observed. During the first exposure to 1000 ppm, female rats showed uncoordinated movements and male dogs showed ethanol-like signs of intoxication during the first weeks. Female and male rats at 1000 ppm and females at 225 ppm showed eye irritation (severe watering of the eyes) from the 37th day of exposure. One dog also showed eye irritation at 1000 ppm. No substance-related histopathological effects were observed in nose, trachea and lungs. The absolute and relative liver weight in dogs and male rats was increased at 1000 ppm. In the exposed dogs, but not in the control group, one dog in each dose group showed cytoplasmic eosinophilic inclusions in the liver. These inclusions likely contained mucopolysaccharides and varied greatly in size and number per cell. The authors considered this alteration as unclear with respect to their toxicological relevance. The MAK commission regarded the effect as substance-related but, since there was no dose-dependence,

as not adverse and not relevant for human risk assessment. Considering the local effects on the eyes, the NOAEC was 50 ppm (182 mg/m^3) (Hartwig and MAK Commission, 2016; MAK Commission, 2008).

Dermal application of 0, 344 or 3440 mg/(kg bw x d) of **2-methylbutan-2-ol** to the skin of rabbits (5 M + 5 F/group) on 5 d/week for 4 weeks produced only local irritant effects on the skin in the low-dose group. Depression of the central nervous system occurred in addition to the local irritation effects at the high dose, and as a result three animals per sex died or had to be killed in a moribund state after several applications. Histopathological examination of these animals did not reveal any abnormal findings. A slight reduction in body weight and a change in fat reserves and glycogen content in the hepatocytes were detected (no details reported) (MAK Commission, 2008).

3.5.3 Genotoxicity and carcinogenicity

Genotoxicity

In vitro

Pentan-1-ol was not mutagenic in bacterial assays with different strains of *Salmonella typhimurium* (Ames test) in the presence or absence of S9 mix when tested up to cytotoxic concentrations. Similarly, the substance was not mutagenic in an assay (only tested without S9-mix) with *Escherichia coli* Sd-4-73. No DNA damage (or a weak effect at cytotoxic concentrations only) was observed after treatment of mammalian cells (A549 human lung carcinoma epithelia cells, human peripheral blood cells, and V79 Chinese hamster lung fibroblasts) with pentan-1-ol in the absence of S9-mix. Pentan-1-ol did not induce micronuclei formation in V79 cells in the absence or presence of S9-mix (ECHA Dissemination, 2022c; MAK Commission, 2008).

2-Methylbutan-1-ol did not induce DNA damage in a comet assay in mammalian cells (A549 human lung carcinoma epithelia cells, human peripheral blood cells, and V79 Chinese hamster lung fibroblasts) in the absence of S9-mix (a weak effect was noted at cytotoxic concentrations only). The substance was not mutagenic in an HPRT-assay and in a micronucleus assay in V79 cells with and without metabolic activation (ECHA Dissemination, 2022c; MAK Commission, 2008).

3-Methylbutan-1-ol was not mutagenic in the presence or absence of S9-mix in Ames tests with different strains of *Salmonella typhimurium* and in a reverse mutation assay with *Escherichia coli* WP2 uvrA when tested up to concentrations which were cytotoxic to at least some of the strains used. No DNA damage was observed in a comet assay after treatment of mammalian cells (A549 human lung carcinoma epithelia cells, human peripheral blood cells, and V79 Chinese hamster lung fibroblasts) with 3-methylbutan-1-ol in the absence of S9-mix (a weak effect was noted at cytotoxic concentrations only). 3-Methylbutan-1-ol was also not mutagenic in an HPRT-assay and in a micronucleus assay in V79 cells with and without metabolic activation (ECHA Dissemination, 2022c; MAK Commission, 2008).

Branched and linear pentan-1-ols (reaction mass of 2-methylbutan-1-ol and pentan-1-ol, CAS No. 94624-12-1, see chapter 3.2) were not mutagenic in different Salmonella typhimurium strains and in E. coli WP2 uvr A with or without S9-mix when tested up to cytotoxic concentrations. In an HPRT assay without S9-mix on CHO cells, this pentanol mixture led to a numerical increase in the mutation frequency at some concentrations. However, these changes were not significant, not concentration dependent, and no effect was observed in the presence of S9-mix. Therefore, it was concluded that *"primary amyl alcohol did not produce any dose-related or repeatable statistically significant increases in the frequency of mutations of CHO cells at*

concentrations which spanned a cytotoxic to non-cytotoxic range of doses tested with and without an S9 metabolic activation system." No significantly increased frequency of chromosomal aberrations were observed in rat peripheral lymphocyte cultures in the presence or absence of S9-mix (ECHA Dissemination, 2022c; MAK Commission, 2008).

(S)-Pentan-2-ol was not mutagenic in different Salmonella typhimurium strains with or without S9-mix in a Bacterial Reverse Mutation Assay according to OECD Guideline 471 (ECHA Dissemination, 2008).

3-Methylbutan-2-ol led to a slightly increased number of DNA strand breaks in a comet assay with A549, V79 and peripheral blood cells only at cytotoxic but not at lower concentrations (MAK Commission, 2008).

2-Methylbutan-2-ol was not mutagenic in the presence or absence of S9-mix in Ames tests with different strains of *Salmonella typhimurium* and in a reverse mutation assay with *Escherichia coli* WP2 uvrA when tested up to limit concentrations. Similarly, in a mammalian cell mutagenicity assay (HPRT assay in Chinese hamster V79 cells), no reproducible increase in mutant frequency was observed in the main experiments up to the maximum (limit) concentration. The mutant frequency remained within the historical range of solvent controls. A 3fold increase in the mutation frequency compared to the corresponding solvent control was observed in one culture in one experiment at a single concentration and only without metabolic activation. This was judged as *"biologically irrelevant fluctuation as it was neither reproduced in the parallel culture under identical experimental conditions nor dose dependent as indicated by the lacking statistical significance".* Furthermore, the substance did not cause a *"biologically relevant increase in the number of cells carrying structural chromosome aberrations"* in the presence or absence of S9-mix in mammalian chromosome aberration test with V79 cells (ECHA Dissemination, 2022b).

There are no data available for 2,2-dimethylpropanol, pentan-2-ol and pentan-3-ol.

In vivo

3-methylbutan-1-ol was considered not be genotoxic in a micronucleus test (following OECD guideline 474) in mice treated with a single oral dose of 1500 mg/kg bw (which caused clinical signs of toxicity but no mortality). In females, but not in males, a significant but weak increase in the frequency of micronuclei was noted after 24 hours (0,17 %, control: 0.05 % of cells) but not after 48 hours. Since this alteration was only observed in one sex and only at one timepoint, it was considered to be not relevant. (ECHA Dissemination, 2022c) (The MAK commission stated that *"no dose dependence was tested and the dosage used was toxic, so that the positive findings in the female animals could be high-dose effects. It is not reported whether a positive control was included."* (MAK Commission, 2008). According to the registration dossier, a positive control was included (cyclophosphamide) but no results were presented (ECHA Dissemination, 2022c).

No data are available for other pentanols.

Carcinogenicity

3-methylbutan-1-ol was administered to Wistar rats by gavage twice a week (15 M + 15 F) in doses of 0.1 ml/kg bw (~ 81 mg/kg bw) each or subcutaneously to 24 animals once a week in doses of 0.04 ml/kg bw (~ 32 mg/kg bw) each (Gibel et al., 1975; Gibel et al., 1974). The application was carried out until the natural death of the animals. The average survival time was 527 days with oral application and 592 days with subcutaneous application. The control groups (exact number of animals not clearly reported) received 1 ml physiological sodium chloride solution/kg bw orally or subcutaneously twice a week and were killed on day 643. The absolute number of tumours was reported to be higher in the exposed animals of both application types compared to the control animals and distributed to different organs. Malignant tumours

included liver carcinomas and sarcomas, spleen and forestomach carcinomas, myeloid leukaemias, a bladder sarcoma, a renal pelvis carcinoma and an adenocarcinoma of the glandular stomach. The benign tumours were mainly papillomas of the forestomach and fibroadenomas of the mamma. The studies and the reporting suffer from a number of shortcomings (no assignment of the individual findings to the animals or the type of application, only relatively few animals used, treatment only once or twice a week) (MAK Commission, 2008).

3.5.4 Toxicity to reproduction

Pregnant Sprague-Dawley rats (115/group) were exposed to 0 or 14 000 mg/m^3 **pentan-1-ol** (3822 ppm) from day 1 to 19 of gestation. The concentration represented the highest achievable vapour concentration at 25°C. A non-significant and only slight delay in ossification of the lumbar vertebrae, sternum, metatarsals and toes of the hind paws was observed in foetuses of the exposed dams, with no reduction in the body weight of the foetuses. The only tested concentration thus represented a NOAEC for the offspring. In a pre-study, this concentration led to a slight decrease of food intake and weight gain in dams (max. 15 %). No information on irritant effects or clinical findings were presented (ECHA Dissemination, 2022c).

A combined repeated dose/reproductive developmental toxicity study according to OECD 422 was conducted in Wistar rats orally exposed to a **mixture (reaction mass) of 2-methylbutan-1-ol and pentan-1-ol.** The animals (10 M + 10 F/group) were exposed to the mixture via drinking water at concentrations of 0, 1250, 3750 or 12500 ppm. These concentrations corresponded to 0, 77, 254 or 842 mg/(kg bw x d) in males and 0, 117, 372 or 1239 mg/(kg bw x d) in females. After a two-week premating period, the parental animals were paired and the females were allowed to give birth and bring up the offspring until sacrifice on PND 4 or PND 13. In the in-depth investigations including the detailed clinical observation, the functional observational battery and the measurement of motor activity no treatment-related differences to control were observed at any concentration. Water consumption, food consumption and body weights and body weight gain did not show relevant test substance-related changes. Clinical pathology (including thyroid hormone measurement) and histopathology revealed no treatment-related, adverse effects at any dose level. Also, fertility, reproductive performance, and pre- to postnatal development were not affected by treatment. The overall NOAEL was found to be the highest concentration of 12500 ppm in the drinking water, corresponding to a NOAEL of 842 mg/(kg bw x d) in males and 1239 mg/(kg bw x d) in females (ECHA Dissemination, 2022c).

Pregnant Wistar rats (20 – 23/group) or New-Zealand White rabbits (14 or 15/group) were exposed in an OECD guideline 414 developmental study to (analytically confirmed) concentrations of 510, 2500 and 9800 mg/m^3 of **3-methylbutan-1-ol** (136, 675 and 2646 ppm), 6 hours/day, on gestational days 6 – 15 (rats) or 7 – 19 (rabbits) (ECHA Dissemination, 2022c). Maternal toxicity (retarded body weight gain) was observed in both species at the highest concentration. There were no signs of embryotoxicity or teratogenicity up to the highest exposure concentration. A significant increase in foetal soft tissue variations was noted in rabbits. As the incidence for this endpoint was unusually low in the concurrent control and the incidences were within the range of biological variation in the treated group, these findings were considered by the authors as not toxicologically relevant. (NOAEC developmental toxicity: ≥ 9800 mg/m^3 in rats and rabbits; NOAEC maternal toxicity in rats and rabbits: 2500 mg/m^3) (Johanson et al., 2017; MAK Commission, 2008).

In a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (following OECD guideline 422), Wistar rats (10 M + 10 F/group) received 0,

1250, 3750, or 12500 ppm **3-methylbutan-1-ol** in drinking water. The overall mean dose was 0, 71, 229 and 785 mg/(kg bw x d) in males and 0, 116, 359 and 1273 mg/(kg bw x d) in females. No clinical symptoms and no effects in the functional observational battery and motor activity were observed at any dose. Clinical chemistry, haematology, water consumption, food consumption, body weight and weight gain did not show important test substance-related changes. No treatment-related adverse effects were noted regarding clinical pathology (including thyroid hormone measurement), organ weights, and histology in male and female rats. Fertility, reproductive performance, pre and postnatal development showed no signs of toxicity in parental animals or their offspring at any dose. Neither determination of anogenital distance/index nor the count of nipple/areola revealed any treatment-related changes. The NOAEL in the P0- and F1-generation was 12500 ppm, the highest concentration in drinking water tested (ECHA Dissemination, 2022c; 2023).

The same concentrations of **3-methylbutan-1-ol** were used in a EOGRT study (extended one-generation reproduction toxicity study according to OECD guideline 443). Wistar rats (25 M + 25 F/group) were exposed to 0, 1250, 3750 and 12500 ppm in drinking water. F0 animals were treated at least for 10 weeks prior to mating to produce a litter (F1 generation). Mating pairs were from the same dose group. Pups of the F1 litter were selected (F1 rearing animals) and assigned to 2 different cohorts which were subjected to postweaning examinations. The study was terminated by the terminal sacrifice of the F1 rearing animals of cohort 1B. Test drinking water containing 3-methylbutan-1-ol was offered continuously throughout the study. In P0 males, body weights from premating day 28 onwards until the end of the study was decreased (7 % below control), and body weight gain was reduced during pre-mating (9 % below control). Otherwise, no treatment-related effects were observed regarding systemic toxicity, fertility, reproduction and development. The NOAEL for systemic toxicity is 3750 ppm (about 405 mg/(kg bw x d)) in the F0 males, based on decreased body weight/body weight gain at 12500 ppm. The NOAEL in the F0 parental females as well as F1 offspring, for fertility and reproductive performance, and for developmental toxicity is 12500 ppm, corresponding to about 1221 mg/(kg bw x d) in males and 1521 mg/(kg bw x d) in females (ECHA Dissemination, 2023).

3-methylbutan-1-ol was tested in a further combined repeated-dose / reproductive developmental toxicity study similar to OECD TG 422. Sprague-Dawley rats (12 M + 12 F/group) received 0, 30, 100 or 300 mg/(kg bw x d) for a total of 42 days (males) and for a total of 41 to 53 days (females, for 14 days before mating throughout the mating and gestation periods up to day 4 of lactation). At 0 and 300 mg/(kg bw x d), a 14-day recovery period was provided after administration for 42 days to examine reversibility of the toxic changes in two additional groups (5 M + 5 F/group, the females in the recovery group were not subjected to mating). No deaths occurred in any group, and there were no test article-related effects in clinical observation, motor activity, food consumption, urinalysis, haematological examination, blood chemistry examination, organ weight, histopathological findings or gross pathological examination. The body weight gain of high-dose males was reduced but recovered after cessation of exposure. There were no significant differences between the control and the treated groups regarding oestrus cycle or reproductive performance and fertility. Also, no treatment-related effects on pup viability, body weight, pathology or clinical signs were noted at any dose. The NOAEL for the P0- and the F1-generation was identified as 300 mg/(kg bw x d), the highest dose tested (ECHA Dissemination, 2023).

In a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (following OECD guideline 422), Wistar rats (10 M + 10 F/group) were exposed „nose/head only“ to **2-methylbutan-2-ol** via inhalation to analytically confirmed vapour concentrations of 0, 724, 2523 and 6663 mg/m^3 (nominal concentrations: 0, 732, 2561 and

7316 mg/m³) on 6 h/day, 7 d/week. Males were treated for at least 30 days and females for 55 days. At the highest concentration (6663 mg/m³), males and females showed clinical signs (unconsciousness, apathy, piloerection, reduced attention, unsteady gaits, gasping) during and after exposure, indicating a narcotic effect of the test substance. Unspecific signs as alopecia and reduced fur care indicated bad general condition of the animals. Females also showed blood in bedding and vaginal discharge. Regarding histopathology, female animals revealed a reduction of vaginal epithelial height and hypertrophy with increase of mucification. The mean body weights of males and females were significantly lower than the control group (no details presented). Clinical chemistry showed increased cholesterol values in both sexes and lowered glucose level in males at the highest concentration. The absolute and relative liver weights were significantly increased (no details presented) in both sexes at ≥ 2523 mg/m³. Reproduction parameters (mating index, fertility index) and developmental parameters (number of implantation sites, post implantation loss, liveborn pups, pup viability, sex ratio and pup body weights) were not affected by treatment. Also, there was no evidence of teratogenicity. The reported NOAEC were 2561 mg/m³ for systemic toxicity and 6663 mg/m³, the highest concentration tested, for fertility and developmental toxicity (ECHA Dissemination, 2022b).

3.5.5 Odour perception

Measurements using the triangle odour bag method revealed an odour threshold for **pentan-1-ol** of 0.10 ppm (0.363 mg/m³) (Nagata, 2003). A sweet pleasant odour was clearly notable at 0.12 ppm (0.454 mg/m³) (MAK Commission, 2008).

The odour of **3-methylbutan-1-ol** (isoamyl alcohol) was detected at levels of 0.022 to 0.028 ppm (0.080– 0.102 mg/m³) and recognised at levels of 0.044 to 0.072 ppm (0.160 – 0.261) (AIHA, 1997). A much lower threshold of 0.0017 ppm (0.006 mg/m³) was determined by the triangle bag method (Nagata, 2003). An acrid-sharp unpleasant odour was clearly notable at 0.23 ppm (0.835 mg/m³) (MAK Commission, 2008).

An odour threshold of 0.29 ppm (1.053 mg/m³) for **pentan-2-ol** and of 0.088 ppm (0.319 mg/m³) for **2-methylbutan-2-ol** were determined by the triangle bag method (Nagata, 2003).

3.6 Evaluation

3.6.1 Existing regulations and classifications

None of the pentanols is classified (harmonised classification) as carcinogenic, mutagenic or toxic to reproduction according to Annex VI of Regulation (EC) No. 1272/2008. With respect to toxicity, pentan-1-ol, pentan-3-ol and 2-methylbutan-2-ol are classified (harmonised classification) as Skin Irrit. 2 (H315), Acute Tox. 4 (H332), and STOT SE 3 (H335, may cause respiratory irritation) (ECHA C&L Inventory, 2023).

Existing DNEL values for pentanols are summarised in Table 25. No registration dossiers according to REACH and thus no DNEL are available for 2,2-dimethylpropan-1-ol, neopentyl alcohol, pentan-2-ol, sec-amyl alcohol, 3-methylbutan-2-ol, sec isoamyl alcohol and pentan-3-ol.

The DNEL of 13 mg/m³ for **pentan-1-ol** (ECHA Dissemination, 2022c) is derived from the German OEL (MAK value) for the category of pentanol isomers of 20 ppm (= 73.16 mg/m³) which is based on local irritation effects observed in subchronic vapour inhalation studies in rats, mice and dogs with the structurally related tertiary alcohol 2-methyl-2-butanol (MAK Commission, 2008). In humans, concentrations of the structural analogue 3 -methylbutan-1-ol of 100 – 150 ppm (366 - 549 mg/m³) were reported to be irritating to the respiratory tract. The

German OEL for workers was modified for consumers according to the ECHA Guidance, using an intraspecies factor of 2 between the general population and workers. Furthermore, an additional factor of 4.2 was considered for the possible exposure time (8 h/d, 5 d/week for workers vs. 24 h/d and 7 d/week), and for correcting the inhalation volume for light work (workers 10 m^3/ consumers 6.7 m^3). This leads to a DNEL for the general population of 3.55 ppm (13 mg/m^3). The German MAK value of 20 ppm (73.16 mg/m^3) was adopted as DNEL for workers (ECHA Dissemination, 2022c).

Table 25: Guide values for pentanols (for explanation, see text)

Guide value Parameter/ Organisation	(ECHA Dissemination, 2022a; ECHA Dissemination, 2022c; ECHA Dissemination, 2022d; ECHA Dissemination, 2023)	ECHA Dissemination (2022b)
Substance	Pentan-1-ol 2-Methylbutan-1-ol 3-Methylbutan-1-ol Pentanol, branched and linear (reaction mass of 2-methylbutan-1-ol and pentan-1-ol)	2-Methylbutan-2-ol
Name (reference period)	DNEL (chronic)	DNEL (chronic)
Value (mg/m³)	13 mg/m³	4.3
Organ/critical effect	Irritation (respiratory tract)	
Species	Human	
Basis	OEL (MAK): 73.16 mg/m³ (20 ppm)	NOAEC: 640 mg/m³
Adjusted for continuous exposure	-	
Extrapolation factors Time LOAEC to NOAEC Interspecies Intraspecies Other Total	 4.2 - - 2 0.67 5.6	 6 - 2.5 10 - 150
Critical study, remarks	DNEL based on OEL for irritation, POD for OEL: eye irritation in rats, LOAEC 225 ppm (825 mg/m³) in subchronic inhalation study with 2-methylbutan-2-ol (Hartwig and MAK Commission, 2016; MAK Commission, 2008)	Probably based on NOAEC of 2561 mg/m³ in an combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test in rats by adjusting for continuous exposure (6 h/ d to 24 h/d)

The same value of 13 mg/m^3 for consumers and of 73.16 mg/m^3 for workers, respectively, were also derived as DNELs for the reaction mass of pentan-1-ol and 2-methylbutan-1-ol (ECHA Dissemination, 2022d), for 2-methylbutan-1-ol (ECHA Dissemination, 2022a), and 3-methylbutan-1-ol (ECHA Dissemination, 2023).

Although the German OEL (MAK value) is based on a study with 2-methyl-2-butanol (MAK Commission, 2008), the DNEL for consumer for 2-methylbutan-2-ol is not derived from this

MAK value. The DNEL is based on a reported NOAEC of 640 mg/m³. This value is presented without further explanation, but probably is derived from the NOAEC of 2561 mg/m³ obtained in a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (see chapter 3.5.4) with inhalation exposure of rats on 6 h/d (2561 mg/m³ x 6 h/24 h = 640 mg/m³). Assessment factors of six (subacute to chronic), 2.5 (interspecies differences), and 10 (intraspecies differences) were applied (total factor: 150) to derive a DNEL for consumers of 4.3 mg/m³. A DNEL for workers of 17.2 mg/m³ was derived from a NOAEC of 1287 mg/m³ (source of NOAEC not explained) using assessment factors of six (subacute to chronic), 2.5 (interspecies differences), and 5 (intraspecies differences) (total factor: 75) (ECHA Dissemination, 2022b).

3.6.2 Derivation of an EU-LCI value

The animal data in Table 24 present a basis for comparing and ranking the irritation potential of primary alcohols. The data indicate that the thresholds for sensory irritation within this group of substances decrease with increasing chain length. A similar trend of decreasing effect levels for sensory irritation in humans with increasing carbon chain length was described by Johanson et al. (2017) for C_1 to C_6 alcohols including 3-methylbutan-1-ol. Based on these data, the SCOEL (2016) concluded that *"there is a clear trend of decreasing effect levels* [i. e. increased potency of the compound] *with increasing carbon chain length".*

The data basis for other pentanols is limited but does not provide evidence for a higher sensory irritation potency compared to pentan-1-ols.

The LCI derivation is based on sensory irritation observed in humans in controlled studies with short-term exposure against 3-methylbutan-1-ol (isoamyl alcohol).

In one of these studies the subjects complained about throat irritation at 100 ppm (Nelson et al., 1943). In a further study, effects noted by the exposed subjects indicated irritation of the throat at the only exposure concentration of 25 ppm (91 mg/m³) (Kumagai et al., 1999), while 1 mg/m³, the only concentration studied, had no adverse effects (NOAEC) in a third study (Ernstgard et al., 2013).

The concentration of 25 ppm (91 mg/m³) is regarded as a LOAEC for sensory irritation in humans and as a POD for the derivation.

The following assessment factors are used:

- LOAEC to NAEC: 10 (considering the low number of exposed persons and the uncertainty regarding the steepness of the concentration-response curve)
- Adjusted study length factor: 1 (sensory irritation develops within minutes and is reversible)
- Interspecies extrapolation: 1 (study with humans)
- Intraspecies extrapolation (interindividual variability, general population): 10

The total assessment factor is 100, leading to a value of 91 mg/m³ : 100 = for 910 µg/m³ (rounded to 900 µg/m³).

Animal data do not indicate substantial differences in the irritation potency (RD50 values) between different pentanol isomers. Therefore, it is proposed to adopt the value based on sensory irritation of 3-methylbutan-1-ol for (the sum of) all pentanol isomers.

An EU-LCI value of (rounded) 900 µg/m³ is proposed for all pentanol isomers.

The proposed LCI value of 900 µg/m^3 is far above the reported odour thresholds for 3-methyl-butan-1-ol of 0.0017 ppm or 6 µg/m^3 as determined by the triangle bag method (Nagata, 2003) and values reported for other pentanols. Thus, the odour of the compounds will be noticed at the proposed EU-LCI value.

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C Appendix

C.1 Fact and data collection sheet for pentanols

Table 26: Data collection sheet for pentanols

Compound	Pentanols	Data collection sheet
N° CAS: **Pentan-1-ol: 71-41-0** **2-Methylbutan-1-ol (racemate): 137-32-6** **3-Methylbutan-1-ol: 123-51-3** **2,2-Dimethylpropan-1-ol: 75-84-3** **Pentan-2-ol (racemate): 6032-29-7** **3-Methylbutan-2-ol (racemate): 598-75-4** **Pentan-3-ol: 584-02-1** **2-Methylbutan-2-ol: 75-85-4** 1ppm = 3.63 mg/m³		**EU-Classification: yes** **CLP:** Pentan-1-ol, pentan-3-ol, 2-methylbutan-2-ol: Acute Tox. 4 2, STOT SE 3, Skin Irrit. 2
Organisation name	SCOEL	MAK commission
Risk value name	OEL (Occupational Exposure Limit) for **isoamyl alcohol (3-methylbutan-1-ol)**	MAK (Maximale Arbeitsplatzkonzentration) for **all pentanol isomers**
Risk value (mg/m³)	5 ppm (18 mg/m³)	20 ppm (73.16 mg/m³)
Reference period	Chronic (workers)	Chronic (workers)
Risk value (mg/m³) Short term (15 min)	10 ppm (36 mg/m³)	80 ppm (290.4 mg/m³), 15 min
Year	2016	2008
Key study	Nelson et al. (1943), Kumagai et al. (1999)	Dow Chemical (1992)
Study type	Acute inhalation exposure	
Species	Human	Rat
Duration of exposure in key study	3 – 5 min	6 h/d, 5 d/week, for 87 d
Critical effect	Local respiratory tract irritation	Lacrimation
Critical dose value	LOAEC: 25 ppm (90.75 mg/m³)	NOAEC: 50 ppm
Adjusted critical dose	None (local effect, completely reversible)	-
Single assessment factors	UF_S 1 x UF_L 5 x UF_A 1 x UF_H 1 x UF_D 1 = 5	See below
Other effects	-	

Compound	Pentanols	Data collection sheet
Remarks	*…irritation effects at 25 and 100 ppm after 3–5 min of exposure, mild sensory irritation of the more potent alcohols n-octanol and 2-ethyhexanol at 6.4 ppm and 10 ppm, respectively, and irritation effects of the less potent alcohol n-butanol at 25 ppm, an 8-hour OEL of 5 ppm is recommended, with a 15-min STEL of 10 ppm. Systemic toxicity is of no concern at these levels.*	*The irritant effect is in the foreground of the effect of the pentanol isomers. In an 87-day study in rats, mice and male dogs, adverse substance-related effects did not occur in any species at the lowest exposure concentration of 50 ml **2-methyl-2-butanol**/m³. At 225 ml/m³ and above, lacrimation occurred in female rats from the 37th day of exposure, and at 1000 ml/m³ also in male rats and dogs. … A re-examination of the study data showed that there were no findings on the nose. … Compared to 1-butanol, whose MAK value of 100 ml/m³ was also set due to local irritant effects, 2-methyl-2-butanol is somewhat more irritant. The MAK value of 2-methyl-2-butanol is therefore set at 20 ml/m³ at half the animal NOAEC.* *Due to the similar effect spectrum of the different pentanol isomers, the MAK value is provisionally also set at 20 ml/m³ for the other pentanol isomers in analogy to 2-methyl-2-butanol.*

UF_L Used LOAEL; UF_H Intraspecies variability; UF_A interspecies variability; UF_S Used subchronic study UF_D data deficiencies.

Table 27: Data collection sheet for pentan-1-ol

Compound	Pentanols
Nº CAS: 71-41-0 1 ppm = 3.63 mg/m³ (23 °C)	**EU-Classification: yes** **CLP:** Pentan-1-ol, pentan-3-ol, reaction mass of 2-methylbutan-1-ol and pentan-1-ol: Acute Tox. 4 2, STOT SE 3, Skin Irrit. 2
Organisation name	**REACH Registrants**
Risk value name	DNEL
Risk value (mg/m³)	13
Reference period	Chronic (general population)
Risk value (mg/m³) **Short term (15 min)**	-
Year	2022
Key study	Adopted from MAK-value for pentanols
Study type	
Species	
Duration of exposure in key study	
Critical effect	
Critical dose value	
Adjusted critical dose	
Single assessment factors	
Other effects	
Remarks	The DNEL was adopted from the MAK value derived by the MAK commission (see above)

UFH Intraspecies variability; UFA interspecies variability; UFS Used subchronic study

Table 28: Data collection sheet for 2-methylbutan-2-ol

Compound	**2-Methylbutan-2-ol**
Nº CAS: 75-85-4 1 ppm = 3.63 mg/m³ (23 °C)	**EU-Classification: yes** **CLP: Acute Tox. 4 2, STOT SE 3, Skin Irrit. 2**
Organisation name	**REACH Registrants**
Risk value name	DNEL
Risk value (mg/m³)	4.3
Reference period	Chronic (general population)
Risk value (mg/m³) **Short term (15 min)**	-
Year	2022
Key study	OECD guideline 422: combined repeated dose toxicity study with the Reproduction/Developmental Toxicity Screening Test
Study type	Inhalation study
Species	Rat
Duration of exposure in key study	6 h/d, 7 d/week, 30 – 55 d
Critical effect	Systemic toxicity (clinical signs)
Critical dose value	NOAEC: 2561 mg/m³
Adjusted critical dose	adjusted to continuous exposure (see remarks)
Single assessment factors	UFs 6, UF_A 2.5, UF_H 10
Other effects	-
Remarks	Although not explicitly stated, the NOAEC of 2561 mg/m³ was probably adjusted for continuous exposure (6 h/24 h)

UF_H Intraspecies variability; UF_A interspecies variability; UF_S Used subacute study

Table 29: Fact sheet for pentanols

Compound	Pentan-1-ol 2-Methylbutan-1-ol 3-Methylbutan-1-ol 2,2-Dimethylpropan-1-ol Pentan-2-ol 3-Methylbutan-2-ol Pentan-3-ol 2-Methylbutan-2-ol		Fact sheet
Parameter	**Note**	**Comments**	**Value / descriptor**
EU-LCI value and status			
EU-LCI value	1	[µg/m³]	900
EU-LCI status	2	Draft/Final	Draft
EU-LCI year of issue	3	Year when EU-LCI value has been issued	2023
General information			
CLP-Index No.	4	INDEX	Pentan-1-ol: 603-200-00-1 2-Methylbutan-1-ol: - 3-Methylbutan-1-ol: - 2,2-Dimethylpropan-1-ol: - Pentan-2-ol: - 3-Methylbutan-2-ol: - Pentan-3-ol: - 2-Methylbutan-2-ol: 603-007-00-2
EC-No.	5	EINECS	Pentan-1-ol: 200-752-1 2-Methylbutan-1-ol: 205-289-0 3-Methylbutan-1-ol: 204-633-5 2,2-Dimethylpropan-1-ol: 200-907-3 Pentan-2-ol: 227-907-6 3-Methylbutan-2-ol: 209-950-2 Pentan-3-ol: 209-526-7 2-Methylbutan-2-ol: 200-908-9
CAS-No.	6	Chemical Abstract Service number	Pentan-1-ol: 71-41-0 2-Methylbutan-1-ol: 137-32-6 3-Methylbutan-1-ol: 123-51-3 2,2-Dimethylpropan-1-ol: 75-84-3 Pentan-2-ol: 6032-29-7 3-Methylbutan-2-ol: 598-75-4 Pentan-3-ol: 584-02-1 2-Methylbutan-2-ol: 75-85-4
Harmonised CLP classification	7	Human health risk related classification	Pentan-1-ol, pentan-3-ol, reaction mass of 2-methylbutan-1-ol and pentan-1-ol: Acute Tox. 4 2, STOT SE 3, Skin Irrit. 2
Molar mass and conversion factor	8	[g/mol] and [ppm – mg/m³]	88.15 1 ppm = 3.63 mg/m³
Key data / database			

Compound	Pentan-1-ol 2-Methylbutan-1-ol 3-Methylbutan-1-ol 2,2-Dimethylpropan-1-ol Pentan-2-ol 3-Methylbutan-2-ol Pentan-3-ol 2-Methylbutan-2-ol		Fact sheet
Key study, authors, year	9	Critical study with lowest relevant effect level	Kumagai et al., 1999, Ernstgaard et al., 2013
Read across compound	10	Where applicable	
Species	11	Rat, human, etc.	Human
Route / type of study	12	Inhalation, oral feed, etc.	Inhalation
Study length	13	Days, subchronic, chronic, etc.	
Exposure duration	14	h/d, d/w	3 – 5 min
Critical endpoint	15	Effect (s), site of	Sensory irritation
Point of departure (POD)	16	LOAEC, NOAEC, BMD, etc.	LOAEC (3-methylbutan-1-ol)
POD value	17	[mg/m³] or ppm or [mg/kg$_{BW}$×d]	25 ppm (91 mg/m³)
Assessment factors (AF)			
Adjustment for exposure duration	19	Study exposure h/d, d/w	1
Study length	20	sa→sc→c	1
Route-to-route extrapolation factor	21	-	1
Dose-response	22a	Reliability of dose-response, LOAEL to NOAEL	10
	22b	Severity of effect (R8 6d)	1
Interspecies differences	23a	Allometric Metabolic rate (R8-3)	1
	23b	Kinetic + dynamic	1
Intraspecies differences	24	Kinetic + dynamic General population	10
AF (sensitive population)	25		1
Other adjustment factors Quality of database	26	Route-to-route-extrapolation	1
Results			
Summary of assessment factors	27	Total Assessment Factor	100
POD/TAF	28	Calculated value [µg/m³ and ppb]	910 µg/m³ and 250 ppb

Compound	Pentan-1-ol 2-Methylbutan-1-ol 3-Methylbutan-1-ol 2,2-Dimethylpropan-1-ol Pentan-2-ol 3-Methylbutan-2-ol Pentan-3-ol 2-Methylbutan-2-ol		Fact sheet
Molar adjustment factor	29		
Rounded value	30	[µg/m³]	900
Additional comments	31		
Rationale selection	32		

Data compilation and evaluation is based on a project funded by the German Environment Agency (Voss et al., 2024).

Rationale for critical effects

The critical effect for the derivation of an EU-LCI value for pentanols is sensory irritation.

30 healthy volunteers (16 men, 14 women) were exposed in random order to 1 mg/m^3 (0.275 ppm) 3-methylbutan-1-ol (3MB) or clean air for 2 h at controlled conditions. Ratings with visual analogue scales revealed slightly increased perceptions of eye irritation and smell compared with control exposure (The median rating of eye irritation during exposure to isoamyl alcohol reached 5 mm at 1 hour of exposure, versus 3 mm for clean air. Zero - 0 - mm on the visual analogue scale corresponds to “Not at all” and 6 mm to “Hardly at all”.) The other ratings were not significantly affected (irritation in nose and throat, dyspnoea, headache, fatigue, dizziness, nausea, and intoxication). No significant exposure-related effects were found in blinking frequency, tear film break-up time, vital staining of the eye, nasal lavage biomarkers, lung function, and nasal swelling. Thus, the study revealed no irritation effects at 1 mg/m^3 (Ernstgard et al., 2013). The SCOEL also considered that 1 mg/m^3 can be regarded as the NOAEC for eye irritation in this study as the effect was minimal (below “hardly at all”) (Johanson et al., 2017).

In a toxicokinetic study (Kumagai et al., 1999), three volunteers inhaling 25 ppm (91 mg/m^3, concentration analytically monitored) 3-methylbutan-1-ol via a mouth piece for 3–5 minutes complained about throat irritation. The mean respiratory rate was slightly increased (15.3/min, as compared to 12.1 – 14.0/min for the other compounds). 3-methylbutan-1-ol was considered to be the causative agent, as no irritation and no effect on the respiratory rate were reported during exposure to nine other polar oxygen-containing solvent vapours (50 ppm methyl isobutyl ketone, up to 100 ppm methanol, methyl acetate, methyl propyl ketone, ethylene glycol monobutyl ether, and propylene glycol monomethyl ether, and up to 200 ppm acetone, ethyl acetate and isopropanol) using the same protocol (Johanson et al., 2017; Kumagai et al., 1999).

Short-term exposure for 3 to 5 minutes of volunteers (about ten subjects, males and female) with 100 ppm (363 mg/m^3) 3-methylbutan-1-ol gave slight throat irritation to some subjects, whereas a majority estimated that this level would not be acceptable for an 8-hour exposure period. Exposure to 150 ppm (545 mg/m^3) evoked irritation of eyes and nose in the majority of subjects and 200 ppm (726 mg/m^3) was objectionable to all (Nelson et al., 1943). It must be noted that only nominal but no analytically confirmed concentrations were reported in this study.

Rationale for starting point

The concentration of 25 ppm (91 mg/m^3) reported in the study of Kumagai et al. (1999) is regarded as a LOAEC for sensory irritation in humans and as a POD for the derivation.

Rationale for assessment factors

No assessment factors are applied for study length or exposure duration, since sensory irritation develops within minutes and is fully reversible. Further, no assessment factor is necessary for interspecies extrapolations, since the POD is based on inhalation exposure of humans. However, since the LOAEC is obtained from a study with a very limited number of healthy volunteers, an assessment factor of 10 is used to extrapolate from the LOAEC to a NAEC.

The following assessment factors are used:

- LOAEC to NAEC: 10 (considering the low number of exposed persons and the uncertainty regarding the steepness of the concentration-response curve)
- Adjusted study length factor: 1 (sensory irritation develops within minutes and is reversible)
- Interspecies extrapolation: 1 (study with humans)
- Intraspecies extrapolation (interindividual variability, general population): 10

The total assessment factor (TAF) is 100. The calculated EU-LCI value for 3-methylbutan-1-ol is POD/TAF = 25 ppm/100 = 0.25 ppm (910 µg/m^3 at 23 °C). The rounded and proposed value is 900 µg/m^3.

Animal data do not indicate substantial differences in the irritation potency (RD50 values) between different pentanol isomers. Therefore, it is proposed to adopt the value based on sensory irritation of 3-methylbutan-1-ol for (the sum of) all pentanol isomers.

The proposed LCI value of 900 µg/m^3 is above the reported odour thresholds for 3-methyl-butan-1-ol and values reported for other pentanols. Thus, the odour of the compounds will probably be noticed at the proposed EU-LCI value.

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4 Toxicological evaluation of 5-chloro-2-methyl-4-isothiazolin-3-one (CIT) and 2-methyl-2H-isothiazol-3-one (MIT) as basis for the derivation of EU-LCI values

4.1 Substance identification

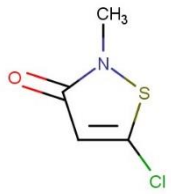
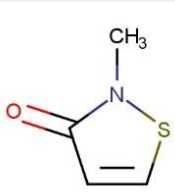
5-Chloro-2-methyl-2H-isothiazol-3-one (CIT) is a crystal-forming 1,2-thiazole (see Table 30). The substance is not registered under REACH and no harmonised classification is available. On the ECHA page "Registry of CLH intentions until outcome" there is a declaration of intent from the year 2020 by the French authority ANSES to submit a CLH dossier (ECHA Dissemination, 2023).

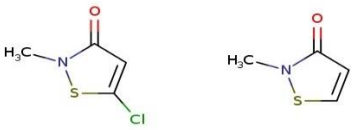
2-Methyl-2H-isothiazol-3-one (MIT) is a colourless, clear liquid, which also belongs to the 1,2-isothiazoles (see Table 30). Since 2000, MIT has increasingly been used as individual substance. For MIT, both a REACH registration dossier and a harmonised classification is available. It is registered under the REACH Regulation in the tonnage band ≥ 10 to < 100 tonnes per annum (ECHA Dissemination, 2023).

The CIT/MIT mixture (3:1 mixture of 5-chloro-2-methyl-2,3-dihydro-1,2-thiazol-3-one and 2-methyl-2,3-dihydro-1,2-thiazol-3-one) has a REACH registration in the tonnage band of ≥ 10 to < 100 tonnes per year with the EC no. 911-418-6 (ECHA Dissemination, 2023). The CIT/MIT mixture has a harmonised classification.

In the following Table 30 an overview of the substances is provided.

Table 30: Substance identification of 5-chloro-2-methyl-4-isothiazolin-3-one (CIT), 2-methyl-2H-isothiazol-3-one (MIT) and 5-chloro-2-methyl-2,3-dihydro-1,2-thiazol-3-one; 2-methyl-2,3-dihydro-1,2-thiazol-3-one (CIT/MIT mixture) (ECHA Dissemination, 2023)

CAS-No. EU-No. CLP-Index-No.	Systematic name, common name	Sum formula	Structural formula
CIT CAS no.: 26172-55-4 EC no.: 247-500-7 -	5-chloro-2-methyl-2H-isothiazol-3-one (CIT)	C4H4ClNOS	
MIT CAS no.: 2682-20-4 EC no.: 220-239-6 CLP index no.: 613-326-00-9	2-methyl-2H-isothiazol-3-one (MIT)	C4H5NOS	

CAS-No. EU-No. CLP-Index-No.	Systematic name, common name	Sum formula	Structural formula
CIT/MIT mixture CAS no.: 55965-84-9 EC no.: 911-418-6 EC no.: 611-341-5 CLP index no.: 613-167-00-5	5-chloro-2-methyl-2,3-dihydro-1,2-thiazol-3-one; 2-methyl-2,3-dihydro-1,2-thiazol-3-one	C8H9ClN2O2S2	

4.2 Substance properties and uses

CIT, MIT, and the CIT/MIT mixture have antimicrobial and antifungal effects and are used as active substances in biocidal products mainly as preservatives in different products (see also section 4.3.2). The physicochemical properties are summarised in Table 31.

Table 31: Physicochemical properties of CIT, MIT, and CIT/MIT mixture

Molar mass (g/mol)	Mp. (°C)	Boiling point (°C)	Vapour pressure (hPa) (at 20 °C)	Conversion 1 ppm = x mg/m³ (23 °C)	log p_{ow}	Solubility in water (g/L)
CIT 149.6	54-55°C[#]	106.5°C[###]	20.8 hPa at 20°C[###]	6.16	0.401 (As determined by SCCS (2009) from a CIT/MIT mixture with 14C labelled CIT)	> 5000 mg/L bei 20°C[###]
MIT 115.2	50-51°C[#]	100°C[##]	0.026 hPa bei 25°C[##]	4.74	-0.486[##] (as determined by SCCS (2009) from a CIT/MIT mixture with 14C labelled CIT)	completely soluble in water, largely soluble in acetonitrile, methanol, and hexane, sparingly soluble in xylene ##
CIT/MIT mixture -	-	100°C bzw. 106.5°C[####]	0.00108 hPa bei 20°C bzw. 20.8 hPa bei 20°C[####]	-	WTE: soluble in water CG: miscible with water, lower alcohols, and glycols #	0.63-0.75[####] (Different values depending on pH value and temperature)

#: (NLM, 2022); ##: (Burnett et al., 2010); ##: (ECB, 2000); ####: (SCCS, 2009)

4.3 Exposure

4.3.1 Indoor air

Multiple studies were published in the past years concerning the indoor concentration of CIT and MIT, after painting activities or due to air conditioning systems containing biocides (see

Table 32 for references). Taken together, the indoor air concentration of both CIT and MIT depends on the CIT/MIT concentration in the applied paint or air conditioning systems products, but also on the air humidity, the temperature, the room ventilation or in case of paints, the ink thickness.

The EU eco-labelling directive set out criteria for indoor paints at a maximum total content of isothiazolinones of 500 ppm. A limit for MIT of 200 ppm was adopted by the Member States for implementation in 2014 (Danish EPA, 2015).

The German Eco-label "Blue Angel" has revised its requirements for the addition of biocides in wall paints and paint mixing systems. From the beginning of 01.01.2021 the isothiazolinone content must not exceed 1.5 ppm for MIT, 0.5 ppm for CIT, 10 ppm for BIT, and 2 ppm for all other isothiazolinones (BMUV, 2019).

Weese (2019) analysed the indoor room concentration of CIT, MIT and OIT (2-octyl-2H-isothiazol-3-one) by collecting 499 air samples. The indoor room measurement was based on health issues including headache and malaise. However, no detailed description of adverse effects in association with indoor room concentrations of MIT, CIT and OIT is available. In summary, the percentage of positive findings (values > limit of detection of 0.02 µg/m^3) showed 74 % for MIT, 4 % for CIT and 2 % for OIT. Indoor air pollution with MIT was most likely found in a concentration range between 4 µg/m^3 to 6 µg/m^3 (Weese, 2019).

Moreover, in South Korea several types of disinfectants, including CIT/MIT mixture, that have been used in indoor humidifiers since 1994, were associated with humidifier disinfectant - associated lung injury (HDLI). A total of 1199 HD exposed patients were enrolled. Out of those, 99 patients used a humidifier disinfectant (HD) containing a CIT/MIT mixture. From these 99 patients, 26 were assessed with the diagnosis of HDLI related to CIT/MIT mixture (Ryu et al., 2019). However, this study must be discussed with caution, since estimated inhalation exposure levels were used to describe a correlation between the use of HD and the diagnosis of HDLI. No indoor measurement values are available.

Table 32: Data on the occurrence of CIT, MIT, and CIT/MIT mixture in indoor air

Rooms	Substance	N	LoD (µg/m³)	N > LoD	Measured value (µg/m³)	Median (µg/m³)	P95 (µg/m³)	Max. (µg/m³)	Ref.
Indoor, Germany	CIT	66	1	0	n.a.	0.5	0.5	0.5	(Hofmann and Plieninger, 2008)
Office room and living room, Germany	CIT	3	0.12	-	16-30 (directly after painting) <LoD (4 months after painting	-	-	-	(Horn et al., 2002)
Apartments, Switzerland	CIT	42	-	-	2-10 (directly after painting) 0.5 (2 weeks after painting)	-	-	-	(Reinhard et al., 2001)
Indoor, Germany	MIT	20 (not relevant)*	1	-	-	-	-	-	(Hofmann and Plieninger, 2008)
Office room and living room, Germany	MIT	3	0.12	-	5 (Directly after painting) < 0.12 (5 weeks after painting)	-	-	-	(Horn et al., 2002)
Apartment, Denmark	MIT	1	-	-	3 (7 days after painting)	-	-	-	(Lundov et al., 2014)
Indoor room 1, after painting, Germany	CIT/MIT	1	-	-	1.64 (7 hrs after painting) 0.5 (4 days after painting) 0.3 (27 days after painting)	-	-	-	(Binder et al., 2001)
Indoor room 2, after painting, Germany	CIT/MIT	1	-	-	1.9 (Direct-ly after painting) 1 (9 days after painting)	-	-	-	(Binder et al., 2001)

Rooms	Substance	N	LoD (µg/m³)	N > LoD	Measured value (µg/m³)	Median (µg/m³)	P95 (µg/m³)	Max. (µg/m³)	Ref.
Indoor air, air conditioning systems containing biocides, Germany	CIT/MIT	> 1	-	-	≤ 3	-	-	-	(Roßkamp, 1990)
Indoor air, Germany	CIT MIT OIT	499	0.02	CIT: 21 MIT: 371 OIT: 8	CIT: 0.04 - 0.47 MIT: 0.02 - 6.31 OIT: 0.03 - 0.64	CIT: 0.09 MIT: 0.48 OIT: 0.09	-	CIT: 0.47 MIT: 6.31 OIT: 0.64	(Weese, 2019)

4.3.2 Other sources

CIT, MIT and the CIT/MIT mixture are widely used preservatives, which inhibit bacterial and fungal growth. Next to the use in biocidal products (e.g., machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners), the substances are also used in cosmetic products (see section 4.3.2.2), personal care products, child toys (see section 4.3.2.3), and medical devices (ECHA Dissemination, 2023).

4.3.2.1 Biocidal products

4.3.2.1.1 CIT

Only limited data is available for CIT (ECHA Dissemination, 2023). An initial application for approval as active substance in biocidal products is ongoing for CIT. National authorisations for CIT in biocidal products of type PT06 - Preservatives for products during storage have been granted (e.g. Acticide® C 1) since 2021 in various EU countries (EU, 2020). The opinion is under development by the Biocidal Products Committee (BPC) (ECHA, 2023).

4.3.2.1.2 MIT

In the EU MIT is an approved active substance for biocidal product use (PT13 (metal working fluids) (EU, 2014); PT11 (preservation for liquid systems) (EU, 2017a) ; PT12 (controlling slimes) (EU, 2017b)). An initial application for approval is in progress for PT06 (product preservation). MIT can be found in products for indoor use (e.g., machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners) and outdoor use as processing aid (ECHA Dissemination, 2023).

4.3.2.1.3 CIT/MIT mixture

The CIT/MIT mixture is approved for the use in biocidal products in the EEA and Switzerland, for PT02 (disinfection) (EU, 2015a), PT04 (food and animals feeds), PT06 (product preservation) (EU, 2015b) , PT11 (preservation for liquid systems), PT12 (controlling slimes), and for preservation for working/cutting fluids.

4.3.2.2 Cosmetics products

In 2005 the use of MIT in leave-on and rinse-off cosmetics was restricted in the EU up to a maximum concentration of 100 ppm (Aerts et al., 2015). In 2015 the final opinion of the

Scientific Committee on Consumer Safety (SCCS) on the use of MIT in cosmetics was published stating that MIT should be abandoned in leave-on products and that the concentration in rinse-off cosmetics should be lowered to 15 ppm (0.0015 %) (SCCS, 2015). Moreover, for the CIT/MIT mixture (3:1), the maximum threshold is also restricted to 15 ppm in rinse- off cosmetics. The two entries are mutually exclusive: the use of the mixture of CIT/MIT is incompatible with the use of MIT alone in the same product (ECHA Dissemination, 2023).

4.3.2.3 Toys

CIT, MIT and CIT/MIT mixture (3:1) is restricted in the European Union in accordance with points 8 and 13, Part III, and Appendices A and C of Annex II (Particular Safety Requirements) to Directive 2009/48/EC on toy safety (EC, 2009). The use in toys for children under 36 months or in other toys intended to be placed in the mouth is limited to the following content(ECHA Dissemination, 2023).

- CIT: 0.75 mg/kg
- MIT: 0.25 mg/kg
- CIT/MIT mixture: 1 mg/kg

4.4 Toxicokinetics

Several studies are available describing the toxicokinetic properties of CIT, MIT and the CIT/MIT mixture. In the following a summary of the key studies is provided.

Absorption

Currently there are no toxicokinetic data available regarding absorption after inhalation of MIT. According to information of the CIT/MIT mixture product with the brand name Nipaguard® CG, the inhalation absorption rate is 100 % (ECHA Dissemination, 2023).

Different studies showed a fast and extensive dermal absorption. For MIT it has been shown *in vitro,* that up to 50 % of the applied concentration was resorbed within 24 h (Burnett et al., 2010; Hartwig, 2013). For CIT/MIT mixtures an absorption rate of 15.6 % CIT and 41.7 % MIT after 24 h was determined (ECHA Dissemination, 2023). In its assessment, the SCCS assumes a default value of 100 % dermal absorption for CIT/MIT (SCCS, 2009). However, the SCCS concludes that no reliable values can be derived from the available data and that the high variability between the individual studies does not allow a clear determination of the actual systemic absorption after dermal exposure. For oral intake, rapid absorption was detected for MIT and CIT/MIT mixture (ECHA Dissemination, 2023).

Distribution

After oral administration (gavage), MIT is rapidly distributed in the body; at early time points (3 and 6 hours), the highest radioactivity was found in the liver. After 24 hours, the radioactivity concentrations in the liver decreased significantly (Burnett et al., 2010; Hartwig, 2013). Also after i.v. application of CIT/MIT, the highest radioactivity levels were measured in the kidney and liver (radiolabelled CIT) (Henschler, 1991).

Metabolism

Up to 31 different metabolites were detected in the urine of rats after oral administration of MIT. The main metabolite is N-methylmalonamic acid (NMMA). Other metabolites include the 3-mercapturic acid derivative of 3-thiomethyl-N-methylpropionamide and N-methyl-3-hydroxyl-propionamide with 21 – 23 %, 10 – 23 % and 4 – 5 % of the administered dose detectable,

respectively (Burnett et al., 2010; Hartwig, 2013). A recently published study from Schettgen et al. (2021) investigated the human metabolism and renal excretion of CIT and MIT by monitoring (labelled) 3-mercapturic acid conjugate of 3-thiomethyl-N-methyl-propionamide (M-12). M-12 has previously been reported as a major metabolite of MIT in rats. As biomarkers of renal excretion, a combination of the quantification of NMMA as common metabolite of CIT and MIT (Henschler, 1991) together with the quantification of the mercapturate M-12 might allow to discriminate between exposures to the standalone biocide MIT and the mixture of CIT/MIT (3:1) (Schettgen et al., 2021).

Elimination

MIT as well as CIT/MIT mixture are almost completely and fast eliminated. They are mainly eliminated via urine and faeces. From the experimental data after oral application, an initial elimination half-life of 3-6 hours can be calculated for MIT, which is dose- and gender independent (Burnett et al., 2010; Hartwig, 2013). For the CIT/MIT mixture the half-life (calculated from radiolabelled CIT) was 300 hours in blood, 38 hours in plasma, and between 80 and 120 hours in liver, kidney and testes, indicating an overall slower excretion of CIT than MIT, after intravenous exposure (Henschler, 1991).

4.5 Health effects

While the toxicological effects of MIT have been partially investigated, no toxicological studies have been identified for CIT. Most of the reported studies were conducted with commercial products, which contain isothiazolinones as active ingredients. The relevant data on both MIT and the CIT/MIT mixture (hereafter referred to as CIT/MIT) are documented below. Where indicated, it is also reported which commercial product was tested. The reporting is based on information from secondary sources, as most of the studies have not been published.

4.5.1 Acute toxicity, sensory irritation, and local effects

4.5.1.1 Acute toxicity

4.5.1.1.1 Studies with MIT

The LD50 values after oral exposure of rats ranged from 120 – 285 mg a.i./kg bw with female rats being more susceptible to the effects. Physical signs included lethargy, few faeces, soiling of the anogenital area, sagging eyelids, emaciation, ataxia, diarrhoea, piloerection, flaccid muscle tone and prostration were observed (ECHA Dissemination, 2023).

After dermal application of MIT a LD50 value of 242 mg/kg bw was determined in rats. This value was calculated from the combined male and female mortality data. Skin effects were observed in both sexes at all levels beginning on day 1. These effects included: blanching, oedema, darkened areas, eschar, sloughing, scabbed areas, and desiccation. Necropsy of the decedents revealed gastrointestinal changes. Necropsy of the survivors revealed no gross changes (ECHA Dissemination, 2023).

Inhalation LC50 values ranged from 110 mg MIT/m^3 air (combined for male and female rats) with “RH-573“ to 770 mg MIT/m^3 (also combined for male and female rats) with a different product (Acticide® SR 3267) (ECHA Dissemination, 2023). During gross pathology slight to severe redness in lung, scattered incidences of red pinpoint foci on lungs, and gas-filled stomachs were found. These findings are consistent with the clinical signs of respiratory irritation.

4.5.1.1.2 Studies with CIT/MIT

An oral LD50 of 60 mg a.i./kg bw for the rat and 80 mg a.i./kg bw for the rabbit is reported for the CIT/MIT mixture. Symptoms of poisoning in the rat were lethargy, ptosis, diarrhoea, lacrimation, and salivation, and lethargy, erythema, and oedema were observed in the rabbit (ECHA Dissemination, 2023; Henschler, 1991).

Acute dermal toxicity studies with Kathon™ 886 in male rabbits showed a LD50 of 87 mg a.i./kg bw. After dermal application of the CIT/MIT mixture (commercial product Acticide® 14) to rats, a LD50 of 141 mg a.i./kg bw was determined (ECHA Dissemination, 2023; SCCS, 2009).

No deaths occurred in a 4 h inhalation test with saturated vapor of a Kathon™ 886F solution containing approximately 14 % a.i. in rats. The LC50 was reported to be > 650 mg a.i./m^3. In another acute inhalation toxicity study of a product containing 14 % a.i., a LC50 of 200-300 mg a.i./m^3 was determined. Symptoms of poisoning included dyspnoea, salivation, pulmonary oedema, and haemorrhages (ECHA Dissemination, 2023; Henschler, 1991). In the REACH registration dossier, it is outlined that "the effects observed are consistent with the clinical signs of respiratory irritation. It is likely that the deaths resulted from excess fluids in the respiratory tract due to the irritant/corrosive nature of C(M)IT/MIT."

4.5.1.2 Irritation (skin and eye)

4.5.1.2.1 Studies with MIT

MIT was applied to the backs of volunteers at concentrations of 0, 0.01 %, 0.03 %, and 0.06 % (0, 100, 300, 600 mg/kg bw) for 24 hours (15 µL). No irritant effects were visible after one and 24 hours Also in comparable experiments with volunteers exposed to hair shampoo, body lotion or sunscreen with a MIT concentration of 0.01 % (100 mg/kg bw) for 24 hours, no irritant effect was observed (Burnett et al., 2010). In a modified human repeated insult patch test (HRIPT), the cumulative irritant effect of MIT was tested in volunteers exposed to concentrations of 50, 100, 250, 500, and 1000 mg/kg bw MIT for 21 consecutive days (23 hours per day, occlusive, 15-17 individuals per group). After a 10-14-day rest period without exposure, a 24-hour challenge at a different skin site was initiated. In the 50, 100, and 250 mg/kg bw groups, the induction and trigger concentrations were the same. Subjects who received an induction concentration of 500 mg/kg bw were treated with trigger concentrations of 100, 250, and 500 mg/kg bw. In the 1000 mg/kg bw induction concentration group, trigger concentrations of 250, 500, and 1000 mg/kg bw were used (see Table 33). Mild, transient irritant reactions were observed during the induction phase in both the treatment and control groups. Cumulative irritation was observed in only one subject in the 1000 mg/kg bw group (SCCS and Giménez-Arnau, 2016).

Table 33: Cumulative irritant and sensitising effects of MIT in a HRIPT test in volunteers (SCCS, 2014)

Group	Number of subjects	MIT induction concentration (mg/kg bw)	Total number of reactions during induction	Cumulative irritation	MIT Trigger concentration (mg/kg bw)	Reactions to the trigger treatment
I	16	50	11/16	0/16	50	0/16
II	15	100	4/15	0/15	100	0/15
III	17	250	6/17	0/17	250	0/17
IV	15	500	7/15	0/15	500	1/15
					250	1/15
					100	0/15
V	16	10000	15/16	1/16	1000	2/16
					500	1/4
					250	1/4

In the ECHA registration dossier, two key studies in rabbits are reported (test materials: RH-573 and Acticide® SR 3267). Both studies according to OECD TG 404 concluded on corrosive properties of the substance. Based on the results of the skin irritation/corrosion studies, it is assumed that the test substance will produce similar effects in the eyes (ECHA Dissemination, 2023). Therefore, MIT has a harmonised classification according to Regulation (EC) No 1272/2008 (CLP Regulation) for Skin Corr. 1B (H314) and Eye Dam. 1 (H318) (ECHA C&L Inventory, 2022).

4.5.1.2.2 Studies with CIT/MIT

Reliable animal studies with various CIT/MIT products have shown corrosive effects on skin and eyes (ECHA Dissemination, 2023; Henschler, 1991). Therefore, CIT/MIT has a harmonised classification according to Regulation (EC) No 1272/2008 (EC, 2021) for Skin Corr. 1C (H314) (ECHA C&L Inventory, 2022). As outlined in the RAC opinion proposing a harmonised classification for the MIT/CIT mixture, a classification with skin corrosion means it is implicit that the substance will also cause serious damage to the eyes (RAC, 2016).

4.5.1.3 Sensitisation

In the past experimental and human data indicated that CIT and consequently CIT/MIT (3:1) mixture showed a higher sensitisation potency compared to MIT. However, recent data show, that both substances CIT and MIT have an equal sensitising potential (Weese, 2019).

4.5.1.3.1 MIT

In several guideline studies (Buehler-test, LLNA, GPMT) MIT was identified as a substance with skin sensitising properties (see also HRIPT reported in section 4.5.1.2.1). Therefore, MIT has a harmonised classification according to Regulation (EC) No 1272/2008 (EC, 2021) for Skin Sens. 1A (H317) (ECHA C&L Inventory, 2022).

4.5.1.3.2 CIT/MIT

The sensitising effect of CIT/MIT was investigated in various assays. Even though there are some negative findings, especially in the GPMT (Guinea Pig Maximization Test according to

Magnusson Kligman), the CIT/MIT mixture was evaluated as strongly sensitising overall. This is mainly based on the positive findings obtained in the Buehler test and especially in the Local Lymph Node Assays (LLNA) (ECHA Dissemination, 2023). CIT/MIT was used as a standard in the validation of the LLNA. The sensitising potency of the substance differed greatly depending on the solvent used. The substance had the strongest sensitising effect in acetone-olive oil (AOO; EC3 value of 0.0082 %; the MIT EC3 value was 0.4 % in comparison). During the validation of the LLNA, CIT was shown to be a much more potent allergen than MIT. The EC3 for CIT is reported to be 2 µg/cm^3, while the EC3 value for MIT is 100-fold higher (200 µg/cm^3)(SCCS, 2009).

CIT/MIT has a harmonised classification according to Regulation (EC) No 1272/2008 (EC, 2021) for Skin Sens. 1A (H317) (ECHA C&L Inventory, 2022). In the (RAC, 2016), the classification was refined from Skin Sens 1 to Skin Sens 1A with a specific concentration limit of C ≥ 0.0015 %.

4.5.1.4 Allergic contact dermatitis (MIT and CIT/MIT)

The results of the Information Network of Departments of Dermatology (IVDK) indicated a marked upward trend for positive patch-test results regarding dermal exposure to MIT and/or CIT/MIT ranging from 2.42 % to > 5 % between 2007 and 2014 among 125436 patients. Between 2015-2018 the percentage for positive results decreased slightly to approximately 4 %. The rise and fall of contact allergy to CIT/MIT, following regulation in the European Union in 2017, reflected the MIT contact allergy epidemic (Uter et al., 2022). The same pattern was seen in Belgium (2014-2019) (Herman et al., 2021) and in Denmark (Havmose et al., 2021).

4.5.1.5 Airborne allergic contact dermatitis (MIT and CIT/MIT)

Many case reports and literature reviews of airborne allergic contact dermatitis secondary to MIT in paints have been reported. A European multicentre study, which analysed isothiazolinones in paints including MIT, summarised the case reports of the past 30 years related to contact allergy to isothiazolinones resulting from paint exposure. The authors concluded, that in many of those case reports, allergic contact dermatitis has developed at directly exposed skin sites, whereas some case reports have shown that emissions of MIT can elicit airborne allergic contact dermatitis at indirectly exposed skin sites, for example the face or arms, even asthmatic symptoms were reported. Additionally, some case reports have described systemic symptoms and generalized dermatitis resulting from exposure to MIT and/or BIT in paints, and a few studies even reported that emergency treatment was necessary, owing to severe asthmatic reactions (Schwensen et al., 2015). Breuer et al. (2015) performed a retrospective analysis (1994–2013) of data from the Information Network of Departments of Dermatology (IVDK), including 201344 consecutively patch-tested patients. The analysis showed that airborne contact dermatitis is more common in patients with occupational dermatitis than in patients with non-occupational dermatitis. Moreover, next to epoxy resin systems, CIT/MIT and Compositae allergens were the most important contact of airborne contact dermatitis. From 201344 patients, which were patch tested between 1994-2013, 1203 showed airborne contact dermatitis. Out of the 1203 patients with airborne contact dermatitis, 5.7 % showed a positive reaction against CIT/MIT mixture (100 ppm in water) in the patch test.

Amsler et al. (2017) investigated airborne allergic contact dermatitis, caused by paints containing isothiazolinones. Epidemiological, clinical and patch test data on airborne allergic contact dermatitis caused by isothiazolinone-containing paints in France and Belgium were collected and evaluated. Fourty-four retrospective case reports were analysed and showed, that patients sensitised to MIT or CIT/MIT can develop airborne allergic contact dermatitis in rooms, painted with water-based paints containing isothiazolinones. Besides cutaneous lesions, 22.7 %

of the patients also reported mucosal symptoms, such as dyspnoea, rhinitis, cough, and/or conjunctivitis, indicating possible respiratory allergy (Amsler et al., 2017). The study identified in most of the cases a previous sensitisation to MIT and/or CIT/MIT from cosmetics, wet wipes, or household detergents. Prior to the occurrence of airborne allergic contact dermatitis, about 20 % of the patients already knew that they were sensitised to MIT and/or CIT/MIT, but they were not aware of the potential presence of isothiazolinones in non-cosmetic products such as paints, or of the risk of airborne exposure (Amsler et al., 2017).

Numerous case reports regarding airborne contact dermatitis caused by MIT and/or CIT/MIT can be found in the literature. Three recently published articles are presented in the following section to give an impression on the various individual cases.

Filippi et al. (2020) described a paediatric case of airborne contact dermatitis caused by water-based poster paints for children containing MIT (>50 ppm (0.005 %)). The patient had a medical history of mild atopic dermatitis (AD). One day after using water-based poster paints for children acute exacerbation of AD occurred. At 6 months follow-up, after the child stopped using paint, the acute episode of AD was healed.

Moreover, Kerre and Aerts (2021) describe the case of a man, with an angioedema-like airborne dermatitis against MIT. Since no pervious medical history of contact allergy against MIT was reported, it is hypothesised, that the patient might have become actively sensitised by MIT through airborne exposure to water-based wall paints.

In addition, a recently published case report describes a patient with a medical history of an occupational contact dermatitis due to working with metalworking fluid containing CIT/MIT. This patient experienced a severe eyelid eczema after using and living in a freshly painted room, whereas the water-based wall paint contained CIT/MIT (Özkaya et al., 2020).

4.5.1.6 Respiratory sensitisation

No data is available for respiratory sensitisation in the registration dossier (ECHA Dissemination, 2023). Recently, it has been shown *in vitro,* that Kathon™ CG (CIT/MIT mixture) induced apoptotic cell death along with membrane damage at 24 h post-exposure with 0, 0.25, 0.5, and 1.0 µg/mL in bronchial epithelial cell line isolated from humans (BEAS-2B cells). Additionally, on day 14 after a single instillation with Kathon™ CG *in vivo* in mice, the total number of pulmonary cells and the levels of TNF-α, IL-5, IL-13, MIP-1α, and MCP-1α in bronchial alveolar lavage (BAL) was analysed. The proportion of natural killer cells and eosinophils were significantly elevated in the spleen and the bloodstream, respectively, and the level of immunoglobulin (Ig) A, but not IgG, IgM, and IgE, dose-dependently increased. According to these results, the authors suggest, that inhaled Kathon™ CG may induce eosinophilia-mediated disease in the lung by disrupting homeostasis of pulmonary surfactants (Park et al., 2020).

Studies on irritant and sensitising effects of CIT/MIT associated with exposure to UV light did not reveal any evidence of phototoxicity (SCCS, 2009).

4.5.2 Repeated dose toxicity

4.5.2.1 Studies with MIT

No studies with inhalation or dermal exposure to MIT are available in the disseminated registration dossier available at ECHA´s website.

In a reliable drinking water study, rats were exposed for three months to doses of 0, 75, 250 or 1000 ppm MIT (males: 0, 6.51, 19.0, 65.7 mg/(kg bw x d), females: 0, 9.78, 24.6, 93.5 mg/(kg bw x d), RH-573, purity of the substance 97.6 %). In the highest dose group reduced feed intake was

observed in male animals and reduced body weight gain in both sexes. In males of all dose groups and in females of the middle and high dose group reduced drinking water intake was noted. No neurotoxic nor other systemic compound-related effects were observed. Macroscopic and microscopic examinations, ophthalmoscopy, haematology, and clinical chemistry were also without findings. In the registration dossier a NOAEL of 250 ppm was derived (19.0-24.6 mg/(kg bw x d)) (ECHA Dissemination, 2023). According to Burnett et al. (2010), who describe the unpublished study in detail, the effects on body weight gain, water and food intake were attributed by the study authors to the bad taste of the substance. In the absence of systemic effects, the authors derived a NOAEL of 1000 mg/kg in drinking water (65.7-93.5 mg/(kg bw x d)).

Moreover, in a subacute 28-day oral toxicity study in Wistar rat considered as reliable in the registration dossier doses of 10.03, 28.59 and 71.21 mg MIT/(kg bw x d) (Acticide® M 50) were applied via gavage. Based on clinical signs, mortalities, clinical pathology and histopathological examination, the NOAEL is reported with 28.59 mg MIT/(kg bw x d) (no further useful information available) (ECHA Dissemination, 2023).

A second 90-day oral toxicity study in rats is available. Three doses of Acticide® M 50 (50.7 % MIT) were tested in Wistar rats via gavage (7.52, 15.05 and 30.09 mg MIT/kg bw). No effects were observed in the study, therefore the NOAEL is 30.09 mg MIT/kg bw (ECHA Dissemination, 2023).

Furthermore, a subchronic feeding study in dogs is available. The animals were exposed to 0, 100 (changed to 130 on test day 22), 400 or 1500 mg MIT/kg feed in the diet for 3 months (0, 2.8, 9.9, and 40.6 mg/(kg bw x d) in males, and 0, 2.7, 11.1, and 40.9 mg/(kg bw x d) in females; Kordek™ 573F, purity 51.4 %). Insufficient recovery shown in the analytical examination of the feed in the low dose group (~70 %) resulted in an increase of the dose from 100 to 130 mg/kg in the diet. MIT exposure had no effect on mortality, clinical signs, organ weights and histopathology. Animals in the high dose group showed reduced body weight gain in the first week, an effect that was reversible in the third and fourth week of treatment. Feed intake was decreased throughout the treatment period in the highest dose group animals, but not significantly. No changes in haematological parameters were observed in this dose group either. A NOAEL of 1500 ppm in male/female (40.6-40.9 mg/(kg bw x d)) was established based on decreased body weight and food consumption. The NOEL is 400 ppm (9.9-11.1 mg/(kg bw x d)) (ECHA Dissemination, 2023).

4.5.2.2 Studies with CIT/MIT

Two studies are available on the toxic effects of CIT/MIT after repeated inhalation exposure.

In a 90-day study in rats, animals were exposed to a Kathon™ 886 aerosol at concentrations of 0, 0.34, 1.15, and 2.64 mg a.i./m^3 (6 h/d, 5 d/w, 14 % active ingredient with 11 % CIT and 3 % MIT). Aerosol concentrations of 0, 0.027, 0.23, and 0.89 mg/m^3 were originally assumed. However, since the analytical method did not include the vapour fraction, the experimental conditions were readjusted at a later time and the concentrations used were redetermined (Greim, 1999). In the highest dose group, feed intake, body weight, and weight gain were reduced in both sexes. Animals in the highest dose group showed symptoms as typically observed upon exposure to a sensory irritant: Discharge of partially coloured nasal secretions, eye blinking, bradypnea, and dyspnoea. Evaluations of urinary and hematologic parameters and ocular examinations were without findings. A decrease in serum proteins was observed in females of the high dose group and a decrease in spleen weight in males. The latter was not observed in the females and was not accompanied by histopathological changes. Histopathologic examination revealed findings in the upper respiratory tract (mild to moderate eosinophilic

droplets in the mucosa of the turbinate and mild rhinitis in the anterior nasal cavity) in animals in the high dose group. The findings were minimal and were interpreted as reversible physiologic responses to exposure to an irritating substance in the upper respiratory tract. In the intermediate dose group, only mild rhinitis was histopathologically detectable (ECHA Dissemination, 2023; Henschler, 1991). A NOAEL for local effects of 0.34 mg a.i./m^3 can be established based on the results of the study.

A reliable 14 day-inhalation toxicity study with CMI 14 (CIT/MIT mixture; 14.2 % a.i.) in rat was performed with nose-only exposure. The mean actual concentrations (± standard deviation) of CMI 14 in the test aerosol were 50 (± 10), 110 (± 10) and 210 (± 10) mg/m^3. The animals were exposed for 6 h/d on 5 days per week, a second high dose group was held for an additional two weeks to monitor potential recovery effects. In the high dose group, a male animal died on exposure day 11. Nasal discharge, laboured breathing and hypoactivity was observed in the animals of the high dose group during days 7 to 14. All these clinical signs were found to be reversible during the recovery period. The average daily food consumption per animal of the high dose group was decreased. As a result, slight body weight reductions were observed. No effects on haematology or clinical chemistry parameters were found. Incidental and non-specific findings were observed in the histopathological analysis. Based on the findings of this study, a NOAEC of 110 mg /m^3 was derived (ECHA Dissemination, 2023).

Next to the inhalation studies, CIT/MIT was tested in several sub-acute and sub-chronic oral toxicity studies in rabbits, rats, and dogs. Additionally, two 90-day dermal repeated dose toxicity studies were performed with CIT/MIT in rabbit and rat (ECHA Dissemination, 2023). In summary, CIT/MIT caused local toxicity at the site of contact e.g., irritation/corrosion of the forestomach, skin or upper respiratory tract following repeated exposure via oral, dermal or inhalation routes. Secondary toxicities may be observed which are typically bodyweight reductions and effects on haematological parameters as a result of primary local toxicity (ECHA Dissemination, 2023).

In the following Table 34 an overview of relevant studies with repeated exposure to MIT and to the CIT/MIT mixture is provided.

Table 34: Summary of relevant repeated exposure studies for MIT and the CIT/MIT mixture

Study type	Doses (mg a.i./(kg bw x d))	Effects	NOEL	NOAEL (local effects)	NOAEL (systemic effects)	Ref.
Studies with MIT						
Rat, 90-day study, drinking water (RH-573, 97.5 %)	0, 75, 250 or 1000 ppm MIT Males: 0, 6.51, 19.0, 65.7 mg/(kg bw x d) Females: 0, 9.78, 24.6, 93.5 mg/(kg bw x d)	High dose group: Males: feed intake ↓ Both sexes: body weight gain ↓ Middle dose group: Both sexes: drinking water intake ↓ Low dose group:		65.7 mg/(kg bw x d)	65.7 mg/(kg bw x d) According to the authors observed effects are considered secondary to palatability	(Burnett et al., 2010; ECHA Dissemination, 2023)

Study type	Doses (mg a.i./(kg bw x d))	Effects	NOEL	NOAEL (local effects)	NOAEL (systemic effects)	Ref.
		Males: Drinking water intake ↓				
Rat, 28-day study, gavage (Acticide® M 50)	10.03, 28.59 and 71.21	Clinical signs, mortalities, clinical pathology, and histopathological examination (no further details available)			28.59 mg/(kg bw x d)	(ECHA Dissemination, 2023)
Rat, 90-day study, gavage (Acticide® M 50, 50.7 %)	7.52, 15.05 and 30.09	No effects observed		30.09 mg/(kg bw x d)	30.09 mg/(kg bw x d)	(ECHA Dissemination, 2023)
Dog, 90-day feeding study (Kordek™ 573F, 51.4 %)	0, 100/130, 400 or 1500 mg/kg feed Males: 0, 2.8, 9.9, 40.6 mg/(kg bw x d) Females: 0, 2.7, 11.1, 40.9 mg/(kg bw x d)	High dose group: Body weight gain ↓ in the first week Feed intake ↓ throughout the treatment period (not significant)	10 mg/(kg bw x d)	41 mg/(kg bw x d) according to study authors	41 mg/(kg bw x d) according to study authors	(ECHA Dissemination, 2023)
Studies with CIT/MIT (inhalation exposure)						
Rat, 90-day study (6 h/d, 5d/w), inhalation, nose-only (Kathon® 886, 14 % a.i.)	0, 0.34, 1.15, 2.64 mg/m³	High dose group: Feed intake ↓, body weight ↓, weight gain ↓ in both sexes Symptoms of sensory irritation: nasal secretions, eye blinking, bradypnea, and dyspnoea Females: serum proteins ↓ Males: spleen weight ↓ Local effects in upper respiratory tract Middle dose group: Mild rhinitis	0.34 mg/m³	0.34 mg/m³	1.15 mg/m³	(ECHA Dissemination, 2023; Henschler, 1991)

Study type	Doses (mg a.i./(kg bw x d))	Effects	NOEL	NOAEL (local effects)	NOAEL (systemic effects)	Ref.
Rat, 14-day study (6 h/d, 5d/w), inhalation, nose-only (CMI® 14, 14.2 % a.i)	50 (± 10), 110 (± 10) and 210 (± 10) mg/m³	High dose group: One male died on day 11 Nasal discharge, laboured breathing and hypoactivity during days 7 to 14, average daily food consumption per animal ↓, body weight ↓ No effects on haematology or clinical chemistry parameters			110 mg/m³	(ECHA Dissemination, 2023)
Rat, 90-day study (6 h/d, 5d/w), inhalation, nose-only (Kathon® 886, 14 % a.i.)	0, 0.34, 1.15, 2.64 mg/m³	High dose group: Feed intake ↓, body weight ↓, weight gain ↓ in both sexes Symptoms of sensory irritation: nasal secretions, eye blinking, bradypnea, and dyspnoea Females: serum proteins ↓ Males: spleen weight ↓ Local effects in upper respiratory tract Middle dose group: Mild rhinitis	0.34 mg/m³	0.34 mg/m³	1.15 mg/m³	(ECHA Dissemination, 2023; Henschler, 1991)

4.5.3 Genotoxicity and carcinogenicity

4.5.3.1 Genotoxicity

4.5.3.1.1 Studies with MIT

MIT (Kordek™ 573T and Acticide® SR 3267) has been tested for *in vitro* gene mutations in bacteria. Two Ames tests (OECD TG 471) were performed, with no indication of a mutagenic potential. Moreover, two HGPRT studies in CHO cells (OECD TG 476) were performed with Kordek™ 573T and Acticide® SR 3267 to study gene mutations in mammalian cells. Results were in line with the Ames test results, showing no mutagenic potential. In addition, cytogenic study in CHO cells (OECD TG 473) with Kordek™ 573F showed no mutagenic potential, either.

However, a remark was provided indicating a high level of cytotoxicity at the highest dose tested *in vitro.* Increase in number of chromosomal aberrations is considered a false negative result, observed at doses that induce cytotoxicity. Furthermore, Acticide® M50 did not induce chromosomal aberration in human lymphocyte culture. Mutagenicity studies *in vivo* including micronucleus tests (OECD TG 474) and unscheduled DNA synthesis (OECD TG 486) indicated no mutagenic potential. The conclusion on classification and labelling is, that MIT is not genotoxic and no classification is required (ECHA, 2015a).

4.5.3.1.2 Studies with CIT/MIT

Several *in vitro* studies of genotoxicity were performed with CIT/MIT. Positive results were observed in three Ames assays and in three tests in mammalian cells (one chromosomal aberration test and two mouse lymphoma assays), with or without S9 activation. In contrast, CIT/MIT was not mutagenic in primary culture of rat hepatocytes (UDS) and in a mouse cell transformation test. A test was also performed with the major metabolite of CIT/MIT, N-(methyl)malonamic acid (NMMA), which appeared not to be mutagenic when tested in a bacterial gene mutation assay test (Ames assay) (ECHA Dissemination, 2023).

In vivo CIT/MIT was tested in one chromosomal aberration assay in mice, and one micronucleus test in mice. Negative results were observed in these *in vivo* studies. In the studies on tissue distribution of radiolabel substance in mice presented in the dossier for CIT/MIT (ECHA Dissemination, 2023), radioactivity has been detected in bone marrow tissue following a single oral dose of the test material to adult male and female animals. This information provides support to the validity of the chromosome aberration test on bone marrow in mice and the micronuclei on bone marrow in mice, since it determines the extent of CIT and MIT distribution to bone marrow of mice after oral exposure. In the absence of genotoxicity, additional tests were carried out in tissue other than bone marrow. Two UDS assays in rats confirmed the absence of genotoxicity of CIT/MIT when tested *in vivo* (ECHA Dissemination, 2023).

Overall, CIT/MIT is not classified for genotoxic effects in accordance with Annex VI Regulation EC 1272/2008 (ECHA Dissemination, 2023). This non-classification was confirmed in the RAC opinion proposing a harmonised classification for the MIT/CIT mixture from 2016.

4.5.3.2 Carcinogenicity

4.5.3.2.1 Studies with MIT

There are no human findings or animal experimental studies on MIT.

4.5.3.2.2 Studies with CIT/MIT

The carcinogenic effect of a CIT/MIT mixture (Kathon™ 886 with 14.2 % active ingredient) was studied in rats exposed via drinking water (0, 30, 100, or 300 ppm; 0, 2.0, 6.6, 17.2 mg/(kg bw x d) in males and 0, 3.1, 9,8, 25.7 mg/(kg bw x d) in females) for 2 years (Burnett et al., 2010; ECHA Dissemination, 2023). No evidence of systemic toxicity or carcinogenic effects were observed. In the animals of the medium and high dose groups morphological changes in the stomach were found, which could be attributed to the corrosive effect of the substance. The animals in the high dose group exhibited decreased body weight and body weight gain which is interpreted as a secondary effect to reduced drinking water intake (Burnett et al., 2010).

Also, no carcinogenic effects were observed in a dermal carcinogenicity study using a CIT/MIT mixture (approximately 3.8 % MIT) where mice were exposed to 25 µl of an aqueous solution containing 400 mg/kg a.i. three times per week for 30 weeks (ECHA Dissemination, 2023; Henschler, 1991).

4.5.4 Toxicity to reproduction

4.5.4.1 Studies with MIT

Reproduction toxicity was investigated in a two-generation reproduction toxicity study according to OECD TG 416. Animals received concentrations of 0, 50, 200 and 1000 mg/L a.i. (Kordek™ 5732, 51.5 % a.i.) in drinking water. The respective doses in females were 0, 6-13, 22-26, 93-115 mg/(kg bw x d) and in males 0, 4-7, 15-19, 69-86 mg/(kg bw x d) (Burnett et al., 2010). In the mid and high dose groups decreased water intake was observed in P0 males and in F0 and F1 females during pregnancy and lactation. The authors of the study attributed this effect to the taste and odour of the test substance. Decreased body weight gain and feed intake were observed in the highest dose group, which were considered secondary to decreased water intake. No lethality, clinical or other signs of general or reproductive toxicity were observed in the study. The NOAEL for reproductive toxic effects for the F0 generation is 69-93 mg/(kg bw x d), for the F1 generation it is 86-115 mg/(kg bw x d) (1000 ppm in the drinking water, the highest concentration applied). The NOAEL for parental systemic toxicity is 200 ppm based on reduced body weight and weight gain, 200 ppm is also the NOAEL for neonatal toxicity with decreased body weights during the latter part of the pre-weaning period in the F1 and F2 pups (Burnett et al., 2010; ECHA Dissemination, 2023).

In an oral developmental toxicity study according to OECD TG 414 MIT (Kordek 573, 51.4 % a.i.) was given to rats via gavage in doses of 0, 5, 20, and 40 mg/(kg bw x d) (the highest dosage of 60 mg/(kg bw x d) was reduced between GD 6 and 9). In the highest dose group mortality in 3 dams was observed between GD 8-15 and two animals were euthanised in a moribund state. At necroscopy these animals showed red areas in the glandular stomach and the lungs. In the highest dose group, body weight gain and feed intake were reduced from gestation day 6-9. This effect was reversible after dose reduction. Apart from this, no compound-related effects were observed, no influence on resorptions, live foetuses per litter, foetal body weight, sex ratios, or on external, visceral, or skeletal parameters. Thus, the NOAEL for maternal toxicity is 20 mg/(kg bw x d) and for developmental toxicity it is 40 mg/(kg bw x d) (Burnett et al., 2010).

In another reliable developmental toxicity study in rats performed according to OECD TG 414, rats were exposed via gavage to 0, 33.4, 50 and 75 mg MIT/(kg bw x d) (Acticide® SR 3267, 49.6 % a.i.). Maternal toxicity was observed at 50 and 75 mg/(kg bw x d), dams showed reduced mean body weight gain (16 and 30 % respectively) and reduced feed consumption. Also, at 50 and 75 mg/(kg bw x d) developmental toxicity was seen. Increased incidences of anomaly (dilated cerebral ventricles) and incomplete ossification were observed at maternally toxic doses. The NOAEL for maternal as well as developmental toxicity can be set at 33.4 mg/(kg bw x d) (ECHA, 2015c; ECHA Dissemination, 2023).

Developmental toxicity was also studied in a second species. In a guideline-conform study with pregnant females rabbits the animals were exposed via gavage from GD 6-28 to 0, 3, 10 or 30 mg/(kg bw x d) MIT (Kordek™ 573F, 51,4 % a.i.). In the highest dose group decreased defecation, dark red areas in the stomach, body weight loss and reduced mean food consumption were noted. In one animal abortion on day 25 of gestation was observed. No other effects on the foetuses were found. Therefore, the NOAEL for maternal toxicity is 10 mg/(kg bw x d) while the NOAEL for developmental toxicity is the highest dose applied, 30 mg/(kg bw x d) (Burnett et al., 2010; ECHA, 2015c; ECHA Dissemination, 2023).

4.5.4.2 Studies with CIT/MIT

In a two-generation reproduction toxicity study according to OECD TG 416, rats were exposed to Kathon™ 886 via drinking water (0, 30, 100, 300 mg/kg a.i.; corresponding to 0, 2.8-4.4, 8.5-

11.8, 22.7-28.0 mg/kg/d for the P1- and 0, 4.3-5.5, 13.4-16.0, 35.7-39.1 mg/kg/d for the P2-generation). Survival rate, and clinical parameters were not affected. Males in the first generation exposed to 300 ppm showed a treatment-related decrease (5 %) in mean body weight during weeks 1 through 6 of treatment. A dose-dependently decreased water consumption was observed in both parent generations, which was probably caused by the inherent taste of the test substance. The decreased body weight gain observed at baseline in P1 was probably due to decreased water intake. The only treatment-related effects observed in the middle and high dose groups were local effects in the stomach and forestomach (including oedema, inflammation, hyperplasia, and hyperkeratosis). Fertility and reproductive behaviour and offspring were not affected by treatment. The SCCS derived a NOAEL of 2.8 mg/(kg bw x d) from this study based on decreased water intake by animals and local effects in the stomach, which was used as the basis for the MOE (margin of exposure) consideration. In the REACH registration dossier, a NOAEL for parental animal toxicity of 30 ppm (2.8-4.4 mg/(kg bw x d) in the P1 animals and 4.3-5.5 mg/(kg bw x d) in the P2 animals) is reported. The reproductive and developmental NOAEL was 300 ppm (22.7-28.0 mg/kg/day in the P1 animals and 35.7-39.1 mg/kg/day in the P2 animals) (ECHA Dissemination, 2023; SCCS, 2009).

In a developmental toxicity study the CIT/MIT mixture was applied orally via gavage to rats between GD 6-15 (0, 1.4, 4.2 and 14 mg/(kg bw x d), Kathon™ 886, 13.9 % a.i.). Up to the highest dose tested, no effects were observed in dams (body weight, pregnancy rates, implantations, resorptions, etc.) or in the offspring (organ, skeletal abnormalities)(Greim and MAK Commission, 1991; Greim and MAK Commission, 2007). The secondary source does not indicate whether a histopathological examination of the stomach was performed.

Moreover, three key studies and one supporting study are listed in the ECHA registration dossier for CIT/MIT regarding developmental toxicity. For Acticide® 14 (14 % a.i.) minimal to slight effect in all dosed females was observed (0, 28, 70, and 139 mg/(kg bw x d)). A maternal LOAEL of 28 mg/(kg bw x d) was established (clinical signs, moderately reduced body weight gain, slightly reduced food consumption). No embryotoxic and teratogenic effects were observed, the NOAEL in the registration dossier is reported with ≥ 19.6 mg/(kg bw x d) (unclear how this was determined) (ECHA Dissemination, 2023).

In a second key study pregnant rats were exposed via gavage to 0, 10, 30, and 100 ppm Kathon™ 886. In the absence of toxic effects a maternal and a developmental NOAEL of 15 mg/(kg bw x d) are reported, which is according to the registration dossier the highest dose tested (ECHA Dissemination, 2023).

Kathon™ 886 was also tested in a developmental toxicity study in rabbits (0, 1.5, 4.4 und 13.3 mg a.i./(kg bw x d) via gavage, exposure from GD 6-18). The medium and high doses were lethal for approximately 80 % of dams until GD 20. Even in the lowest dose group, approximately 30 % of dams died, showing hypoactivity, ataxia, salivation, and diarrhoea before death. At autopsy, erosions of the gastric mucosa and associated haemorrhages were observed. In the lowest dose group, an increase in early resorptions (post-implantation losses) and a small decrease in the number of surviving foetuses were observed. The surviving foetuses did not exhibit malformations or other abnormalities. In the highest dose group, no surviving foetuses were found. Furthermore, the number of implantations and corpora lutea, as well as foetal weight and sex ratio, were not altered compared to control. The maternal NOEL reported in the registration dossier is 1.4 mg/(kg bw x d) based on mortality and reduced body weight gain. The developmental NOEL is 13.3 mg/(kg bw x d) (ECHA Dissemination, 2023; Greim and MAK Commission, 1991; Greim and MAK Commission, 2007). Overall, the MAK Commission concludes that rabbits are not suitable for this type of testing due to the biocidal properties of the substance.

In the following Table 35 an overview of relevant reproductive toxicity studies with MIT and the CIT/MIT mixture is provided.

Table 35: Summary of relevant reproductive toxicity studies with MIT and the CIT/MIT mixture

Study type	Doses mg a.i./(kg bw x d)	Effects	NOEL mg/(kg bw x d)	NOAEL (local effects) mg/(kg bw x d)	NOAEL (systemic effects) mg/(kg bw x d)	Ref.
Studies with MIT						
Rat, two-generation, drinking water (Kordek™ 5732, 51.5 %)	Females: 0, 6-13, 22-26, 93-115 Males: 0, 4-7, 15-19, 69-89	High and middle dose group: Drinking water intake ↓ (males F0, females F0+F1). Highest dose: feed intake ↓ and body weight gain ↓ (males and females). No reproductive effects	4	n.a.	15	(Burnett et al., 2010; Dow Chemical Company, 2003; ECHA Dissemination, 2023)
Rat, developmental toxicity, oral gavage GD 6-19 (Kordek™ 573, 51.4 % a.i.)	0, 5, 20, 60/40 (highest dose was reduced to 40 mg/(kg bw x d) between GD 6 and 9)	High dose group: Mortality in 3 animals between GD 8-15, two animals killed in moribund condition at GD 8 and 9, respectively; local effects in stomach and lungs; after dose reduction, feed intake and body weight development were no longer impaired. No further effects	20	20	Maternal toxicity: 20	(Burnett et al., 2010; ECHA Dissemination, 2023)
Rat, developmental toxicity, oral gavage GD 6-15 (Acticide® SR, purity 49.8 % a.i.)	0, 33.4, 50, and 75	High and middle dose group (dams): mean body weight gain ↓ and food consumption ↓. Highest dose group (pups): incidence of dilated cerebral ventricles ↑, incomplete ossification ↑.	n.a.	n.a.	Maternal toxicity: 33.4	(ECHA, 2015c; ECHA Dissemination, 2023)
Rabbit, developmental toxicity, oral gavage	0, 3, 10, 30	High dose group: Maternal animals: faecal output ↓, dark red areas visible in stomach,	10	10	Maternal toxicity: 10	(Burnett et al., 2010; ECHA, 2015b; ECHA Dissemination, 2023)

Study type	Doses mg a.i./(kg bw x d)	Effects	NOEL mg/(kg bw x d)	NOAEL (local effects) mg/(kg bw x d)	NOAEL (systemic effects) mg/(kg bw x d)	Ref.
GD 6-28 (Kordek™ 573F, 51.4 % a.i.)		abortion in one animal on day 25 of GD. No further effects				
Studies with CIT/MIT						
Rat, two-generation study, drinking water (Kathon™ 886)	P0: 0, 2,8-4,4, 8,5-11,8, 22,7-28,0 P1: 0, 4,3-5,5, 13,4-16,0, 35,7-39,1	High and middle dose group: water consumption ↓, local effects in the stomach	2.8	2.8	2.8-4.4	(ECHA Dissemination, 2023; SCCS, 2009)
Rat, developmental toxicity, gavage GD 6-15 (Kathon™ 886, 13.9 % a.i.)	0, 1.4, 4.2, 14	No adverse effects observed, unclear whether histopathological examinations of the GI were performed.	n.a.	n.a.	14	(Greim and MAK Commission, 1991; Greim and MAK Commission, 2007)
Rat, developmental toxicity, oral gavage (Acticide® 14, 14 % a.i.)	0, 28, 70, 139	Treatment with the test article resulted in maternal toxicity with clearly distinguished dose-dependent grades of severity (clinical signs, moderately reduced body weight gain, slightly reduced food consumption). No effects on foetuses	n.a.	n.a.	Maternal toxicity (LOAEL): 28 Developmental toxicity: ≥ 19.6	(ECHA Dissemination, 2023)
Rat, developmental toxicity, oral gavage (Kathon™ 886)	0, 10, 30, and 100 ppm	No effects were observed	n.a.	n.a.	Maternal toxicity: 15	(ECHA Dissemination, 2023)
Rabbit, developmental toxicity, oral gavage	0, 1.5, 4.4 und 13.3	Middle dose group (dams): body weight gain ↓ No developmental toxicity effects observed.	Maternal toxicity: 1.4	n.a.	n.a.	(ECHA Dissemination, 2023; Greim and MAK Commission, 1991; Greim

Study type	Doses mg a.i./(kg bw x d)	Effects	NOEL mg/(kg bw x d)	NOAEL (local effects) mg/(kg bw x d)	NOAEL (systemic effects) mg/(kg bw x d)	Ref.
(Kathon™ 886)						and MAK Commission, 2007)

4.5.5 Odour perception

Pure CIT/MIT (3:1) has a weakly sweet and pungent odour at 20 - 25 °C (ECHA, 2015b). No thresholds are reported (AIHA, 1997; Amoore and Hautala, 1983; Burnett et al., 2010; Ruth, 1986).

4.6 Evaluation

4.6.1 Existing regulations and classifications

Both CIT/MIT (3:1) mixture and MIT as single substance are included as active substances in several biocidal product types (substance/product-type combination) and are authorised under the Biocidal Products Regulation ((EU) No 528/2012 (BPR)). MIT (CAS no. 2682-20-4) is included as active substance in four different biocidal product types which fall into the main group of preservatives (see section 4.3.2.1.2). Three MIT/product-type combinations are approved since 2016, whereas one initial application of approval is in progress for MIT/P06 at the moment (ECHA Dissemination, 2023). CIT/MIT (3:1) mixture (CAS no. 55965-84-9) is included as active substance mixture in six different and approved biocidal product types either in the main group as disinfectants or in the main group as preservatives (see section 4.3.2.1.3). All products are approved since 2017. For CIT (CAS no. 26172-55-4) the initial application for approval is in progress for preservatives for products during storage (PT06) (see section 4.3.2.1.1). However, several products are authorised in different European countries since 2021. According to the available product assessment report of Acticide® C1 (PT06) the risk characterisation to determine the Accepted Exposure Level (AEL) and Acceptable Exposure Concentration (AEC) are based on a cross-referencing approach using already available toxicological information from the CIT/MIT (3:1) mixture (CAS no. 55965-84-9; EC no. 911-418-6) (EU, 2015b). For details see section 4.6.2.

Neither CIT nor MIT nor the mixture are listed in the current TRGS 900 (edition: January 2006 last amended and supplemented: GMBl 2023 p. 626-627 of 20.4.2023 [No. 30]; cf. http://www.baua.de/de/Themen-von-A-Z/Gefahrstoffe/TRGS/TRGS-900.html (accessed on 7.11.2023). In an older version of the TRGS (version 2000), the CIT/MIT mixture (3:1) was still listed with the old MAK value (from 1991) of 0.05 mg/m^3 with the note "H" (skin absorption). This old MAK value was based on the same study as the current MAK value, only the improved exposure estimation was not yet used for its derivation.

For the CIT/MIT mixture, the MAK Commission derived a new MAK value in 1999 of 0.2 mg/m^3 I (inhalable aerosol fraction). Due to the corrosive effects, the peak limitation is according to category I, the exceedance factor is 2 (Greim and MAK Commission, 1991; Greim and MAK Commission, 2007). The MAK value was derived based on a repeated inhalation study in rats (NOAEL 0.34 mg a.i./m^3). Furthermore, the substance is labelled as skin sensitising ("Sh") and

categorised in Pregnancy Group C (prenatal toxic effects are unlikely at the MAK- or the BAT value) with the 2007 revision.

Since MIT has corrosive properties and no studies are available on the toxicity of the substance after repeated inhalation exposure, the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) did not derive a MAK value. The substance has been assessed as skin sensitising ("Sh") (Hartwig and MAK Commission, 2015). The MAK Commission does not give a statement on the individual substance CIT.

The Committee for Health-related Evaluation of Building Products (AgBB) has set NIK values (lowest concentration of interest) for CIT and MIT of 1 and 100 µg/m^3, respectively (AgBB, 2018). These values are derived from individual substance assessments (individual case justification of NIK AG, unpublished). The NIK AG concluded that the available toxicological data from animals for CIT/MIT (3:1) mixture and MIT indicated, that local and systemic irritative adverse effects can be expected for both substances, but with higher severity for CIT/MIT mixture compared to MIT, irrespective of the exposure route. According to the NIK AG this sequence is also applicable to describe the sensitisation properties of the CIT/MIT (3:1) mixture and MIT both from animal and human data. Compared to the CIT/MIT (3:1) mixture, MIT shows a sensitisation potential which is lower by about a factor of 200. Consequently, the NIK value for MIT is higher by a factor of 100 compared to the either CIT/MIT (3:1) mixture or CIT.

In the 1/1998 issue of a publication of the German Environment Agency (Umweltmedizinischer Informationsdienst des Instituts für Wasser-, Boden- und Lufthygiene des Umweltbundesamtes), a provisional indoor guide value RW II of 0.5 µg/m^3 was derived, based on the old MAK value considering the old basic scheme. Correspondingly, a RW I of 0.05 µg/m^3 was stated for the general population. The author expressly pointed out the insufficient data on which this derivation was based on (UBA, 1998).

The German Committee on Indoor Air Guide Values has not yet officially derived indoor air guide values I and II. However, according to the minutes of the meetings available at the homepage of the Committee[3], provisional values are currently available. Based on a LOAEC of 1.15 mg/m^3 from the 90-day inhalation toxicity study with CIT/MIT an RW II of 0.002 mg/m^3 (2 µg/m^3) was calculated (converting to continuous exposure (x 6/24 x 5/7), factor 2 subchronic/chronic, 2.5 interspecies variability, 10 intraspecies variability, 2 child factor; total factor 100). Based on the NOAEC of 0.34 mg/m^3 from the same study, converting to continuous exposure and applying the standard factors a RW I of 0.0006 mg/m^3 (0.6 µg/m^3) results.

For CIT no harmonised classification is available. The reaction product of CIT and MIT is classified according to Regulation (EC) No 1272/2008 (EC, 2021) for (only classifications regarding human toxicity listed):

- Acute Tox. 2, H330, 310
- Acute Tox. 3, H301
- Skin Corr. 1C, H314
- Skin Sens. 1A, H317.

[3] https://www.umweltbundesamt.de/themen/gesundheit/kommissionen-arbeitsgruppen/ausschuss-fuer-innenraumrichtwerte (accessed on 7.11.2023)

For MIT a harmonised classification is available, too. The substance is classified for (only classifications regarding human toxicity listed):

- Acute Tox. 2, H330
- Acute Tox. 3, H311, and H301
- Skin Corr. 1B, H314
- Eye Dam. 1, H318
- Skin Sens. 1A, H317.

4.6.2 Derivation of an EU-LCI value

Only studies on the toxicity of MIT or the CIT/MIT mixture are available, no studies on toxicity after administration of CIT could be identified (except for skin sensitisation).

The toxic effects of MIT and CIT/MIT are dominated by their sensitising effects. Both MIT and CIT/MIT lead to skin sensitisation after dermal exposure, with the CIT/MIT mixture proving to be more potent than MIT in the LLNA (SCCS, 2014). Data from the HRIPT assay also indicate an approximately 30-fold greater sensitising potential of CIT/MIT over MIT. From the data of the Open Epicutaneous Test, the effect of CIT/MIT is approximately 50-fold stronger (SCCS, 2014). After opening the ring structure of isothiazolinones, a sensitising effect is no longer observed (SCCS, 2014). Several case reports document the occurrence of contact dermatitis also after exposure to MIT or CIT/MIT via indoor air. However, the corresponding exposure concentrations are not known, so that it is not possible to estimate the air concentration at which sensitising effects must be expected. The available measured values for the indoor air concentration after application of paints containing CIT and/or MIT indicate concentrations of a few (well below 10) µg/m^3 in the freshly painted rooms, which drop into the range of around 1 µg/m^3 after only a few days.

There are only two studies available with the CIT/MIT mixture in which repeated inhalation exposure toxicity was investigated (a 90 day and a 14-day study in rats). For MIT alone, only acute inhalation exposure studies are available. These studies demonstrate that local effects are predominant after inhalation exposure unless lethal concentrations were applied.

Local effects in the gastrointestinal tract are observed after oral administration. The local effects are accompanied by non-specific effects on feed and drinking water intake, which lead to effects on body weight. The latter is probably secondary to the reduced water or feed intake, which are presumably related to the palatability of the substance. Because of these local effects, an assessment of systemic, inhalation toxicity based on oral data using pathway-to-pathway transfer is not possible.

With regard to specific endpoints, the available studies show that the CIT/MIT mixture was not carcinogenic in studies with rats receiving the substance at concentrations up to 300 ppm via drinking water (corresponding to 17.2 and 25.7 mg/(kg bw x d) in males and females, respectively). No carcinogenic effect was observed after dermal application of a solution containing 400 mg/kg CIT/MIT to mice. Genotoxicity studies of MIT and CIT/MIT also produced mostly negative results. In multigeneration and developmental toxicity studies of MIT and CIT/MIT, in which the test substance was administered orally, toxic effects on the parent animals but no impairment of reproductive ability or embryotoxic or teratogenic effects were observed. For MIT, a NOAEL of 15 mg/(kg bw x d) was established in a two-generation study

with rats. The corresponding value for CIT/MIT (also from a two-generation toxicity study) was 2.8 mg/(kg bw x d).

Derivation of an EU-LCI value for CIT/MIT (3:1) mixture[4]

A derivation of an EU-LCI value for the CIT/MIT mixture is possible on the basis of the 90-day inhalation study in rats with a NOAEC of 0.34 mg/m³ and a LOAEC of 1.15 mg/m³ (with the product Kathon™ 886).

The following assessment factors can be used:

- Adjustment for continuous exposure (6 h/d, 5 d/week): 5.6
- Adjusted study length factor: 2
- Interspecies extrapolation: 2.5 (allometric scaling not performed since route of exposure is inhalation)
- Intraspecies extrapolation (interindividual variability, general population): 10

The total assessment factor is 280, leading to a value of 0.34 mg/m³: 280 = 0.0012 mg/m³ 1.2 µg/m³) for CIT/MIT (**rounded to 1 µg/m³).**

Derivation of an EU-LCI value for the individual substances CIT and MIT

To derive EU-LCI values for CIT and MIT as individual substances the same approach was applied which was used to derive an Acceptable Exposure Concentration (AEC) for biocidal products (EU, 2014; EU, 2017a; EU, 2017b; EU, 2020):

In the absence of repeated experimental data on inhalation exposure for MIT and CIT, the derivation of the AEC for MIT and CIT is based on the NOAEC of 0.34 mg/m³ from the CIT/MIT (3:1) mixture (product Kathon™ 886) (EU, 2015b) (see Table 34).

For MIT the unchanged NOAEC of 0.34 mg/m³ of the CIT/MIT (3:1) mixture is used. This approach is feasible since toxicological data indicate that both local and systemic irritative adverse effects can be expected in the following severity sequence; CIT/MIT mixture > MIT irrespective of the exposure route (see sections 4.5.1.1 and 4.5.2). The same was shown for the sensitising properties (see 4.5.1.3).

In analogy to the MIT AEC derivation for biocidal products (EU, 2014; EU, 2017a; EU, 2017b; EU, 2020), the EU-LCI value for MIT is therefore the same as the value for the CIT/MIT mixture.

Proposed EU-LCI value for MIT: 1 µg/m³.

For CIT however, an adaption of the NOAEC of 0.34 mg/m³ by a factor of 0.75 is performed (0.34 * 0.75). In the absence of toxicological data for CIT and in a conservative approach, all toxicity caused by the CIT/MIT mixture (3:1), is attributed to CIT alone. This is reflected in the factor 0.75. This is in line with the biocidal product assessment report for CIT (EU, 2020).

- NOAEC of 0.34 mg/m³ from CIT/MIT (3:1) mixture (product Kathon™ 886) x 0.75 = 0.26 mg/m³
- Adjustment for continuous exposure (6 h/d, 5 d/week): 5.6

[4] The LCI value for the CIT/MIT is used as a basis for the derivation of LCI values for CIT and MIT. It cannot be used in practice since, normally, individual substances (not specific mixtures) are measured in emission tests.

- Adjusted study length factor: 2
- Interspecies extrapolation: 2.5 (allometric scaling not performed since route of exposure is inhalation)
- Intraspecies extrapolation (interindividual variability, general population): 10

The total assessment factor is 280, leading to a value of 0.26 mg/m^3: 280 = 0.0009 mg/m^3 0.9 µg/m^3) for **CIT** (**rounded to 1 µg/m^3 which is in the end the same value as for CIT/MIT and MIT).**

The derived EU-LCI values would provide protection from systemic and local irritant effects. According to available airborne contact dermatitis case reports and measured indoor air concentrations of CIT and MIT after application of wall paint, the occurrence of contact dermatitis cannot be ruled out at present with the proposed EU-LCI values of 1 µg/m^3.

4.7 List of references

The links listed in the bibliography were last checked and confirmed on 3 November 2023.

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D Appendix

D.1 Fact and data collection sheet for 5-chloro-2-methyl-2H-isothiazol-3-one (CIT), 2-methyl-2H-isothiazol-3-one (MIT), and 5-chloro-2-methyl-2,3-dihydro-1,2-thiazol-3-one; 2-methyl-2,3-dihydro-1,2-thiazol-3-one (CIT/MIT)

Table 36: Data collection sheet for 5-chloro-2-methyl-2,3-dihydro-1,2-thiazol-3-one; 2-methyl-2,3-dihydro-1,2-thiazol-3-one (CIT/MIT)

Compound	**CIT/MIT mixture**		
Nº CAS: 55965-84-9	EU-Classification: yes CLP, harmonised classification: Acute Tox. 2, H330, 310; Acute Tox. 3, H301; Skin Corr. 1C, H314; Skin Sens. 1A, H317		
Organisation name	**REACH Registrants**	**MAK commission**	**Biocidal Authorisation Holders**
Risk value name	DNEL (general population)	DNEL (worker)	AEC (Acceptable Exposure Concentration) long term
Risk value (mg/m³)	0.02 mg/m³ (inhalation local/long term)	0.2 mg/m³ I (inhalable aerosol fraction)	0.02 mg a.i/m³
Reference period	Chronic	Chronic	Chronic
Risk value (mg/m³) Short term (15 min)	-	-	-
Year	-	1999	2015
Key study	OECD Guideline 413 (repeated dose 90-day inhalation toxicity study in rodents)	OECD Guideline 413 (repeated dose 90-day inhalation toxicity study in rodents)	OECD Guideline 413 Rat, 90-day study (6 h/d, 5d/w), inhalation, nose-only (Kathon 886, 14 % a.i.)
Study type	90-day inhalation study	90-day inhalation study	90-day inhalation study
Species	Rat, Crl:CD BR	Rat, Crl:CD BR	Rat, Crl:CD BR
Duration of exposure in key study	90 days	90 days	90 days
Critical effect	Irritation (respiratory tract)	Irritation (respiratory tract)	Irritation (respiratory tract)
Critical dose value	NOAEC: 0.34 mg/m³	NOAEC: 0.34 mg/m³	NOAEC: 0.34 mg/m³
Adjusted critical dose		-	-

Compound	CIT/MIT mixture		
Single assessment factors	UFs 2, UF_A 2.5, UF_H 3.2	-	UFs 2, UF_A 2.5, UF_H 3.2
Other effects	-	-	-
Remarks	-	-	-

UF_H Intraspecies variability; UF_A interspecies variability; UF_S Used subchronic study;

Table 37: Data collection sheet for 2-methyl-2,3-dihydro-1,2-thiazol-3-one (MIT)

Compound	2-Methyl-2H-isothiazol-3-one (MIT)		
Nº CAS: 2682-20-4 1 ppm = 4.74 mg/m³ at 23 °C	EU-Classification: yes CLP, harmonised classification: Acute Tox. 2, H330; Acute Tox. 3, H311, and H301; Skin Corr. 1B, H314; Eye Dam. 1, H318; Skin Sens. 1A, H317		
Organisation name	**REACH Registrants**	**AgBB**	**Biocidal Authorisation Holders**
Risk value name	DNEL (general population)	NIK ('Lowest Concentration of Interest')	AEC (Acceptable Exposure Concentration) long term
Risk value (mg/m³)	0.021 mg/m³ (inhalation local/long term)	0.1	0.021 mg/m³
Reference period	Chronic	Chronic (general population)	Chronic
Risk value (mg/m³) Short term (15 min)	-	-	0.043 mg/m³ (acute, medium-term AEC)
Year	-	2018	2014
Key study	OECD Guideline 413 (repeated dose 90-day inhalation toxicity study in rodents)	-	OECD Guideline 413 Rat, 90-day study (6 h/d, 5d/w), inhalation, nose-only (Kathon886™ 886, 14 % a.i.)
Study type	90-day inhalation study	-	90-day inhalation study
Species	Rat, Crl:CD BR	-	Rat, Crl:CD BR
Duration of exposure in key study	90 days	-	90 days
Critical effect	Irritation (respiratory tract)	-	Irritation (respiratory tract)

Compound	**2-Methyl-2H-isothiazol-3-one (MIT)**		
Critical dose value	NOAEC: 0.34 mg/m³	-	NOAEC: 0.34 mg/m³
Adjusted critical dose	-	-	-
Single assessment factors	UFs 2, UF_A 2.5, UF_H 3.2	-	UFs 2, UF_A 2.5, UF_H 3.2
Other effects	-	-	-
Remarks	**-**	Individual substance assessments	Conservative approach to use NOAEC from CIT/MIT mixture without adaptation

UFH Intraspecies variability; UFA interspecies variability; UFS Used subchronic study

Table 38: Data collection sheet for 5-chloro-2-methyl-2H-isothiazol-3-one (CIT)

Compound	**5-Chloro-2-methyl-2H-isothiazol-3-one (CIT)**	
N° CAS: 26172-55-4 1 ppm = 6.16 mg/m³ at 23 °C	EU-Classification: no CLP, harmonised classification: no	
Organisation name	**AgBB**	**Biocidal Authorisation Holders**
Risk value name	NIK ('Lowest Concentration of Interest')	AEC (Acceptable Exposure Concentration) long term (8 h TWA)
Risk value (mg/m³)	0.001	0.012 mg pure CIT /m³
Reference period	Chronic (general population)	Chronic
Risk value (mg/m³) Short term (15 min)	-	
Year	2018	2020
Key study	-	OECD Guideline 413 Rat, 90-day study (6 h/d, 5d/w), inhalation, nose-only (Kathon 886™, 14 % a.i.)
Study type	-	90-day inhalation study
Species	-	Rat, Crl:CD BR
Duration of exposure in key study	-	90 days
Critical effect	-	Irritation (respiratory tract)
Critical dose value	-	NOAEC: 0.34 mg/m³ x 0.75 x (6h/8h) = 0.26 mg/m³
Adjusted critical dose	-	
Single assessment factors	-	UFs 2, UF_A 2.5, UF_H 3.2
Other effects	-	chronic
Remarks	Individual substance assessments	

UF_H Intraspecies variability; UF_A interspecies variability; UF_S Used subchronic study

Table 39: Fact sheet for 5-chloro-2-methyl-2,3-dihydro-1,2-thiazol-3-one; 2-methyl-2,3-dihydro-1,2-thiazol-3-one (CIT/MIT)

Compound	**5-Chloro-2-methyl-2,3-dihydro-1,2-thiazol-3-one; 2-methyl-2,3-dihydro-1,2-thiazol-3-one (CIT/MIT)**		**Fact sheet**
Parameter	**Note**	**Comments**	**Value / descriptor**
EU-LCI value and status			
EU-LCI value	1	[µg/m³]	1
EU-LCI status	2	Draft/Final	Draft
EU-LCI year of issue	3	Year when EU-LCI value has been issued	2023
General information			
CLP-Index No.	4	INDEX	613-167-00-5
EC-No.	5	EINECS	911-418-6
CAS-No.	6	Chemical Abstract Service number	55965-84-9
Harmonised CLP classification	7	Human health risk related classification	Acute Tox. 2, H330, 310; Acute Tox. 3, H301; Skin Corr. 1C, H314; Skin Sens. 1A, H317
Molar mass and conversion factor	8	[g/mol] and [ppm – mg/m³]	-
Key data / database			
Key study, authors, year	9	Critical study with lowest relevant effect level	OECD Guideline 413 Rat, 90-day study (6 h/d, 5d/w), inhalation, nose-only (Kathon 886™, 14 % a.i.)
Read across compound	10	Where applicable	-
Species	11	Rat, human, etc.	Rat, Crl:CD BR
Route / type of study	12	Inhalation, oral feed, etc.	Inhalation
Study length	13	Days, subchronic, chronic, etc.	90-days
Exposure duration	14	h/d, d/w	d/w
Critical endpoint	15	Effect (s), site of	Irritation (respiratory tract)
Point of departure (POD)	16	LOAEC, NOAEC, BMD, etc.	NOAEC
POD value	17	[mg/m³] or ppm or [mg/kg$_{BW}$×d]	0.34 mg/m³
Assessment factors (AF)			
Adjustment for exposure duration	19	Study exposure h/d, d/w	5.6

Compound	**5-Chloro-2-methyl-2,3-dihydro-1,2-thiazol-3-one; 2-methyl-2,3-dihydro-1,2-thiazol-3-one (CIT/MIT)**		**Fact sheet**
Study length	20	sa→sc→c	2
Route-to-route extrapolation factor	21	-	1
Dose-response	22a	Reliability of dose-response, LOAEL to NOAEL	1
	22b	Severity of effect (R8 6d)	1
Interspecies differences	23a	Allometric Metabolic rate (R8-3)	1
	23b	Kinetic + dynamic	2.5
Intraspecies differences	24	Kinetic + dynamic General population	10
AF (sensitive population)	25		-
Other adjustment factors Quality of database	26	Route-to-route-extrapolation	1
Results			
Summary of assessment factors	27	Total Assessment Factor	280
POD/TAF	28	Calculated value [µg/m³ and ppb]	1.2 µg/m³
Molar adjustment factor	29		-
Rounded value	30	[µg/m³]	1
Additional comments	31		-
Rationale selection	32		

Table 40: Fact sheet for 2-methyl-2,3-dihydro-1,2-thiazol-3-one (MIT)

Compound	**2-methyl-2,3-dihydro-1,2-thiazol-3-one (MIT)**		**Fact sheet**
Parameter	**Note**	**Comments**	**Value / descriptor**
EU-LCI value and status			
EU-LCI value	1	[µg/m³]	1
EU-LCI status	2	Draft/Final	Draft
EU-LCI year of issue	3	Year when EU-LCI value has been issued	2023
General information			
CLP-Index No.	4	INDEX	613-326-00-9
EC-No.	5	EINECS	220-239-6
CAS-No.	6	Chemical Abstract Service number	2682-20-4
Harmonised CLP classification	7	Human health risk related classification	Acute Tox. 2, H330; Acute Tox. 3, H311, and H301; Skin Corr. 1B, H314; Eye Dam. 1, H318; Skin Sens. 1A, H317
Molar mass and conversion factor	8	[g/mol] and [ppm – mg/m³]	115.2 g/mol 1 ppm = 4.74 mg/m³
Key data / database			
Key study, authors, year	9	Critical study with lowest relevant effect level	OECD Guideline 413 Rat, 90-day study (6 h/d, 5d/w), inhalation, nose-only (Kathon 886™, 14 % a.i.)
Read across compound	10	Where applicable	CIT/MIT without molar adjustment
Species	11	Rat, human, etc.	Rat, Crl:CD BR
Route / type of study	12	Inhalation, oral feed, etc.	Inhalation
Study length	13	Days, subchronic, chronic, etc.	90-days
Exposure duration	14	h/d, d/w	d/w
Critical endpoint	15	Effect (s), site of	Irritation (respiratory tract)
Point of departure (POD)	16	LOAEC, NOAEC, BMD, etc.	NOAEC
POD value	17	[mg/m³] or ppm or [mg/kg$_{BW}$×d]	0.34 mg/m³
Assessment factors (AF)			
Adjustment for exposure duration	19	Study exposure h/d, d/w	5.6
Study length	20	sa→sc→c	2

Compound	**2-methyl-2,3-dihydro-1,2-thiazol-3-one (MIT)**		**Fact sheet**
Route-to-route extrapolation factor	21	-	1
Dose-response	22a	Reliability of dose-response, LOAEL to NOAEL	1
	22b	Severity of effect (R8 6d)	1
Interspecies differences	23a	Allometric Metabolic rate (R8-3)	1
	23b	Kinetic + dynamic	2.5
Intraspecies differences	24	Kinetic + dynamic General population	10
AF (sensitive population)	25		1
Other adjustment factors Quality of database	26	Route-to-route-extrapolation	1
Results			
Summary of assessment factors	27	Total Assessment Factor	280
POD/TAF	28	Calculated value [µg/m³ and ppb]	1.2 µg/m³ and 0.25 ppb
Molar adjustment factor	29		-
Rounded value	30	[µg/m³]	1
Additional comments	31		Conservative approach to use NOAEC from CIT/MIT mixture without adaptation
Rationale selection	32		

Table 41: Fact sheet for 5-Chloro-2-methyl-4-isothiazolin-3-one (CIT)

Compound	**5-Chloro-2-methyl-4-isothiazolin-3-one (CIT)**		**Fact sheet**
Parameter	**Note**	**Comments**	**Value / descriptor**
EU-LCI value and status			
EU-LCI value	1	[µg/m³]	1
EU-LCI status	2	Draft/Final	Draft
EU-LCI year of issue	3	Year when EU-LCI value has been issued	2023
General information			
CLP-Index No.	4	INDEX	-
EC-No.	5	EINECS	247-500-7
CAS-No.	6	Chemical Abstract Service number	26172-55-4
Harmonised CLP classification	7	Human health risk related classification	-
Molar mass and conversion factor	8	[g/mol] and [ppm – mg/m³]	149.6 g/mol 1 ppm = 6.16 mg/m³
Key data / database			
Key study, authors, year	9	Critical study with lowest relevant effect level	OECD Guideline 413 Rat, 90-day study (6 h/d, 5d/w), inhalation, nose-only (Kathon 886™, 14 % a.i.)
Read across compound	10	Where applicable	CIT/MIT without molar but potency adjustment
Species	11	Rat, human, etc.	Rat, Crl:CD BR
Route / type of study	12	Inhalation, oral feed, etc.	Inhalation
Study length	13	Days, subchronic, chronic, etc.	90-days
Exposure duration	14	h/d, d/w	d/w
Critical endpoint	15	Effect (s), site of	Irritation (respiratory tract)
Point of departure (POD)	16	LOAEC, NOAEC, BMD, etc.	NOAEC
POD value	17	[mg/m³] or ppm or [mg/kg$_{BW}$×d]	0.34 mg/m³ x 0.75 = 0.26 mg/m³
Assessment factors (AF)			
Adjustment for exposure duration	19	Study exposure h/d, d/w	5.6
Study length	20	sa→sc→c	2

Compound	5-Chloro-2-methyl-4-isothiazolin-3-one (CIT)		Fact sheet
Route-to-route extrapolation factor	21	-	1
Dose-response	22a	Reliability of dose-response, LOAEL to NOAEL	1
	22b	Severity of effect (R8 6d)	1
Interspecies differences	23a	Allometric Metabolic rate (R8-3)	1
	23b	Kinetic + dynamic	2.5
Intraspecies differences	24	Kinetic + dynamic General population	10
AF (sensitive population)	25		1
Other adjustment factors Quality of database	26	Route-to-route-extrapolation	1
Results			
Summary of assessment factors	27	Total Assessment Factor	280
POD/TAF	28	Calculated value [µg/m³ and ppb]	0.9 µg/m³ and 0.14 ppb
Molar adjustment factor	29		-
Rounded value	30	[µg/m³]	1
Additional comments	31		Correction factor for NOAEC = 0.75 since NOAEC derived from CIT/MIT (3:1) mixture data
Rationale selection	32		

Data compilation and evaluation for CIT/MIT and MIT and CIT individually is based on a project funded by the German Environment Agency (Voss et al., 2024).

Rationale for critical effects

2-Methyl-2,3-dihydro-1,2-thiazol-3-one (MIT) and 5-chloro-2-methyl-2,3-dihydro-1,2-thiazol-3-one (CIT) are belonging to the 1,2-thiazoles which are liquids with boiling points between 100°C and 106.5°C.

Toxicological effects have been either investigated for MIT or the mixture of CIT/MIT (3:1). No toxicological studies have been identified for CIT.

The acute toxicity inhalation studies in rats (males and females) showed LD50 concentrations between 110 mg MIT/m^3 air to 770 mg MIT/m^3 air with clinical signs of respiratory irritations. For MIT/CIT mixture the LC50 was reported to be between 200-300 mg a.i./m^3 or > 650 mg a.i./m^3.

Available human data including epidemiological studies show that CIT, MIT and/or the mixture of CIT/MIT are highly potent skin sensitisers, including case reports showing airborne contact dermatitis. Recent data indicate that both substances CIT and MIT have an equal sensitising potential (Weese, 2019). Both MIT and CIT/MIT mixture have a harmonised classification for Skin Sens. 1A (H317) (ECHA C&L Inventory, 2022).

Furthermore, MIT has a harmonised classification for Skin Corr. 1B (H314) and Eye Dam. 1 (H318) (ECHA C&L Inventory, 2022). CIT/MIT mixture has a harmonised classification for Skin Corr. 1C (H314) (ECHA C&L Inventory, 2022) also indicating that the mixture will cause serious eye damage after exposure (RAC, 2016).

The substances did neither show a genotoxic or carcinogenic potential nor is there a concern for reproductive or developmental toxicity.

There are only two studies available with the CIT/MIT mixture in which repeated inhalation exposure toxicity was investigated (a 90-day and a 14-day study in rats). For MIT as single substance, only acute inhalation exposure studies are available. These studies demonstrate that local effects are predominant after inhalation exposure unless lethal concentrations were applied.

A derivation for EU-LCI values for CIT and MIT as well as for the mixture of CIT/MIT (3:1) are based on toxicological data for the CIT/MIT mixture obtained from the 90-day inhalation study in rats with a NOAEC of 0.34 mg/m³ and a LOAEC of 1.15 mg/m³ (product Kathon™ 886)[5]. Adjustment for continuous exposure (6 h/d, 5 d/week) was performed by using the factor of 5.6. Additionally, the study length was adjusted by the factor of 2. For interspecies extrapolation a factor of 2.5 (allometric scaling not performed since route of exposure is inhalation) was applied and for intraspecies extrapolation (interindividual variability, general population) a factor of 10 was used. The total assessment factor is 280.

- NOAEC CIT/MIT (3:1) mixture 0.34 mg/m³: 280 = 0.0012 mg/m³ (1.2 µg/m³ for CIT/MIT, rounded to 1 µg/m³).

In a conservative approach and in analogy to the MIT AEC derivation for biocidal products (EU, 2014, 2017a, b), the EU-LCI value for MIT is the same as the value for the mixture. Consequently, it is derived as follows:

- NOAEC CIT/MIT (3:1) mixture 0.34 mg/m³: 280 = 0.0012 mg/m³ (1.2 µg/m³ for MIT, rounded to 1 µg/m³).

However, for the EU-LCI value for CIT an adaption of the NOAEC of 0.34 mg/m³ by a factor of 0.75 is performed. This approach follows the procedure as suggested in the AEC derivation for biocidal products (EU, 2020). In the absence of toxicological data for CIT and in a conservative approach, all toxicity caused by the CIT/MIT mixture (3:1), is attributed to CIT alone. This is reflected in the factor 0.75.

- NOAEC of 0.34 mg/m³ from CIT/MIT (3:1) mixture (product Kathon™ 886) x 0.75 = 0.26 mg/m³: 280 = 0.0009 mg/m³ 0.9 µg/m³ for CIT (rounded to 1 µg/m³).

With the rounding rules as suggested in the ECA Report 29 (EC, 2013) the EU-LCI values for the CIT/MIT (3:1) mixture, and the individual substances MIT and CIT all result in the same value: 1 µg/m³.

[5] The LCI value for the CIT/MIT is used as a basis for the derivation of LCI values for CIT and MIT. It cannot be used in practice since, normally, individual substances (no specific mixtures) are measured in emission tests.

Remarks:

In case that both individual substances (MIT and CIT) are measured the total concentration of both substances should not exceed 1 µg/m³.

Based on the cases of airborne contact dermatitis and considering the measured values for indoor areas after application of wall paint, the occurrence of contact dermatitis cannot be ruled out at the proposed EU-LCI values of 1 µg/m³.

Systematic studies on humans to get more information on the endpoint "airborne contact dermatitis" are ethically unacceptable.

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