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Toxicological basic data for the derivation of EU-LCI values for 5 substances from building products

Final Report

For our Environment

Umwelt Bundesamt

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Toxicological basic data for the derivation of EU-LCI values for 5 substances from building products

Final Report

by

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On behalf of the German Environment Agency

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Kurzbeschreibung

Gegenstand des Berichts ist die Erstellung von Stoffberichten für die Ableitung von EU-LCI-Werten für die im Titel genannten Stoffe. 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, einer Expertengruppe mit Fachleuten aus zehn europäischen Ländern, 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 hin gearbeitet, dass die Europäische Kommission diese Harmonisierungsinitiative weiter voran bringt. Im November 2015 hat die Europäische Kommission das Mandat zur Fertigstellung der EU-LCI Liste an die EU-LCI-Arbeitsgruppe erteilt. Eine vollständig harmonisierte EU-LCI Liste soll bis Ende 2019 erarbeitet und veröffentlicht werden. Die im Rahmen dieses Forschungsvorhabens ausgearbeiteten Stoffdossiers unterstützen und beschleunigen diesen Prozess.

Abstract

The subject of this report is the preparation of substance reports for the derivation of EU-LCI values for the substances 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 compilation 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, a group of experts from ten European countries. This Working Group is developing a harmonized 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. A completely harmonised EU-LCI list shall be prepared and published by the end of 2019. The substance dossiers prepared within the scope of this project will add in and accelerate this process.

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List of abbreviations

Abbreviation	Explanation
ABS	Acrylonitrile-butadiene-styrene
AGÖF	Arbeitsgemeinschaft ökologischer Forschungsinstitute (Association of Ecological Research Institutes)
CARB	California Air Resources Board
DNEL	Derived no effect level
DPGME	Dipropylene glycol monomethyl ether
DPGnB	Dipropylene glycol mono-n-butyl ether
F	Females
FDA	Food and Drug Administration
GD	Gestation Day
GI tract	Gastrointestinal tract
GPT	Glutamate-pyruvate transaminase
GSH	Glutathion
HPRT	Hypoxanthine-guanine phosphoribosyltransferase
IRIS	Integrated risk information system
IUPAC	International union of pure and applied chemistry
LCI	Lowest concentration of interest
LOAEC	Lowest observed adverse effect concentration
LoD	Limit of detection
Log Pow	Logarithm of octanol/water partition coefficient
M	Males
Mp	Melting point
MTD	Maximum tolerated dose
NAEC	No adverse effect concentration
NIK	Niedrigste Interessierende Konzentration (Lowest concentration of interest)
NOAEC	No observed adverse effect concentration
NOEL	No observed effect level
n. r.	Not reported
OECD	Organization for economic cooperation and development
OEL	Occupational exposure limit
PGME	Propylene glycol monomethyl ether
PND	Postnatal day
POD	Point of departure

Abbreviation	Explanation
RD_{50}	concentration inducing a 50% decrease in respiratory frequency
REL	Reference Exposure Level
RIVM	Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven, NL
SCE	Sister Chromatid Exchange
TCA	Tolerable concentration in air
TPGME	Tripropylene glycol monomethyl ether
TWA	Time-weighted Average

Zusammenfassung

Stoffprofil und EU-LCI-Wert für 2-Phenylpropen

2-Phenylpropen ist eine klare, farblose Flüssigkeit mit einem durchdringenden unangenehmen Geruch, der bereits bei niedrigen Konzentrationen wahrnehmbar ist. Verwendet wird 2-Phenylpropen bei der Herstellung von Kopolymeren aus Acrylonitril-Butadien-Styrol (ABS) und anderen Polymeren und Harzen, in Weichmachern von Farben sowie in Wachsen und Klebstoffen.

Zum Vorkommen von 2-Phenylpropen in der Innenraumluft liegen nur wenige Angaben vor. Demnach sind die Konzentrationen dieses Stoffs in Innenräumen allgemein sehr gering. In Büros, Wohnungen, Vorschulen und Schulen in Deutschland lagen Median- und Maximalwerte unterhalb von 10 µg/m^3.

Systemische Wirkungen nach inhalativer oder oraler Exposition belegen die Resorption der Substanz über diese Pfade. Verlässliche quantitative Angaben liegen jedoch nicht vor. 2-Phenylpropen wird rasch in Form von Metaboliten mit dem Urin ausgeschieden (76% binnen 24 h, etwa 90% binnen 72 h).

Zu toxischen Wirkungen von 2-Phenylpropen beim Menschen liegen nur sehr wenige Angaben vor. Es wird angegeben, dass bei kurzzeitiger Exposition gegenüber 200 ppm (975 mg/m^3) unangenehmer Geruch und Augenreizung auftreten, höhere Konzentrationen verursachten außerdem starke nasale Reizungen. Bei 50 ppm (245 mg/m^3) wurde kein Reizeffekt angegeben, der Geruch ist noch unterhalb von 1 ppm (5 mg/m^3) wahrnehmbar.

Für eine gentoxische Wirkung von 2-Phenylpropen liegen keine klaren Belege vor. Bei parental nicht toxischen Dosierungen traten bei Ratten in einer kombinierten Studie zur Reproduktions- und Entwicklungstoxizität keine reproduktions- oder entwicklungstoxischen Effekte auf.

Der kritische Effekt einer inhalativen Exposition gegenüber 2-Phenylpropen besteht in der Reizwirkung auf die nasalen Epithelien. In einer chronischen Inhalationsstudie an Ratten traten in allen exponierten Gruppen bei Männchen und Weibchen erhöhte Inzidenzen von Basalzellhyperplasien auf (LOAEC 100 ppm oder 487 mg/m^3, kein NOAEC); die Inzidenz für eine Degeneration des olfaktorischen Epithels war ab1470 mg/m^3 (300 ppm) bei Weibchen und bei 4900 mg/m^3 (1000 ppm) auch bei Männchen erhöht. In einer parallel durchgeführten Untersuchung an Mäusen war die Inzidenz olfaktorischer epithelialer Metaplasien und Hyperplasien von Drüsen im olfaktorischen Epithel bei allen exponierten Gruppen von Männchen und Weibchen erhöht. Außerdem entwickelten Männchen ab 300 ppm eine Atrophie des olfaktorischen Epithels. Weder bei Mäusen noch bei Ratten wurden nach chronischer Exposition erhöhte Inzidenzen von Tumoren der nasalen Epithelien beobachtet.

Mechanistische Überlegungen sprechen dafür, dass die bei Mäusen beobachteten Veränderungen der nasalen Epithelien für die Risikobewertung beim Menschen weniger relevant sind als die Effekte bei Ratten. Es bleibt aber festzuhalten, dass grundsätzlich das olfaktorische Epithel in beiden Spezies das kritische Zielgewebe der toxischen Wirkung von 2-Phenylpropen darstellt.

Die LOAEC von 100 ppm (490 mg/m^3) 2-Phenylpropen aus der chronischen Inhalationsstudie an Ratten wird als POD für die vorgeschlagene Ableitung eines EU-LCI-Wertes herangezogen.

Die folgenden Extrapolationsfaktoren werden herangezogen:

- Adjustierung auf kontinuierliche Exposition (von 6 h/d, 5 d/Woche): 5,6
- LOAEC→ NAEC: 3
- Zeitextrapolation (chronische Exposition): 1
- Interspeziesextrapolation: 2.5
  (Gemäß ECA-Bericht 29 ist für die Interspeziesextrapolation keine Korrektur für Unterschiede im systemischen Metabolismus vorgesehen, wenn der POD auf lokalen Effekten beruht. Für verbleibende Unsicherheiten wird ein Wert von 1 für verbleibende Speziesunterschiede für

Wirkungen auf Haut, Auge oder Gastrointestinaltrakt gewählt, sofern die Wirkungsweise nur eine einfache Zerstörung der Membranen impliziert. Ein Standardwert von 2,5 wird für Effekte auf Haut. Auge und Gastrointestinaltrakt gewählt, sofern lokale Metabolisierung oder Rezeptorbindungsreaktionen beteiligt sind. Für 2-Phenylpropen wird ein Faktor von 2,5 verwendet, da bekannt ist, dass die Metabolisierung bei der Toxizität strukturell verwandter Verbindungen beteiligt ist und dies wahrscheinlich auch für 2-Phenylpropen zutrifft.)

- Intraspeziesextrapolation (interindividuelle Variabilität, Allgemeinbevölkerung): 10

Gesamtextrapolationsfaktor: 420.

Als EU-LCI-Wert (gerundet) für 2-Phenylpropen wird somit eine Konzentration von 1200 µg/m^3 vorgeschlagen.

Der vorgeschlagene EU-LCI-Wert liegt im Bereich der weiten Spanne berichteter Geruchschwellenwerte (0,1-244 mg/m^3; 0,02-49,7 ppm). Da außerdem angegeben wird, dass der penetrante Geruch bereits unterhalb von 5 mg/m^3 (1 ppm) oder sogar bei noch niedrigeren Konzentrationen von etwa 1,5 mg/m^3 (0,3 ppm) wahrgenommen werden kann, sind Geruchswahrnehmung und –belästigung beim EU-LCI-Wert nicht auszuschließen.

Stoffprofil und EU-LCI-Wert für Vinyltoluol

Vinyltoluole sind klare, farblose Flüssigkeiten mit einem ausgeprägten und sehr unangenehmen Geruch. Vinyltoluole sind großtechnische Produkte; die Verbindungen werden allein oder zusammen mit anderen zur Herstellung von Polymeren, in Klebstoffen, Harzen, Oberflächenanstrichen und zur Herstellung anderer Chemikalien und Insektizide verwendet. Das mit der CAS-Nr. 25013-15-4 bezeichnete üblicherweise in der Industrie eingesetzte Produktgemisch enthält 60-70% m-Vinyltoluol und 30-40% p-Vinyltoluol. Sofern nicht anders angegeben, wurden die Untersuchungen zur Toxizität mit derartigen Gemischen durchgeführt.

Zum Vorkommen von Vinyltoluolen in der Innenraumluft liegen nur wenige Angaben vor. Vinyltoluole konnten in insgesamt 66 Messungen in unterschiedlichen Innenräumen in Deutschland nicht nachgewiesen werden. In anderen Untersuchungen konnten Vinyltoluole in Wohnräumen nachgewiesen werden, wurden jedoch nicht quantifiziert.

Systemische Wirkungen nach inhalativer oder oraler Exposition belegen die Resorption der Vinyltoluole über diese Pfade. Verlässliche quantitative Angaben liegen jedoch nicht vor. Angaben zur Verteilung von Vinyltoluol im Organismus liegen nicht vor. Vinyltoluole werden in Form verschiedener Metabolite rasch mit dem Urin ausgeschieden. Als Hauptmetaboliten im Urin wurden Thioether identifiziert, weiterhin Methylmandelsäure und Derivate. Insgesamt weist die Metabolisierung von Vinyltoluol (Methylstyrol) starke Parallelen zu der des Styrols auf.

Zu toxischen Wirkungen von Vinyltoluolen beim Menschen liegen nur sehr wenige Angaben vor. Eine kurzfristige Exposition mit 50 ppm (245 mg/m^3) Vinyltoluol wurde am Geruch wahrgenommen. Bei 300 ppm (1460 mg/m^3) wurde der Geruch stark und unangenehm, und ab 400 ppm (1950 mg/m^3) berichteten die Probanden außerdem starke Augen- und Nasenreizung.

Tierversuche weisen darauf hin, dass der kritische Effekt einer inhalativen Vinyltoluolexposition in einer Reizung der nasalen Epithelien besteht. In einer chronischen Inhalationsstudie verursachte Vinyltoluol bei Ratten bei allen untersuchten Konzentrationen degenerative und nicht-neoplastische proliferative Läsionen. Die Läsionen betrafen sowohl das respiratorische als auch das olfaktorische Epithel. Im respiratorischen Epithel entwickelten sich diffuse Hyperplasien und intraepitheliale Schleimzysten. Im olfaktorischen Epithel wurden Zysten, fokale Erosionen, eosinophile Hyperplasien und fokale epitheliale respiratorische Metaplasien beschrieben. Eine NOAEC konnte in der Studie nicht ermittelt werden, die LOAEC lag in der Rattenstudie bei 100 ppm (490 mg/m^3). Läsionen der nasalen Epithelien und außerdem in der Lunge wurden in einer ähnlichen Studie auch bei Mäusen festgestellt, wobei die Inzidenzen höher waren und die Effekte bereits bei deutlich niedrigeren Konzentrationen als bei Ratten auftraten (LOAEC 49 mg/m^3, keine NOAEC).

Für eine gentoxische Wirkung der Vinyltoluole liegen keine klaren Belege vor. Das technische Gemisch der Vinyltoluole (65-71% *meta*- und 32-35% *para*-Isomer) verursachte weder bei Ratten noch bei Mäusen nach Inhalation Tumoren.

Begrenzte Daten einer unveröffentlichten 2-Generationenstudie mit oraler Exposition von Ratten mit p-Vinyltoluol weisen nicht auf reproduktionstoxische Effekte bei Dosierungen hin, die nicht bereits parental systemisch toxisch wirken. In ähnlicher Weise liefern Studien mit oraler Exposition von Kaninchen und Ratten keine überzeugenden Hinweise auf entwicklungstoxische Effekte von Vinyltoluol.

Somit weisen sowohl Befunde an Ratten als auch an Mäusen darauf hin, dass die nasalen Epithelien die kritischen Ziele einer inhalativen Exposition gegenüber Vinyltoluolen sind. Mechanistische Aspekte sprechen dafür, dass die in nasalen Epithelien von Mäusen auftretenden Effekte für die Risikobewertung beim Menschen von geringerer Relevanz sind als die Befunde an Ratten. Aus diesem Grund wird die LOAEC von 100 ppm (490 mg/m^3) Vinyltoluol (Gemisch von m- und p-Vinyltoluol) aus der chronischen Inhalationsstudie an Ratten als POD für die vorgeschlagene Ableitung eines EU-LCI-Wertes herangezogen.

Die folgenden Extrapolationsfaktoren werden herangezogen:

- Adjustierung auf kontinuierliche Exposition (von 6 h/d, 5 d/Woche): 5,6
- LOAEC→ NAEC: 3
- Zeitextrapolation (chronische Exposition): 1
- Interspecies extrapolation: 2.5
  (Gemäß ECA-Bericht 29 ist für die Interspeziesextrapolation keine Korrektur für Unterschiede im systemischen Metabolismus vorgesehen, wenn der POD auf lokalen Effekten beruht. Für verbleibende Unsicherheiten wird ein Wert von 1 für verbleibende Speziesunterschiede für Wirkungen auf Haut, Auge oder Gastrointestinaltrakt gewählt, sofern die Wirkungsweise nur eine einfache Zerstörung der Membranen impliziert. Ein Standardwert von 2,5 wird für Effekte auf Haut. Auge und Gastrointestinaltrakt gewählt, sofern lokale Metabolisierung oder Rezeptorbindungsreaktionen beteiligt sind. Für Vinyltoluol wird ein Faktor von 2,5 verwendet, da bekannt ist, dass die Metabolisierung bei der Toxizität strukturell verwandter Verbindungen beteiligt ist und dies wahrscheinlich auch auf Vinyltoluole zutrifft.)
- Intraspeziesextrapolation (interindividuelle Variabilität, Allgemeinbevölkerung): 10

Gesamtextrapolationsfaktor: 420.

Als EU-LCI-Wert (gerundet) für Vinyltoluole wird somit eine Konzentration von 1200 µg/m^3 vorgeschlagen.

Der POD entstammt einer Untersuchung, die mit einem Gemisch von 65–71% *meta*-Vinyltoluol und 32–35% *para*-Vinyltoluol durchgeführt wurde. Daten aus Studien mit wiederholter Exposition gegenüber o-Vinyltoluol liegen nicht vor; aus der begrenzten Datenlage ergeben sich jedoch keine Hinweise auf deutliche Unterschiede in der Toxizität der drei Isomere.

Technisches Vinyltoluol enthält ein Gemisch von m- und p-Vinyltoluol mit nur geringen oder fehlenden Anteilen des o-Isomeren. Von daher ist bei Bauprodukten der Nachweis von m- und p-Vinyltoluol in der Luft möglich.

Der Geruch von Vinyltoluolen wurde als sehr unangenehm und widerwärtig beschrieben. Zu Geruchsschwellen von Vinyltoluolen (Methylstyrolen) liegen keine verlässlichen Angaben vor. Aufgrund der strukturellen Ähnlichkeit zu Styrol, für das in anderen Veröffentlichungen Geruchschwellen um 70 µg/m^3 berichtet werden, können Geruchswahrnehmung und –belästigung durch Vinyltoluole beim vorgeschlagenen EU-LCI-Wert nicht ausgeschlossen werden.

Stoffprofil und EU-LCI-Wert für n-Heptan

Heptan ist eine farblose, flüchtige Flüssigkeit mit einem schwachen kohlenwasserstoffartigen Geruch. Technisches Heptan ist ein Isomerengemisch. Heptan ist Bestandteil von Kraftstoffen, es wird als Lösemittel für Kleber, Lacke und Tinten verwendet, weiterhin als Extraktionsmittel, in der Herstellung von Kunststoffschäumen sowie bei der Synthese von Toluol und anderen Alkylbenzolen. Die gegenwärtige Produktionsmenge von n-Heptan in der EU wird mit mehr als 1000 t/a angegeben.

In der Innenraumluft von Wohnungen, Büros und Schulen wurden geringe Konzentrationen von n-Heptan /und anderen Isomeren) gemessen (Mediane im Bereich von 1-2 µg/m^3). Neuere Daten lassen im Vergleich zu Messungen aus den 1980er Jahren einen Rückgang der Konzentration von Heptan erkennen.

In Untersuchungen an Probanden wurden pulmonale Retentionswerte im Bereich von 30 % ermittelt. In den Körper aufgenommenes n-Heptan wird zu einem hohen Anteil (80 %) metabolisiert. Dabei werden neben dem Hauptprodukt 2-Heptanol verschiedene weitere Heptanole, Hydroxyketone und Diketone gebildet und im Urin ausgeschieden. Die Diketonbildung ist etwa 40-mal geringer als beim n-Hexan.

Für eine gentoxische Wirkung von n-Heptan liegen keine Hinweise vor. Untersuchungen zur Kanzerogenität liegen nicht vor, für reproduktionstoxische Wirkungen ergeben sich bei insgesamt unvollständiger Datenlage keine Hinweise.

Als Grundlage für die Ableitung eines EU-LCI-Wertes ist eine subchronische Studie an Ratten zu diskutieren. Trotz geringer konzeptioneller Mängel der Studie wird als Point of Departure (POD) die NOAEC von 3000 ppm (12510 mg/m^3) (höchste getestete Konzentration) gewählt. Diese Studie ist nicht öffentlich verfügbar, jedoch in ausreichendem Detailgrad in mehreren Übersichtsarbeiten referiert.

Bei der Extrapolation der auf kontinuierliche Exposition korrigierten NOAEC in Höhe von 2235 mg/m^3 auf eine lebenslange Exposition der Allgemeinbevölkerung werden folgende Faktoren angewendet:

- LOAEC→ NAEC Extrapolation: entfällt, Basis ist eine NOAEC
- Interspeziesextrapolation: Faktor 2,5
- Berücksichtigung der intraindividuellen Variabilität bei der Allgemeinbevölkerung: Faktor 10
- Berücksichtigung der nur subchronischen Expositionszeit: Faktor 2
- Berücksichtigung der unvollständigen Histopathologie sowie des Fehlens von Studien zu reproduktionstoxischen Effekten: Faktor 3

Der Gesamtextrapolationsfaktor beträgt damit 150.

Als EU-LCI (gerundet) für n-Heptan wird somit eine Konzentration von 15000 µg/m^3 vorgeschlagen.

Der vorgeschlagene EU-LCI-Wert von 15 000 µg/m^3 liegt über der in einer Untersuchung referierten Geruchsschwelle von 2,8 mg/m^3, jedoch berichten andere Autoren deutlich höhere Geruchsschwellen von 167-1668 mg/m^3. Bei sensiblen Personen sind deshalb geruchliche Wahrnehmungen beim EU-LCI-Wert nicht auszuschließen.

Stoffprofil und EU-LCI-Wert für Hexylenglykol

Hexylenglykol (2-Methyl-2,4-pentandiol) ist eine farblose, hygroskopische Flüssigkeit mit einem leicht süßlichen Geruch. Der Stoff wurde als flüchtige Aroma- und Geschmackskomponente in Äpfeln nachgewiesen. Hexylenglykol ist ein großtechnisches Produkt, das in Farben, Lacken und Anstrichen als Lösevermittler für Oberflächenbeschichtungen sowohl in lösemittel- als auch in wasserbasierten Produkten eingesetzt wird. Weiterhin wird der Stoff in Kosmetika, in der Leder- und Textilverarbeitung, in Frostschutzmitteln sowie als Dispersionsmittel in Reinigern, Desinfektionsmitteln und Pestizidzubereitungen eingesetzt.

Zum Vorkommen von Hexylenglykol in der Innenraumluft liegen nur sehr wenige Angaben vor. Es wird berichtet, dass Hexylenglykol qualitativ als untergeordneter flüchtiger Bestandteil der Emission neuer Teppichböden nachgewiesen wurde. In 66 Proben aus unterschiedlich genutzten Innenräumen in Deutschland konnte nur in einem Fall Hexylenglykol nachgewiesen werden.

Systemische Wirkungen nach oraler sowie dermaler Exposition belegen die Aufnahme des Stoffs über diese Pfade. Verlässliche quantitative Angaben liegen jedoch nicht vor. Es wird angegeben, dass Hexylenglykol von der Schleimhaut des Atemtrakts sowie des Gastrointestinaltrakts absorbiert wird. Allgemein ist bekannt, dass gesättigte aliphatische Glykole über alle Aufnahmepfade gut resorbiert werden.

In Untersuchungen an Probanden sowie in Tierversuchen an Ratten und Kaninchen wurde gefunden, dass etwa die Hälfte des oral verabreichten Hexylenglykols als Glucuronid im Urin ausgeschieden wurde. Hexylenglykol wurde in der Muttermilch säugender Ratten und in den so gestillten Jungtieren nachgewiesen.

Zu toxischen Wirkungen von Hexylenglykol beim Menschen liegen nur sehr wenige Angaben vor. Eine kurze inhalative Exposition gegenüber 50 ppm Hexylenglykoldampf (245 mg/m^3) führte bei den Probanden nach den Angaben der Studie zu Augen- nicht aber Atemwegreizungen. Humandaten mit wiederholter inhalativer Exposition liegen nicht vor. Tierversuchsdaten mit wiederholter Exposition liegen mit Ausnahme einer unzureichend dokumentierten subakuten Studie nicht vor. In einer subchronischen Studie mit oraler Exposition von Ratten beschränkten sich die Wirkungen auf die Leber bis zur höchsten getesteten Dosis von 450 mg/(kg KG x d) auf eine adaptive hepatozelluläre Hypertrophie ohne histopathologisch nachweisbare Schäden. In einer Screeningstudie zur Reproduktions- und Entwicklungstoxizität wurde jedoch die Entstehung von Foci veränderter Leberzellen bei einer etwas höheren Dosis von 500 mg/(kg KG x d) berichtet. Diese Befunde wurden von den Studienautoren als advers eingestuft, da sie als konsistent mit präneoplastischen Schädigungen angesehen wurden.

Für eine gentoxische Wirkung von Hexylenglykol *in vitro* bestehen keine Anzeichen. Untersuchungen zur Gentoxizität *in vivo* liegen ebenso wenig vor wie Studien zur chronischen Toxizität / Kanzerogenität. Angesichts vorliegender Daten zur fehlenden Gentoxizität *in vitro* kann ein nicht-gentoxischer Mechanismus bei der Entstehung der Foci veränderter Leberzellen als plausibel betrachtet werden. Für einen derartigen nicht-gentoxischen Mechanismus kann ein Schwellenwert angegeben werden. In einer Studie mit subchronischer Exposition wurden bei 450 mg/(kg KG x d) keine derartigen Foci berichtet. Diese Dosis liegt jedoch zu nahe an derjenigen, die adverse Wirkungen verursachte. Daher wird der NOAEL von 200 mg/(kg KG x d) Hexylenglykol für Hepatotoxizität bei männlichen Ratten aus der Screeningstudie zur Reproduktions- und Entwicklungstoxizität als POD für die vorgeschlagene Ableitung eines EU-LCI herangezogen. Es wird eine Pfad-zu-Pfad-Übertragung vorgenommen. Dabei wird davon ausgegangen, dass die Resorption von Hexylenglykol bei Inhalation und oraler Aufnahme ähnlich hoch ist, sodass kein Faktor zur Berücksichtigung unterschiedlich hoher Resorptionsquoten herangezogen wird.

Die folgenden Extrapolationsfaktoren werden herangezogen:

- Pfad-zu-Pfad-Extrapolation: 1,15 m^3/(kg KG x d) (Ratte)

- Zeitextrapolation (subchronische Exposition): 2
- Allometrisches Scaling: bereits im Pfadextrapolationsfaktor enthalten
- Interspeziesextrapolation: 2,5
- Intraspeziesextrapolation (interindividuelle Variabilität, Allgemeinbevölkerung): 10

Gesamtextrapolationsfaktor: 57.5 m^3/(kg KG x d).

Als EU-LCI (gerundet) für Hexylenglykol (2-Methylpentan-2,4-diol) wird somit eine Konzentration von 3500 µg/m^3 vorgeschlagen.

Der Geruch von Hexylenglykol wurde als schwach süßlich beschrieben. Verlässliche Angaben zur Geruchsschwelle liegen nicht vor, so dass über die Geruchswahrnehmung beim EU-LCI keine Aussage getroffen werden kann.

Stoffprofil und EU-LCI-Wert für Tripropylenglykolmonomethylether (TPGME)

Tripropylenglykolmonomethylether (TGPME) wird technisch als Gemisch von Isomeren produziert, die für gewöhnlich nicht weiter aufgetrennt oder als Einzelsubstanzen vermarktet werden. Tripropylenglykolmonomethylether können in 8 unterschiedlichen Isomeren vorliegen. Die CAS Nr. 25498-49-1 bezeichnet Isomerengemisch, die CAS Nr. 20324-33-8 hingegen das Haupt-alpha-Isomer.

Tripropylenglykolmonomethylether (TPGME) ist eine klare, viskose, farblose und nahezu geruchlose Flüssigkeit mit einem sehr niedrigen Dampfdruck. Natürliche Quellen von TPGME sind nicht bekannt. Es handelt sich um ein großtechnisches Produkt. Wegen des hohen Lösevermögens für Polymere bei zugleich niedriger Verdunstungsrate wird TPGME in Tinten, Stiften und Stempelkissen zum Schutz vor Austrocknen benutzt, außerdem wird der Stoff in Reinigern und Beschichtungsmitteln eingesetzt.

Zum Vorkommen von TPGME in Innenräumen liegen so gut wie keine Daten vor. Auf der Grundlage von 615 Messungen von Proben aus unterschiedlichen Innenräumen wird als "Normalwert" (der dem Median entspricht) eine Konzentration von < 1 µg/m^3 TPGME (CAS Nr. 20324-33-8) angegeben.

Zur Aufnahme von TPGME bei inhalativer Exposition oder über andere Pfade liegen keine Angaben vor. Von Propylenglykolethern als Substanzklasse ist bekannt, dass sie inhalativ und oral rasch aufgenommen und im Körper verteilt werden. Glykolether können außerdem leicht durch die Haut aufgenommen werden, sogar im gasförmigen Zustand. Einmal aufgenommen werden sie im Körper rasch verteilt. Die Metabolisierung von Glykolethern erfolgt über zwei Hauptwege. Der erste Stoffwechselweg beinhaltet die Oxidation durch Alkoholdehydrogenase und weiterhin durch Aldehyddehydrogenase unter Bildung von Alkoxyalkansäuren. Dieser Weg erfordert eine primäre Hydroxylgruppe. Isomere, die über keine primäre, sondern über eine sekundäre Hydroxylgruppe verfügen, können auf diesem Weg nicht zu Alkoxyalkansäuren oxidiert werden, sondern nur zu den entsprechenden Ketonen, die durch andere Stoffwechselreaktionen weiter oxidiert werden. Der zweite Weg beinhaltet die Oxidation durch mikrosomale Cytochrom-P450-Monooxygenasen an der Etherbrücke unter O-Dealkylierung. Dies führt zur Bildung des betreffenden Glykols (im Falle von TPGME also Tripropylenglykol) und stellt den Hauptabbauweg für Di- und Tripropylenglykole dar. Tripropylenglykol kann in weiteren Oxidationsschritten unter oxidativer Spaltung und finaler Oxidation der Kohlenstoffkette bis zu Kohlenstoffdioxid abgebaut werden. Alternativ dazu können Propylenglykolether oder deren partielle Oxidationsprodukte konjugiert und als Glukuronide oder Sulfate über die Nieren in den Urin ausgeschieden werden. Metabolismusstudien an Ratten ergaben eine rasche Metabolisierung und Elimination der Metaboliten mit dem Urin, daneben auch von CO_2 in der Ausatemluft

Die Datenbasis zu TPGME ist sehr limitiert. Zusätzliche Informationen können jedoch aus seiner Reihe von Untersuchungen mit verschiedenen strukturell ähnlichen Glykolethern gewonnen werden.

Angaben zur Toxizität von TPGME beim Menschen liegen nicht vor. Daten aus Inhalationsstudien mit Versuchstieren weisen auf eine geringe lokale und systemische Toxizität von TPGME hin. In einer subakuten Inhalationsstudie mit Ratten und Mäusen wurden keine adversen toxischen Wirkungen beobachtet. Der einzige Effekt in dieser Untersuchung bestand in einer Erhöhung des Lebergewichts bei Ratten und Mäusen ohne jede histologische Veränderung sowie einer veränderten Anfärbung der Leberlappen von Mäusen bei der höchsten Konzentration. Diese Veränderungen wurden als adaptiver und nicht als degenerativer Prozess bewertet. Aus dieser Studie kann eine NOAEC von 1010 mg/m^3, der höchsten Testkonzentration, abgeleitet werden. Inhalationsstudien mit Dipropylenglykolmonomethylether (DPGME) an Ratten und Kaninchen und anderen, strukturell verwandten Propylenglykolethern unterstützen die Bewertung, dass die systemische Toxizität dieser Verbindungen einschließlich TPGME gering ist.

Eine begrenzte Zahl von *In-vitro*-Befunden ergibt keine Hinweise auf ein gentoxisches Potential von TPGME in Prokaryonten oder in Säugerzellen. *In-vivo*-Daten liegen zu TPGME und den meisten anderen Propylenglykolethern nicht vor.

Kanzerogenitätsstudien mit TPGME oder DPGME liegen nicht vor. Monopropylenglykolmethylether (PGME) wirkte in einer Untersuchung an Ratten nicht kanzerogen.

Fertilitätsstudien mit TPGME liegen nicht vor. In einer 2-Generationen-Studie mit Monopropylenglykolmethylether (PGME) lag der NOEL für Fertilität und reproduktionstoxische Effekte bei 1000 ppm (3710 mg/m^3). Bei dieser Konzentration traten schwache maternal toxische Wirkungen auf.

Die Entwicklungstoxizität, insbesondere die Teratogenität, stellt einen kritischen Endpunkt bei der Bewertung der Toxizität einiger Glykolether dar, die eine primäre Hydroxylgruppe und eine Methoxy- oder Ethoxyseitenkette aufweisen. Auch 2-Methoxypropan-1-ol (beta-Isomer des Propylenglykolmethylethers, ß-PGME) hat sich als wirksam erwiesen. Als ultimal entwicklungstoxisch wirksame Metaboliten werden die entsprechenden Alkoxysäuren angesehen, die bei der Oxidation der primären Hydroxylgruppe gebildet werden. Dementsprechend wird Methoxypropansäure (MPA) als aktiver Metabolit angesehen, der bei der Oxidation von ß-PGME entsteht. Es wurde festgestellt, dass Kaninchen erheblich empfindlicher reagieren als Ratten, vermutlich wegen der längeren Halbwertszeit der Elimination bei dieser Art. MPA kann prinzipiell auch beim oxidativen Abbau von Di- und Tripropylenglykolethern gebildet werden. Bei Ratten wurden nach inhalativer Exposition mit bis zu 118 ppm TPGME (1000 mg/m^3) keine entwicklungstoxischen Effekte festgestellt. Befunde aus Untersuchungen mit TPGME an Kaninchen, die als empfindlichere Spezies gelten, liegen nicht vor. Eine Studie an Kaninchen mit DPGME erbrachte jedoch keine Hinweise auf entwicklungstoxische Effekte dieser Verbindung bis zur höchsten getesteten Konzentration von 300 ppm (2728 mg/m^3). Daraus lässt sich schließen, dass die verfügbaren Daten insgesamt keinerlei Anzeichen für eine entwicklungstoxische Wirkung von TPGME liefern.

Als geeignete Schlüsselstudie zur Ableitung eines EU-LCI-Wertes für TPGME wird die subakute Inhalationsstudie eingeschätzt. Die NOAEC in dieser Studie von 1010 mg/m^3 (120 ppm) TPGME für Ratten und Mäuse dient als POD für die Ableitung. Die Studie ist zwar nicht veröffentlicht, in genügender Detaillierung jedoch im Registrierungsdossier nach REACH sowie im OECD SIDS beschrieben.

Die folgenden Extrapolationsfaktoren werden herangezogen:

- Adjustierung auf kontinuierliche Exposition (von 6 h/d, 5 d/Woche): 5,6
- Zeitextrapolation (subakute Exposition): 6
- Interspeziesextrapolation: 2,5 (Faktor für systemische Effekte bei inhalativer Exposition)
- Intraspeziesextrapolation (interindividuelle Variabilität, Allgemeinbevölkerung): 10

Gesamtextrapolationsfaktor: 840.

Als EU-LCI (gerundet) für Tripropylenglykolmonomethylether (TPGME) wird somit eine Konzentration von 1200 µg/m^3 vorgeschlagen.

Angaben zur Geruchsschwelle von TPGME liegen nicht vor. Es wird jedoch berichtet, dass TPGME nahezu geruchlos sei.

Summary

Substance profile and EU-LCI value for 2-phenylpropene

2-Phenylpropene is a clear colourless liquid with a penetrating unpleasant odour which is detectable at low concentration. 2-Phenylpropene is used in the production of acrylonitrile-butadiene-styrene (ABS) copolymers and other polymers and resins, in plasticizers in paints, waxes and adhesives.

Few data are available regarding the concentration of 2-phenylpropene in indoor air. These data indicate that exposure concentrations are generally very low. In offices, homes, preschools and schools in Germany median and maximum values were below 10 µg/m^3.

Systemic effects observed after inhalation or oral exposure show that the substance is absorbed via these pathways. However, no reliable quantitative data are available. 2-Phenylpropene is rapidly eliminated as metabolites in urine (76% within 24 h, about 90% within 72 h).

Very few data are available regarding toxic effects of 2-phenylpropene in humans. Brief acute exposure to 200 ppm (975 mg/m^3) was reported to have an unpleasant odour and to cause eye irritation, higher concentrations also caused strong nasal irritation. No irritation was noted at 50 ppm (245 mg/m^3), the odour is detectable below 1 ppm (5 mg/m^3).

There is no clear evidence of genotoxicity of 2-phenylpropene. At non-parentally toxic doses, 2-phenylpropene had no effect on reproductive and developmental parameters in a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in rats.

The critical effect of 2-phenylpropene inhalation is irritation of the nasal epithelia. In a chronic inhalation study with rats, incidences of basal cell hyperplasia were significantly increased in all exposed groups of males and females (LOAEC 100 ppm or 487 mg/m^3, no NOAEC), and the incidences of degeneration of the olfactory epithelium were increased in 1470 mg/m^3 (300 ppm) females and 4900 mg/m^3 (1000 ppm) males and females. No olfactory epithelial degeneration was observed in rats at 490 mg/m^3 (100 ppm). In the parallel study with mice, the incidences of olfactory epithelial metaplasia and hyperplasia of the glands overlying the olfactory epithelium were significantly increased in all exposed groups of males and females. In addition, atrophy of the olfactory epithelium was significantly increased in 300 and 600 ppm males. No increased incidences of neoplastic lesions were observed in the nasal epithelia of rats and mice after chronic exposure.

Mechanistic aspects indicate that the effects observed in the nasal epithelia of mice seem of less relevance for humans than the effects described in rats. Nevertheless, studies in both species indicate that the olfactory nasal epithelium is the critical target of inhalation exposure to 2-phenylpropene.

The LOAEC of 100 ppm (490 mg/m^3) 2-phenylpropene from the chronic inhalation exposure study with rats is used as the POD for the proposed derivation of a EU-LCI value.

The following adjustment factors are used:

- Adjustment for continuous exposure (6 h/d, 5 d/week): 5.6
- LOAEC→ NAEC: 3
- Adjusted study length factor (chronic exposure study): 1
- Interspecies extrapolation: 2.5
  (According to the ECA report No. 29, no correction has to be made for differences in systemic metabolism when the POD is related to local effects. For remaining uncertainties, a value of 1 is used for remaining specific differences for effects on skin, eye and GI tract if the mode of action implies only a simple destruction of membranes, and a default value of 2.5 is used for effects on the skin, eye and GI tract if local metabolism or receptor binding reactions are involved. A factor of 2.5 for 2-phenylpropene is used because metabolism is known to be involved in the toxicity of structurally related compounds and likely so for 2-phenylpropene)

- Intraspecies extrapolation (interindividual variability, general population): 10

Total assessment factor: 420.

A EU-LCI value (rounded) for 2-phenylpropene of 1200 µg/m^3 is proposed.

The proposed EU-LCI value is within the reported wide range of odour thresholds of 0.1-244 mg/m^3 (0.02-49.7 ppm). Since it is also reported that the penetrating odour may be perceived below 5 mg/m^3 (1 ppm) or at even lower concentrations of about 1.5 mg/m^3 (0.3 ppm), odour perception and annoyance cannot be excluded at the EU-LCI.

Substance profile and EU-LCI value for vinyl toluene

Vinyl toluenes are clear colourless liquids with a strong and very unpleasant odour. Vinyl toluenes are large-scale industrial products. These substances are used alone or in combination with others in the production of polymers, as adhesives, resins, surface coating and paints and in the production of other chemicals and insecticides. The CAS No. 25013-15-4 usually describes the commercial technical product containing 60-70% m-vinyl toluene and 30-40% p-vinyl toluene. If not stated otherwise, toxicity studies were performed with such mixtures.

Very few data are available regarding the occurrence of vinyl toluene in indoor air. Vinyl toluenes were not detectable in 66 measurement performed in various indoor rooms in Germany. Vinyl toluenes were detected but not quantified in homes in other investigations.

Systemic effects observed after inhalation or oral exposure show that the substance is absorbed via these pathways. However, no reliable quantitative data are available. Data on the distribution of vinyl toluenes are not available. Vinyl toluenes are rapidly eliminated as metabolites in urine. Thioethers have been identified as main metabolites, other metabolites were methylmandelic acid and related substances. Altogether, the metabolism of vinyl toluenes (methylstyrenes) is very similar to that of styrene.

Very few data are available regarding toxic effects of vinyl toluenes in humans.

Brief acute exposure to 50 ppm (245 mg/m^3) vinyl toluene were detected by the odour. At 300 ppm (1460 mg/m^3), the odour became strong and objectionable, and at ≥ 400 ppm (1950 mg/m^3), the subjects additionally noted strong eye and nasal irritation.

Animal studies indicate that the critical effect of vinyl toluene inhalation is irritation of the nasal epithelia. In a chronic inhalation study with rats, degenerative and non-neoplastic proliferative lesions were observed at all exposure concentrations. These lesions included effects in both the respiratory and the olfactory epithelium. Diffuse hyperplasia and intraepithelial mucous cysts were observed in the respiratory epithelium. In the olfactory epithelium, cysts, focal erosion, eosinophilic hyperplasia and a focal respiratory epithelial metaplasia were observed. No NOAEC could be derived from the study; the LOAEC for rats was 100 ppm (490 mg/m^3). Lesions of the nasal epithelia and in the lung were also observed in a similar study with mice with higher incidences and at much lower concentrations (LOAEC 49 mg/m^3, no NOAEC) than in rats.

There is no clear evidence of genotoxicity of vinyl toluenes. No carcinogenicity in any tissue or organ was observed in rats and mice exposed to commercial mixtures of vinyl toluenes (65-71% *meta*- and 32-35% *para*-isomer) by inhalation or to p-vinyl toluene by gavage.

Limited data from an unpublished two-generation study with oral exposure of rats to p-vinyl toluene do not indicate reprotoxic effects at doses that do not also lead to parental systemic toxicity. Similarly, studies with oral exposure of rats and rabbits do not provide convincing evidence for developmental toxicity of p-vinyl toluene.

Thus, studies in both species, mice and rats, indicate that the nasal epithelia are the critical target of inhalation exposure to vinyl toluenes. Mechanistic aspects indicate that the effects observed in the nasal epithelia of mice are of less relevance for humans than the effects described in rats. Therefore, the LOAEC of 100 ppm (490 mg/m^3) vinyl toluene (mixture of m- and p-vinyl toluene) from the chronic inhalation exposure study with rats is used as the POD for the derivation of a EU-LCI value.

The following adjustment factors are used:

- Adjustment for continuous exposure (6 h/d, 5 d/week): 5.6
- LOAEC→ NAEC: 3
- Adjusted study length factor (chronic exposure study): 1

- Interspecies extrapolation: 2.5
  (According to the ECA report No. 29, no correction has to be made for differences in systemic metabolism when the POD is related to local effects. For remaining uncertainties, a value of 1 is used for remaining specific differences for effects on skin, eye and GI tract if the mode of action implies only a simple destruction of membranes, and a default value of 2.5 is used for effects on the skin, eye and GI tract if local metabolism or receptor binding reactions are involved. A factor of 2.5 for vinyl toluenes is used because metabolism is known to be involved in the toxicity of structurally related compounds and likely so for vinyl toluenes)
- Intraspecies extrapolation (interindividual variability, general population): 10

Total assessment factor: 420.

A EU-LCI value (rounded) for vinyl toluenes of 1200 µg/m^3 is proposed.

The POD is derived from a study performed with a mixture of 65–71% *meta*-vinyl toluene and 32–35% *para*-vinyl toluene. No data from repeated inhalation studies are available for o-vinyl toluene, but the limited data base does not indicate gross differences in the toxicity of the three isomers.

The commercially used vinyl toluenes contain a mixture of m- and p-vinyl toluenes, but no or only trace amounts of the ortho-isomer. Thus, release from building products may lead to the detection of m- and p-vinyl toluene in air.

The odour of vinyl toluenes has been described as very unpleasant and disagreeable. No reliable odour thresholds are available for vinyl toluenes (methylstyrenes). Because of the structural similarity with styrene for which odour thresholds as low as 70 µg/m^3 have been reported, odour perception and annoyance from vinyl toluenes cannot be excluded at the proposed EU-LCI.

Substance profile and EU-LCI value for n-heptane

Heptane is a colourless volatile liquid with a faint gasoline-like odour. Technical heptane is a mixture of isomers. Heptane is a component of fuels. It is used as solvents in adhesives, lacquers and inks and as extractant, in the production of polymer foams and for the synthesis of toluene and other alkyl benzenes. The current production of n-heptane in the European Union is in the order of more than 1000 t/a.

Low concentrations of n-heptane and/or isomers have been determined in indoor air from homes, offices and schools (median values around 1-2 µg/m^3). More recent data indicate a reduction in the concentration of n-heptane compared to data obtained in the 1980s.

Studies with humans have shown that about 30% of n-heptane inhaled are pulmonary retained. Once absorbed, n-heptane is efficiently metabolised in the body (about 80%). The main metabolic product is 2-heptanol, besides that, other heptanols, hydroxyketones and diketones are formed. The formation of diketones is about 40fold lower than from n-hexane.

There is no evidence for genotoxic effects of n-heptane. Carcinogenicity studies with n-heptane are not available. Limited data do not provide evidence for toxicity to reproduction.

A subchronic study in rats is discussed as the basis for the derivation of a EU-LCI value. Despite conceptual limitations, the NOAEC of 3000 ppm (12510 mg/m^3, the highest concentration tested) from this study is used as POD. The study has not been published, but is described in sufficient detail in several reviews. Adjusting for continuous exposure leads to a NOAEC of 2235 mg/m^3.

The following other extrapolation factors are used:

- Adjustment for continuous exposure (6 h/d, 5 d/week): 5.6
- Adjusted study length factor (subchronic to chronic): 2
- Interspecies extrapolation: 2.5
- Intraspecies extrapolation (interindividual variability, general population): 10
- Quality of whole database (no complete histopathology and lack of studies regarding toxicity to reproduction): 3

Total assessment factor: 5.6 x 150.

A EU-LCI value for n-heptane of 15 000 µg/m^3 is proposed.

The proposed EU-LCI value is higher than a reported odour threshold of 2.8 mg/m^3; however, other authors reported considerable higher thresholds of 167-1668 mg/m^3. Therefore, perception of odour by sensitive subjects may not be excluded at the EU-LCI value.

Substance profile and EU-LCI value for hexylene glycol

Hexylene glycol (2-methyl-2,4-pentanediol) is a colourless hygroscopic liquid with a mild sweet odour. The substance has been detected as a volatile aroma and flavour component of apples. Hexylene glycol is a large-scale industrial product which is used in paints, lacquers and varnishes as a solvent plasticizer in surface coatings, both in water- and solvent-based paints. It is also used in cosmetics, in leather and textile processing and in antifreezes and as dispersant agent in cleaners, disinfectants and pesticide formulations.

Very few data are available regarding the occurrence of hexylene glycol in indoor air. It is reported that hexylene glycol was qualitatively detected as a minor volatile emission component from new carpets. The substance was detected in only one of 66 samples from various indoor rooms in Germany.

Systemic effects observed after oral and dermal exposure show that the substance is absorbed via these pathways. However, no reliable quantitative data are available. It is stated that hexylene glycol is absorbed through the mucosa of the airways and the gastrointestinal tract. Generally, saturated aliphatic glycols are known to be well absorbed by all routes of administration. Studies with humans and animals have found that about half of the orally administered dose of hexylene glycol is excreted as glucuronide in urine. Hexylene glycol was shown to be excreted into the milk of rat dams after oral administration and could be detected in mother milk-nursed pups.

Few data are available regarding toxic effects of hexylene glycol in humans. Brief inhalation exposure of volunteers to 50 ppm hexylene glycol vapour (245 mg/m^3) was reported to cause eye irritation but not irritation of the respiratory tract. There are no data available for the effects of repeated inhalation of hexylene glycol in humans. No animal studies are available with repeated inhalation exposure, except for a subacute study with insufficient documentation. In a subchronic study with oral exposure of rats, effects on the liver were restricted to an adaptive hepatocellular hypertrophy but no pathological lesions were observed up to 450 mg/(kg bw x d), the highest dose tested. However, the development of altered liver cell foci in male rats was described at a slightly higher oral dose of 500 mg/(kg bw x d) in a screening reproduction/developmental toxicity test with at least 10 weeks of oral exposure. These findings were considered by the study authors to be adverse since these changes could be consistent with pre-neoplastic lesions.

There is no evidence of genotoxicity of hexylene glycol *in vitro. In vivo* genotoxicity data are not available. Also, no carcinogenicity/chronic toxicity studies are available.

With the available data indicating no genotoxic effects of hexylene glycol, a non-genotoxic mechanism may be considered as plausible for the development of altered liver cell foci. A threshold may be defined for such a non-genotoxic mechanism. No liver cell foci were described in the subchronic study at 450 mg/(kg bw x d). However, this dose is too close to the adverse effect level. Therefore, the NOAEL of 200 mg/(kg bw x d) hexylene glycol for hepatotoxicity in male rats from the screening study for reproductive/development toxicity is used as the POD for the proposed derivation of a EU-LCI value. A route-to-route extrapolation is performed. It is assumed that the absorption of hexylene glycol by inhalation and oral exposure are similar, so that no adjustment factor for differences in absorption is taken into account.

The following adjustment factors are used:

- Route-to-route extrapolation: 1.15 m^3/(kg bw x d) (rat)
- Adjusted study length factor (subchronic exposure study): 2
- Allometric scaling: already included in route-to-route extrapolation
- Interspecies extrapolation: 2.5
- Intraspecies extrapolation (interindividual variability, general population): 10

Total assessment factor: 57.5 m^3/(kg bw x d).

A EU-LCI value (rounded) for hexylene glycol (2-methylpentane-2,4-diol) of 3500 µg/m^3 is proposed.

The odour of hexylene glycol has been described as mild sweetish. No reliable odour thresholds are available, so no conclusion can be drawn about the perception of an odour at the proposed EU-LCI.

Substance profile and EU-LCI value for tripropylene glycol monomethyl ether (TPGME)

Tripropylene glycol monomethyl ether (TGPME) is produced commercially as a mixture of isomers which normally are not further separated or marketed as individual substances. The tripropylene glycol ethers may form up to 8 isomeric forms. The CAS No. 25498-49-1 is for the isomeric mixture, while the CAS No. 20324-33-8 is for the main alpha-isomer.

Tripropylene glycol monomethyl ether (TPGME) is a clear, viscous, colourless and nearly odourless liquid with a very low vapour pressure.

TGPME has no known natural sources. It is a large-scale industrial product. Because of its high polymer solvency and low evaporation rate, TGPME is used in inks, pens and inkpads to prevent drying; it is also used in cleaners and coatings.

Hardly any data are available regarding the occurrence TPGME in indoor air. Based on 615 measurements of samples from various indoor air sources, a "normal value" (presenting the median) of $< 1\ \mu g/m^3$ for TPGME (CAS No. 20324-33-8) has been reported.

There are no data available on the absorption of TPGME after inhalation or other routes of exposure. Propylene glycol ethers as a class are known to be rapidly absorbed and distributed throughout the body when introduced by inhalation or exposure. Glycol ethers may also be well absorbed via the skin, even in the vapour state. Once absorbed, glycol ethers are readily distributed through the body.

The metabolism of glycol ethers follows two main oxidative pathways. The first pathway involves oxidation by alcohol dehydrogenase and further oxidation by aldehyde dehydrogenase with the formation of alkoxyalkanoic acids. This pathway requires a primary hydroxyl (OH) group. Isomers which do not contain a primary but a secondary free hydroxyl group cannot be oxidized via this pathway to alkoxyalkanoic acids but only to the corresponding ketones which are further oxidized by other pathways. The second pathway involves oxidation by microsomal cytochrome P450 monooxygenases at the ether bond via O-dealkylation. This leads to the production of the corresponding glycol (tripropylene glycol in case of TPGME) and is the main pathway for di- and tripropylene glycols. Tripropylene glycol may undergo further metabolism with oxidative cleavage of ether bonds and final oxidation of the carbon chain to carbon dioxide. Alternatively, propylene glycol ethers or their partially metabolised products may be conjugated with glucuronide or sulfate and excreted via the kidneys into the urine. Metabolism studies in rats revealed a rapid metabolic oxidation and elimination of the metabolites with urine and also of CO_2 in breath.

The data base for TGPME is very limited. However, additional data available from a number of studies with various structurally related glycol ethers.

No data are available on the toxicity of TPGME in humans. Limited data from inhalation studies with animals indicate a low systemic and local toxicity of TPGME. No adverse effects were observed in a subacute inhalation study with rats and mice. The only effects observed in this study were increased liver weights without any histological changes in rats and mice and altered tinctorial properties in hepatic lobules of mice at the highest concentration. These effects are considered to be an adaptive response rather than a degenerative phenomenon. Thus, a NOAEC of 1010 mg/m^3, the highest concentration tested, can be obtained from this study. Inhalation studies with dipropylene glycol monomethyl ether (DPGME) in rats and rabbits and other, structurally related propylene glycol ethers support the view that the systemic toxicity of these compounds including TPGME is low.

Limited *in vitro* data provide no evidence for genotoxic effects of TPGME in prokaryotic and mammalian assays. *In vivo* data are not available for TPGME and most other propylene glycol ethers. Carcinogenicity studies are not available for tripropylene or dipropylene glycol methyl ether. Monopropylene glycol methyl ether was not carcinogenic in a study with rats.

No data are available from fertility studies with TPGME. A two-generation reproductive toxicity study with propylene glycol methyl ether (PGME) provided a no-observed-effect-level (NOEL) for fertility and reproductive effects of 1000 ppm (3710 mg/m^3). Mild parental toxicity was noted at this concentration.

Developmental toxicity, especially teratogenicity, is a critical endpoint in the evaluation of the toxicity of some glycol ethers which contain a primary hydroxyl group and a methoxy or ethoxy side chain. 2-methoxypropan-1-ol (beta isomer of propylene glycol methyl ether, ß-PGME) was also shown to be effective. The corresponding alkoxy acids which are produced by oxidation of the primary hydroxyl group are considered the ultimate developmental toxins. Accordingly, methoxypropanoic acid (MPA) is considered the active metabolite formed by oxidation of ß-PGME. Rabbits were observed to be a species much more sensitive to these effects than rats, probably because of the long half-life of elimination in this species. MPA may also be formed by the oxidative degradation of di- and tripropylene glycol ether. No developmental toxicity was observed in rats exposed by inhalation to TPGME concentrations of up to 118 ppm (1000 mg/m^3). No data are available for TPGME from studies with rabbits which are considered more sensitive than rats. However, an inhalation study in rabbits with dipropylene glycol methyl ether (DPGME) provided no evidence for a developmental toxicity of this compound up to the highest tested concentration of 300 ppm (2728 mg/m^3). It is concluded that the available data do not provide any evidence for a developmental toxicity of TPGME.

The subacute inhalation toxicity study is considered a suitable key study for the derivation of a EU-LCI value for TPGME. The NOAEC of 1010 mg/m^3 (120 ppm) TPGME from the subacute inhalation study with rats and mice is used as the POD for the calculation. The study is not published but described in sufficient detail in the REACH registration dossier and the OECD SIDS.

The following adjustment factors are used:

- Adjustment for continuous exposure (6 h/d, 5 d/week): 5.6
- Adjusted study length factor (subacute exposure study): 6
- Interspecies extrapolation: 2.5 (factor for systemic effects at inhalation exposure)
- Intraspecies extrapolation (interindividual variability, general population): 10

Total assessment factor: 840.

A EU-LCI value (rounded) for TPGME of 1200 µg/m^3 is proposed.

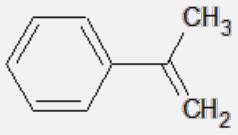
Data on odour thresholds are not available. However, TPGME is reported to be nearly odourless.

1 Toxicological evaluation of 2-phenylpropene as basis for the derivation of a EU-LCI value

1.1 Substance identification

Substance identification data and physicochemical properties are shown in Table 1.1 and Table 1.2.

Table 1.1: Substance identification of 2-phenylpropene

CAS-No. EU-No. CLP-Index-No.	Systematic Name, common names	Summary formula	Structural formula
98-83-9 202-705-0 601-027-00-6	2-Phenylpropene, α-methylstyrene, 1-methylethenylbenzene, isopropenyl benzene	C_9H_{10}	

1.2 Substance properties and uses

2-Phenylpropene is a clear colourless liquid with a penetrating unpleasant odour which is detectable at low concentrations (SCOEL, 1995). Further data regarding the odour are reported in chapter 1.5.5. 2-Phenylpropene is nearly insoluble in water but is soluble in most organic solvents. Commercially available 2-phenylpropene may be stabilised with *tert*-butyl catechol to inhibit oxidation and polymerization during storage (NTP, 2007).

Table 1.2: Physicochemical properties of 2-phenylpropene (ECHA Dissemination, 2018)

Molar mass (g/mol)	Mp. (° C)	Boiling point (° C)	Vapour pressure (hPa)	Conversion 1 ppm = x mg/m³ (23 °C)	log pow (at pH)	Solubility in water (mg/L)
118.2	-23.2	165.4	2.53 (20 °C)	4.87	3.48	100

2-Phenylpropene has no known natural sources. It is used as a large-scale industrial product (tonnage band in the EU: 100000 – 1000000 t/a). It is used in the production of acrylonitrile-butadiene-styrene (ABS) copolymers and other polymers and resins, in plasticizers in paints, waxes and adhesives (ECHA Dissemination, 2018; NTP, 2007).

1.3 Exposure

1.3.1 Indoor air

Few data are available regarding the concentration of 2-phenylpropene in indoor air (Table 1.3). These data indicate that exposure concentrations are generally very low. In offices, homes, preschools and schools in Germany median and maximum values were below 10 µg/m^3.

Table 1.3: Data on the occurrence of 2-phenylpropene in indoor air from homes, schools, children day care centres and offices

Rooms	N	LoD (µg/m³)	N > LoD (% > LoD)	Median (µg/m³)	P95 (µg/m³)	Maximum (µg/m³)	Reference
Houses in Finland (no further data)	26	n. r.	8	n. r.	n. r.	n. r.	(HSDB, 2005)
Offices, homes, (pre)-schools, Germany	441	4	3	2.0	2.0	5	(Hofmann and Plieninger, 2008)

1.3.2 Other sources

Very few data are available. Regarding the migration of 2-phenylpropene from ABS into food, a migration of about 5-14 µg/kg food was observed in test cells (Anon., 2004).

Headspace analyses from copier toner cartridges showed concentrations of 22-33 ng 2-phenylpropene/ml in the air taken from emission test chambers. The emission rate of 2-phenylpropene from dry-process photocopiers ranged from <10-24 ug/hr per copier while idle and <50-330 ug/hr per copier during operation. 2-Phenylpropene or an isomer was detected but not quantified in 2 of 12 breast milk samples. It was identified in the 1980s in 2 of 14 treated water supplies in England and in a groundwater aquifer which served as the drinking water supply for Milan, Italy, at a depth of 30 m beneath a paint factory where organics were stored in leaking tanks (HSDB, 2005).

1.4 Toxicokinetics

Systemic effects observed after inhalation or oral exposure show that the substance is absorbed via these pathways. However, no reliable quantitative data are available. It was reported that about two thirds of the vapour was retained in the respiratory tract of humans during an 8 h inhalation and that 2-phenyl lactic acid (atrolactic acid) was found as metabolite in urine. However, the documentation is insufficient for assessment (no data about exposure concentrations, number of volunteers and applied method) (ECHA Dissemination, 2018). For structurally related compounds (C_9-C_{15} alkylbenzenes) uptake of 50-70% was reported in studies with humans (Ad-hoc AG, 2012).

The distribution, metabolism, and excretion of 2-phenylpropene was studied in male F344 rats after intravenous administration of ^{14}C-labelled 2-phenylpropene (11 mg/kg bw) and also from limited additional experiments with inhalation (300 or 900 ppm, 6 h, nose only) and oral exposure (1000 mg/kg bw) (De Costa et al., 2001; DFG, 2004; ECHA Dissemination, 2018; NICNAS, 2017). 2-Phenylpropene was rapidly eliminated. 72 h after administration, only 0.3% of the administered activity was still present in tissues, the highest values were observed in spleen, followed by kidney, bladder, lung, liver, heart and skin and adipose tissue. The lowest values were found in muscle, testis and brain. The elimination half-life of 2-phenylpropene was calculated to be 3-5 h after inhalation exposure (De Costa et al., 2001). Depletion of hepatic glutathione was observed in another study after inhalation exposure of rats to 2-phenylpropene (Morgan et al., 1999). The excretion of mercapturic acids (see below) is in line with this observation. About 76 % of the administered activity was excreted in urine within 24 h and about 90% in 72 h, only small amounts were excreted in faeces and breath (1-3%). The main metabolites found in blood were 2-phenyl-1,2-propanediol and 2-phenylpropanoic acid. The main metabolites in urine were identified as 2-phenyl-1,2-propanediol (3%) and its glucuronide (50 %), 2-phenyl lactic acid (27%), S-(2-hydroxy-2-phenylpropyl)-N-acetylcysteine (13%) and 2-phenylpropanoic acid (1%) (De Costa et al., 2001).

The presence of both of the diastereomeric isomers of the mercapturates and of the glucuronides suggested that the initial epoxidation of 2-phenylpropene is not stereoselective. Incubation of 2-phenyl-

propene with human liver slices produced the same metabolites as those excreted in rat urine, with 2-phenyl-1,2-propanediol present as the predominant metabolite (De Costa et al., 2001).

1.5 Health effects

1.5.1 Sensory irritation and local effects

In a study with human volunteers, the subjects were briefly exposed (no details reported) to 2-phenylpropene in a test room and noted their reactions with respect to odour, eye irritation, and nasal irritation. No response to the odour was noted by the subjects below 10 ppm (49 mg/m^3). At 50 ppm (245 mg/m^3), the odour was detectable, but no mucous membrane irritation was noted by the subjects. At 100 ppm (490 mg/m^3), the odour was strong, but considered as tolerable, at 200 ppm (975 mg/m^3), the odour became strong and objectionable, and slight eye irritation was noted. At ≥ 600 ppm (2920 mg/m^3), the subjects noted a very strong odour and strong eye and nasal irritation (Wolf et al., 1956).

An RD_{50} of 273 ppm (1330 mg/m^3) is reported for Swiss mice (no details available) (DFG, 2004).

1.5.2 Repeated dose toxicity

In a repeated exposure inhalation study, 10-25 rats, 5-10 guinea pigs, 1-2 rabbits and 1-2 monkeys were exposed 7 h/d, 5 d/week for up to seven months against 200 or 600 ppm 2-phenylpropene (974 mg/m^3 or 2920 mg/m^3). Rats and guinea pigs were additionally exposed to 800 ppm and 3000 ppm (3900 mg/m^3 and 14600 mg/m^3) (Wolf et al., 1956). Lethality was observed in rats at 3000 ppm and in guinea pigs at 600 ppm. Adverse effects, e. g. reduced weight gain and increased liver- and kidney weight, was observed at 600 and 800 ppm. 200 ppm was reported to present a NOAEC. No details about histological examinations were presented.

F344 rats (5 M + 5 F/concentration) were exposed to 0, 600 or 1000 ppm 2-phenylpropene (0, 2920, 4870 mg/m^3) 6 h/d, 5 d/week for two weeks (12 exposures). No mortality was observed. The relative liver weight was significantly increased in males and females at both concentrations. Males showed an accumulation of hyaline droplets in the renal tubuli at both concentrations. There were no histopathological changes in other organs (including nose, lung, spleen and liver) (Morgan et al., 1999).

In a subsequent study specifically aimed at renal effects, rats (4 male and female F344 and male NBR) were exposed to 0, 125, 250 or 500 ppm 2-phenylpropene (0, 610, 1220, 2440 mg/m^3) for 6 h/d, 5 d/week for 9 days. Hyaline droplet formation was confirmed at concentrations of ≥ 250 ppm in male F344 rats but was not observed in female F344 and male NBR rats which are α2u-globulin deficient (Morgan et al., 1999).

In B6C3F1 mice (18 M + 18 F/concentration) exposed to 0, 600, 800 or 1000 ppm 2-phenylpropene (0, 2920, 3900, 4870 mg/m^3) for 6 h/d, 5 d/weeks, 12 d, a concentration-dependent increase of initial mortality was observed within the first 3-4 d in females. Relative spleen weight decreased, relative liver weight increased and hepatic glutathione concentration decreased in both sexes at all concentrations. Histopathological changes were not observed in any organ. No mortality or organ weight changes occurred in a separate experiment with exposure up to 500 ppm (2440 mg/m^3) (Morgan et al., 1999).

In a subchronic NTP-study with F344 rats, 10 M + 10 F/concentration were exposed by whole-body inhalation to 2-phenylpropene at concentrations of 0, 75, 150, 300, 600, or 1000 ppm (0, 360, 725, 1450, 2900, 4840 mg/m^3) for 6 h/d, 5 d/week for 14 weeks (NTP, 2007). Additional clinical pathology groups of 10 male and 10 female rats were exposed to the same concentrations for 23 days. No mortality was observed, and mean body weights of all exposed groups were similar to those of the controls. Kidney weights were significantly increased in 1000 ppm males and 600 and 1,000 ppm females. Statistically significant increases in liver weights occurred at ≥ 150 ppm in males and at ≥ 600 ppm in females. The incidences of renal hyaline droplet accumulation were similar between exposed groups

and control, but the severity of hyaline droplet accumulation in males was greater at ≥ 600ppm. Consistent with the hyaline droplet accumulation, an exposure-related increase in α2u-globulin was detected in the kidneys of males exposed to 2-phenylpropene. No morphologic changes were detected in the liver.

In the subchronic NTP-study with B6C3F1 mice, 10 M + 10 F/concentration were exposed by whole-body inhalation 0, 75, 150, 300, 600, or 1000 ppm 2-phenylpropene (0, 360, 725, 1450, 2900, 4840 mg/m^3) for 6 h/d, 5 d/week for 14 weeks (NTP, 2007). Two female mice in the 1000 ppm group died after two exposures. Final mean body weights of males at ≥ 600 ppm males and 75, 300, and 1000 ppm females were significantly less than those of the controls; body weight gains of mice exposed to ≥ 300 ppm were also significantly less. The highest concentration led to sedation and ataxia. The absolute liver weights of females exposed to ≥ 600 ppm and the relative liver weights of males at ≥ 300 ppm were significantly increased. The oestrous cycle lengths of females at ≥ 600 ppm females were significantly longer than that of the controls. Minimal to mild centrilobular hypertrophy was present in the livers of both sexes at ≥ 600 ppm. The incidences of exposure-related nasal lesions, including atrophy and hyperplasia of Bowman's glands and atrophy and metaplasia of the olfactory epithelium, were significantly increased in all exposed groups of both sexes. Eosinophilic globules in the cytoplasm of the respiratory epithelium were significantly increased in females exposed to ≥ 150 ppm.

Non-carcinogenic and carcinogenic effects of chronic exposure to 2-phenylpropene are described in the following chapter 1.5.3 (see "carcinogenicity").

The results of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test with oral administration of the test substance are reported in chapter 1.5.4.

1.5.3 Genotoxicity and carcinogenicity

Genotoxicity

In vitro, 2-phenylpropene was not mutagenic in several bacterial mutation assays tested at up to cytotoxic concentrations with and without exogenous metabolic activation system (S9 mix from rat liver) in all tested strains of *Salmonella typhimurium* (TA97, TA98, TA100, TA1535, TA1537, TA1538) and in *Escherichia coli* WP2uvr A. In mammalian cells, the substance did not induce mutations in a HPRT assay (in Chinese Hamster Ovary Cells, CHO) or chromosomal aberrations (in Chinese Hamster Lung and CHO Cells) with and without metabolic activation. 2-phenylpropene induced sister chromatid exchanges (SCE) in CHO cells in the presence but not in the absence of metabolic activation. A further study in human lymphocytes showed a weakly positive result (increase of SCE less than twofold) (ECHA Dissemination, 2018).

In vivo, inhalation exposure of B6C3F1 mice (10 M + 10 F/concentration) with up to 1000 ppm (4900 mg/m^3) 2-phenylpropen for 6 h/d, 5 d/week for 13 weeks did not induce micronuclei in erythrocytes in peripheral blood 24 after the last exposure. A significant increase in micronucleated normochromatic erythrocytes (NCE) was observed in female mice at the highest exposure concentration. 2 of the 10 female mice died at this concentration indicating that the exposure was in the lethally toxic range. No increase in micronucleated polychromatic immature erythrocytes (PCE) was observed in males or females, indicating that the observed effect in NCE was reflective of long-term accumulation of damage and was not detectable immediately after exposure by analysing PCE (ECHA Dissemination, 2018; NTP, 2007). Further *in vivo* genotoxicity studies are not available.

Carcinogenicity

A carcinogenicity study was conducted with F344 rats (NTP, 2007). The animals (50 M + 50 F/concentration) were exposed to 0, 100, 300 or 1000 ppm 2-phenylpropene (0, 487, 1460 or 4870 mg/m^3) by inhalation for 6 h/d, 5 d/week for a total of 105 weeks. Survival rates of exposed animals were similar to those of the control group. The mean body weights of 1000 ppm males and females were 5% to 10% less than those of the control groups during the second year of the study. No clinical

symptoms related to exposure were observed. The incidence of neoplasms of the kidney showed a concentration-dependent increase: Two males at 1000 ppm and one at 600 ppm had renal tubule carcinomas, and one additional male at 300 ppm had a renal tubule adenoma. Because of the observed neoplasms after chronic exposure and the hyaline droplet nephropathy with α2u-globulin accumulation at the end of the subchronic study, and the known association between both, additional kidney step sections were performed. The incidences of renal tubule adenoma and carcinoma combined in the 1000 ppm males were significantly greater than those in the controls when the single and step sections were combined. Additionally, the incidence of mineralization of the renal papilla was significantly increased in 1000 ppm males.

The incidence of mononuclear cell leukemia was significantly increased in males at 1000 ppm (38/50) compared to the control group (26/50). The increase was slightly above the range observed in historical controls (32-66%). It was concluded that there was "some evidence of carcinogenic activity" in male rats based on the increased incidences of renal tumours, and that the increased incidence of leukemia in male rats may have been related to the exposure. There was no evidence of carcinogenic activity in female rats (NTP, 2007).

Non-neoplastic effects were observed in the nasal olfactory epithelium of the exposed animals. The incidences of basal cell hyperplasia of the olfactory epithelium were significantly increased in all exposed groups of males and females, and the incidences of degeneration of the olfactory epithelium were increased in 1470 mg/m^3 (300 ppm) females and 4900 mg/m^3 (1000 ppm) males and females. No significantly increased olfactory epithelial degeneration was observed in rats at 490 mg/m^3 (100 ppm). The observed lesions were rated as minimal (degree 1) to less than mild (2) on a scale ranging from 1 to 4. No exposure-related effects were observed in the nasal respiratory epithelium or in other organs and tissues of exposed rats (NTP, 2007). No NOAEC for non-neoplastic effects can be derived from this study; the LOAEC is 100 ppm (487 mg/m^3).

Table 1.4: Incidence of non-neoplastic lesions in the nasal epithelia of rats after chronic inhalation of 2 phenylpropene (NTP, 2007)

Organ/ effect	Concentration of 2-phenylpropene (ppm)			
	0	100	300	1000
Males				
Nose, olfactory epithelium				
hyperplasia of basal cells	0/50	17/50	18/50	43/49
degeneration	1/50	3/50	3/50	16/50
Females				
Nose, olfactory epithelium				
hyperplasia of basal cells	0/49	14/49	30/50	49/50
degeneration	1/49	1/49	7/50	24/50

In the correspondent study with BFC3F1 mice (50 M + 50 F/concentration), the animals were exposed to 0, 100, 300 or 600 ppm 2-phenylpropene (0, 487, 1460 or 2920 mg/m^3) by inhalation for 6 h/d, 5 d/week for a total of 105 weeks (NTP, 2007). Survival rates of exposed animals were similar to those of the control group. The mean body weight of males at 600 ppm was less than that of the control group throughout the study, and that of 600 ppm females was less after week 13. No clinical findings related to chemical exposure were observed. The incidences of hepatocellular adenoma or carcinoma (combined) were significantly increased in the 100 and 600 ppm males (control: 28/50, 100 ppm: 36/50, 300 ppm: 33/50, 600 ppm: 37/50) and in all exposed groups of females (control: 13/50, 100 ppm: 26/50, 300 ppm: 24/50, 600 ppm: 33/50). Females also showed increased incidences of hepatocellular adenoma in all exposed groups and of hepatocellular carcinoma at 600 ppm. The incidence of

eosinophilic foci was significantly increased in 600 ppm females. It was concluded that there was "equivocal evidence of carcinogenic activity" in male mice and "clear evidence of carcinogenic activity" in female mice, each based on the incidences of hepatocellular adenomas and carcinomas (NTP, 2007).

Non-neoplastic effects were observed the kidney of female mice. At 600 ppm, the incidence and the severity of nephropathy were increased. Generally, nephropathy is a spontaneously occurring lesion in this strain of mice, but the higher incidence in 600 ppm females was considered related to 2-phenylpropene. A higher incidence of forestomach epithelial hyperplasia and inflammation was observed in exposed male rats, the effects were significant at ≥ 300 ppm for hyperplasia and at 600 ppm for inflammation.

As in rats, non-neoplastic effects were observed in the nasal olfactory epithelium of the exposed animals. The incidences of olfactory epithelial meta- and hyperplasia were significantly increased in all exposed groups of males and females. Atrophy of the olfactory epithelium was also observed, but the increase was only significant in males at ≥ 300 ppm (Table 1.5). The severity of the lesions increased with concentration and was rated as minimal (degree 1) to moderate (2) on a scale ranging from 1 to 4. No exposure-related effects was observed in the nasal respiratory epithelium of exposed mice (NTP, 2007). No NOAEC for non-neoplastic effects can be derived from this study; the LOAEC is 100 ppm (487 mg/m^3).

Table 1.5: Incidence of non-neoplastic lesions in the nasal epithelia of mice after chronic inhalation of 2 phenylpropene (NTP, 2007)

Organ/ effect	Concentration of 2-phenylpropene (ppm)			
	0	100	300	1000
Males				
Nose, olfactory epithelium				
metaplasia	6/50	47/50	49/50	49/50
hyperplasia of glands	4/50	50/50	50/50	50/50
atrophy	0/50	2/50	8/50	12/50
Females				
Nose, olfactory epithelium				
metaplasia	2/49	49/49	47/50	50/50
hyperplasia of glands	3/49	49/49	50/50	50/50
atrophy	1/49	6/49	4/50	3/50

1.5.4 Toxicity to reproduction

A combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (according to OECD Guideline 422) was performed with Sprague-Dawley rats (ECHA Dissemination, 2018). The animals (10 M + 10 F/Dose) were exposed to 0, 40, 200 or 1000 mg/(kg bw x d) of 2-phenylpropene in olive oil by gavage from 14 d prior to mating for a total of 43 d (M) or (F), including the mating period, until day 3 of lactation. Parental toxicity was observed at the highest dose: Male and female rats showed a suppression of body weight gain and a decrease in food consumption, one animal died. Increases of liver and kidney sizes, acidophilic change of hepatocytes and increase of fatty droplets in the fascicular zone of the adrenals were observed in both sexes, increase of hyaline droplets and basophilic change in the renal tubular epithelium, formation of urinary calculi and hyperplasia of the mucosal epithelium occurred in the urinary bladder in male rats, and vacuolation and infiltration of lymphocytes in the renal tubular epithelium and atrophy of the thymus occurred in female rats. Clinical chemistry revealed increases in GPT, urea nitrogen and potassium, and a decrease in triglycer-

ide in male rats. At 200 mg/(kg bw x d) , similar histological changes were found in the liver and kidneys of both sexes, and the thymus of female rats, and an increase in GPT was observed in male rats.

The test substance had no effects on any reproductive parameter. However, two dams at the highest dose lost all their offspring during the lactation period. Neonates from the highest dose group showed a decrease of body weight and a slightly lower viability index on PND 4 due to the total litter losses of the two dams. No significant differences in other developmental parameters or on clinical signs, body weight gain after birth or at necropsy were observed in the offspring.

A LOAEL for repeated dose toxicity of 200 mg/(kg bw x d) can be derived from this study (NOAEL 40 mg/(kg bw x d)). For reproductive and developmental toxicity, a LOAEL of 1000 mg/(kg bw x d) can be derived (NOAEL 200 mg/(kg bw x d)).

1.5.5 Odour perception

The odour of 2-phenylpropene has been described as penetrating and displeasing. It is reported that the odour is detectable below 5 mg/m^3 (1 ppm) (SCOEL, 1995) or at even lower concentrations of about 1.5 mg/m^3 (0.3 ppm) (DFG, 1997). Overall, the reported odour threshold concentrations cover a wide range of 0.1-244 mg/m^3 (0.02-49.7 ppm) (AIHA, 2013).

1.5.6 Mechanistic aspects and structure-activity relationships

A comparison of the effects observed in mice and rats indicates species-specific differences in the toxicity of 2-phenylpropene, i.e. a higher mortality and more pronounced lesions in the nasal epithelia of mice compared to rats. Mechanistic aspects underlying these differences have been summarised by the German MAK-Commission (DFG, 2004).

The metabolism of 2-phenylpropene (= α-methylstyrene) proceeds in a way similar to that of styrene, and a similar species difference in sensitivity of the nasal epithelium of mice and rats was also observed for styrene. Both rats and mice oxidize styrene to styrene epoxide, which is considered the toxic metabolite causing the nasal lesions. However, rats are able to detoxify the epoxide more rapidly than mice (via epoxide hydrolase and GSH-transferase). *In vitro* data suggest that human nasal tissue has a low capacity to oxidize styrene but contains epoxide hydrolase and GSH-transferase activity. It was concluded that styrene has low toxicity to human nasal epithelia and, by analogy, that the effects observed in the nasal epithelia of mice after exposure to 2-phenylpropene seem of little relevance for humans (DFG, 2004). Similar conclusions were also presented in the evaluation of the toxicity of alkylbenzenes and related compounds including 2-phenylpropene (Ad-hoc AG, 2012).

Some aromatic solvents are known to cause ototoxicity in rats. A comparison of the ototoxicity of 21 different compounds was performed by Gagnaire and Langlais (2005). Sprague-Dawley rats were exposed by gavage to 8.47 mmol/(kg bw x d) of the test compound for 5 d/week for 2 weeks (i.e. 1000 mg 2-phenylpropene/(kg bw x d)). Eight compounds including 2-phenylpropene and styrene showed some ototoxicity as seen in morphological investigations of the cochlea. However, 2-phenylpropene was the least active compound tested in this assay.

1.6 Evaluation

1.6.1 Existing regulations and classifications

2-Phenylpropene is classified in the EU with respect to its toxicity as eye irritant (H319) and as respiratory irritant (H335), but not as mutagenic, carcinogenic or toxic to reproduction (ECHA C&L Inventory, 2018).

General population

A DNEL of 41 mg/m^3 (8.4 ppm) is reported in the REACH registration dossier for 2-phenylpropene (ECHA Dissemination, 2018). A total extrapolation factor is reported to have been used, but no details are presented.

Table 1.6: Guide values for 2-phenylpropene in indoor air (for explanation, see text)

Guidance value Parameter/ Organisation	ECHA Registered Substances (2017)	SCOEL (1995)
Name (reference period)	DNEL (chronic)	OEL (8-h TWA) (workers)
Value (mg/m³)	41 (8.4 ppm)	246 (50 ppm)
Organ/critical effect	Not indicated	Irritation (eye and respiratory tract), systemic toxicity
Species	Not indicated	Human, several animal species
Basis	Not indicated	NOAEC 50 ppm
Adjusted for cont. exposure	Not indicated	Not performed
Extrapolation factors **Time** **LOAEC to NOAEC** **Interspecies** **Intraspecies** **Total**	2	1

Workplace

An OEL of 246 mg/m^3 was derived by SCOEL (1995). This value is based on an "overall approach", taking into account a NOAEC for eye irritation reported in an acute study with humans and a NOAEC of 984 mg/m^3 (200 ppm) for systemic toxicity in several animal species in the same publication (Wolf et al., 1956), and also by analogy with styrene (SCOEL, 1995).

The OEL for 2-phenylpropene in Germany is also set to 50 ppm (250 mg/m^3). This value corresponds to the OEL derived by SCOEL and the same values for the maximum allowable concentration derived by the MAK-commission (AGS, 2018). The MAK-value is based on the NOAEC of 75 ppm from a sub-chronic NTP-study in rats, also taking into account the data from the same acute study in humans as SCOEL (DFG, 2004). The same value of 50 ppm has also been derived for OEL in most other European countries except France (25 ppm) and Sweden (20 ppm) (IFA, 2018).

1.6.2 Derivation of a EU-LCI value

Very few data are available regarding toxic effects of 2-phenylpropene in humans. Brief acute exposure to 200 ppm (975 mg/m^3) was reported to have an unpleasant odour and to cause eye irritation, higher concentrations also caused strong nasal irritation (Wolf et al., 1956). No irritation was noted at 50 ppm (245 mg/m^3), the odour is detectable below 1 ppm (5 mg/m^3).

Accumulation of hyaline droplets in renal tubules, renal tubule lesions and, after chronic exposure, an increased incidence of renal tubule tumours were observed in male F344 rats exposed to 2-phenylpropene (Morgan et al., 1999; NTP, 2007). This effect is related to the α2u-globulin associated nephropathy in male rats and is not relevant for risk assessment in humans (Swenberg and Lehman-McKeeman, 1999). Similarly, the observed increased incidence of hepatocellular tumours in the used strain of mice is generally considered not relevant for risk assessment.

There is no clear evidence of genotoxicity of 2-phenylpropene. At non-parentally toxic doses, 2-phenylpropene had no effect on reproductive and developmental parameters in a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in rats (ECHA Dissemination, 2018).

The critical effect of 2-phenylpropene inhalation is irritation of the nasal epithelia. In a chronic inhalation study with rats, incidences of basal cell hyperplasia were significantly increased in all exposed

groups of males and females (LOAEC 100 ppm or 487 mg/m³, no NOAEC), and the incidences of degeneration of the olfactory epithelium were increased in 1470 mg/m³ (300 ppm) females and 4900 mg/m³ (1000 ppm) males and females. No olfactory epithelial degeneration was observed in rats at 490 mg/m³ (100 ppm) (NTP, 2007). In the parallel study with mice, the incidences of olfactory epithelial metaplasia and hyperplasia of the glands overlying the olfactory epithelium were significantly increased in all exposed groups of males and females. In addition, atrophy of the olfactory epithelium was significantly increased in 300 and 600 ppm males. Increased incidences of exposure-related nasal lesions, including atrophy and hyperplasia of Bowman's glands and atrophy and metaplasia of the olfactory epithelium, were also observed in all exposed groups of male and female mice after subchronic inhalation for 14 weeks (LOAEC: 368 mg/m³, 75 ppm). No nasal epithelial lesions were observed in rats after subchronic inhalation at concentrations ranging from 368 to 4900 mg/m³ (75 -1000 ppm). No increased incidences of neoplastic lesions were observed in the nasal epithelia of rats and mice after chronic exposure (NTP, 2007).

Mechanistic aspects as described in chapter 1.5.6 indicate that the effects observed in the nasal epithelia of mice seem of less relevance for humans than the effects described in rats. Nevertheless, studies in both species indicate that the olfactory nasal epithelium is the critical target of inhalation exposure to 2-phenylpropene.

The LOAEC of 100 ppm (490 mg/m³) 2-phenylpropene from the chronic inhalation exposure study with rats is used as the POD for the proposed derivation of a EU-LCI value (see Table 1.7).

Table 1.7: Derivation of a EU-LCI value for 2-phenylpropene (for explanation, see text)

Endpoint	POD (mg/m³)	Adjustment factor				Value (mg/m³)	Reference
		LOAEC → NAEC	Time	Interspecies	Intraspecies		
Local toxicity (nasal epithelium)	LOAEC: 490	3	1	2.5	10	1.17	(NTP, 2007)

The following adjustment factors are used:

- Adjustment for continuous exposure (6 h/d, 5 d/week): 5.6
- LOAEC→ NAEC: 3
- Adjusted study length factor (chronic exposure study): 1
- Interspecies extrapolation: 2.5
  (According to the ECA report No. 29 (EC, 2013), no correction has to be made for differences in systemic metabolism when the POD is related to local effects. For remaining uncertainties, a value of 1 is used for remaining specific differences for effects on skin, eye and GI tract if the mode of action implies only a simple destruction of membranes, and a default value of 2.5 is used for effects on the skin, eye and GI tract if local metabolism or receptor binding reactions are involved. A factor of 2.5 for 2-phenylpropene is used because metabolism is known to be involved in the toxicity of structurally related compounds (Ad-hoc AG, 2012; DFG, 2004) and likely so for 2-phenylpropene)
- Intraspecies extrapolation (interindividual variability, general population): 10

Total assessment factor: 420. This leads to a concentration of 490 mg/m³: 420 = 1.17 mg/m³.

A EU-LCI value (rounded) for 2-phenylpropene of 1200 µg/m³ is proposed.

The proposed EU-LCI value is within the reported wide range of odour thresholds of 0.1-244 mg/m³ (0.02-49.7 ppm) (AIHA, 2013). Since it is also reported that the penetrating odour may be perceived below 5 mg/m³ (1 ppm) (SCOEL, 1995) or at even lower concentrations of about 1.5 mg/m³ (0.3 ppm) (DFG, 1997), odour perception and annoyance cannot be excluded at the proposed EU-LCI.

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1.8 Fact and data sheet for 2-phenylpropene

Table 1.8: Data collection sheet 2-phenylpropene (α-methylstyrene)

Rapporteur, Date		
Compound	**2-PHENYLPROPENE**	***Data collection sheet***
N° CAS 98-83-9 1 ppm = 4.9 mg/m³	**EU-Classification:** **CLP:** Flam. Liq. 3 (H226); Eye Irrit. 2 (H319); STOT SE 3 (H335); Aquatic Chronic 2 (H411)	
Organization Name	SCOEL	Reach registrants
Risk Value Name	OEL (8-h TWA)	DNEL
Risk Value (mg/m³)	246 (50 ppm)	41 (8.5 ppm)
Reference period	Chronic (worker)	Chronic
Risk Value (mg/m³) Short Term (15 min)	492 (100 ppm)	-
Year	1995	2018
Key Study	Wolf et al. (1956)	Not reported
Study type	Exposure chamber study	
Species	Human	
Duration of exposure in key study	"brief exposures"	
Critical effect	Irritation (eye and respiratory tract)	Not reported
Critical dose value	NOAEC 246 mg/m³ (50 ppm)	Not reported
Adjusted critical dose	No dose adjustment (conc. dependent local effect)	Not reported
Single Assessment factors	No factors used	Not reported, total factor: 2
Other effects	Data from 6 month inhalation exposure study with rats, guinea pigs rabbits and monkeys indicating a NO-AEC of 984 mg/m³ (200 ppm) (Wolf et al., 1956) and analogy of irritation and odour effects with styrene were taken into account	
Remarks		
UF_L Used LOAEL; UF_H Intraspecies variability; UF_A interspecies variability; UF_S Used subchronic study UF_D data deficiencies		

Table 1.9: Fact sheet 2-Phenylpropene (α-methylstyrene)

Rapporteur, Date			
Compound	**2-PHENYLPROPENE**		***Factsheet***
Parameter	**Note**	Comments	Value / descriptor
EU-LCI Value and Status			
EU-LCI value	1	Mass/volume [µg/m³]	1200
EU-LCI status	2	Draft/Final	Draft
EU-LCI year of issue	3	Year when EU-LCI value has been issued	2018
General Information			
CLP-Index No.	4	INDEX	601-027-00-6
EC-No.	5	EINECS	202-705-0
CAS-No.	6	Chemical Abstract Service number	98-83-9
Harmonised CLP classification	7	Human health risk related classification	Eye Irrit. 2 (H319); STOT SE 3 (H335)
Molar mass and conversion factor	8	[g/mol] and [ppm – mg/m³]	118.2 1 ppm = 4.9 mg/m³
Key Data / Database			
Key study, Authors, Year	9	Critical study with lowest relevant effect level	NTP (2007)
Read across compound	10	Where applicable	
Species	11	Rat	Sprague-Dawley rats and B6C3F1 mice
Route / type of study	12	Inhalation, oral feed….	Inhalation
Study length	13	Days, subchronic, chronic…	Chronic (2 years)
Exposure duration	14	h/d, d/w	6 h/d, 5 d/week
Critical endpoint	15	Effect (s), site of	Lesions of nasal olfactory epithelium
Point of Departure (POD)	16	LOAEC, NOAEC, BMD…	LOAEC
POD value	17	[mg/m³] or [ppm] or [mg/kg_{BW}×d]	490 mg/m³ (100 ppm)
Assessment Factors (AF)	18		
Adjustment for exposure duration	19	Study exposure h/d, d/w	5.6
AF study length	20	sa→sc→c	1
Route-to-route extrapolation factor	21		1
AF Dose-response	22a	Reliability of dose-response, LOAEL to NOAEL	3
	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		1
Result			
Summary of assessment factors	27	Total Assessment Factor	420
POD/TAF	28	Calculated value [µg/m³ and ppb]	1166 µg/m³ (240 ppb)
Molar adjustment factor	29		
Rounded value	30	[µg/m³]	1200
Additional comments	31		
Rationale Section	32		

Rationale for critical effects

In a study with human volunteers, brief exposures to a concentration of 975 mg/m³ (200 ppm) was reported to have an unpleasant odour and to cause eye irritation, higher concentrations also caused strong nasal irritation. No irritation was reported at 245 mg/m³ (50 ppm), but the odour is detectable below 5 mg/m³ (1 ppm).

The critical effect of 2-phenylpropene inhalation is respiratory tract irritation. In a chronic inhalation study with rats (50 M + 50 F/concentration, 0, 100, 300 or 1000 ppm 2-phenylpropene (0, 487, 1460 or 4870 mg/m³), 6 h/d, 5 d/week for a total of 105 weeks), incidences of basal cell hyperplasia were significantly increased in all exposed groups of males and females, and the incidences of degeneration of the olfactory epithelium were increased in 1470 mg/m³ (300 ppm) females and 4900 mg/m³ (1000 ppm) males and females. No olfactory epithelial degeneration was observed in rats at 490 mg/m³ (100 ppm). In the parallel study with mice, the incidences of olfactory epithelial metaplasia and hyperplasia of the glands overlying the olfactory epithelium were significantly increased in all exposed groups of males and females. In addition, atrophy of the olfactory epithelium was significantly increased in 300 and 600 ppm males. Increased incidences of exposure-related nasal lesions, including atrophy and hyperplasia of Bowman's glands and atrophy and metaplasia of the olfactory epithelium, were also observed in all exposed groups of male and female mice after subchronic inhalation for 14 weeks (LOAEC: 368 mg/m³, 75 ppm). No nasal epithelial lesions were observed in rats after subchronic inhalation at concentrations ranging from 368 to 4900 mg/m³ (75 -1000 ppm).

In the study with chronic exposure, renal tubule lesions and an increased incidence of renal tubule tumours in male rats were also observed. This effect is related to the α2u-globulin associated nephropathy in male rats and is not relevant for risk assessment in humans. Similarly, the observed increased incidence of hepatocellular tumours in the used strain of mice is considered not relevant for risk assessment. No increased incidences of neoplastic lesions were observed in the nasal epithelia of rats and mice.

There is no clear evidence of genotoxicity of 2-phenylpropene. At non-parentally toxic doses, 2-phenylpropene had no effect on reproductive and developmental parameters in a one-generation study with rats.

Rationale for starting point

The derivation of the EU-LCI value is based on the observed lesions of the nasal epithelia in rats, at the same concentration, similar effects were also observed in mice. Slight effects were already observed at 490 mg/m³, the lowest concentration tested. This LOAEC serves as the starting point for the derivation of the LCI.

Rationale for Extrapolation factors

- Factor for adjustment for exposure duration: 5.6
- Adjusted study length factor: 1 (chronic exposure)
- LOAEC → NOAEC extrapolation: 3
- Interspecies differences: 2.5 (According to the ECA report No. 29, no correction has to be made for differences in systemic metabolism when the POD is related to local effects. For remaining uncertainties, a value of 1 is used for remaining specific differences for effects on skin, eye and GI tract if the mode of action implies only a simple destruction of membranes, and a default value of 2.5 is used for effects on the skin, eye and GI tract if local metabolism or receptor binding reactions are involved. A factor of 2.5 for 2-phenylpropene is used, because metabolism is known to be involved in the toxicity of structurally related compounds (Ad-hoc AG, 2012; DFG, 2004) and likely so for 2-phenylpropene.)
- Intraspecies differences: 10

Total extrapolation factor is: 420, leading to a value of 490 000 µg/m³ : 420 = 1200 µg/m³.

The following EU-LCI is proposed for 2-phenylpropene (α-methylstyrene): 1200 µg/m³. The derived EU-LCI is within the reported wide range of odour thresholds of 0.1-244 mg/m³ (0.02 – 49.7 ppm) reported by AIHA (2013) and, according to SCOEL (1995), the penetrating and unpleasant odour of 2-phenylpropene is detectable below 5 mg/m³ (1 ppm). Thus, odour perception and annoyance cannot be excluded at the proposed EU-LCI.

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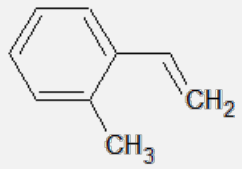
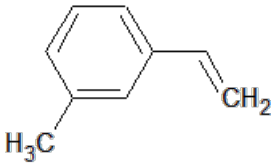
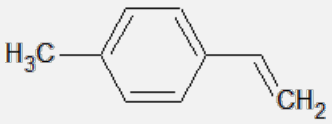
2 Toxicological evaluation of vinyl toluenes as basis for the derivation of a EU-LCI value

2.1 Substance identification

Substance identification data and physicochemical properties are shown in Table 2.1 and Table 2.2. The mixture described by the CAS No. 25013-15-4 contains about 60-70% m-vinyl toluene and 30-40% p-vinyltoluene (DFG, 2017; NTP, 1990). If not stated otherwise, toxicity studies were performed with such mixtures.

Table 2.1: Substance identification of vinyl toluenes (methylstyrenes) (DFG, 2017; ECHA C&L Inventory, 2018)

CAS-No. EU-No. CLP-Index-No.	Systematic Name, common names	Summary formula	Structural formula
611-15-4 210-256-7 601-028-00-1	o-vinyl toluene, o-methylstyrene, 2-methylstyrene, 2-vinyl toluene, 1-methyl-2-vinylbenzene	C_9H_{10}	
100-80-1 202-889-2 -	m-vinyl toluene, m-methylstyrene, 3-methylstyrene, 1-methyl-3-vinyl benzene	C_9H_{10}	
622-97-9 210-762-8 -	p-vinyl toluene, p-methylstyrene, 4-methylstyrene, 1-methyl-4-vinyl benzen	C_9H_{10}	
25013-15-4 215-292-7 -	mixture of o-, m-, p-vinyl toluene	C_9H_{10}	

2.2 Substance properties and uses

Vinyl toluenes are clear colourless liquids with a strong and very unpleasant odour (DFG, 2017). Further data regarding the odour are reported in chapter 2.5.5. Vinyl toluenes are nearly insoluble in water but are soluble in most organic solvents. Commercially available vinyl toluenes may be stabilized with *tert*-butyl catechol to inhibit oxidation and polymerization during storage (NTP, 1990).

Ortho-vinyl toluene has been identified in the essential oil of the gras *Distichlis spicata* (HSDB, 2015b). However, the dominant source of vinyl toluenes is commercial industrial production. Vinyl toluenes are large-scale industrial products (tonnage band in the EU: 1000–10000 t/a) (ECHA Dissemination, 2017; ECHA Dissemination, 2018). These substances are used alone or in combination with others in the production of polymers, as adhesives, resins, surface coating, paints and in the production of other chemicals and insecticides (DFG, 2017).

Table 2.2: Physicochemical properties of vinyl toluenes (DFG, 2017; DGUV, 2017)

Molar mass (g/mol)	Mp. (° C)	Boiling point (° C)	Vapour pressure (hPa) (at 20 °C)	Conversion 1 ppm = x mg/m³ (23 °C)	log pow (at pH)	Solubility in water (mg/L)
118.2	o-isomer: -69	o-isomer: 171	o-isomer: 3.5	4.87	o-isomer: no data	o-isomer: ca. 89
118.2	m-isomer: -86.3	m-isomer: 164	m-isomer: 2.3	4.87	m-isomer: 3.35	m-isomer: 151
118.2	p-isomer: -34.1	p-isomer: 172.8	p-isomer: 2.4	4.87	p-isomer: 3.35	p-isomer: 89
118.2	mixture: -76.7	mixture: 170	mixture: 1.47-2	4.87	mixture: 3.58	mixture: 89

2.3 Exposure

2.3.1 Indoor air

Very few data are available regarding the occurrence of vinyl toluene in indoor air (Table 2.3). Vinyl toluenes were not detectable in 66 measurement performed in various indoor rooms in Germany (Hofmann and Plieninger, 2008). Vinyl toluenes were detected but not quantified in homes in Washington and Chicago, USA (HSDB, 2015b).

Table 2.3: Data on the occurrence of vinyl toluenes in indoor air from homes, schools, children day care centres and offices

Rooms	N	LoD (µg/m³)	N > LoD (% > LoD)	Median (µg/m³)	P95 (µg/m³)	Maximum (µg/m³)	Reference
Offices, homes, (pre)-schools, Germany	66	1	0	0.5	0.5	0.5	(Hofmann and Plieninger, 2008)

2.3.2 Other sources

Very few data are available.

Vinyl toluene (o-, m- and p-isomers) has been identified as a component of tobacco smoke (HSDB, 2015b). 4-Vinyl toluene was listed as a contaminant found in drinking water for a survey of US cities in the 1980s (HSDB, 2015a). Well water sampled in northern Spain, in the vicinity of prior industrial pollution, contained p-vinyl toluene concentrations of not-detectable to 58 ng/L (HSDB, 2015b).

2.4 Toxicokinetics

Systemic effects observed after inhalation or oral exposure show that the substance is absorbed via these pathways. However, no reliable quantitative data are available.

Data on the distribution of vinyl toluenes are not available. The available data on the toxicokinetics of vinyl toluenes and the excretion of metabolites have been summarised (DFG, 2017; ECHA Dissemination, 2017; IARC, 1994; NTP, 1990). 55% of a single dose administered by intraperitoneal injection (50 mg/kg bw) was found as urinary metabolites, mainly in the first 6 hours; at higher doses, slightly smaller percentages were found (Heinonen, 1984). Thioethers were detected as the principal urinary metabolites (25%), other metabolites identified were p-methylmandelic acid (5.7%), p-methylphenylglyoxylic acid (11.9%), p-methylbenzoyl glycine (9.3%), p-methylphenylacetyl glycine (2.5%), and p-vinyl benzoyl glycine (1%). Pretreatment with an inhibitor of the cytochrome P450

monooxygenase inhibited the excretion of these metabolites with urine. Further, vinyl toluene was found to bind to hepatic cytochrome P450, and the hepatic and renal content of reduced glutathione (GSH) was decreased in rats after a single intraperitoneal injection (Heinonen and Vainio, 1980). These findings suggest that metabolism of vinyl toluene is catalysed by cytochrome P450, producing vinyl toluene-7,8-oxide as the main reactive intermediate, with subsequent conjugation to glutathione or hydration to diols (Heinonen, 1984). Overall, the metabolism of the vinyl toluenes (methylstyrenes) is very similar to that of styrene, both, styrene and p-methylstyrene (p-vinyl toluene) are oxidized at the vinyl group *in vitro* by CYP450 enzymes at comparable rates (Hanzlik et al., 1978). The detection of p-vinyl benzoyl glycine indicates that a small percentage of vinyl toluene is oxidized at the methyl group.

2.5 Health effects

2.5.1 Sensory irritation and local effects

In a study with human volunteers, the subjects were briefly exposed (no details reported) to a mixture containing 55-70% m- and 30-45% p-vinyl toluene in a test room and noted their reactions with respect to odour, eye irritation, and nasal irritation. No response to the odour was noted by the subjects below 10 ppm (49 mg/^{3}). At 50 ppm (245 mg/m^3), the odour was detectable, but no mucous membrane irritation was noted by the subjects. At 200 ppm (975 mg/m^3), the odour was strong, but considered as tolerable without excessive discomfort. At 300 ppm (1460 mg/m^3), the odour became strong and objectionable. At ≥ 400 ppm (1950 mg/m^3), the subjects noted a very strong odour and strong eye and nasal irritation (Wolf et al., 1956).

2.5.2 Repeated dose toxicity

In a repeated exposure inhalation study, 10-25 rats, 5-10 guinea pigs, 1-2 rabbits and 1-2 monkeys were exposed 7 h/d, 5 d/week for up to 139 days against 580, 1130 or 1350 ppm vinyl toluene (2825, 5500, 6575 mg/m^3, mixture containing 55-70% m- and 30-45% p-vinyl toluene). All concentrations were tolerated by monkeys and 580 ppm was tolerated by all species without pathological changes. Exposure to 1130 and 1350 ppm led to reduced weight gain (rats, guinea pigs) and increased weight of the kidney (guinea pigs, rabbits) or liver (rats, guinea pigs). Fatty degeneration occurred in the liver of rats, guinea pigs and rabbits. No details about histological examinations were presented. A "moderate amount of mortality" was seen in rats at the highest concentration (no details reported) (Wolf et al., 1956).

In a subacute study, F344 rats (5 M + 5 F/group) were exposed to 0, 200, 400, 800, or 1,300 ppm vinyl toluene (0, 975, 1950, 3900, 6330 mg/m^3, mixed isomers: 65-71% *meta*- and 32-35% *para*-isomer) for 6 h/d, 5 d/week for two weeks (NTP, 1990). Histopathology was only performed in the highest exposure and in the control group. No mortality was observed. Lethargy, lachrymation, red discolouration around the nose were noted at 1300 ppm. The mean body weights at necropsy of rats exposed to 400-1300 ppm were 13-19% lower than that of controls for males and 9-13% lower for females. Most male rats exposed to 1300 ppm had centrilobular necrosis and focal inflammatory cell infiltration of the liver, whereas minimal centrilobular vacuolization of the liver was seen in all female rats exposed to 1300 ppm. Dysplasia of the bronchial epithelial lining, chronic bronchitis, and lymphoid hyperplasia of the lung were also observed in all rats exposed to 1,300ppm.

In the correspondent study with B6C3F1 mice (5 M + 5 F/group), the animals were exposed to 0, 10, 25, 50, 100 or 200 ppm vinyl toluene (0, 49, 122, 245, 490, 975 mg/m^3, mixed isomers: 65-71% *meta*- and 32-35% *para*-isomer) for 6 h/d, 5 d/week for two weeks (NTP, 1990). Histopathology was only performed in the highest exposure and in the control group. Ataxia was observed at 100 ppm. At 200 ppm, animals were lethargic and had their eyes closed during exposure. Three of five male mice exposed to 200 ppm died before the end of the studies. Four of five male mice exposed to 200 ppm had moderate to severe hepatocellular necrosis; all female mice exposed to 200 ppm had hyperplasia of

the epithelium of the intrapulmonary bronchi and centrilobular necrosis, vacuolization, and inflammatory cell infiltrates in the liver.

In a subchronic NTP-study with F344 rats, 10 M + 10 F/concentration were exposed by inhalation to vinyl toluene (mixed isomers: 65-71% *meta*- and 32-35% *para*-isomer) at concentrations of 0, 25, 60, 160, 400, or 1000 ppm (0, 122, 292, 780, 1950 or 4870 mg/m^3) for 6 h/d, 5 d/week for 14 weeks (NTP, 1990). Histopathology was only performed in the highest exposure and in the control group. No mortality was observed. Ruffled fur, closed eyes and severe lacrimation were noted at 1000 ppm during the exposure. The final mean body weight of rats exposed to ≥ 400 ppm was 8-19% lower than that of controls for males and 6-12% lower for females. The relative liver weight was significantly increased at 1000 ppm by 27% (M) or 34% (F). The severity of nephropathy was increased in male rats exposed to ≥ 160 ppm. Compound-related lesions were not observed in female rats.

In the correspondent subchronic study with B6C3F1 mice (10 M + 10 F/group), the animals were exposed by inhalation to vinyl toluene (mixed isomers: 65-71% *meta*- and 32-35% *para*-isomer) at concentrations of 0, 10, 25, 60 or 160 ppm (0, 49, 122, 290, 780 mg/m^3) (NTP, 1990). Histopathology was only performed in animals exposed to 0, 25, 60 or 160 ppm. No exposure-related effect of exposure on the mortality could be observed. Lethargy was observed at ≥ 60 ppm and the animals had their eyes closed at exposure to 160 ppm. The final mean body weights of mice exposed to 25-160 ppm were 12-20% lower than that of controls for males and 13-16% lower for females. Inflammation of the lung was observed in 5/10 male and 3/9 female mice exposed to 160 ppm. Metaplasia of the nasal turbinates was seen in all exposed groups.

A subchronic inhalation study with *para*-vinyl toluene (97%, 3% m-vinyl toluene) was performed with Sprague-Dawley rats (ECHA Dissemination, 2017). The animals (15 M + 15 F/ group) were exposed to 0, 100, 500, 1600/1300 ppm (0, 490, 2435, 7780/6330 mg/m^3), 6 h/d, 5 d/week for 13 weeks. There were notable clinical signs at 1300 and 1600 ppm including: ocular and oral secretions, respiratory abnormalities, prostration, neuromuscular impairment, tremors, hypoactivity. At 500 ppm clinical signs were restricted to increased secretions and respiratory abnormalities. Body weight remained comparable to the controls. Liver weight was increased at 500 and 1600 ppm, and alkaline phosphatase levels were increased at 1600 ppm, but there were no pathological changes. It was stated that there were no histopathological changes, but no data were presented in the report.

Non-carcinogenic and carcinogenic effects of chronic inhalation exposure to vinyl toluene (mixed isomers: 65-71% *meta*- and 32-35% *para*-isomer) are described in the following chapter 2.5.3 (see "carcinogenicity").

In a 90-d study with **oral exposure,** Sprague-Dawley rats (15 M + 15 F/dose) received 0, 91, 273, 547 mg/(kg bw x d) p-vinyl toluene by gavage. No substance-related mortality occurred. After gavage treatment, animals showed increased salivation and motor activity. Body weight was lower in treated males only. There were no organ weights or clinical pathology findings which were considered related to treatment. Histopathological findings were restricted to aspiration pneumonia and perioral dermatitis, both resulting from direct contact irritation during dosing (ECHA Dissemination, 2017).

In a further subchronic study with **oral exposure** to p-vinyl toluene**,** F344 rats (15 M + 15 F/dose) were given 0, 50, 100, 300, 700 or 1500 mg/(kg bw x d) in olive oil by gavage for 90 days (All animals received 1 mL/kg olive oil and then the test substance was mixed with the olive oil at various dose volumes to give the appropriate dose level; i.e. the highest dose received the greatest dose volume). The highest dose was associated with notable mortality and exceeded the maximum tolerated dose (MTD). Male body weight was reduced in all treated groups. There were no clinical pathology findings of note. At necropsy there was an increase in liver weight without pathological changes at ≥ 300 mg/(kg bw x d) and in kidney weight at ≥ 700 mg/(kg bw x d). In the lung, multifocal chronic pneumonitis and focal hyperplasia of bronchial and bronchiolar epithelium was observed in all treated groups at a greater severity than in the controls. A LOAEL of 50 mg/(kg bw x d) is derived from this

study (no NOAEL) based on reduced male body weight and histopathological changes in the lungs (ECHA Dissemination, 2017). It is likely that the effects on the lungs, which are already observed at the lowest tested dose, are related to the aspiration of the test substance (as in the study described above), since no pulmonary effects were observed in rats after inhalation exposure to higher systemic dose.

Neurotoxicity studies

Wistar rats (20 M/concentration) were exposed in the dark to vinyl toluene (mixed isomers) by inhalation to 0, 50, 100 or 300 ppm (0, 245, 490, 1460mg/m^3) for 6 h/d, 5 d/week for up to 15 weeks (Seppalainen and Savolainen, 1982; U.S.EPA, 2010). Motor conduction velocity (MCV) of the tail nerve of immobilized rats was measured at the start of the study and after 4, 8, 12 and 15 weeks. Myelin-deprived axons from the spinal cord were analysed for protein composition. Exposed animals were inactive and body weights at 300 ppm were lower than controls. At ≥ 100 ppm, MCV were slightly lower than controls at 12 and 15 weeks. The amplitude of evoked muscle action potential was lower at both of these concentrations than the controls at 12 weeks, and protein composition of the axons at these concentrations differed from controls at 15 weeks. No effect was observed at 50 ppm.

In a similar study, Sprague-Dawley rats (10 M/concentration) were exposed to vinyl toluene (mixed isomers) by whole-body inhalation to 100 or 300 ppm (490 and 1460 mg/m^3) for 6 h/d, 5 d/week for up to 21 weeks. Body weights at 300 ppm were lower than for controls, but the effect not statistically significant. Motor and sensory nerve conduction velocities of the tail nerve were significantly lower than controls at 300 ppm during weeks 15 and 20. Histopathology of the sciatic nerve was no different from controls at 21 weeks (Gagnaire et al., 1986; U.S.EPA, 2010).

2.5.3 Genotoxicity and carcinogenicity

Genotoxicity

In vitro, vinyl toluenes (commercial mixture of m- and p-isomer, if not otherwise stated) was not mutagenic in several bacterial mutation assays tested at up to cytotoxic concentrations with and without exogenous metabolic activation system (S9 mix from rat liver) in all tested strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) and in *Escherichia coli* WP2uvr A (DFG, 2017; ECHA Dissemination, 2018). In mammalian cells, the substance did not induce mutations in a thymidine kinase mutagenicity assay with L5178Y lymphoma cells of mice in the absence of exogenous metabolic activation at non-cytotoxic concentrations. A positive result was obtained in two of three tests at cytotoxic concentrations. No test with exogenous metabolic activation was performed (NTP, 1990). No chromosomal aberrations or sister chromatid exchanges (SCE) were induced with and without metabolic activation in Chinese Hamster Ovary Cells (CHO) (NTP, 1990). In other tests using higher concentrations than in the NTP-tests, induction of SCE and chromosomal aberrations were reported in CHO cells and human lymphocytes without metabolic activation. However, no positive control was run and no data on cytotoxicity were presented. Tests with metabolic activation system were not conducted (Norppa, 1981a; Norppa et al., 1981; Norppa and Vainio, 1983).

In vivo, vinyl toluene was not mutagenic in a sex-linked recessive lethal (SLRL) assay in the fruit fly *Drosophila melanogaster* after oral or inhalation exposure (Norppa et al., 1981). No chromosomal aberrations were induced in the bone marrow of male Sprague-Dawley rats after oral exposure with up to 1340 mg/(kg bw x d) of p-vinyl toluene for 5 d (ECHA Dissemination, 2017). An increase of micronuclei in polychromatic erythrocytes was described for male mice having received 200, 300 or 500 but not at 100 mg/kg body weight. The highest concentration was lethal for 3 of 5 animals (Norppa, 1981b). 4-methylstyrene (p-vinyl toluene) was not mutagenic in a dominant-lethal assay with male rats at doses of up to 1.5 mg/(kg bw x d) for 5 d (ECHA Dissemination, 2017).

Carcinogenicity

A carcinogenicity study was conducted with F344 rats (NTP, 1990). The animals (50 M + 50 F/concentration) were exposed to 0, 100 or 300 ppm (0, 490, 1460 mg/m^3) of vinyl toluene (mixed

isomers: 65-71% *meta*- and 32-35% *para*-isomer) by inhalation for 6 h/d, 5 d/week for 103 weeks. Survival rates of exposed animals were not significantly different from the control group animals. Mean body weights of male rats exposed to 300 ppm vinyl toluene and those of female rats exposed to 100 and 300 ppm were generally 4%-11% lower than those of controls.

Degenerative and non-neoplastic proliferative lesions of the nasal mucosa were observed at increased incidences in exposed rats (Table 2.4). These lesions included diffuse hyperplasia (goblet cell) of the respiratory epithelium with intraepithelial mucous cysts and focal erosion of the olfactory epithelium with cystic dilatation (cysts) of the Bowman's glands. Focal respiratory epithelial metaplasia of the olfactory epithelium was seen in some exposed males, and cells with homogeneous eosinophilic cytoplasm in the olfactory epithelium occurred at increased incidences in exposed female rats.

Neoplasms of the nasal mucosa were not observed. Furthermore, there were no chemically related increases in neoplasm incidence in other organs of exposed male or female rats. It was concluded that there was "no evidence of carcinogenic activity" in male and female rats (NTP, 1990). No NOAEC for non-neoplastic effects can be derived from this study; the LOAEC is 100 ppm (490 mg/m^3).

Table 2.4: Incidence of non-neoplastic lesions in the nasal epithelia of rats after chronic inhalation of vinyl toluenes (NTP, 1990)

Organ/ effect	Concentration of vinyl toluene (ppm)		
	0	100	300
Males			
Nose, olfactory epithelium			
cysts	0/48	4/50	6/50
focal erosion	0/48	8/50	1/50
eosinophilic hyperplasia	1/48	0/50	0/50
metaplasia	0/48	6/50	4/50
Nose, respiratory epithelium			
intraepithelial cysts	2/48	13/50	9/50
diffuse hyperplasia	12/48	24/50	28/50
Females			
Nose, olfactory epithelium			
cysts	0/50	5/49	13/50
focal erosion	0/50	3/49	4/50
eosinophilic hyperplasia	2/50	9/49	21/50
metaplasia	0/50	1/49	0/50
Nose, respiratory epithelium			
intraepithelial cysts	0/50	6/49	10/50
diffuse hyperplasia	8/50	19/49	19/50

In the correspondent study with BFC3F1 mice (50 M + 50 F/concentration), the animals were exposed to 0, 10 or 25 ppm (0, 49, 122 mg/^{3}) of vinyl toluene (mixed isomers: 65-71% *meta*- and 32-35% *para*-isomer) by inhalation for 6 h/d, 5 d/week for 103 weeks (NTP, 1990). Survival rates of exposed animals were not reduced compared to the control group animals (The survival of male mice at 25 ppm was significantly greater than that of controls). Mean body weights of mice exposed to 25 ppm were 10-23% lower than those of controls after two months, at 10 ppm, the weight decrement was generally < 10%.

Degenerative and inflammatory lesions of the nasal mucosa were observed at increased incidences in exposed mice (). These lesions included focal chronic active inflammation and diffuse hyperplasia of

the respiratory epithelium. Chronic active inflammation of the bronchioles occurred in many exposed mice but not in controls.

Table 2.5: Incidence of non-neoplastic lesions in the nasal epithelia of mice after chronic inhalation of vinyl toluenes (NTP, 1990)

Organ/ effect	Concentration of vinyl toluene (ppm)		
	0	10	25
Males			
Nose, respiratory epithelium			
chronic active inflammation	2/50	47/48	48/49
hyperplasia	5/50	48/48	49/49
Lung/bronchioles			
chronic active inflammation	0/50	15/49	30/49
Females			
Nose, respiratory epithelium			
chronic active inflammation	3/48	49/49	47/48
hyperplasia	5/48	49/49	47/48
Lung/bronchioles			
chronic active inflammation	0/48	14/49	37/49

Neoplasms of the nasal passage were not observed in mice. Furthermore, there were no chemically related increases in neoplasm incidence in exposed male or female mice (Actually, incidences of alveolar/bronchiolar neoplasms and malignant lymphomas were lower in exposed males and that of hepatocellular neoplasms was lower in exposed females). It was concluded that there was "no evidence of carcinogenic activity" in male and female mice (NTP, 1990). No NOAEC for non-neoplastic effects can be derived from this study; the LOAEC is 10 ppm (49 mg/m^3).

In a study with **oral** exposure of Sprague-Dawley rats (60-90 M + 60-90 F/dose), treatment by gastric intubation with p-vinyl toluene (97%, 3% m-isomer) in olive oil with doses up to 250 mg/(kg bw x d), 5 d/week, for 108 weeks did not lead to a treatment-related increase in the incidence of tumours. Also, no such increase was observed in a similar study with female Swiss mice after oral administration of up to 250 mg/(kg bw x d) of p-vinyl toluene (97%, 3% m-isomer) for 78 weeks (IARC, 1994).

2.5.4 Toxicity to reproduction

There are no studies available with inhalation exposure to vinyl toluenes.

Fertility

A summary of the results from a two-generation study with oral exposure of rats to p-vinyl toluene is presented in the registration dossier, the full study was not available. Sprague-Dawley rats received 0, 25, 200, 500, and 600 mg/(kg bw x d) by oral gavage for 404 days. There were no effects on the viability of pups from dams dosed at 25 or 200 mg/(kg bw x d). There was also no effect on mating, fertility, gestation, delivery of pups, or lactation index at these dose levels. Mortality, reduced weight gain in adults and slight increase in pup mortality (first generation only) was observed at 500 mg/(kg bw x d). A NOAEL of 200 mg/(kg bw x d) is reported (ECHA Dissemination, 2017).

Development

Pregnant CD rats (25 F/dose) were treated with p-vinyl toluene by gavage on GD 6-19. The animals received 0, 50, 300 or 600 mg/(kg bw x d) by gavage (ECHA Dissemination, 2017). Caesarean sections were conducted on GD 20. No mortality or clinical signs were observed at any dose. Maternal weight gain was reduced at all dose levels compared to control. Fetal weights in all treated groups were also

statistically significantly lower than the controls. It is not clear whether this could be (partially) related to the unusually high fetal weight in the control group, which was above the normal background range in this laboratory. There were no biologically meaningful differences in the mean number of corpora lutea, total implantations, early or late resorptions, post implantation loss, viable fetuses, fetal sex distribution, mean fetal body weight or number of fetuses (and litters) with malformations. A single case of a meningocele was observed in the highest dose group. This malformation is extremely rare in rats. However, studies with chemical-induced meningocele indicate that this effect is accompanied by other malformations and that incidences are relatively high when this type malformation is induced at all. Therefore, the occurrence of a single meningocele in this study was considered not related to treatment but by chance (DFG, 2017). Thus, the NOAEL for teratogenicity was 600 mg/(kg bw x d); the NOAEL for maternal toxicity was not identified.

Treatment of pregnant Sprague-Dawley rats (20 F/dose) with 0, 60, 190 or 600 mg/(kg bw x d) p-vinyl toluene by gavage on GD 6-15 had no maternal toxic effects. No dose-dependent treatment-related effects were observed in offsprings (NOAEL ≥ 600 mg/(kg bw x d)). The study is only available as a summary report (DFG, 2017; U.S.EPA, 2010).

No maternal or developmental toxicity was observed in a similar study with pregnant Dutch white rabbits (16 F/dose) after treatment with 0, 60, 100 or 150 mg/(kg bw x d) p-vinyl toluene by gavage on GD 6-27 (NOAEL ≥ 150 mg/(kg bw x d). The study is only available as a summary report (DFG, 2017; U.S.EPA, 2010).

2.5.5 Odour perception

The odour of vinyl toluenes has been described as very unpleasant and disagreeable (Ruth, 1986). The odour of a mixture of vinyl toluenes (55-70% meta- and 30-45% para-isomer) was detectable at 50 ppm (245 mg/m^3) but not below 10 ppm (49 mg/m^3); the result was reported to be similar to that obtained for styrene (DFG, 2017; Wolf et al., 1956) It must, however, be noted that this value is several orders of magnitude higher than the threshold of 70 µg/m^{3}cited for styrene by other sources (IARC, 1994) and for other structurally related chemicals (toluene, ethyl toluenes) (Nagata, 2003). No further data on odour thresholds of vinyl toluenes are available.

2.5.6 Mechanistic aspects and structure-activity relationships

A comparison of the effects observed in mice and rats indicates species-specific differences in the toxicity of vinyl toluenes, i.e. a higher sensitivity of mice compared to rats, with lesions in the nasal epithelia of mice at much lower concentrations than in the nasal epithelia of rats (Table 2.4 and Table 2.5).

In the correspondent study with BFC3F1 mice (50 M + 50 F/concentration), the animals were exposed to 0, 10 or 25 ppm (0, 49, 122 mg/^{3}) of vinyl toluene (mixed isomers: 65-71% meta- and 32-35% para-isomer) by inhalation for 6 h/d, 5 d/week for 103 weeks (NTP, 1990). Survival rates of exposed animals were not reduced compared to the control group animals (The survival of male mice at 25 ppm was significantly greater than that of controls). Mean body weights of mice exposed to 25 ppm were 10-23% lower than those of controls after two months, at 10 ppm, the weight decrement was generally < 10%.

Degenerative and inflammatory lesions of the nasal mucosa were observed at increased incidences in exposed mice (Table 2.5). These lesions included focal chronic active inflammation and diffuse hyperplasia of the respiratory epithelium. Chronic active inflammation of the bronchioles occurred in many exposed mice but not in controls.). Mechanistic aspects underlying these differences have been summarised by the German MAK-Commission (DFG, 2017).

The metabolism of vinyl toluenes (=methylstyrenes) proceeds in a way similar to that of styrene, and a similar species difference in sensitivity of the nasal epithelium of mice and rats was also observed for styrene. Both rats and mice oxidize styrene to styrene epoxide, which is considered the toxic metabolite causing the nasal lesions. However, rats are able to detoxify the epoxide more rapidly than mice

(via epoxide hydrolase and GSH-transferase). *In vitro* data suggest that human nasal tissue has a low capacity to oxidize styrene but contains epoxide hydrolase and GSH-transferase activity with a similar activity as in rat nasal tissue. It was concluded that styrene has low toxicity to human nasal epithelia and, by analogy, that the effects observed in the nasal epithelia of mice after exposure to vinyl toluenes seem of little relevance for humans. A lower sensitivity of humans compared to mice is also likely with respect to pulmonary effects (DFG, 2017). Similar conclusions were also presented in the evaluation of the toxicity of alkylbenzenes and related compounds (Ad-hoc AG, 2012).

Some aromatic solvents are known to cause ototoxicity in rats. A comparison of the ototoxicity of 21 different compounds was performed by Gagnaire and Langlais (2005). Sprague-Dawley rats were exposed by gavage to 8.47 mmol/(kg bw x d) of the test compound for 5 d/week for 2 weeks (i.e. 1000 mg 2-, 3- or 4-vinyl toluene/(kg bw x d)). In contrast to styrene, no signs of ototoxicity were observed for the vinyl toluenes in morphological investigations of the cochlea in this assay.

2.6 Evaluation

2.6.1 Existing regulations and classifications

2-Vinyl toluene (o-vinyl toluene) is classified in the EU with respect to its toxicity as harmful if inhaled (H332), but not as mutagenic, carcinogenic or toxic to reproduction (ECHA C&L Inventory, 2018).

General population

No DNEL or other values are available which have been derived for the protection of the general population.

Table 2.6: Guide values for vinyl toluenes in indoor air (for explanation, see text)

Guidance value Parameter/ Organisation	ECHA Registered Substances (ECHA Dissemination, 2017)	DFG (2017)
Name (reference period)	DNEL (chronic, workers)	OEL (8-h TWA) (workers)
Value (mg/m³)	37 (7.5 ppm)	98 (20 ppm)
Organ/critical effect	Not indicated	Nasal irritation
Species	Not indicated	Rat
Basis	NOAEC (not further indicated)	LOAEC 490 mg/m³ (100 ppm)
Adjusted for cont. exposure	Not indicated	Not performed
Extrapolation factors **Time** **LOAEC to NAEC** **Interspecies** **Intraspecies** **Total**	 4	 3 1

Workplace

A DNEL of 37 mg/m³ (7.5 ppm) is reported in the REACH registration dossier for vinyl toluene (ECHA Dissemination, 2017). A total extrapolation factor of 4 has been used, but no details are presented. In a second REACH registration dossier for vinyl toluene, no DNEL have been derived (ECHA Dissemination, 2018).

The OEL for vinyl toluene (all isomers) in Germany is set to 20 ppm (98 mg/m³) (AGS, 2018), adopting the MAK-value which is based on the LOAEC of 100 ppm (490 mg/m³) from a chronic NTP-study in rats, extrapolating to a NAEC of 33 ppm by using a factor of 3 (DFG, 2017). A similar OEL of 25 ppm has been reported for Denmark. A lower value of 10 ppm (49 mg/m³) has been derived as OEL in Fin-

land and Sweden, and OEL of 50 or 100 ppm have been reported for various other European countries (IFA, 2018).

2.6.2 Derivation of a EU-LCI value

Very few data are available regarding toxic effects of vinyl toluenes in humans.

Brief acute exposure to 50 ppm (245 mg/m^3) vinyl toluene were detected by the odour. At 300 ppm (1460 mg/m^3), the odour became strong and objectionable, and at ≥ 400 ppm (1950 mg/m^3), the subjects additionally noted strong eye and nasal irritation (Wolf et al., 1956).

Animal studies indicate that the critical effect of vinyl toluene inhalation is irritation of the nasal epithelia. In a chronic inhalation study with rats, degenerative and non-neoplastic proliferative lesions were observed at all exposure concentrations (Table 2.4). These lesions included effects in both the respiratory and the olfactory epithelium. Diffuse hyperplasia and intraepithelial mucous cysts were observed in the respiratory epithelium. In the olfactory epithelium, cysts, focal erosion, eosinophilic hyperplasia and a focal respiratory epithelial metaplasia were observed. No NOAEC could be derived from the study; the LOAEC for rats was 100 ppm (490 mg/m^3) (NTP, 1990).

There is no clear evidence of genotoxicity of vinyl toluenes. No carcinogenicity in any tissue or organ was observed in rats and mice exposed to commercial mixtures of vinyl toluenes (65-71% *meta*- and 32-35% *para*-isomer) by inhalation or to p-vinyl toluene by gavage.

Limited data from an unpublished two-generation study with oral exposure of rats to p-vinyl toluene do not indicate reprotoxic effects at doses that do not also lead to parental systemic toxicity (ECHA Dissemination, 2017). Similarly, studies with oral exposure of rats and rabbits do not provide convincing evidence for developmental toxicity of p-vinyl toluene (DFG, 2017; ECHA Dissemination, 2017; U.S.EPA, 2010).

Thus, studies in both species, mice and rats, indicate that the nasal epithelia are the critical target of inhalation exposure to vinyl toluenes. Mechanistic aspects as described in chapter 2.5.6 indicate that the effects observed in the nasal epithelia of mice are of less relevance for humans than the effects described in rats. Therefore, the LOAEC of 100 ppm (490 mg/m^3) vinyl toluene (mixture of m- and p-vinyl toluene) from the chronic inhalation exposure study with rats is used as the POD for the proposed derivation of a EU-LCI value (see Table 2.7).

Table 2.7: Derivation of EU-LCI value for vinyl toluenes (for explanation, see text)

Endpoint	POD (mg/m³)	Adjustment factor				Value (mg/m³)	Reference
		LOAEC → NAEC	Time	Interspecies	Intraspecies		
Local toxicity (nasal epithelium)	LOAEC: 490	3	1	2.5	10	1.17	(NTP, 1990)

The following adjustment factors are used:

- Adjustment for continuous exposure (6 h/d, 5 d/week): 5.6
- LOAEC→ NAEC: 3
- Adjusted study length factor (chronic exposure study): 1
- Interspecies extrapolation: 2.5
  (According to the ECA report No. 29 (EC, 2013), no correction has to be made for differences in systemic metabolism when the POD is related to local effects. For remaining uncertainties, a value of 1 is used for remaining specific differences for effects on skin, eye and GI tract if the mode of action implies only a simple destruction of membranes, and a default value of 2.5 is used for effects on the skin, eye and GI tract if local metabolism or receptor binding reactions

are involved. A factor of 2.5 for vinyl toluenes is used because metabolism is known to be involved in the toxicity of structurally related compounds (Ad-hoc AG, 2012; DFG, 2004; DFG, 2017) and likely so for vinyl toluenes)

- Intraspecies extrapolation (interindividual variability, general population): 10

Total assessment factor: 420. This leads to a concentration of 490 mg/m^3: 420 = 1.17 mg/m^3.

A EU-LCI value (rounded) for vinyl toluenes of 1200 µg/m^3 is proposed.

The POD is derived from a study performed with a mixture of 65–71% *meta*-vinyl toluene and 32–35% *para*-vinyl toluene. No data from repeated inhalation studies are available for o-vinyl toluene, but the limited data base does not indicate gross differences in the toxicity of the three isomers.

The commercially used vinyl toluenes contain a mixture of m- and p-vinyl toluenes, but no or only trace amounts of the ortho-isomer. Thus, release from building products may lead to the detection of m- and p-vinyl toluene in air.

The odour of vinyl toluenes has been described as very unpleasant and disagreeable. No reliable odour thresholds are available for vinyl toluenes (methylstyrenes). A statement that the odour of vinyl toluene was not detectable at less than 10 ppm (49 mg/m^3) (Wolf et al., 1956) cannot be understood as to represent a defined threshold. It must be noted that thresholds of about 70 µg/m^3, i.e. several orders of magnitude lower, have been reported by other sources for styrene (IARC, 1994) and for other structurally related chemicals (toluene, ethyl toluenes) (Nagata, 2003). It is concluded that odour perception and annoyance from vinyl toluenes cannot be excluded at the proposed EU-LCI.

2.7 References

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2.8 Fact and data sheet for vinyl toluenes

Table 2.8: Data collection sheet Vinyl toluenes (methylstyrene, all isomers, mixture)

Rapporteur, Date		
Compound	**Vinyl toluenes**	***Data collection sheet***
N° CAS 25013-15-4 (mixture of isomers) 1 ppm = 4.9 mg/m³	**EU-Classification:** - **CLP:** no harmonised classification for mixture, 3- and 4-vinyl toluene 2-vinyltoluene: Acute Tox 4 (H332), Aquatic Chronic 2 (H411)	
Organization Name	DFG	Reach registrants
Risk Value Name	MAK	DNEL
Risk Value (mg/m³)	98 mg/m³ (20 ppm)	37 (7.5 ppm)
Reference period	Chronic (worker)	Chronic (worker)
Risk Value (mg/m³) Short Term (15 min)	196 mg/m³ (40 ppm)	-
Year	2016	2017
Key Study	NTP (1990) NTP Technical Report on the Toxicology and Carcinogenesis of vinyl toluene (mixed isomers) (65%-71% meta-isomer and 32%-35% para-isomer) (CAS no. 25013-15-4) in F344/N rats and B6C3f1 mice (inhalation studies). U.S. Department of Health and Human Services PHS, National Institutes of Health	Not indicated
Study type	Inhalation	Not indicated
Species	Rat	Not indicated
Duration of exposure in key study	Chronic (2 years)	Not indicated
Critical effect	Irritation (respiratory tract)	Irritation (respiratory tract)
Critical dose value	NAEC: 162 mg/m³ (33 ppm)	Not indicated
Adjusted critical dose	-	Not indicated
Single Assessment factors	UF_L 3 x UF_H 10 x UF_A 1 = 30	Not indicated (total factor: 4)
Other effects		
Remarks	Value derived using "preferred value approach", taking into account the assessment factors noted above	No DNELs derived in a further dossier (ECHA Dissemination, 2018)
UF_L Used LOAEL; UF_H Intraspecies variability; UF_A interspecies variability; UF_S Used subchronic study; UF_D data deficiencies		

Table 2.9: Fact sheet Vinyl toluene (methylstyrene, mixture)

Rapporteur, Date			
Compound	TPGME		***Factsheet***
Parameter	**Note**	Comments	Value / descriptor
EU-LCI Value and Status			
EU-LCI value	1	[µg/m³]	1200
EU-LCI status	2	Draft/Final	Draft
EU-LCI year of issue	3	Year when EU-LCI value has been issued	2018
General Information			
CLP-Index No.	4	INDEX	-
EC-No.	5	EINECS	246-562-2
CAS-No.	6	Chemical Abstract Service number	25013-15-4 (mixture) 611-15-4 (2-vinyltoluene) 100-80-1 (3-vinyltoluene) 622-97-9 (4-vinyltoluene)
Harmonised CLP classification	7	Human health risk related classification	none for mixture, 3- and 4-vinyl toluene; 2-vinyltoluene: Acute Tox 4 (H332)
Molar mass and conversion factor	8	[g/mol] and [mg/m³ - ppm]	118.2 4.87
Key Data / Database			
Key study, Authors, Year	9	Critical study with lowest relevant effect level	NTP (1990)
Read across compound	10	Where applicable	
Species	11	Rat	Rat
Route / type of study	12	Inhalation, oral feed….	Inhalation
Study length	13	Days, subchronic, chronic…	Chronic (2 years)
Exposure duration	14	h/d, d/w	6 h/d, 5 d/week
Critical endpoint	15	Effect (s), site of	Lesions of nasal epithelia
Point of Departure (POD)	16	LOAEC, NOAEC, BMD…	LOAEC
POD value	17	[mg/m³] or ppm or [mg/kg$_{BW}$×d]	490 mg/m³ (100 ppm)
Assessment Factors (AF)	18		
Adjustment for exposure duration	19	Study exposure h/d, d/w	5.6
AF study length	20	sa→sc→c	1
Route-to-route extrapolation factor	21	-	1
AF Dose-response	22a	Reliability of dose-response, LOAEL to NOAEL	3
	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		1
Result			
Summary of assessment factors	27	Total Assessment Factor	420
POD/TAF	28	Calculated value [µg/m³ and ppb]	1166 µg/m³ (240 ppb)
Molar adjustment factor	29		

Rounded value	30	[µg/m³]	1200
Additional comments	31		

Rationale Section	32		

Rationale for critical effects

In humans, vinyl toluene is irritating to the eyes and upper respiratory tract at concentrations ≥ 1960 mg/m³ (400 ppm), strong odour was noticed at 980 mg/m³ (200 ppm), and a NOAEC for irritation at 245 – 490 mg/m³ (50 – 100 ppm) is reported (DFG, 2017).

The critical effect of vinyl toluene inhalation is respiratory tract irritation. In a chronic inhalation study with F344 rats (50 M + 50 F/concentration, exposed to 0, 100 or 300 ppm (0, 490, 1460 mg/m³) of vinyl toluene (mixed isomers: 65-71% meta- and 32-35% para-isomer), increased incidences of degenerative and non-neoplastic proliferative lesions of the nasal mucosa were observed. The lesions included diffuse hyperplasia of the respiratory epithelium and focal erosion of the olfactory epithelium. Focal respiratory epithelial metaplasia of the olfactory epithelium was seen in some exposed males, and eosinophilic cytoplasm inclusions in the olfactory epithelium occurred at increased incidences in exposed female rats. The effects were already observed at the lower exposure concentration tested (490 mg/m³, 100 ppm) (NTP, 1990).

Similar to rats, respiratory tract irritation as in the nasal epithelia and, additionally, the lung were observed in mice but at much lower concentrations (LOAEC 49 mg/m³, 10 ppm). Metabolism studies provide strong evidence that mice are much more sensitive to the toxic effects of styrene and similar compounds than rats and also humans (DFG, 1997). Therefore, the data obtained in the exposure study with mice are not considered relevant for the quantitative risk evaluation for humans.

There was no evidence of carcinogenicity in rats or mice (NTP, 1990).

The described NTP-study was conducted using a mixture of 65 – 71 % 3-vinyl toluene and 32 – 35 % 4-vinyl toluene. No data from repeated inhalation studies are available for 2-vinyl toluene, but the limited data base from studies with other exposure paths does not indicate gross differences in the toxicity of the three isomers.

Rationale for starting point

The derivation of the EU-LCI value is based on the observed lesions of the nasal epithelia in rats. Effects were already observed at 490 mg/m³, the lowest concentration tested. This LOAEC serves as the starting point for the derivation of the LCI.

Rationale for Extrapolation factors

- Factor for adjustment for exposure duration: 5.6
- Adjusted study length factor: 1 (chronic exposure)
- LOAEC → NAEC extrapolation: 3
- Interspecies differences: 2.5 (According to the ECA report No. 29, no correction has to be made for differences in systemic metabolism when the POD is related to local effects. For remaining uncertainties, a value of 1 is used for remaining specific differences for effects on skin, eye and GI tract if the mode of action implies only a simple destruction of membranes, and a default value of 2.5 is used for effects on the skin, eye and GI tract if local metabolism or receptor binding reactions are involved. Metabolism is known to be involved in the toxicity of vinyl toluenes and structurally related compounds. There are metabolism data for styrene indicating that humans may be no more or even less sensitive than rats regarding effects on the nasal epithelia (DFG, 2017). However, data for vinyl toluenes (methylstyrenes) are not available, and therefore the extrapolation factor of 2.5 is retained.
- Intraspecies differences: 10

Total extrapolation factor is: 420, leading to a value of 490 000 µg/m³ : 420 = 1200 µg/m³.

The following EU-LCI is proposed for vinyl toluenes (mixture): 1200 µg/m³.

The derived EU-LCI is below the concentration of 245 mg/m³ (50 ppm) which was reported to be tolerated upon brief exposure in a study with volunteers (Wolf et al., 1956). No reliable odour threshold for vinyl toluenes is available. For styrene, a wide range of odor thresholds of 0.012 - 263 mg/m³ (0.0028 - 61 ppm) is reported (AIHA, 2013), and the odour of styrene and vinyl toluenes are described similarly as strong and disagreeable (NTP, 1990). It is concluded that odour perception cannot be excluded at the proposed EU-LCI.

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NTP (1990) NTP Technical Report on the Toxicology and Carcinogenesis of vinyl toluene (mixed isomers) (65%-71% meta-isomer and 32%-35% para-isomer) (CAS no. 25013-15-4) in F344/N rats and B6C3f1 mice (inhalation studies). U.S. Department of Health and Human Services PHS, National Institutes of Health. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr375.pdf

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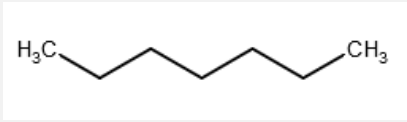
3 Toxicological evaluation of n-heptane as basis for the derivation of a EU-LCI value

3.1 Substance identification

Substance identification data and physicochemical properties are shown in Table 3.1 and Table 3.2.

Table 3.1: Substance identification of n-heptane

CAS-No. EU-No. CLP-Index-No.	Systematic Name, common names	Summary formula	Structural formula
142-82-5 205-563-8 601-008-00-2	n-heptane	C_7H_{16}	

3.2 Substance property and uses

Heptane is a colourless volatile liquid with a faint gasoline-like odour. Further data regarding the odour are reported in chapter 3.5.5. Heptane occurs naturally in petroleum gas and mineral oil and in the essential oil of conifers. Commercial heptane is a mixture of isomers. Heptane is a component of fuels. It is used as solvents in adhesives, lacquers and inks and as extractant, in the production of polymer foams and for the synthesis of toluene and other alkyl benzenes. It is also used as standard in the determination of the octane index (ACGIH, 2001; Nordic Council of Ministers, 1999; SCOEL, 1995). The current production of n-heptane in the European Union is in the order of more than 1000 t/a (ECHA Dissemination, 2017).

Table 3.2: Physicochemical properties of n-heptane (Greim, 1995; NLM, 2017a; NLM, 2017b)

Molar mass (g/mol)	Mp. (° C)	Boiling point (° C)	Vapour pressure (hPa) (at 20 °C)	Conversion 1 ppm = x mg/m³ (23 °C)	log pow (at pH)	Solubility in water (mg/L)
100.2	- 90.5	98.4	48	4.13	4.66	0.0034

3.3 Exposure

3.3.1 Indoor air

Data on the occurrence of n-heptane in indoor air from offices, homes, schools and children day care units are reported in the current AGÖF list (2013) (Table 3.4 and Table 3.5). These values, as those from Schulz et al. (2010) and Ostendorp et al. (2009), indicate a reduction of the contamination compared to previous measurement programs, e. g. the German environmental survey conducted 1985-1986 (Krause et al., 1991). In these investigations, higher concentrations of n-heptane and isoheptanes in indoor air were associated especially with new flooring and furniture and with the use of heaters.

Determinations in new building or in rooms with new floorings showed concentrations around 370-4900 µg/m³. Without known contamination sources indoor air concentrations were in the range of 0.5-18 µg/m³ (These data were collected in the USA in the 1980s and 1990s (NLM, 2017b)).

Table 3.3: Normal, attention and guidance values for heptanes in indoor air

Substance	N	LoD (µg/m³)	N > BG (% > BG)	Normal value P50 (µg/m³) 1)	Attention value P90 (µg/m³) 1)	Guidance value (µg/m³) 1)	Reference
n-Heptane	3624	1)	no data	2.0	9.0	9.0	(AGÖF, 2013)
2-Methyl-hexane	1196	1)	no data	1.0	4.0	4.0	(AGÖF, 2013)
3-Methyl-hexane	1832	1)	no data	1.0	6.3	6.3	(AGÖF, 2013)
2,3-Dimethyl-pentane	750	1)	no data	<1	4.4	4.4	(AGÖF, 2013)

1) Concentrations below the limit of detection (LoD) were taken into account using 0.5 x LoD when deriving the normal, the attention and the guidance values. If the derived value is below the LoD, the corresponding percentile is presented with the prefix „<" (LoD)..

Table 3.4: Data on the occurrence of heptanes in indoor air from homes, schools, children day care centres and offices

Substance	N	LoD (µg/m³)	N > BG (% > BG)	Normal value P50 (µg/m³) 1)	Attention value P90 (µg/m³) 1)	Guidance value (µg/m³) 1)	Reference
n-Heptane	479	2)	no data	5.1 (M). 5.95 ± 1.97 (geom. MV)	25.6	168.2	(Krause et al., 1991)
Isoheptane	479	2)	no data	7.2 (M). 7.40 ± 1.95 (geom. MV)	22.9	242.2	(Krause et al., 1991)
n-Heptane	246	3)	no data	2 (M). 10 ± 54 (arithm. MV)	23	800	(Schleibinger et al., 2001)
n-Heptane	555	1	63	1.4 (M). 2.09 (1.88 - 2.31. geom. MV)	22.8	414	(Schulz et al., 2010)
n-Heptane	285	13)	59	1.50 (M). 1.1 (geom. MV)	5.0	33	(Ostendorp et al., 2009)

2) Concentrations below the limit of detection (LoD) were taken into account using 0.7 x LoD.
3) Concentrations below the limit of detection (LoD) were taken into account using 0.5 x LoD.

3.3.2 Other sources

Mean ambient air concentrations in the 1980s were in the range of 5-60 µg/m³ in US cities (maximum values 233 µg/m³). Concentrations in rural areas were around 0.01-3.5 µg/m³ (NLM, 2017b).

3.4 Toxicokinetics

The inhalation clearance for n-heptane, determined over 4 h in a closed-chamber system at a starting concentration of 110 pm (459 mg/m³), was 54 % for alveolar ventilation and 44 % for alveolar retention, i. e. a value of 29% for the whole respiratory tract. A further study observed volunteers exposed to 0.24 or 0.66 ppm (1.0 or 2.8 mg/m³) for 40 min and calculated a pulmonal retention of 25 % (Greim, 1995).

In an open exposure system (using face masks with separate currents for inhalation and exhalation) a mean pulmonary retention of 35% was determined for the 7 subjects, independent of the exposure concentration within the range of 101 and 503 ppm (421-2098 mg/m³) (Filser et al., 1996).

A closed-chamber exposure of rats (6 h, up to 10000 ppm (about 41700 mg/m³)) gave a value of 61% for alveolar ventilation and 51 % for retention.

The dermal penetration of n-heptane through the isolated skin of rats was determined to be low (Greim, 1995).

At concentrations below 35 ppm (146 mg/m³), 80% of the absorbed amount of n-heptane was metabolised by humans and rats. The half-time of n-heptane in the body is about 0.17 h in rats and 1.88 h in humans (Csanády et al., 1992).

Volunteers and workers exposed to n-heptane excreted 2-heptanol as main metabolite in urine, small amounts were excreted as 3-heptanol, 2- and 4-heptanone and (0.006-0.009% of the total amount metabolised in humans and rats) as 2,5-heptanedione. Thus, the formation of diketones is about 40fold lower than in case of n-hexane (Filser et al., 1996; Greim, 1995).

Following an exposure of rats to 2000 ppm (8340 mg/m³) n-heptane for 6 h/d, 5 d/week for 12 weeks, or to 1800 ppm (7506 mg/m³) for 6 h, the following oxidation products could be determined in urine: 1-, 2-, 3- and 4-heptanol and further oxidation products, i.e. diols, ketones, hydroxyketones, 2,5- and 2,6-heptanedione (EPA, 2016; Greim, 1995).

In humans and animals, small amounts of 2,5-heptanedione could be detected in urine (5,5 ± 2,6 mg/L) without known exposure to n-heptane (Filser et al., 1996).

The linearily increasing concentration of heptanedione in urine could serve as an appropriate metabolite for the determination of the inner exposure to heptane, however, the amount of this metabolite is too low to produce neurotoxic effects at the MAK-value of 500 ppm. Thus, a biomonitoring as indicator for this endpoint is not necessary (Drexler and Greim, 2006).

3.5 Health effects

3.5.1 Sensory irritation and local effects

Volunteers exposed to 5000 ppm (20850 mg/m³) n-heptane for 4 min reported no irritation of eyes, mucous membranes and respiratory tract. Exposure to a mixture of 65% heptane and 33% toluene (51-122 ppm (213-509 mg/m³) heptane) for 15 min or to 130 ppm (542 mg/m³) for 30 min caused slight eye irritation in 1/5 or 4/7 subjects, respectively. Dermal exposure of the forearm caused itching, erythema, pigmentation, swelling a painful sensation within 1 h (DGMK, 1986; Snyder, 1987).

An RD_{50} of 17400 ppm (72558 mg/m³) was determined in CF-1 mice which were exposed "head only" for up to 10 min. The threshold for irritation (R_0) was calculated as 5450 ppm (22727 mg/m³). The authors of the study estimated an irritation threshold in the order of 1000-1200 ppm (4170-5004 mg/m³) (Greim, 1995; Greim, 2000).

3.5.2 Repeated dose toxicity

Haematological alterations (anaemia, leukopenia, neutropenia) described in tyre production workers cannot causally be attributed to n-heptane because of mixed exposure with other solvents (DGMK, 1986; Snyder, 1987). The same holds true for the appearance of vertigo, paresthesia in the extremities, leg pain, a reduction in nerve conduction velocity and signs of bilateral denervation of leg muscles which had been observed in a female shoemaker following exposure to 36 ppm (150 mg/m³) n-heptane and several other solvents including methyl ethyl ketone. Workers in shoe production exposed to mean concentrations of 45 ppm n-heptane (188 mg/m³) complained more often than non-exposed controls about dysesthesia, seizures and vertigo. Neurological examinations revealed subclinical neuropathy reduced distal sensory conduction velocity and increased latency of sensory action

potentials of the nervus medianus. Mixed exposure had occurred in these cases, too, and the extent of exposure prior to the examination is unclear (Greim, 1995).

In a study not available as original document Sprague-Dawley rats (15 M + 15 F/group) were exposed 6 h/d, 5 d/week for 26 weeks to n-heptane (98.5% pure) at concentrations of 400 or 3000 ppm (1668 or 12510 mg/m³). During the initial weeks the animals showed increased respiration and languor, especially at the higher concentration. These effects ceased during the later phase of the study. Histological examinations were performed in 3, 5 and 4 males and females from the groups after 9, 18 and 27 weeks and in the remaining animals (maximum 3 M + 3 F/group) after a postexposure recovery phase of two weeks. One female of each exposure group died prior to the end of the study, both cases were judged as non-exposure related. Body weight gain, haematology and urine analysis showed no changes; the only clinical chemical parameter altered was an increased phosphatase activity in the serum of females (significant at the high dose, increase 1.6 fold). Except for the nervous system no other organs were histologically examined. No pathological or neurochemical alterations could be detected in the peripheral and central nervous system. This unpublished study is referred to in different ways: Bio/Dynamics (1980) (A 26-week inhalation toxicity study of heptane in the rat, Project no. 78-7233), API Med. Res. Publ. No. 28-31209 or EPA (1981) (A 26-week inhalation toxicity study of heptane in the rat with cover letter. Bio Dynamics Inc. EPA/OTS #FYI-AX-1081-0135), but is described in detail in several reviews (DGMK, 1986; EPA, 2016; Snyder, 1987) including the registration dossier (ECHA Dissemination, 2017) where the study is rated as reliable with restrictions (RL2 according to Klimisch et al. (1997)). Nevertheless, EPA (2016) criticised the study because of its reduced extent of examination.

In the study of Frontali et al. (1981) exposure of male Sprague-Dawley rats (9 h/d, 5 d/week, 30 weeks) to 1500 ppm (6255 mg/m³) n-heptane (99.5% pure) neither led to a reduction of body weight gain nor behavioural changes or pathological alterations of the nervous systems. Other organs were not examined.

Takeuchi et al. (1980; Takeuchi et al., 1981) studied the effects of a subchronic exposure of male Wistar rats (7/group; 12 h/d, 7 d/week, 16 weeks) to 3000 ppm (12510 mg/m³) n-heptane. The body weight of the exposed animals was slightly lower than that of control animals throughout the study, but statistical significance was reached only in week 8. Compared to group exposed to n-hexane, the animals exposed to n-heptane neither showed behavioural changes nor other neurotoxic effects (histological alterations, reduction of peripheral nerve conduction velocity). This study served as key study in the REACH registration dossier (rated with reliability 2) using the NOAEC of 3000 ppm (ECHA Dissemination, 2017).

The latter two studies demonstrate that the neurotoxicity of n-heptane is lower than that of n-hexane; however, no conclusions with respect to the toxicity in other organs may be drawn from these studies.

Male Long-Evans rats (9-11/group) exposed to 800 or 4000 ppm (3336 or 16680 mg/m³) 6 h for 28 d did not show signs of poisoning. Two months after exposure to the higher concentration (which reduced body weight gain) reduced amplitudes of auditory evoked potentials in the brain stem were described. These authors state that this effect provides evidence for lesions of the hair cells in the cochlea (Simonsen and Lund, 1995). Additional studies indicate that the peripheral neuropathy typical for n-hexane does not develop following n-heptane exposure at concentrations up to 3000 ppm (12510 mg/m³) and exposure times of up to 30 months but does develop with commercial heptane containing 52% n-heptane after exposure to 1500 ppm (6255 mg/m³), 5 h/d, 5 d/week for up to 6 months (DGMK, 1986; Greim, 1995; Nordic Council of Ministers, 1999).

2,5-heptanedione, a minor metabolite of n-heptane in the body, was found to be neurotoxic to the peripheral nerves of rats in a subchronic oral study at doses ≥ 1000 mg/(kg bw x d). Its toxicity was 2.5-5fold lower than that of 2,5-hexanedione (TPHCWG, 1997).

3.5.3 Genotoxicity and carcinogenicity

Genotoxicity

All published studies reported negative results throughout. The substance had been tested in Ames tests using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 and in *Escherichia coli* WP2/WP2uvr A. Furthermore, tests for mitotic gene conversion in *Saccharomyces cerevisiae* and for the induction of chromosomal aberrations in rat liver cells had been performed *in vitro* (Brooks et al., 1988). *In vivo,* a dominant-lethal assay in rats and mice using 1ml/kg bw (688 mg/kg bw) „50 Thinner" (containing 68.4 % n-heptane) showed a negative result (DGMK, 1986).

Carcinogenicity

No data are available for this endpoint, including the registration dossier for n-heptane according to REACH (ECHA Dissemination, 2017).

3.5.4 Toxicity to reproduction

No studies are available regarding reproductive or developmental toxic effects of n-heptane.

Read-across: n-Hexane had no effect on reproduction in a 2-generation study in rats (according to OECD guideline 416) up to the highest concentration tested (9000 ppm = 32220 mg/m³). The only observed effect was a reduced weight gain in animals of the F1 and F2 generation (NOAEL 3000 ppm, 10740 mg/m³). A mixture of C_7-C_9 alkanes neither caused maternal nor developmental toxicity in a segment II study (following FDA guideline) up concentrations up to 1200 ppm (5004 mg/m³) (ECHA Dissemination, 2017).

3.5.5 Odour perception

Reported odour thresholds for n-heptane are in the range of 40-546 ppm (167-2277 mg/m³) (Greim, 1995). SCOEL (1995) refer to a threshold of 400 ppm (1668 mg/m³) and characterise the odour as weakly petroleum like. An odour threshold of 150 ppm (626 mg/m³) has also been reported (Amoore and Hautala, 1983), and a range of 200 to 1280 mg/m³ (Ruth, 1986). The latter author characterised the odour as gasoline-like.

A considerable (about 60 fold) lower odour detection threshold of 0.67 ppm (2.8 mg/m³) has been determined by Nagata (2003). This value was determined by the "triangle bag method" in which the subjects inhale the the test substance in air directly in comparison to pure air.

3.6 Evaluation

3.6.1 Existing regulations and classifications

General population

A DNEL of 447 mg/m³ was derived for inhalation exposure of the general population in the registration dossier ECHA, 2017). No further details of the derivation is presented, it is, however, likely that this value is based on the key study for repeated exposure (Takeuchi et al., 1980; Takeuchi et al., 1981).

The NIK value for n-heptane is 21 mg/m³; this value is based on the MAK value described below using a divisor of 100 (AgBB, 2015).

n-Heptane is listed in IRIS (EPA, 2017), but no reference concentration (RfC) or dose (RfD) was derived.

An Ontario's Ambient Air Quality Criteria (AAQC) of 11 mg/m³ has been published in 2016 (Anon., 2016). Furthermore, RIVM has reported a tolerable concentration in air (TCA) of 18.4 mg/m³ in 2007 (Dusseldorp et al., 2007). Background documents for both values are not available.

The Total Petroleum Hydrocarbon Working Group (TPHCWG, 1997) has derived a reference dose for oral exposure of 2 mg/(kg bw x d). This value is based on a comparison with n-hexane: Pharmacokinetic calculations provided evidence that the formation of 2,5-heptanedione in rats and humans is 38fold lower than for 2,5-hexanedione. Assuming conservatively that both diketones are equally potent, this factor was used for the derivation of the reference dose. Since the oral reference dose for n-hexane was derived from the inhalation reference concentration of 0.2 mg/m^3, a reference dose for n-heptane can be derived based on the n-hexane data as 38 x 0.2 mg/m^3 = 7.6 mg/m^3 for n-heptane.

EPA (2016), based on the ototoxicity study of Simonsen and Lund (1995), derived a reference concentration of 0.4 mg/m^3 by calculating a human equivalent benchmark concentration ($BMCL_{1SD}$) of 1170 mg/m^3, taking into account an extrapolation factor of 3000.

Other reference doses for oral exposure are without the scope of this evaluation and are not reported here.

Workplace

SCOEL (1995) derived an 8-h TWA of 500 ppm (2085 mg/m^3) for n-heptane. This value is based on the NOAEC of 3000 ppm from the study of Takeuchi et al. (1980; Takeuchi et al., 1981), taking into account an extrapolation factor of 5. Some European countries have established considerably lower values (e. g., 200 ppm in Denmark and Sweden; 400 ppm in Belgium and France) (IFA, 2017); no justifications are available.

The German MAK value is at the same level as the TWA of SCOEL (1995); no specific study is mentioned as key study. Supportive evidence for the value which was already derived in 1958 is reported to come from studies on respiratory depression, a subchronic inhalation study with no effect at 3000 ppm and the lower formation of 2,5-heptanedione compared to 2,5-hexanedione in humans and rats (Greim, 1995; Greim, 2000).

An OEL of 500 ppm for all isomers of heptane is reported in the German TRGS 900, referring to the MAK value (BMAS, 2017).

The TLV of 400 ppm (1668 mg/m^3) derived by ACGIH (2001) has been justified with the narcotic and irritating effect of n-heptane, without assigning the mentioned effects to selected studies.

3.6.2 Derivation of a EU-LCI value

The 26-weeks study from Bio/Dynamics is to be discussed as the basis for the derivation of a EU-LCI value. The study is not available as original report, but described in reviews in sufficient details to allow for an evaluation. This study with rats is rated in all reviews with a NOAEC of 3000 ppm (12510 mg/m^3), except for the registration dossier where this concentration is judged as a LOAEC for acute neurotoxicity, because of the symptoms (increased respiration, weakness) observed during the first week. The histological evaluation was restricted to the nervous system; however, blood and urine analyses revealed no relevant disorders of liver and kidney. The exposure was performed 6 h/d, 5 d/week, leading to a NOAEC of 536 ppm (2235 mg/m^3) adjusted for continuous exposure. The quality of the study is rated as acceptable with limitations (reliability 2) (ECHA Dissemination, 2017).

Furthermore, the study of Takeuchi et al. (1980; Takeuchi et al., 1981) has to be taken into account, in which 16-week exposure (12 h/d, 7 d/week) of rats at 3000 ppm (12510 mg/m^3) had no effects, including no neurotoxicity, besides a marginal reduced body weight gain which was only statistically significant in week 8. Adjusting for continuous exposure leads to a concentration of 1500 ppm (6255 mg/m^3). This study is rated as key study in the registration dossier with a NOAEC of 3000 ppm (ECHA Dissemination, 2017) and was also judged as acceptable with limitations (RL2). This study substantiates the lower neurotoxicity of n-heptane compared to n-hexane, but does not allow to draw conclusions with respect to the toxicity to other organs.

A somewhat higher LOAEC of 4000 ppm (16680 mg/m³) is documented in the ototoxicity study of Simonsen und Lund (1995) which was also performed with rats (28 d, 6 h/d), using pure n-heptane (purity 99.5%). The NOAEC in this study was 800 ppm (3336 mg/m³). It must, however, be noted that such effects have up to now not been described for other aliphatic hydrocarbons but only for chlorinated or aromatic hydrocarbons (Greim, 1995). Only in case of the simultaneous co-exposure of toluene and n-hexane, the toxicity of toluene was increased by n-hexane (Simonsen and Lund, 1995). n-Hexane was considered as ototoxic in a review dealing with this endpoint (Vyskocil et al., 2012). However, the authors of this review do not consider that this observation may be transferred to n-heptane, but conclude in analogy with Greim that the observations described by Simonsen und Lund (1995) are a single finding which cannot be finally evaluated. Malley et al. (2000) observed a reduced or lacking reaction to an auditory stimulus following exposure of rats and mice to ≥ 2000 ppm (7000 mg/m³) cyclohexane. Similar effects have been reported by Kreckmann et al. (2000) in adult animals in a 2-generation study on reproductive toxicity, again at concentrations of ≥ 2000 ppm (7000 mg/m³). However, the authors of both of these studies concluded that these effects do not indicate specific ototoxicity but are the results of a sedation effect.

The narcotic effect observed at high acute exposure has to be considered as a typical non-specific effect of liphophilic hydrocarbons but not as specific neurotoxic effect (e. g. see DGMK, 1986; Greim, 1995; Snyder, 1987). Therefore, these observations of a specific ototoxicity of n-heptane need confirmation and can only be seen as hypothesis. Adjusting for continuous exposure would lead to a concentration of 1000 ppm (4170 mg/m³) as LOAEC and 200 ppm (834 mg/m³) as NOAEC.

Under these circumstances the NOAEC of 3000 ppm (12510 mg/m³) from the study of Bio/Dynamics will be used as POD despite of its conceptual limitations. This study has not been published but is described in sufficient detail in several reviews. Although signs of acute toxicity appeared in the initial phase of the study, no such effects were noted in other studies with repeated exposure of two other strains of rats at the same or even higher exposure concentrations (see chapter 3.5.2 and the summary of data presented above). Taking into account the intraspecies extrapolation of 10 should provide sufficient protection against acute effects of n-heptane. Alternatively, the concentration of 3000 ppm could be regarded as minimal LOAEC for a sensitive strain concluding that it could be justified to reduce the interspecies extrapolation factor but finally ending up with the same LCI. The reduced scope of examination in this study is taken into account in the "quality of whole database factor" (see below).

The reference concentration of 0.4 mg/m³ derived by the US EPA (2016) is based on the ototoxicity study of Simonsen and Lund (1995). This value is by a factor of 100 lower than the EU LCI value proposed here. However, the discrepancy is at least partially resolved when one considers that the EPA authors (in line with their methodology) used higher extrapolation factors of 10 for data base gaps (in this evaluation: 3, according to EC, 2013) and also of 10 for the extrapolation to chronic exposure (this evaluation: 6, according to EC, 2013). Furthermore, it is concluded that a specific ototoxic effect of n-heptane is not yet sufficiently supported by the overall database (see above) to serve as reliable basis for the derivation of a EU-LCI.

Table 3.5: Derivation of a EU-LCI value for n-heptane (for explanation, see text)

Endpoint	POD (mg/m³)	Extrapolation factor					Value (mg/m³)	Reference
		LOAEC→ NAEC	Inter-species	Intra-species	Time	Data gaps		
Systemic toxicity	12510 (2235[1)]) (NOAEC)	-	2.5	10	2	3	15	Bio/Dynamics (1980), unpublished

1): Already adjusted for continuous exposure (6 h/24 h x 5 d/7 d)

The following adjustment factors are used:

- Adjustment for continuous exposure (6 h/d, 5 d/week): 5.6
- Adjusted study length factor (subchronic to chronic): 2
- Interspecies extrapolation: 2.5
- Intraspecies extrapolation (interindividual variability, general population): 10
- Quality of whole database (no complete histopathology and lack of studies regarding toxicity to reproduction): 3

Total assessment factor: 5.6 x 150. This leads to a concentration of 2235 : 150 = 15 mg/m^3.

A EU-LCI value (rounded) for n-heptane of 15 000 µg/m^3 is proposed.

The proposed value is a little lower than the NIK value of 21 000 µg/m^3. However, that value is not established on a toxicological evaluation but corresponds to a 1/100 of the MAK/AGW value.

The proposed EU-LCI value is higher than the odour threshold of 2.8 mg/m^3 reported by Nagata (2003); however, other authors reported considerable higher thresholds of 167-1668 mg/m^3 (see chapter 3.5.5). Therefore, perception of odour by sensitive subjects may not be excluded at the proposed EU-LCI value.

3.7 References

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3.8 Fact and data sheet for n-heptane

Table 3.6: Data collection sheet n-heptane

Rapporteur, Date						
Compound	**n-HEPTANE**			***Data collection sheet***		
N° CAS 142-82-5 1 ppm = 4,1 mg/m³	**EU-Classification:** F; R11, R67, Xn; R65, Xi; R38 **CLP:** Flam. Liq.2 (H225), Skin Irrit. 2 (H315), Asp. Tox. 1 (H304), STOT SE 3 (H336)					
Organization Name	ACGIH	DFG	SCOEL	TPHCWG	EPA	Reach registrants
Risk Value Name	TLV/STEL	MAK	TWA / STEL	Inhalation RfC	Inhalation RfC	DNEL
Risk Value (mg/m³)	1640 (400 ppm)	2085 (500 ppm)	2085 (500 ppm)	7.6 (1.8 ppm)*	0.4 mg/m³ (0.1 ppm)*	447 (107 ppm)*
Reference period	Chronic (worker)	Chronic (worker)	Chronic (worker)	Chronic	Chronic	Chronic ($DNEL_{Gen.\ Pop.\ long\ term}$)
Risk Value (mg/m³) Short Term (15 min)	2050 (500 ppm)	2085 (500 ppm)	-	-	-	-
Year	2001	1958, updated 1995 and 2000	1995	1997	2016	2011, updated 2017
Key Study	No key study, comparison to pentane, hexane and octane	Confirmation of value of 1958 by more recent data, no key study	Takeuchi et al (1980, 1981)	No key study, comparison to neurotoxicity of n-hexane	Simonsen und Lund (1995)	Not indicated. Study by Takeuchi et al (1980, 1981) reported as „key“ study for repeated dose inhalation
Study type	-	-	16 weeks inhalation study (0, 3000 ppm = 12510 mg/m³)	-	28 days inhalation study (0, 800 and 4000 ppm, 3336 und 16680 mg/m³)	16 weeks inhalation study (0, 3000 ppm = 12510 mg/m³)
Species	-	-	Wistar rats (7 males/group)	-	Long-Evans rats (9-11 males per group)	Wistar rats (7 males/group)
Duration of exposure in key study	-	-	12 h/d, 7 d/w for 16 weeks	-	6 h/d for 28 days	12 h/d, 7 d/w for 16 weeks
Critical effect	Narcotic and irritative effects	-	No critical effect	Neurotoxicity	Ototoxicity	No critical effect
Critical dose value	-	-	$NOAEC_{systemic}$: 12510 mg/m³	38-times lower formation of 2,5-heptandione compared to 2,5-hexandione	$BMCL_{1SD}$	$NOAEC_{systemic}$: 12510 mg/m³

		-	$LOAEC_{systemic}$: not determined			$LOAEC_{systemic}$: not determined
Adjusted critical dose	-	-	Not indicated	0.2 mg/m³ for n-hexane	Human equivalent $BMCL_{1SD}$: 1170 mg/m³	$NOAEC_{timeadjusted}$: 6150 mg/m³ (12 h/d, 7 d/w)
Single Assessment factors	Not indicated	-	Overall factor of 5	-	UF_A: 3 UF_H: 10 UF_D: 10 UF_S: 10	Not indicated
Other effects						
Remarks			No time adjust	Read-across	Endpoint not sufficient-ly confirmed	
UF_H Intraspecies variability; UF_A interspecies variability; UF_S Used subchronic study UF_D data deficiencies						

*calculated in the context of this evaluation

Table 3.7: Fact sheet n-heptane

Rapporteur, Date			
Compound	**n-HEPTANE**		***Factsheet***
Parameter	**Note**	Comments	Value / descriptor
EU-LCI Value and Status			
EU-LCI value	1	[µg/m³]	15000
EU-LCI status	2	Draft/Final	Draft
EU-LCI year of issue	3	Year when EU-LCI value has been issued	2018
General Information			
CLP-Index No.	4	INDEX	601-008-00-2
EC-No.	5	EINECS	205-563-8
CAS-No.	6	Chemical Abstract Service number	142-82-5
Harmonised CLP classification	7	Human health risk related classification	Skin Irrit. 2 (H315), Asp. Tox. 1 (H304), STOT SE 3 (H336)
Molar mass and conversion factor	8	[g/mol] and [mg/m³ - ppm]	100.2 4.13
Key Data / Database			
Key study, Authors, Year	9	Critical study with lowest relevant effect level	Bio/Dynamics (1980)
Read across compound	10	Where applicable	-
Species	11	Rat	Sprague-Dawley rats (15/sex/ group)
Route / type of study	12	Inhalation, oral feed….	Inhalation
Study length	13	Days, subchronic, chronic…	Subchronic, up to 26 weeks (two weeks of recovery)
Exposure duration	14	h/d, d/w	6 h/d, 5 d/w
Critical endpoint	15	Effect (s), site of	Slight transient narcotic effects at start of study, not considered relevant in view of other studies
Point of Departure (POD)	16	LOAEC, NOAEC, BMD…	NOAEC
POD value	17	[mg/m³] or ppm or [mg/kg_{BW}×d]	12510 mg/m³ (3000 ppm)
Assessment Factors (AF)	18		
Adjustment for exposure duration	19	Study exposure h/d, d/w	5.6
AF study length	20	sc→c	2
Route-to-route extrapolation factor	21		1
AF Dose-response	22a	LOAEC→NOAEC	1
	22b		1
Interspecies differences	23a	Remaining differences	2.5
	23b		1
Intraspecies differences	24	Kinetic + dynamic Worker - general population	10
AF (sensitive population)	25		1
Other adjustment factors Quality of database	26		3
Result			
Summary of assessment factors	27	Total Assessment Factor	840
POD/TAF	28	Calculated value [µg/m³ and ppb]	15 000 µg/m^3, 3.6 ppb
Molar adjustment factor	29		
Rounded value	30	[µg/m³]	15000
Additional comments	31		

Rationale Section	32		

Rationale for critical effects

The reliability on data of human effects (haematological changes, unspecific symptoms, neurological effects) is limited due to mixed exposure with other (neurotoxic) solvents. Animal studies did not show clear or doubtless adverse effects up to the highest concentrations tested.

The derivation of the EU-LCI is based on the NOAEC of a 26-weeks rat study from Bio/Dynamics, which is unpublished, but referred sufficiently in detail in the reviews and the REACH registration dossier (there considered as "reliable with restrictions, RL 2"). In this study Sprague-Dawley rats (15 M + 15 F/group) were exposed 6 h/d, 5 d/week for 26 weeks to n-heptane (98.5% pure) at concentrations of 400 or 3000 ppm (1668 or 12510 mg/m³).Other studies support the lack of adverse effects at comparable exposure concentrations. One study documenting ototoxicity in rats at higher concentrations is a stand-alone result and needs confirmation.

Rationale for starting point

The derivation of the EU-LCI value is based on a subchronic inhalation study (26 weeks) in rats. Slight transient narcotic effects at the start of study were not considered as adverse effect in view of other studies without effects at comparable and even higher concentrations. The NOAEC of 3000 ppm (12510 mg/m³), the highest concentration tested, serves as a POD, adjusted from intermittent (6 h/d, 5 d/w) to continuous exposure of 2235 mg/m^3.

Rationale for Extrapolation factors

- Factor for adjustment for exposure duration: 5.6
- Adjusted study length factor: 2 (subchronic study)
- Interspecies differences: 2.5 (default value for systemic effects)
- Intraspecies differences: 10 (default value)
- Other adjustment factors: 3 (lack of data on reproductive and developmental endpoints)

Total extrapolation factor: 840, leading to a value of 14900 µg/m³ which is rounded to 15000 µg/m³.

The following EU-LCI is proposed for n-heptane: 15000 µg/m³. The derived value is higher than the lowest reported odour threshold of 2.8 mg/m³ (Nagata, 2003), but lower than other reported odour thresholds (≥ 167 mg/m³) (Greim, 1995).

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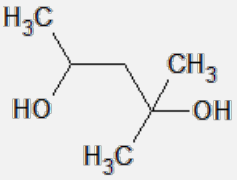
4 Toxicological evaluation of hexylene glycol as basis for the derivation of a EU-LCI value

4.1 Substance identification

Substance identification data and physicochemical properties of hexylene glycol (2-methylpentane-2,4-diol) are shown in Table 4.1 and Table 4.2. The chemical structure includes one chiral centre, the commercial product is a racemic mixture. No toxicity data are available for the two enantiomers.

Table 4.1: Substance identification of hexylene glycol (2-methylpentane-2,4-diol) (ECHA Dissemination, 2017)

CAS-No. EU-No. CLP-Index-No.	Systematic Name, common names	Summary formula	Structural formula
107-41-5 203-489-0 603-053-00-3	2-methylpentane-2,4-diol, 2,4-dihydroxy-2-methylpentane, 2,4-dihydroxy-2-methyl pentane, 2-methyl 2,4 pentanediol, 4-methyl 2,4 pentanediol	C6H14O2	

4.2 Substance properties and uses

Hexylene glycol is a colourless hygroscopic liquid with a mild sweet odour. Further data regarding the odour are reported in chapter 4.5.5. Hexylene glycol is soluble in water. It is also soluble in alcohol, ether and lower aliphatic and aromatic hydrocarbons and miscible with fatty acids (Anon., 1985; DFG, 1997; HSDB, 2015).

2-Methyl-2,4-pentanediol has been detected as a volatile aroma and flavour component of apples. Hexylene glycol is a large-scale industrial products (tonnage band in the EU: 10000–100000 t/a) (ECHA Dissemination, 2017). Hexylene glycol is used in paints, lacquers and varnishes as a solvent plasticizer in surface coatings, both in water- and solvent-based paints. It is also used in cosmetics, in leather and textile processing and in antifreezes and as dispersant agent in cleaners, disinfectants and pesticide formulations (OECD SIDS, 2001).

Table 4.2: Physicochemical properties of hexylene glycol (2-methylpentane-2,4-diol) (ECHA Dissemination, 2017)

Molar mass (g/mol)	Mp. (° C)	Boiling point (° C)	Vapour pressure (hPa) (at 20 °C)	Conversion 1 ppm = x mg/m³ (23 °C)	log pow	Solubility in water (g/L)
107-41-5	118.18	-50	< 0.1	4.9	0	68.6

4.3 Exposure

4.3.1 Indoor air

Very few data are available regarding the occurrence of hexylene glycol in indoor air (Table 4.3). It is reported that hexylene glycol was qualitatively detected as a minor volatile emission component from new carpets (HSDB, 2015). The substance was detected in only one of 66 samples from various indoor rooms in Germany (Hofmann and Plieninger, 2008). Based on 1244 measurements of samples from various indoor air sources, AGÖF presented a "normal value" (presenting the median) and an "attention value" (presenting the 90th percentile) of < 5 µg/m^3 for hexylene glycol (AGÖF, 2013).

Table 4.3: Data on the occurrence of hexylene glycol in indoor air from homes, schools, children day care centres and offices

Rooms	N	LoD (µg/m³)	N > LoD (% > LoD)	Median (µg/m³)	P95 (µg/m³)	Maximum (µg/m³)	Reference
Offices, homes, (pre)-schools, Germany	451	7.3	1			9	(Hofmann and Plieninger, 2008)

4.3.2 Other sources

There are no data available.

4.4 Toxicokinetics

Systemic effects observed after oral and dermal exposure show that the substance is absorbed via these pathways. However, no reliable quantitative data are available. It is stated that hexylene glycol is absorbed through the mucosa of the airways and the gastrointestinal tract (DFG, 2001b). Generally, saturated aliphatic glycols are known to be well absorbed by all routes of administration (Anon., 2005; ECETOC, 2005; Kumagai et al., 1999).

Five test persons were given oral doses of a 10 % aqueous solution of hexylene glycol, either as single 5 g doses or two 2.5 g doses daily on 5 consecutive days, or 1 g daily for 8 to 11 days followed by 2 g daily for 13 to 14 days; 18% to 35% of the dose was excreted in the urine. According to the authors, half of the excreted substance was in the form of the glucuronide of hexylene glycol. Hexylene glycol was detectable in the urine for 5 to 11 days after the last dose. A half-life was not given. After administration of single doses ≤ 2 g or daily doses ≤ 600 mg for 90 days, with the unspecific photometric method which was used (detection limit about 100 mg in the 24-hour urine) neither free hexylene glycol nor its glucuronide could be detected in the urine (DFG, 2001b; Jacobsen, 1958a; 1958b).

Rats received oral doses of 200 mg/(kg bw x d) for 60-131 d. 32-47% of the dose was excreted in urine, mostly (96 %) as glucuronide. Similar values (37-60%) were obtained at a dose of 100 mg/(kg bw x d) (DFG, 2001b; Larsen, 1958). Rabbits excreted on average 67% (range 49-93%) as glucuronide in urine within 1-2 d after an oral dose of 118 mg/(kg bw x d) (DFG, 2001b; Gessner et al., 1960).

Generally, secondary alcoholic hydroxyl (OH) groups may be oxidized by alcohol dehydrogenase or monooxygenases to ketones. In case of hexylene glycol (2-methylpentane-2,4-diol), metabolic oxidation at the secondary hydroxyl group would lead to the formation of the ketone 4-hydroxy-4-methylpentan-2-one (diacetone alcohol). It is reported that this compound may be excreted as glucuronide or sulfate in urine or enter intermediary metabolic pathways and be excreted as carbon dioxide (DFG, 2001a).

However, this compound could not be detected in the blood of male Sprague-Dawley rats (n=9) which had received a single oral dose of 540 mg hexylene glycol/kg bw within the 24h observation period (ECHA Dissemination, 2017). In this study, the maximum plasma concentration of hexylene glycol was reached after 1.5 h and then decreased for the next 6 h in a monophasic manner.

Hexylene glycol was shown to be excreted into the milk of rat dams after oral administration and ^{14}C-labelled hexylene glycol could be detected in mothermilk-nursed pups (ECHA Dissemination, 2017).

4.5 Health effects

4.5.1 Sensory irritation and local effects

Exposure of 12 male and 12 female test persons to 50 ppm hexylene glycol vapour (245 mg/m^3, approximately the saturation concentration at 25°C) for 15 min caused eye irritation in most of the persons but no irritation of the nose or throat. No details were reported (Silverman et al., 1946).

No signs of irritation or other effects were noted in two studies in which rats (n=6) were either exposed to an atmosphere saturated with hexylene glycol at room temperature (about 310 mg/m^3) for 1 h or for 8 h (DFG, 2001b).

4.5.2 Repeated dose toxicity

There are no data available for the effects of repeated inhalation of hexylene glycol in humans. No effects on the well-being of five subjects were reported who had ingested hexylene glycol as aqueous solution (either 1 g/d for 8-11 d and then 2 g/d for 13-14 d or 5 g/d on 5 consecutive days). Also, the results of urine analysis were normal (DFG, 2001b; Jacobsen, 1958b).

Wistar rats (10 M + 10 F/group) were exposed to an aerosol atmosphere of 700 mg/m^3 (140 ppm) hexylene glycol for 7 h/d, 5 d/week for a total of 9 exposures (UC, 1976). No overt signs of toxicity, effects on body weight gain, or absolute or relative liver or kidney weights were observed. There were no microscopic lesions in the major organs. Upon histological examination, two rats showed congestion of the trachea and one rat had submucosal hemorrhage. The documentation was considered as insufficient for assessment (ECHA Dissemination, 2017).

There are no further studies available with inhalation exposure to hexylene glycol.

Several older studies investigated the toxicity of hexylene glycol in rats and mice after oral exposure. These studies indicate that liver, kidney and adrenal glands may be potential target organs. However, no NOAELs can be derived from these data because these studies do not meet present day requirements (DFG, 2001b). More recently conducted studies, however, are also available which followed or were largely equivalent to OECD guidelines.

In a subacute study with Sprague-Dawley rats (6 M + 6 F/group), the animals received 0, 40, 200 or 1000 mg/(kg bw x d) hexylene glycol by gavage for 2 weeks. No mortality, clinical signs of toxicity or effects on food uptake were observed. At the highest dose, slightly higher absolute and relative liver weights (in both sexes) and slightly higher absolute and relative kidney weights (in males) were noted. Minimum to slight hepatocellular hypertrophy was also observed in both sexes at the highest dose; this was considered to be adaptive and not as an adverse effect. Acidophilic globules in the cortical tubular epithelium of the kidneys were seen in males receiving ≥ 200 mg/(kg bw x d); this change was regarded as related to the accumulation of α2u-globulin (ECHA Dissemination, 2017; Fabreguette, 1999b). These results are in line with those of a dose-finding study with Sprague-Dawley rats (5 M + 5 F/dose) in which the only significant effect after 14 d of gavage exposure with up to 1000 mg/(kg bw x d) was an increase in adrenal, kidney and liver weights in both sexes at 1000 mg/(kg bw x d) and of liver weight in males at 300 mg/(kg bw x d) (ECHA Dissemination, 2017).

A subchronic oral toxicity study was performed in Sprague Dawley rats (20 M + 20 F/control and high dose, 10 M + 10 F/group at other doses). The animals received 0, 50, 150, or 450 mg/(kg bw x d) hexylene glycol by gavage for 13 weeks. There were no effects on mortality, clinical signs, body weight, food or water consumption, food efficiency, ophthalmology, or neurobehaviour. Minor and/or reversible effects associated with treatment were observed on haematology (increased fibrinogen in mid- and high-dose males and in high-dose females, considered secondary to inflammatory lesions in the stomach and forestomach), clinical chemistry (increased cholesterol in high-dose males and females and decreased glucose in mid- and high-dose animals of both sexes, considered related to increased demand in liver function noted histologically, with evidence of reversibility), urine analysis (lower pH and higher specific gravity in high-dose males, considered secondary to male rat-specific excretion of α2u-globulin, with evidence of reversibility), and organ weights (higher liver weight in high-dose males and females and higher kidney weight in high-dose males, with evidence of reversibility). Macroscopic lesions were noted on kidneys of high-dose males at the end of the treatment period, with evidence of reversibility. Microscopic findings in the mid- and high-dose groups included hepatocellular hypertrophy in both sexes (considered a response to an increased demand in liver function), kid-

ney changes in males (acidophilic globules in tubular epithelium, tubular basophilia, and peritubular fibrosis; all considered related to α2u-globulin), and local irritating effects in the stomach and forestomach of both sexes. All of these microscopic effects showed evidence of reversibility. Based on these findings a NOEL of 50 mg/(kg bw x d) and a NOAEL of 450 mg/(kg bw x d) (the highest dose tested) were derived (Fabreguette, 1999a).

4.5.3 Genotoxicity and carcinogenicity

Genotoxicity

In vitro, hexylene glycol was not mutagenic in bacterial mutation assays tested with and without exogenous metabolic activation system (S9 mix from rat liver) in all tested strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) and in *Escherichia coli* WP2uvr A. No cytotoxicity was observed in the assays. Incubation with positive control substances in the presence of metabolic activation resulted in anticipated increases in the reverse mutation rate but did not always do so in the absence of metabolic activation. This study is therefore considered reliable with restrictions (Brooks et al., 1988; ECHA Dissemination, 2017; OECD SIDS, 2001). Hexylene glycol did not induce mitotic gene conversions in a yeast gene mutation assay with *Saccharomyces cerevisiae* JD1 with and without exogenous metabolic activation. No cytotoxicity was observed in the assays. As the post-treatment period was 3 days as opposed to the recommended 4 to 7 days, this study is considered reliable with restrictions (Brooks et al., 1988; ECHA Dissemination, 2017; OECD SIDS, 2001).

In mammalian cells, the substance did not induce mutations in a thymidine kinase mutagenicity assay with L5178Y lymphoma cells of mice in the presence or absence of exogenous metabolic activation. No cytotoxicity was observed in the assay when testing up to limit concentrations (ECHA Dissemination, 2017). Furthermore, hexylene glycol did not induce chromosomal aberrations or cytotoxicity with and without metabolic activation in Chinese Hamster Ovary (CHO) cells when tested up to limit concentrations (CHO) (Brooks et al., 1988; ECHA Dissemination, 2017; OECD SIDS, 2001).

Read-across:

Diacetone alcohol (4-hydroxy-4-methylpentan-2-one), a possible *in vivo* oxidation product of hexylene glycol (2-methylpentane-2-4-diol), was not mutagenic in any of seven *in vitro* tests (3 in prokaryotes with *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) and *Escherichia coli* WP2uvr A), 2 gene mutation assays in mammalian cells (Thymidine Kinase test in L5178Y mouse lymphoma cells) and 2 chromosome aberration tests in mammalian cells (Chinese Hamster lung cells and rat liver RL-4 cells)) in the absence or presence of exogenous metabolic activation system. Except for one bacterial test, incubation with positive control substances in the absence or presence of metabolic activation were reported to result in anticipated increases in the mutation frequencies (ECHA Dissemination, 2018).

In vivo genotoxicity data are not available.

Carcinogenicity

No carcinogenicity / chronic toxicity studies are available.

4.5.4 Toxicity to reproduction

There are no studies available with inhalation exposure to hexylene glycol.

Fertility

A reproduction/developmental toxicity screening test according to OECD Guideline 421 was conducted with Sprague-Dawley rats (10 M + 10 F/group). 0, 200, 500 or 1000 mg/(kg bw x d) hexylene glycol was given by gavage for 4 weeks before mating, through mating, gestation and the beginning of the lactation period (until day 4 after parturition) (total exposure period for M and F at least 10 weeks) (ECHA Dissemination, 2017).

No unscheduled deaths occurred in any male groups during the study. Two F0 females from the high-dose group were prematurely sacrificed on lactation day 2 or 3 following the death of their litter. No relevant clinical signs were recorded for the parent animals. There were no effects of treatment on mating performance or on the fertility index. All pregnant females gave birth to live pups, the length of gestation was similar in all groups and no difficulties at delivery were observed. Pre-implantation loss was similar in all groups. At 1000 mg/(kg bw x d), there was a marked increase in pup mortality after birth and body weight gain of the surviving pups was reduced. At 500 mg/(kg bw x d), there was a slight increase in post-implantation loss, together with a slight increase in pup mortality up to PND 5. Pup sex ratio on PND all groups and pup body weight gain up to PND 5 at 200 and 500 mg/(kg bw x d) were unaffected by the treatment of the parents with the test item. There were no relevant external or macroscopic abnormalities in the pups from any group.

No effects were observed on spermatozoa motility, morphology, or epididymal and testicular sperm count. A dose-related increase in kidney and liver weights was noted in the males at ≥ 200 and in females at 1000 mg/(kg bw x d). Hepatocellular hypertrophy was noted in the males at ≥ 200 and in the females at ≥ 500 mg/(kg bw x d). Histological examination revealed altered liver cell foci (clear cell- and basophilic cell-types) in some males at 500 or 1000 mg/(kg bw x d). These findings were considered by the study authors to be adverse since these changes could be consistent with pre-neoplastic lesions. Hyaline droplets along with basophilic tubules were found in the tubular epithelium of the kidneys in all groups of treated males. Minimal hyperplasia of squamous cells together with hyperkeratosis of the forestomach was seen in some males at ≥ 500 mg/(kg bw x d). The NOAEL for hexylene glycol was considered to be 1000 mg/(kg bw x d) for the parent females and 200 mg/(kg bw x d) for the parent males, based on the adverse microscopic findings in the liver. The NOAEL for reproductive performance was 1000 mg/(kg bw x d), the NOAEL for the F1 offspring was 500 mg/(kg bw x d).

In a subchronic oral toxicity study with rats (see chapter 4.5.2), no effects on the gonads were observed up to 450 mg/(kg bw x d), the highest dose tested.

An extended One-Generation Reproductive Toxicity Study (according to OECD Guideline 443) is currently performed, results are not yet available (ECHA Dissemination, 2017).

Development

The developmental toxicity of hexylene glycol was studied in rats (study according to OECD guideline 414). 0, 30, 300 or 1000 mg/(kg bw x d) hexylene glycol was administered by oral gavage to mated female Sprague-Dawley rats (24 F/dose) on GD 6-15 (Clode, 1997). Animals were killed on gestation day 20 and uterine content/implantation and foetal effects were examined. The test substance had no effect on survival, clinical observations, or necropsy findings in the dams. Body weight gain was initially decreased (GD6-7) in mid- and high-dose dams but returned to normal thereafter, a similar effect was noted on food intake. No other effects were observed in the maternal animals of any dose group. Mean litter and foetal weights in the high-dose group were marginally but not statistically significantly lower than in the control group. There were no adverse effects of treatment on sex ratio or on the incidences of foetal malformations or external/visceral variations. The incidence of skeletal variations in the high-dose group was slightly higher than in the control group but the nature of the specific changes involved (mainly incomplete ossification of cranial, sternebral, or forepaw structures) suggested only marginal delay in the normal ossification process. Moreover, the variations in the high-dose group were considered to be associated with the reduction in maternal body weight gain. Based on these findings a NOAEL of 300 mg/(kg bw x d) was determined for maternal and foetal effects of hexylene glycol (ECHA Dissemination, 2017).

4.5.5 Odour perception

The odour of hexylene glycol has been described as mildly sweet. A single value for an odour threshold of 3.93 ppm (19 mg/m^3) is reported without any details (AIHA, 2013). The validity of this data cannot be assessed. No further data are available.

4.6 Evaluation

4.6.1 Existing regulations and classifications

Hexylene glycol (2-methylpentane-2,4-diol) is classified in the EU with respect to its toxicity as skin irritant 2 (H315) and eye irritant (H319), but not as mutagenic, carcinogenic or toxic to reproduction (ECHA C&L Inventory, 2018).

General population

A DNEL for the protection of the general population has been derived on the basis of a NOAEL of 450 mg/(kg bw x d), the highest dose tested, in a subchronic oral toxicity study in rats (ECHA Dissemination, 2017). A route-to-route extrapolation was performed, assuming no differences in absorption by inhalation and via the gastrointestinal tract (ECHA Dissemination, 2017).

Table 4.4: Guide values for the protection of the general population against hexylene glycol in air (for explanation, see text)

Guidance value Parameter/ Organisation	ECHA Registered Substances (ECHA Dissemination, 2017)	CARB (2010)	CARB (2010)
Name (reference period)	DNEL (chronic)	Draft Acute REL (1 h)	Draft interim 8-h REL
Value (mg/m³)	7.8	3 (0.6 ppm)	0.28 (0.058 pm)
Organ/critical effect	Not indicated	Ocular irritation	Increased liver and kidney weights
Species	Not indicated	Human	Rat
Basis	NOAEC 450 mg/(kg bw x d)	LOAEC 241.5 mg/m³ (50 ppm)	LOAEC 676.2 mg/m³ (140 ppm)
Adjusted for cont. exposure	not necessary	241.5 x 15 min/60 min	676.2 x 7 h/8 h x 5 d/7 d
Extrapolation factors			
Route-to-route	1.14 m³/(kg bw x d)		
Time	2		10
LOAEC to NAEC	-	6	10
Interspecies	2.5	-	2 x √10
Intraspecies	10	3	10
Total	50 x 1.15 m³/(kg bw x d)	72	6000

A "Draft acute REL" (Reference Exposure Level) has been presented by CARB (2010). This value is based on a LOAEC for eye irritation observed in a study with 15-min exposure of volunteers to an atmosphere saturated with hexylene glycol vapour (Silverman et al., 1946).

A "Draft interim REL" has also been presented by CARB (2010). The basis for the derivation of this value is not entirely clear. In the summarised presentation it is stated that the critical effects were "increased organ weights (liver and kidney)". However, in the more detailed explanation of the document

it is reported that the value was based on "lesions of the respiratory epithelium" in a 2-week inhalation study (UC, 1976) (CARB, 2010).

Workplace

A DNEL of 44 mg/m^3 is presented in the registration dossier. The value is based on the same NOAEC as the DNEL for the general population but with a lower intraspecies factor of 5 and after correction for difference in exposure conditions and between respiratory rates under standard conditions and under conditions of light activity (1/0.38 m^3/kg x 7 d/5 d x 6.7 m^3/10 m^3) (ECHA Dissemination, 2017).

An 8-h-TWA for hexylene glycol of 10 ppm (49 mg/m^3) has been proposed by the German MAK commission. This value is based on limited human data from a study with brief exposure. In this study, 10 ppm led to eye irritation which was not considered very severe. Because of the lack of adequate data for the irritant potential of hexylene glycol, the MAK value was considered provisional requiring confirmation in further studies. The commission further notes that, assuming 100% absorption of the inhaled substance, exposure to concentrations in the range of the MAK value could result in a dose of 7.5 mg/(kg bw x d) (DFG, 2001b). An OEL of 10 ppm is also reported for Austria and Switzerland. A higher OEL of 25 ppm has been reported for various other European countries (IFA, 2018).

4.6.2 Derivation of a EU-LCI value

Few data are available regarding toxic effects of hexylene glycol in humans. Brief inhalation exposure of volunteers to 50 ppm hexylene glycol vapour (245 mg/m^3) was reported to cause eye irritation but not irritation of the respiratory tract (Silverman et al., 1946). There are no data available for the effects of repeated inhalation of hexylene glycol in humans. Repeated oral exposure to hexylene glycol (up to 2 g for up to two weeks or 5 g/d on 5 days) had no effects on the "well-being" of the participants (DFG, 2001b; Jacobsen, 1958b).

No animal studies are available with repeated inhalation exposure, except for a subacute study with insufficient documentation. In a subchronic study with oral exposure of rats, kidney lesions consistent with α2u-nephropathy were observed in male rats. These effects are sex- and species-specific and generally considered as not relevant for risk evaluation in humans. In the same study, effects on the liver were restricted to an adaptive hepatocellular hypertrophy but no pathological lesions were observed up to 450 mg/(kg bw x d), the highest dose tested (Fabreguette, 1999a). However, the development of altered liver cell foci in male rats was described at a slightly higher oral dose of 500 mg/(kg bw x d) in a screening reproduction/developmental toxicity test (according to OECD Guideline 421) with at least 10 weeks of oral exposure (ECHA Dissemination, 2017). These findings were considered by the study authors to be adverse since these changes could be consistent with pre-neoplastic lesions (see chapter 4.5.4).

There is no evidence of genotoxicity of hexylene glycol *in vitro. In vivo* genotoxicity data are not available. Also, no carcinogenicity/chronic toxicity studies are available.

Table 4.5: Derivation of EU-LCI value for hexylene glycol (for explanation, see text)

End-point	POD	Adjustment factor				Value (mg/m³)	Reference
		Route to route	Time	Inter-species	Intra-species		
hepato-toxicity	NOAEC: 200 mg/(bw KG x d)	1.15 m³/kg bw x d	2	2.5	10	1.17	(ECHA Dissemination, 2017)

With the available data indicating no genotoxic effects of hexylene glycol, a non-genotoxic mechanism may be considered as plausible for the development of altered liver cell foci. A threshold may be defined for such a non-genotoxic mechanism. No liver cell foci were described in the subchronic study at 450 mg/(kg bw x d). However, this dose is too close to the adverse effect level. Therefore, the NOAEL

of 200 mg/(kg bw x d) hexylene glycol for hepatotoxicity in male rats from the screening study for reproductive/development toxicity is used as the POD for the proposed derivation of a EU-LCI value (see Tab. 4-5). A route-to-route extrapolation is performed. It is assumed that the absorption of hexylene glycol by inhalation and oral exposure are similar, so that no adjustment factor for differences in absorption is taken into account.

The following adjustment factors are used:

- Route-to-route extrapolation: 1.15 m^3/(kg bw x d) (rat)
- Adjusted study length factor (subchronic exposure study): 2
- Allometric scaling: already included in route-to-route extrapolation
- Interspecies extrapolation: 2.5
- Intraspecies extrapolation (interindividual variability, general population): 10

Total assessment factor: 57.5 m^3/(kg bw x d). This leads to a concentration of
200 mg/(kg bw x d) : 57.5 m^3/(kg bw x d) = 3.478 mg/m^3.

A EU-LCI value (rounded) for hexylene glycol (2-methylpentane-2,4-diol) of 3500 µg/m^3 is proposed.

The odour of hexylene glycol has been described as mild sweetish. No reliable odour thresholds are available, so no conclusion can be drawn about the perception of an odour at the EU-LCI.

4.7 References

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4.8 Fact and data sheet for hexylene glycol

Table 4.6: Data collection sheet 2-methylpentane-2,4-diol (hexylene glycol)

Rapporteur, Date			
Compound	**2-METHYLPENTANE-2,4-DIOL**		***Data collection sheet***
N° CAS 107-41-5 1 ppm = 4,9 mg/m³	**EU-Classification:** Xi; R36/38 **CLP:** Skin Irrit. 2 (H315), Eye Irrit. H319		
Organization Name	CARB	DFG	Reach registrants
Risk Value Name	8-hour REL	MAK	DNEL
Risk Value (mg/m³)	0.28 (0.058 ppm)	49 (10 ppm)	7.8 (1.6 ppm)
Reference period	acute (8 h)	Chronic (worker)	Chronic (DNEL $_{\text{Gen. Pop. long term}}$)
Risk Value (mg/m³) Short Term (15 min)		100 (20 ppm)	-
Year	2010	1997, 2000, 2001	2017
Key Study	Union Carbide Corporation (1976)	Silverman et al. (1946)	Fabreguette (1999)
Study type	Subacute exposure study, 2 weeks	Acute exposure study, 15 min (245 mg/m³)	Subchronic study, oral
Species	Rat	Human	Rat
Duration of exposure in key study	7 h/d, 5 d/week, 9 d	15 min	7 d/week, 90 d
Critical effect	Lesions of the respiratory epithelium	Eye irritation	Highest dose tested
Critical dose value	LOAEC 676.2 mg/m³ (140 ppm)	LOAEC: 245 mg/m³	NOAEC 450 mg/(kg bw x d)
Adjusted critical dose	422.6 mg/M³ (676.2 X 7/8 X 5/7), RGDR*: 4 → Human Concentration Adjustment: 1690 mg/m³	-	-
Single Assessment factors	UF_L 10 x UF_H 10 x UF_A 2 x √10 x UF_S 10 x UF_D 1 = 6000	Not indicated	UF_H 10 x UF_A 2.5 x UF_S 2 x= 50
Other effects			
Remarks			Route-to-route-extrapolation factor 1.15 m³/(kg bw x d), no differences in oral and inhalation absorption assumed
UF_L Used LOAEL; UF_H Intraspecies variability; UF_A interspecies variability; UF_S Used subchronic study UF_D data deficiencies			

*RGDR: Regional gas dose ratio for gases with respiratory effects

Table 4.7: Fact sheet 2-methylpentane-2,4-diol (hexylene glycol)

Rapporteur, Date			
Compound	**2-METHYLPENTANE-2,4-DIOL**		***Factsheet***
Parameter	**Note**	Comments	Value / descriptor
EU-LCI Value and Status			
EU-LCI value	1	[µg/m³]	3500
EU-LCI status	2	Draft/Final	Draft
EU-LCI year of issue	3	Year when EU-LCI value has been issued	2018
General Information			
CLP-Index No.	4	INDEX	603-053-00-3
EC-No.	5	EINECS	203-489-0
CAS-No.	6	Chemical Abstract Service number	107-41-5
Harmonised CLP classification	7	Human health risk related classification	Skin Irrit. 2 (H315), Eye Irrit. H319
Molar mass and conversion factor	8	[g/mol] and [mg/m³ - ppm]	118.18 4.9
Key Data / Database			
Key study, Authors, Year	9	Critical study with lowest relevant effect level	ECHA (2017) Reproduction / Developmental Toxicity Screening Test (OECD Guideline 421) (2010)
Read across compound	10	Where applicable	-
Species	11	Rat	Rat, Sprague-Dawley (10/sex/dose)
Route / type of study	12	Inhalation, oral feed….	Oral (gavage)
Study length	13	Days, subchronic, chronic…	Males: 4 weeks before pairing, during the pairing period (3 weeks), until final sacrifice of the females, at least 10 weeks in total
Exposure duration	14	h/d, d/w	once/day, daily
Critical endpoint	15	Effect (s), site of	Hepatotoxicity: minimal altered cell foci were recorded in males given 500 or 1000 mg/(kg b.w. x d). It consisted of clear cell focus or foci, associated with basophilic cell foci in one of the males given 1000 mg/kg/day. These findings were considered to be adverse after 3 months of treatment.
Point of Departure (POD)	16	LOAEC, NOAEC, BMD…	NOAEL
POD value	17	[mg/m³] or ppm or mg/(kg b.w. x d)	200 mg/(kg b.w. x d)
Assessment Factors (AF)	18		
Adjustment for exposure duration	19	Study exposure h/d, d/w	1
AF study length	20	sc→c	2
Route-to-route extrapolation factor	21		1.15 m³/(kg b.w. x d) (rat) (assuming identical resorption rates for oral and inhalation exposure)
AF 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)	According to table R.8-4 in chapter R.8 of the ECHA guidance document, the AF of 4 is already included in the route to route extrapolation
	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		1
Result			
Summary of assessment factors	27	Total Assessment Factor	1.15 m³/(kg b.w. x d) x 50
POD/TAF	28	Calculated value [mg/m³ and ppm]	174 mg/m^3, 35.5 ppm
Molar adjustment factor	29		
Rounded value	30	[µg/m³]	3500
Additional comments	31		
Rationale Section	32		

Rationale for critical effects

The data base of studies regarding effects in humans is extremely limited. A study on volunteers with acute exposure to 245 mg/m³ (50 ppm) of the test substance for 15 min led to eye, but not respiratory tract irritation in most of the 12 exposed males and females. No NOAEC was reported in that study (Silverman et al., 1946). No data are available for the effects of repeated inhalation of hexylene glycol in humans.

In the only available study with repeated inhalation of animals, short-term exposure to 700 mg/m³ of an inhalable aerosol for 9 days (7 h/d, with a two-day break in between after day 5) led to minimal effects on the epithelium in the trachea of exposed rats and the single exposed rabbit (very slight submucosal haemorrhage, congestion, slight hyperplasia). No effects were observed in the lungs or other organs or on body weight.

Several studies with repeated (up to subchronic) oral exposure of rats revealed largely adaptive effects of hexylene glycol on the liver (increased liver weight with hepatocellular hypertrophy). In a reproduction / developmental toxicity screening test (OECD Guideline 421) with Sprague-Dawley rats (10 M + 10 F/group), 0, 200, 500 or 1000 mg/(kg bw x d) hexylene glycol was given by gavage for 4 weeks before mating, through mating, gestation and the beginning of the lactation period (until day 4 after parturition) (total exposure period for M and F at least 10 weeks). Minimal to slight dose-related hepatocellular hypertrophy was recorded in male Sprague-Dawley rats given 200 mg/(kg bw x d) and in males and females given 500 or 1000 mg/(kg bw x d). This correlated with increased liver weights. In addition, minimal altered cell foci were recorded in males given 500 or 1000 mg/(kg bw x d), consisting of clear cell focus or foci, associated with basophilic cell foci in one of the males given 1000 mg/kg/day. These findings were considered to be adverse after 3 months of treatment since these changes could be consistent with pre-neoplastic lesions (ECHA, 2017).

Carcinogenicity studies or *in vivo* genotoxicity studies with hexylene glycol are not available. *In vitro* genotoxicity studies provide no evidence of a genotoxic potential.

Rationale for starting point

The only inhalation toxicity study with repeated exposure may be used as supportive, but in itself is considered insufficient for the evaluation of the toxicity of hexylene glycol and the derivation of a EU-LCI. Therefore, the derivation of a EU-LCI is based on data from a guideline study with oral exposure. This procedure is justified as the critical effect is a systemic-toxic effect and toxicokinetic data do not provide evidence against a route-to-route-extrapolation.

The NOAEL of 200 mg/(kg bw x d) for adverse hepatic effects (minimal altered cell foci) in male rats observed in an oral reproduction / developmental toxicity screening test (ECHA, 2017) served as a POD for the derivation of a EU-LCI-value. The study is not published, but described in sufficient detail in the REACH registration dossier and considered as “reliable without restrictions", RL 1) (ECHA, 2017).

Rationale for Extrapolation factors

- Route-to-route extrapolation factor: 1.15 m³/(kg bw x d) (rat)
- Adjusted study length factor: 2 (subchronic exposure)
- Allometric scaling (rat to human): already included in route-to-route extrapolation
- Interspecies differences: 2.5 (default value for systemic effects)
- Intraspecies differences: 10 (default value)

Total extrapolation factor is: 50 x 1,15 m³/(kg bw x d), leading to a value of 200 mg/(kg bw x d) : 1,15 m³/(kg bw x d): 50 = 3.48 mg/m³ which was rounded to 3.5 mg/m³.

The derived EU-LCI value is supported by derivations from other studies:

In a developmental toxicity study (exposure on GD 6-15) with oral exposure of rats, a dose-dependent transitional reduction of body weight gain was observed on GD 6-7 in pregnant dams with a NOAEL of 300 mg/(kg b.w. x d) (A slight but still significant transitional effect on weight gain at this dose was not considered adverse, therefore, the lowest dose of 30 mg/(kg bw x d) was assessed as a NOEL). From the NOAEL, a value of 10 mg/m³ can be derived; the NOEL would lead to a value of 1 mg/m³.

From the subacute inhalation study in rats, a value of about 1 mg/m³ could be derived (using the following factors: LOAEC to NOAEC: 3; 7h/24 x 5d/7d for continuous exposure; subacute to chronic: 6, Interspecies: 1; Intraspecies: 10; total factor 864). The effects on the respiratory system in this study were mild, and the descried calculation considered to be conservative. There, it is concluded that the derived EU-LCI of 3.5 mg/m³ will also be protective against local effects on the respiratory system.

The following EU-LCI is proposed for 2-methylpentane-2,4-diol (hexylene glycol): 3500 µg/m³. The derived EU-LCI is far below the concentration reported to cause eye irritation in humans at brief exposure. The derived value is also below the reported odour threshold of 19 mg/m³ (3.9 ppm) reported by AIHA (2013). Thus, odour perception seems unlikely at the proposed EU-LCI.

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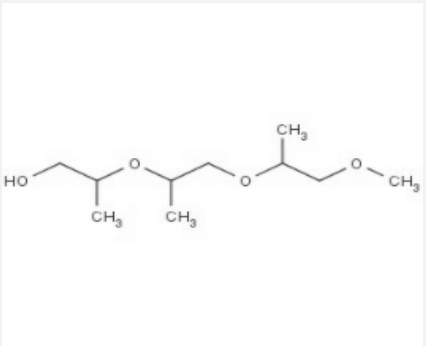
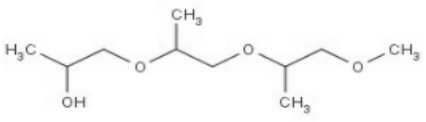
5 Toxicological evaluation of tripropylene glycol monomethyl ether as basis for the derivation of a EU-LCI value

5.1 Substance identification

Tripropylene glycol monomethyl ether (TGPME) is produced commercially as a mixture of isomers which normally are not further separated or marketed as individual substances. The monopropylene glycol ethers may exist in two isomeric forms, alpha or beta. The alpha form, which is thermodynamically favoured during synthesis, consists of a secondary alcohol configuration. The beta form consists of a primary alcohol. The tripropylene glycol ethers may form up to 8 isomeric forms. All isomers exhibit either the "alpha" or "beta" configuration, existing as secondary or primary alcohols, respectively. The distribution of isomeric forms for the tripropylene glycols, as with the mono-propylene glycol ethers, also results in predominantly the alpha form (i.e., the secondary alcohol). Only the alpha isomer and isomeric mixtures (consisting predominantly of alpha isomer) are produced commercially. The CAS No. 25498-49-1 is for the isomeric mixture, while the CAS No. 20324-33-8 is for the main alpha-isomer (CARB, 2010; OECD SIDS, 2003b). Substance identification data and physicochemical properties are shown in Table 5.1 and Table 5.2. Each of the structural isomers contains three chiral centres, the commercial product is a racemic mixture. No data are available for the individual diastereomers.

Table 5.1: Substance identification (ECHA Dissemination, 2018; OECD SIDS, 2003b)

CAS-No. EU-No. CLP-Index-No.	Systematic Name, common names	Summary formula	Structural formula
25498-49-1 247-045-4 -	CAS No. for isomeric mixture containing [2-(2-methoxymethyl-ethoxy)methylethoxy]propanol, 2-[2-(2-methoxy-1-methylethoxy)-1-methylethoxy]propan-1-ol (IUPAC name), methyltripropylene glycol, TPM, TGPME, tripropylene glycol monomethyl ether	$C_{10}H_{22}O_4$	
20324-33-8 243-734-9 -	Main alpha-isomer: 1-[2-(2-methoxy-1-methylethoxy)-1-methylethoxy]-propan-2-ol, 1-[(1-[(1-methoxypropan-2-yl)oxy]propan-2-yl)oxy)]propan-2-ol (IUPAC name), methyltripropylene glycol, TPM, TPGME, tripropylene glycol monomethyl ether	$C_{10}H_{22}O_4$	

5.2 Substance properties and uses

Tripropylene glycol monomethyl ether (TPGME) is a clear, viscous, colourless and nearly odourless liquid with a very low vapour pressure. TGPME is miscible with water.

Table 5.2: Physicochemical properties of TGPME (ECHA Dissemination, 2018; OECD SIDS, 2003b)

Molar mass (g/mol)	Mp. (° C)	Boiling point (° C)	Vapour pressure (hPa)	Conversion 1 ppm = x mg/m³ (23 °C)	log pow (at pH)	Solubility in water (mg/L)
206.32	-77.8	243	0.028	8.5	0.08	miscible

TGPME has no known natural sources. It is a large-scale industrial product (tonnage band in the EU: 1000 – 10000 t/a). Because of its high polymer solvency and low evaporation rate, TGPME is used in inks, pens and inkpads to prevent drying; it is also used in cleaners and coatings (OECD SIDS, 2003b). According to the registration dossier, TPGME is used in anti-freeze products, coating products, lubricants and greases, biocides (e.g. disinfectants, pest control products) and inks and toners (ECHA Dissemination, 2018).

5.3 Exposure

5.3.1 Indoor air

Hardly any data are available regarding the occurrence TPGME in indoor air. Based on 615 measurements of samples from various indoor air sources, AGÖF presented a "normal value" (presenting the median) and an "attention value" (presenting the 90th percentile) of < 1 µg/m^3 for TPGME (CAS No. 20324-33-8) (AGÖF, 2013). No further data are available.

5.3.2 Other sources

No data are available.

5.4 Toxicokinetics and structure-activity relationships

There are no data available on the absorption of TPGME after inhalation or other routes of exposure. Propylene glycol ethers as a class are known to be rapidly absorbed and distributed throughout the body when introduced by inhalation or exposure (OECD SIDS, 2003b). Glycol ethers may also be well absorbed via the skin, even in the vapour state. Once absorbed, glycol ethers are readily distributed through the body (ECETOC, 2005).

The metabolism of glycol ethers follows two main oxidative pathways. The first pathway involves oxidation by alcohol dehydrogenase and further oxidation by aldehyde dehydrogenase with the formation of alkoxyalkanoic acids. This pathway requires a primary hydroxyl (OH) group and thus is observed with the alpha isomers. Beta-isomers, which do not contain a primary but a secondary free hydroxyl group, cannot be oxidized via this pathway to alkoxyalkanoic acids but only to the corresponding ketones which are further oxidized by other pathways (ECETOC, 2005; OECD SIDS, 2003b).

The second pathway involves oxidation by microsomal cytochrome P450 monooxygenases at the ether bond via O-dealkylation. This leads to the production of the corresponding glycol (tripropylene glycol in case of TPGME) and is the main pathway for di- and tripropylene glycols. Tripropylene glycol may undergo further metabolism with oxidative cleavage of ether bonds and final oxidation of the carbon chain to carbon dioxide. Alternatively, propylene glycol ethers or their partially metabolized products may be conjugated with glucuronide or sulfate and excreted via the kidneys into the urine (OECD SIDS, 2003b).

A metabolism study was conducted with [1-^{14}C]-labelled TPGME (CAS No. 25498-49-1) in F344 rats (ECHA Dissemination, 2018). Three male rats were administered oral doses via gavage of approximately 206 or 825 mg TPGME/kg bw. Rats were housed in metabolism cages where urine, feces, and expired air were collected in varying time increments over a total period of 48 hours and monitored for radioactivity. Urine was collected in 12 hour increments, feces in 24 hour increments, and expired air was collected at 4 hour intervals for the first 12 hours and at 12 hour intervals thereafter. In addi-

tion, at the end of 48 hours, brain, muscle, perirenal fat, skin, kidneys, liver and the remaining carcass were analysed for total radioactivity.

TPGME was rapidly distributed and quickly metabolised and eliminated. Approximately 69-75% of the ^{14}C-activity was excreted in urine, 16% was eliminated as $^{14}CO_2$ within 48 hours after dosing and about 5% with the feces. Less than 1% was eliminated as volatile radioactive compounds. Overall, > 94% of the dose was eliminated within 48 h. Tripropylene glycol (isomers, 6-12%), dipropylene glycol and propylene glycol (1.3-3.8%) as well as an oxidation product of dipropylene glycol, dipropylene glycol monomethyl ether (DPGME), TPGME and the sulfate conjugate of TPGME (11-18%) were identified in the urine. A total of 12-25 % was tentatively identified as 4 isomers of DPGME and 6 isomers of TPGME and 54-56 % as 3 isomers of dipropylene glycol and 2 isomers of 2-(1-hydroxy-2-propoxy)propanoic acid. These results indicate that TPGME is metabolised extensively and is comparable to DPGME in disposition and types of metabolites (ECHA Dissemination, 2018; OECD SIDS, 2003b).

The rate of metabolism of propylene glycol methyl ether (PGME), dipropylene glycol methyl ether (DPGME), and tripropylene glycol monomethyl ether (CAS No. 25498-49-1) to methoxypropanoic acid (MPA) was studied *in* vitro in primary cultures of frozen and thawed hepatocytes from female Sprague-Dawley rat, female New Zealand rabbit and a female human donor. The viability of the hepatocytes in culture was not reported, however, PGME-beta (which is oxidized to MPA) was incubated as a positive control for each experiment. The rate of oxidation to MPA was determined via chemical analysis of this metabolite in the media. MPA was only a minor metabolite of the commercial propylene glycol methyl ether (99.7% 1-methoxy-2-propanol). Oxidation of the di- and tripropylene glycol methyl ethers to MPA was also low, with metabolite levels at 24 hours less than 0.5% of initial substrate concentrations. Overall, formation of MPA from any of the glycol ethers was highest from incubation with rat hepatocytes. MPA levels in human cell incubations were approximately 2-fold lower than rat and MPA formation in rabbit hepatocytes was approximately 4 to 6-fold lower than rat for propylene glycol methyl ether. Separate incubations of lactic acid did not produce any metabolic MPA, suggesting that methylation of lactic acid was not a relevant route for MPA formation *in vitro* (ECHA Dissemination, 2018).

5.5 Health effects

5.5.1 Sensory irritation and local effects

No human data are available.

Signs of local irritation were observed in a developmental toxicity study with inhalation exposure of the dams against TPGME (see chapter 5.5.4). No such effects were described in a subacute inhalation exposure study (see chapter 5.5.2).

5.5.2 Repeated dose toxicity

No human data are available.

A subacute inhalation exposure study (comparable to guidelines, reliability 1) was conducted with F344 rats and B6C3F1 mice (ECHA Dissemination, 2018; Miller et al., 1985). The rats and mice (5 M + 5 F/concentration) were exposed to 0, 150, 360 or 1010 mg/m^3 of TPGME (at least partially as aerosol) for 6 h/d, 5 d/week for a total of 9 days. The fur of the animals in high exposure group was slightly wet following each day of exposure as a result of the high concentration of liquid aerosol in that chamber. No substance-related mortality was observed. No clinical signs were noted except for a slight nasal exudate in rats at the high exposure group following the last exposure. There were no statistically significant differences in the mean body weights of control and exposure groups of animals at any time of the study. No substance-related adverse changes of haematological, clinical-chemical or urine parameters were observed. At necropsy the mean absolute and relative liver weights of male and female

rats in the high exposure group, as well as the mean relative liver weight of male rats in the 360 mg/m^3 exposure group, were significantly higher than for the controls. Significant increases in the mean liver weights were also found in mice. The liver weights of male mice were increased an all three exposure groups in an apparent exposure concentration related manner, while the liver weights of female mice were affected only at the highest exposure concentration. The weight of other organs was not affected by exposure. There were no treatment related gross pathologic observations in the liver of rats or mice. Histopathologic examinations revealed no adverse exposure related changes in any organ or tissue in either rats or mice. 3 of 5 male mice at the highest exposure had altered tinctorial properties in peripheral regions of hepatic lobules; this observation was considered to be an adaptive response rather than a degenerative phenomenon (ECHA Dissemination, 2018; Miller et al., 1985). Thus, the highest exposure concentration of 1010 mg/m^3 in this study represents a NOAEC.

A subchronic dermal toxicity study with TPGME was conducted with rabbits (5-8 M + 5-8 F/dose, strain not reported). The animals received topical applications (shaved skin, occluded exposure area 56 cm^2) of TPGME at doses of 0, 1.0, 3, 5 or 10 ml/kg bw x d on 5 d/week for 3 months. The highest dose level produced narcosis and death in all but one animal within 3 weeks of initiation of treatment. The remaining non-survivor died during the 10th week of exposure. The hematology analyses for the rabbits were unremarkable, revealing no differences between control and exposure group of animals. At 3 and 5.0 ml/kg bw x d kidney weights were increased. Gross examination of the skins of the rabbits at the site of TPGME application indicated occasional erythema "scalding" but did not reveal significant severity or incidence differences from water treated controls. Histopathology was largely normal in all organs. Based on the results of this study, a NOAEL$_{dermal}$ of 1.0 ml/kg bw x d (965 mg/(kg bw x d) can be derived (LOAEL is 3.0 ml/kg x d = 2895 mg/(kg bw x d)), based on increased kidney weights (ECHA Dissemination, 2018).

Read-across:

A subchronic inhalation study was performed with dipropylene glycol monomethyl ether (DPGME) in which F344 rats (10 M + 10 F/group) were exposed to 0, 15, 50 and 200 ppm (0, 91, 303 and 1212 mg/m^3) for 6 h/d, 5 d/week for 13 weeks. DPGME exposure had no related adverse effects on body weights. There were no statistically significant differences from control body weight means, no exposure related adverse effects on hematology, clinical chemistry or urinary parameters in either sex of rat. There were no statistically significant differences in organ weights, except for a slight decrease in mean relative liver weight males at 50 ppm. There were no histopathological effects in the liver or other organs of exposed animals. A NOAEC for DPGME of 200 ppm (1212 mg/m^3) is derived from this study (ECHA Dissemination, 2018).

A similar study with DPGME was performed with New Zealand White rabbits (7 M + 7 F/group). The animals were exposed to 0, 15, 50 and 200 ppm (0, 91, 303 and 1212 mg/m^3) 6 h/d, 5 d/week for 13 weeks. As in rats, there were no substance-related adverse effects at any concentrations on any parameter. An observed increase in mean relative kidney weight of female rabbits exposed to 200 ppm and of absolute mean kidney weights of 50 and 200 ppm exposed female rabbits were within the range of historical control values. Additionally, there was no evidence of nephrotoxicity. Thus, the increased kidney weights in the female rabbits were considered unrelated to treatment (ECHA Dissemination, 2018). A NOAEC of 200 ppm (1212 mg/m^3) can be derived from this study.

5.5.3 Genotoxicity and carcinogenicity

Genotoxicity

In vitro, TPGME (Dowanol TPM, CAS No. 25498-49-1) was not mutagenic in a bacterial mutation assay (Ames test) tested at up to cytotoxic concentrations with and without exogenous metabolic activation system (S9 mix from rat liver) in all tested strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) (ECHA Dissemination, 2018).

In mammalian cells, TPGME (Dowanol TPM Glycol Ether, ((2-(2-methoxymethylethoxy)methyl-ethoxy)-propanol)) did not induce mutations in a HPRT assay in Chinese Hamster Ovary Cells (CHO cells) with and without metabolic activation (ECHA Dissemination, 2018). Furthermore, TPGME did not induce unscheduled DNA synthesis in primary cultures of hepatocytes from male F344 rats when tested up to cytotoxic concentrations (OECD SIDS, 2003b).

DPGME was negative in a cytogenetic assay in CHO cells with and without metabolic activation. An ambiguous result was obtained in cytogenetic assays with DPGnB: A positive result was obtained with and without exogenous metabolic activation system in three assays performed in one laboratory, whereas negative results were reported in two of these assays in another laboratory. Ames tests and a HPRT assay in CHO cells were negative (OECD SIDS, 2003b).

No data are available from *in vivo* studies with TPGME. Because of the ambiguous results obtained in cytogenetic assays with DPGnB, a micronucleus tests was performed with CD-1 mice (5 M + 5 F/ group). The animals received a single dose of 0, 250, 833 or 2500 mg/kg bw by gavage. No increase in the incidence of micronuclei was observed at any dose (OECD SIDS, 2003b).

Carcinogenicity

No data are available from studies with TPGME.

Read-across:

No evidence of carcinogenicity was observed in a two-year carcinogenicity study (according to OECD guideline 453) with inhalation exposure of F344 rats (50 M + 50 F/group) to propylene glycol monomethyl ether (PGME, CAS NO. 107-98-2) up to the highest concentration tested (3000 ppm). Non-neoplastic effects observed in this study included decreased activity, incoordination, and transient sedation during and immediately after exposure to 3000 ppm. Body weights were also decreased at the 3000 ppm exposure level. Liver and kidney weights were increased at 3000 ppm in both sexes. Dark foci in the liver were observable in male rats exposed to 1000 and 3000 ppm PGME after 24 months. These subjects also exhibited eosinophilic hepatocellular foci and cystic degeneration microscopically that was not reported in female rats. In the kidney, histopathology revealed that male rats had α2u-globulin nephropathy. The incidence and severity of this condition was increased in males exposed to 1000 and 3000 ppm PGME compared to controls. A NOAEC of 300 ppm based on altered hepatocellular foci in males can be derived from this study (ECHA Dissemination, 2018).

5.5.4 Toxicity to reproduction

Fertility

No data are available from fertility studies with TPGME.

Read-across:

A two-generation reproductive toxicity study (according to OECD guideline) was performed with propylene glycol methyl ether (PGME) (99% 1-methoxy-2-hydroxypropane or propylene glycol methyl ether (alpha isomer), CAS No. 107-98-2 and 1.9% 2-methoxy-1-hydroxypropane or propylene glycol methyl ether (beta isomer)). Sprague-Dawley rats (30 M + 30 F/group) were exposed to 0, 300, 1000 or 3000 ppm PGME (0, 1110, 3710, 11170 mg/m^3) via inhalation, for 6 h/d, 5 d/week prior to mating and 6 h/d, 7 d/week during mating, gestation and lactation for two generations (Carney et al., 1999; ECHA Dissemination, 2018; OECD SIDS, 2003a). Inhalation exposure of adult male and female rats to 1000 (females only) and 3000 (males and females) ppm PGME resulted in dose-related parental effects. Toxicity in 3000 ppm PGME P1 and P2 males and females was evidenced primarily as an increased incidence of sedation for several weeks early in the exposure regimen and significant decreases in body weights. Decreased body weights in the P1 and P2 high concentration females generally persisted throughout the pre-breeding, gestation and lactation phases of the study. Additional effects noted among P1 and P2 adult females exposed to 3000 ppm PGME included lengthened estrous cycles,

decreased fertility, decreased ovary weights and an increased incidence of histologic ovarian atrophy. The effects on fertility, estrous cyclicity and ovarian weight/histology appeared to be interrelated and associated with the significant decreases in 3000 ppm PGME female body weights and general toxicity/nutritional stress throughout the test period. No treatment-related differences in sperm counts or motility were observed among P1 or P2 adult males. Neonatal effects observed at 3000 ppm PGME consisted of decreased pup body weights, reduced pup survival and litter size, increased time to vaginal opening or preputial separation, and histopathologic observations in the liver and thymus of weanling rats. These neonatal effects were considered secondary to maternal toxicity. In the 1000 ppm PGME group, mild parental toxicity was evidenced by slightly decreased pre-mating body weights among P1 and P2 females, but was not accompanied by any statistically significant effects on parental reproduction or neonatal survival, growth or development. There were no treatment-related parental or neonatal effects related to exposure of rats to 300 ppm PGME. In conclusion, the no-observed-effect-level (NOEL) for fertility and reproductive effects in this two-generation inhalation reproduction study was 1000 ppm (3710 mg/m^3) PGME. Mild parental toxicity was noted at this concentration (ECHA Dissemination, 2018).

Development

Female mated Sprague-Dawley rats (25/group) were exposed to aerosol atmospheres of 0, 100, 300, or 1000 mg/m^3 (0, 11.8, 35.6, or 118 ppm) TPGME (Dowanol TPM) on 6 h/d for GD 6-15 and evaluated for maternal and developmental toxicity after delivery on GD20 (Breckenridge et al., 1985; ECHA Dissemination, 2018; OECD SIDS, 2003b). No deaths occurred during the study. Clinical symptoms showed an increased incidence of red staining of the muzzle of the dams at the highest exposure level. The body weight of the exposed dams was similar to that of the control animals. Gross pathology and uterine parameters (numbers of corpora lutea, live fetuses, dead fetuses, resorptions, implantations, the pre- and post-implantation losses, fetal weights and the sex ratios) were not affected by treatment. No treatment related effects were observed regarding the overall incidences of fetal findings, malformations and minor visceral and skeletal anomalies. From this study, a LOAEC for maternal toxicity of 1000 mg/m^3 (NOAEC 300 mg/m^3) can be derived. No developmental toxicity was observed up to the highest concentration.

Read-across: The developmental toxicity of DPGME was studied in rats and rabbits via the inhalation route of exposure at concentrations of 0, 50, 150, or 300 ppm (0, 303, 909, or 2728 mg/m^3) (Breslin, 1990; ECHA Dissemination, 2018; OECD SIDS, 2003b).

Mated F344 rats (32-37/group) were exposed for 6 h/d on GD 6-15. On day 21 of gestation, all animals were euthanized prior to cesarean section and examined. No treatment-related effects were observed on any of the maternal, embryonal and fetal parameters evaluated.

In a similar study, New Zealand rabbits (16 mated F/group) were exposed for 6 h/d on GD 7-19. On day 28 of gestation, all animals were euthanized and examined. No treatment related effects were observed on any of the maternal and embryonal/fetal parameters evaluated at any exposure level.

Thus, the highest vapour concentration of 300 ppm (2728 mg/m^3) which was practically attainable at normal room temperature represents a NOAEC for DPGME for the studies in both species.

5.5.5 Odour perception

TPGME is described to be practically odourless at ambient temperature. No data on odour thresholds are available.

5.6 Evaluation

5.6.1 Existing regulations and classifications

TPGME (CAS No. 25498-49-1 and CAS No. 20324-33-8) are not classified in the EU and not listed in the C&L Inventory with a harmonized classification (ECHA C&L Inventory, 2018).

A NIK-value of 2000 µg/m³ is listed in the list of NIK values (AGBB, 2015). No details of the derivation of this value are available. In an unpublished discussion paper, a value of 1200 µg/m³ has been proposed as NIK. The proposed derivation is equivalent to that for the proposed derivation of a EU-LCI value in this report (see chapter 5.6.2).

General population

A DNEL of 19 mg/m³ is reported in the REACH registration dossier for TPGME (CAS NO. 25498-49-1) (ECHA Dissemination, 2018). The DNEL is based on a NOAEC but the study from which this value was derived is not reported.

An "Acute draft interim REL" (Reference Exposure Level) has been presented by CARB (2010). This value is based on liver effects (increased weight, eosinophilic foci) in mice observed at 1010 mg/m³ but not at 360 mg/m³ in a subacute inhalation study (see chapter 5.5.2). CARB evaluated the effects at the highest concentration as adverse and not as adaptive (no further details presented). With a total extrapolation factor of 2000, a REL of 0.1 mg/m³ was derived.

The German Ad-hoc Working Group on Indoor Guidelines has evaluated the toxicity of glycol ethers and glycol esters and derived substance-specific guide values for substances with sufficient data. A preliminary (health-based) guide value I for DPGME of 2 mg/m³was derived based on a NOAEC of 200 ppm (1210 mg/m³) in subchronic inhalation studies with rats and rabbits (see "read-across" in chapter 5.5.2). No substance-specific value was derived by the working group for TPGME. A default guide value I of 0.005 ppm was recommended for glycol ethers and glycol esters with insufficient data basis (Ad-hoc AG, 2013). This recommendation was based on a statistical analysis of the available data of all glycol ethers, not taking into account substance-specific structural criteria for individual compounds. In case of TPGME, the recommended guide value I of 0.005 ppm corresponds to a mass-based concentration of 42.5 µg/m³.

Table 5.3: Guide values for TPGME in air (for explanation, see text)

Guidance value Parameter/ Organisation	CARB (2010)	ECHA Disseminati-on (2018)	ECHA Dissemination (2018)
Name (reference period)	8-h REL	DNEL (chronic, general population)	DNEL (chronic, workers)
Value (mg/m³)	0.1 (0.012 ppm)	19 (2.2 ppm)	187 (22 ppm)
Organ/critical effect	Liver, eosinophilia	Not indicated	Not indicated
Species	Mouse	Not indicated	
Basis	NOAEC 360 mg/m³ (42 ppm)	NOAEC 133 mg/m³ (16 ppm)	NOAEC 758 mg/m³ (89 ppm)
Adjusted for cont. exposure	1.9 (6 h/8h, 5 d/7 d)	Not indicated	
Extrapolation factors			
Time	10	1.4	1.4
LOAEC to NOAEC	-	-	-
Interspecies	2 x √10 = 6.3	1	1
Intraspecies	10 x √10 = 30	5	3
Total	2000 (rounded)	7	4.2

Workplace

A DNEL of 187 mg/m^3 for workers is reported in the REACH registration dossier for TPGME (CAS NO. 25498-49-1) (ECHA Dissemination, 2018). The DNEL is based on a NOAEC for "repeated dose inhalation" but the study from which this value was derived is not reported.

There are no further OEL available for TPGME.

5.6.2 Derivation of a EU-LCI value

The data base for TGPME is very limited. However, additional data available from a number of studies with various structurally related glycol ethers.

No data are available on the toxicity of TPGME in humans. Limited data from inhalation studies with animals indicate a low systemic and local toxicity of TPGME. No adverse effects were observed in a subacute inhalation study with rats and mice (ECHA Dissemination, 2018; Miller et al., 1985). The only effects observed in this study were increased liver weights without any histological changes in rats and mice and altered tinctorial properties in hepatic lobules of mice at the highest concentration. These effects are considered to be an adaptive response rather than a degenerative phenomenon. Thus, a NOAEC of 1010 mg/m^3, the highest concentration tested, can be obtained from this study. It must be noted that the no-effect level may indeed be even higher for two reasons. First, 1010 mg/m^3 was the highest concentration tested, so there is no information about the concentration-response curve at higher concentrations. Second, the whole-body exposure of the animals was conducted with a mixture of a vapour and an aerosol. Since glycol ethers are known to be well absorbed through the skin, additional dermal exposure would have increased the systemic dose.

Inhalation studies with dipropylene glycol monomethyl ether (DPGME) in rats and rabbits (see chapter 5.5.2) (ECHA Dissemination, 2018) and other, structurally related propylene glycol ethers (OECD SIDS, 2003b) support the view that the systemic toxicity of these compounds including TPGME is low.

Limited *in vitro* data provide no evidence for genotoxic effects of TPGME in prokaryotic and mammalian assays. *In vivo* data are not available for TPGME and most other propylene glycol ethers. DPGnB had no clastogenic activity in the bone marrow of mice.

Carcinogenicity studies are not available for tripropylene or dipropylene glycol methyl ether. Monopropylene glycol methyl ether was not carcinogenic in a study with rats (ECHA Dissemination, 2018).

No data are available from fertility studies with TPGME. A two-generation reproductive toxicity study with propylene glycol methyl ether (PGME) provided a no-observed-effect-level (NOEL) for fertility and reproductive effects of 1000 ppm (3710 mg/m^3). Mild parental toxicity was noted at this concentration (ECHA Dissemination, 2018).

Developmental toxicity, especially teratogenicity, is a critical endpoint in the evaluation of the toxicity of some glycol ethers which contain a primary hydroxyl group and a methoxy or ethoxy side chain. The most active substances are methoxyethanol and ethoxyethanol, but 2-methoxypropan-1-ol (beta isomer of propylene glycol methyl ether, ß-PGME) was also shown to be effective (Hanley et al., 1984; Hellwig et al., 1994). The corresponding alkoxy acids which are produced by oxidation of the primary hydroxyl group are considered the ultimate developmental toxins (Ad-hoc AG, 2013; Carney et al., 2003; OECD SIDS, 2003b). Accordingly, methoxypropanoic acid (MPA) is considered the active metabolite formed by oxidation of ß-PGME. Rabbits were observed to be a species much more sensitive to these effects than rats, probably because of the long half-life of elimination in this species. ß-PGME showed concentration-dependent increases in the incidence of developmentally toxic and teratogenic effects in rabbits after inhalation through GD 6-18 at concentrations ≥ 225 ppm but not at 145 ppm (540 mg/m^3) (Hellwig et al., 1994). Comparative studies of MPA and methoxyethanoic acid (MEA), the oxidation product of methoxyethanol, indicated that the potency of MPA to induce developmental lesions was about tenfold lower than that of MEA (Carney et al., 2003).

MPA may also be formed by the oxidative degradation of di- and tripropylene glycol ether. No developmental toxicity was observed in rats exposed by inhalation to TPGME concentrations of up to 118 ppm (1000 mg/m^3) (Breckenridge et al., 1985; ECHA Dissemination, 2018; OECD SIDS, 2003b). No data are available for TPGME from studies with rabbits which are considered more sensitive than rats. However, an inhalation study in rabbits with dipropylene glycol methyl ether (DPGME) provided no evidence for a developmental toxicity of this compound up to the highest tested concentration of 300 ppm (2728 mg/m^3). It is concluded that the available data do not provide any evidence for a developmental toxicity of TPGME.

The subacute inhalation toxicity study is considered a suitable key study for the derivation of a EU-LCI value for TPGME. The NOAEC of 1010 mg/m^3 (120 ppm) TPGME from the subacute inhalation study with rats and mice is used as the POD for the calculation (see Table 5.4). The study (Miller et al., 1985) is not published but described in sufficient detail in the REACH registration dossier (ECHA Dissemination, 2018) and the OECD SIDS (2003b).

Table 5.4: Derivation of EU-LCI value for TPGME (for explanation, see text)

Organ/endpoint	POD (mg/m³)	Adjustment factor				Value (mg/m³)	Reference
		LOAEC→ NAEC	Time	Inter-species	Intra-species		
(Liver/ no effect at highest test concentration)	NOAEC: 1010	-	6	2.5	10	1.2	(Miller et al., 1985)

The following adjustment factors are used:

- Adjustment for continuous exposure (6 h/d, 5 d/week): 5.6
- Adjusted study length factor (subacute exposure study): 6
- Interspecies extrapolation: 2.5 (factor for systemic effects at inhalation exposure)
- Intraspecies extrapolation (interindividual variability, general population): 10

Total assessment factor: 840. This leads to a concentration of 1010 mg/m^3: 840 = 1.20 mg/m^3.

A nearly identical value may be derived from local irritation effects observed in dams in a developmental inhalation toxicity study with rats (see chapter 5.5.4). In that study, a red staining around the muzzle of dams was observed at 1000 mg/m^3 (LOAEC) but not at 300 mg/m^3 (NOAEC). Adjusting for exposure duration (6 h/24 h), study length (factor 6), interspecies (factor 1: direct local irritation effect without metabolism or receptor binding) and intraspecies variability (standard factor 10) results in an overall assessment factor of 240 and a value of 300 mg/m^3 : 240 = 1.25 mg/m^3 which corroborates the value presented above.

A EU-LCI value (rounded) for TPGME of 1200 µg/m^3 is proposed.

Data on odour thresholds are not available. However, TPGME is reported to be nearly odourless.

5.7 References

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5.8 Fact and data sheet for tripropylene glycol monomethyl ether

Table 5.5: Data collection sheet tripropylene glycol monomethyl ether (TPGME)

Rapporteur, Date		
Compound	**TRIPROPYLENE GLYCOL MONOMETHYL ETHER**	***Data collection sheet***
N° CAS 25498-49-1[a] **N° CAS 20324-33-8[b]** 1 ppm = 8.5 mg/m³	**EU-Classification:** - **CLP:** no harmonized classification	
Organization Name	CARB	Reach registrants
Risk Value Name	8-hour REL	DNEL
Risk Value (mg/m³)	0.1 (0.012 ppm)	19
Reference period	acute (8 h)	Chronic ($DNEL_{Gen.\ Pop.\ long\ term}$)
Risk Value (mg/m³) Short Term (15 min)		19
Year	2010	2017
Key Study	Miller R, Lomax LG, Calhoun L, Kociba R (1985) Tripropylene Glycol Monomethyl Ether: 2-Week aerosol inhalation toxicity study in rats and mice. Confidential report of the Dow Chemical Company, November 12, 1985. Unpublished report. Cited in OECD SIDS (2003) and ECHA (2017)	Not indicated, probably subacute inhalation study with rats and mice
Study type	Subacute exposure study, 2 weeks	Probably subacute exposure study, 2 weeks
Species	Mouse	Not indicated
Duration of exposure in key study	6 h/d, 5 d/Week, 9 d	Probably 6 h/d, 5 d/week, 9 exposures
Critical effect	Eosinophilia in the liver	Not indicated
Critical dose value	NOAEC 360 mg/m³	NOAEC 133 mg/m³
Adjusted critical dose	193 mg/m³ (360 X 6/8 X 5/7), RGDR*: 1 → Human Concentration Adjustment: 193 mg/m³	
Single Assessment factors	UF_L 1 x UF_H 10 x √10 x UF_A 2 x √10 x UF_S 10 x UF_D 1 = 2000	UF_H 5 x UF_A 1 x UF_S 1.4 = 7
Other effects		
Remarks	Derived for [2-(2-methoxy-methylethoxy)-methylethoxy]-propanol (CAS 25498-49-1)	DNEL derivation not presented in detail
UF_L Used LOAEL; UF_H Intraspecies variability; UF_A interspecies variability; UF_S Used subchronic study UF_D data deficiencies		

*RGDR: Regional gas dose ratio for gases with systemic effects.

a: for [2-(2-methoxymethylethoxy)methylethoxy]propanol; b: for 1-[2-(2-methoxy-1-methylethoxy)-1-methylethoxy]propan-2-ol

Table 5.6: Fact sheet tripropylene glycol monomethyl ether (TPGME), all isomers (CAS No. 25498-49-1 and CAS No 20324-33-8)

Rapporteur, Date			
Compound	**TRIPROPYLENE GLYCOL MONOMETHYL ETHER**		***Factsheet***
Parameter	**Note**	Comments	Value / descriptor
EU-LCI Value and Status			
EU-LCI value	1	[µg/m³]	1200
EU-LCI status	2	Draft/Final	Draft
EU-LCI year of issue	3	Year when EU-LCI value has been issued	2018
General Information			
CLP-Index No.	4	INDEX	-
EC-No.	5	EINECS	247-045-4 and 243-734-9
CAS-No.	6	Chemical Abstract Service number	25498-49-1 and 20324-33-8
Harmonised CLP classification	7	Human health risk related classification	none
Molar mass and conversion factor	8	[g/mol] and [mg/m³ - ppm]	206.32 8.5
Key Data / Database			
Key study, Authors, Year	9	Critical study with lowest relevant effect level	Miller et al. (1985)
Read across compound	10	Where applicable	
Species	11	Rat	Rat, Sprague-Dawley, Mouse, B6C3F1 (5 M + 5 F/group)
Route / type of study	12	Inhalation, oral feed….	Inhalation
Study length	13	Days, subchronic, chronic…	subacute
Exposure duration	14	h/d, d/w	6 h/d, 5 d/week, 2 weeks (9 exposures)
Critical endpoint	15	Effect (s), site of	No adverse effect up to highest test concentration
Point of Departure (POD)	16	LOAEC, NOAEC, BMD…	NOAEC
POD value	17	mg/m³ or ppm or mg/(kg b.w. x d)	1010 mg/m³ (120 ppm)
Assessment Factors (AF)	18		
Adjustment for exposure duration	19	Study exposure h/d, d/w	5.6
AF study length	20	sa→sc→c	6
Route-to-route extrapolation factor	21		1
AF 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		1
Result			
Summary of assessment factors	27	Total Assessment Factor	840
POD/TAF	28	Calculated value [µg/m³ and ppb]	1200 µg/m³ (141 ppb)
Molar adjustment factor	29		

Rounded value	30	[µg/m³]	1200
Additional comments	31		

Rationale Section	32		

Rationale for critical effects

No relevant human data are available for the derivation of a EU-LCI for TPGME.

No adverse effects were observed in a subacute inhalation study with TPGME in rats and mice (5 M + 5 F/concentration, exposed to 0, 150, 360 or 1010 mg/m³ (at least partially as aerosol) for 6 h/d, 5 d/week for a total of 9 days) after exposure of up to 1010 mg/m³ (Miller et al., 1985). Slight alterations in the liver of male mice were considered to be an adaptive response and not considered adverse.

There is no evidence for genotoxic effects of TPGME from limited *in vitro* data or from read-across with other propylene glycol ethers. Propylene glycol methyl ether (1-methoxypropan-2-ol) was not carcinogenic in an inhalation study with rats and mice.

Depending on the structure of the isomer, propylene glycol ethers may cause developmental toxicity and teratogenicity, mediated by the metabolic formation of 2-methoxypropionic acid.

In a prenatal developmental toxicity study (equivalent or similar to OECD guideline 414) with inhalation exposure of pregnant Sprague-Dawley rats on GD 6-15 to an aerosol of TPGME (CAS No. 25498-49-1 or 20324-33-8), no evidence of developmental toxicity or systemic toxicity to dams were observed up to the highest concentration of 1000 mg/m³. However, a high incidence (15/25) of muzzle staining in dams at the highest concentration indicated a local irritation effect (Breckenridge et al., 1985).

No developmental toxicity study is available with TPGME with a second species, especially with rabbits which are known to be more sensitive than rats to developmental and teratogenic effects of teratogenic isomers of glycol ethers. However, a prenatal developmental toxicity study with dipropylene glycol methyl ether (DPGME, CAS No. 34590-94-8) with rabbits revealed no such effects up to the highest tested concentration of 1850 mg/m³ (300 ppm), which is even higher than the highest tested concentration of TPGME in rats (ECHA Dissemination, 2018).

Rationale for starting point

The NOAEC of 1010 mg/m³ (120 ppm) from a subacute inhalation toxicity study with rats and mice (Miller et al., 1985) served as a POD for the derivation of a EU-LCI value. The only effects observed in this study were regarded as adaptive and not as adverse. The study is not published, but described in sufficient detail in OECD SIDS (2003) and in the REACH registration dossier (ECHA Dissemination, 2018).

Rationale for Extrapolation factors

- Factor for adjustment for exposure duration: 5.6
- Adjusted study length factor: 6 (subacute exposure)
- Interspecies differences: 2.5 (interspecies factor for systemic effects)
- Intraspecies differences: 10 (default value)

Total extrapolation factor is: 840, leading to a value of 1010000 µg/m³: 840 = 1200 µg/m³.

A very similar value of 1250 µg/m³ may be derived from the NOAEC of 300 mg/m³ (75 mg/m³ for continuous exposure) for slight local irritation in pregnant rats observed in a prenatal developmental toxicity study, taking into account an extrapolation of 6 for study length (subacute to chronic), of one for interspecies differences (local irritating effect) and of 10 for intraspecies extrapolation. This study is not published, but described in sufficient detail in the OECD SIDS (2003) and in the REACH registration dossier (ECHA Dissemination, 2018).

The following EU-LCI is proposed for tripropylene glycol monomethyl ether: 1200 µg/m³.

No odour thresholds are available for TPGME. However, TPGME is described to be nearly odourless.

References

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