Australian Government
Department of Health
Australian Industrial Chemicals Introduction Scheme

1,6-Octadiene, 7-methyl-3-methylene- (myrcene)

Evaluation statement

30 May 2022

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AICIS evaluation statement

Subject of the evaluation

1,6-Octadiene, 7-methyl-3-methylene- (myrcene)

Chemical in this evaluation

Name	CAS Registry Number
1,6-Octadiene, 7-methyl-3-methylene-	123-35-3

Reason for the evaluation

The Evaluation Selection Analysis indicated a potential risk to human health.

Parameters of evaluation

The chemical, myrcene is listed on the Australian Inventory of Industrial Chemicals (the Inventory). This evaluation is a human health risk assessment of all identified industrial uses of the chemical.

d-Limonene (CAS No. 5989-27-5) is structurally similar to β-myrcene. It has a similar metabolism with epoxide formation and metabolism via cytochrome CYP450 enzymes (NTP, 2010). Data for d-limonene was used as read across where data are not available for the chemical and to support the conclusions of the hazard assessment.

Summary of evaluation

Summary of Introduction, Use and End Use

The chemical has reported commercial use in Australia as a component of industrial cleaning products.

Based on international use information, the chemical is used in wide range of industrial applications. The chemical is a fragrance ingredient in cosmetic and domestic products including air fresheners and cleaning products. The reported concentration of the chemical as a fragrance ingredient in cosmetic products is 0.021% (95th percentile in aftershaves and perfumes) and concentrations in domestic products up to 5% have been identified. The chemical is a major component of many essential oils including hops, lemongrass, verbena oil, galbanum oil and bay oils.

The chemical is used as an intermediate for the production of other chemicals.

The chemical has non-industrial uses as a flavouring agent.

Human Health

Summary of health hazards

The critical health effects for risk characterisation include local effects (skin and eye irritation and potential respiratory irritation). The chemical may cause adverse effects in the kidney. There is evidence of non-genotoxic carcinogenic effects in experimental animals; however, the relevance to humans is uncertain.

Based on the available data for the chemical and its structurally similar analogue, d-limonene (CAS No. 5989-27-5), the chemical is readily absorbed via oral and dermal routes.

The chemical has low acute oral toxicity with a median lethal dose (LD50) of >2000 mg/kg bw in mice; LD50 >5000 mg/kg bw in rats and low acute dermal toxicity with LD50 of >5000 mg/kg bw in rats.

The chemical may be fatal if swallowed and enters airways (aspiration hazard). This would be dependent on the viscosity of the chemical as introduced. The threshold kinematic viscosity value for classification as an aspiration hazard is 20.5 mm^2/s at 40 °C.

Based on the limited data available the chemical is irritating to skin and eyes. Available in vitro test data for skin irritation does not distinguish between irritation and corrosion. Based on the weight of evidence, corrosive effects are not expected. The chemical is reported to cause moderate eye irritation (mean conjunctival redness score was 2, in 2 out of 3 animals) in a single animal study. Observed effects were reversible by day 8. Although no inhalation data are available, the chemical caused inflammation of the nasal cavity in repeat dose studies.

The chemical is not expected to be a skin sensitiser based on the negative results observed in a local lymph node assay (LLNA) (Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 429). However, upon autoxidation of the chemical, sensitising chemicals may form. Autoxidation and formation of autoxidation products is known to cause sensitisation for other terpenes including limonene and terpinolene (NICNAS 2002; NICNAS 2018).

Based on the available data, the chemical is potentially harmful following repeated oral exposure to high doses. Effects in the kidneys were reported in several rat studies at doses ≥250 mg/kg bw/day. In sub-chronic and chronic rat studies, complex renal pathology including both α2u-globulin nephropathy, and an unusual nephrosis involving the outer stripe of outer medulla (OSOM) were observed (Cesta 2013). Alpha2u-globulin nephropathy is specific to male rats and considered not relevant to humans. The main effect in female rats was renal tubule necrosis. Nephrotoxicity was not observed in mice.

Based on the available data, the chemical is not considered to be genotoxic. The chemical was reported to be negative in bacterial and mammalian gene mutation assays, mammalian chromosome aberration assays and in an in vivo mammalian erythrocyte micronucleus assay.

The available data are insufficient to determine whether the chemical has carcinogenic potential relevant to humans. Observed clinical effects from 2 year chronic oral gavage studies included increased incidences of renal tubule neoplasms in male rats and liver tumours in male mice. There was equivocal evidence of kidney tumours at high doses in female rats. Kidney tumours reported in male rats are likely to be predominantly α2u-globulin

nephropathy, which is specific to male rodents and is; therefore, not relevant to humans. However, histopathological analysis indicate that although the kidney tumours at lower doses are likely associated with α2u-globulin induced nephropathy, the kidney tumours at higher doses may be due to nephrosis. The liver tumours observed in male mice are typical in a strain (B6C3F1) known to have a high background incidence of spontaneous liver tumours.

The chemical is not expected to cause specific adverse effects on fertility or development following oral exposure. Several reproductive and developmental toxicity studies showed adverse effects on birth weight, peri and post-natal mortality and foetal development at high dose oral exposures in rats and mice. A no observed adverse effect level (NOAEL) of 300–500 mg/kg bw/day for reproductive toxicity and a NOAEL of 500 mg/kg bw/day for developmental toxicity in rats were calculated. Peri and post-natal adverse effects were considered to be secondary to maternal toxicity.

Health Hazard Classification

The chemical satisfies the criteria for classification according to the Globally Harmonised system of Classification and Labelling of Chemicals (GHS) (UNECE 2017) for hazard classes relevant for work health and safety as follows. This does not consider classification of physical hazards and environmental hazards.

Health Hazards	Hazard Category	Hazard Statement
Aspiration	Asp. Tox. 1	H304: May be fatal if swallowed and enters airways
Skin Irritation	Skin Irrit. 2	H315: Causes skin irritation
Eye Irritation	Eye Irrit. 2A	H319: Causes serious eye irritation

Summary of Health Risk

Public

Based on the available use pattern, the public may be exposed to the chemical at concentrations up to 5% by:

- direct application of the chemical to the skin
- direct skin contact during use of domestic products
- incidental skin and eye contact with the chemical during use of domestic products
- inhaling aerosols/vapours.

Consumer products containing the chemical may oxidise over time and form sensitising derivatives. However, no information is available on the extent of autoxidation upon exposure to air of the commercial products and the auto-oxidation is expected to be limited by the presence of an anti-oxidant additives.

Systemic effects are only observed in animal studies at relatively high doses and not expected at the current use concentrations. In comparison to NOAEL values for kidney effects, reported margins of exposure using a total estimated systemic exposure of 0.63 µg/kg bw/day were >39000. Irritant effects are expected to be minimal at concentrations reported to be in use.

Based on the available hazard information the chemical has low toxicity at typical exposure concentrations. Therefore, there are no identified risks to the public that require management.

Workers

During product formulation and packaging, dermal, ocular and inhalation exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment.

Worker exposure to the chemical at lower concentrations could also occur while using formulated products for professional cleaning. The level and route of exposure will vary depending on the method of application and work practices employed. Good hygiene practices to minimise incidental oral exposure are expected to be in place.

Given the critical local health effects, the chemical could pose a risk to workers. Control measures to minimise dermal, ocular and inhalation exposures are required to manage the risk to workers (see **Proposed means for managing risk** section).

Proposed means for managing risk

Workers

Recommendation to Safe Work Australia

It is recommended that Safe Work Australia (SWA) update the Hazardous Chemical Information System (HCIS) to include classifications relevant to work health and safety.

The recommended classification and labelling entry should have the following note appended. 'Note 9: The aspiration hazard classification should only be applied if the kinematic viscosity criteria for aspiration classification in the GHS is met'.

Information relating to safe introduction and use

The information in this report, including recommended hazard classifications, should be used by persons conducting a business or undertaking at workplace (such as an employer) to determine the appropriate controls.

Recommended control measures that could be implemented to manage the risk arising from occupational exposure to the chemical include, but are not limited to:

- using local exhaust ventilation to prevent the chemical from entering the breathing zone of any worker
- minimising manual processes and work tasks through automating processes
- adopting work procedures that minimise splashes and spills
- cleaning equipment and work areas regularly
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

Measures required to eliminate, or manage risks arising from dermal, ocular and inhalation exposure arising from storing, handling and using a hazardous chemical depend on the physical form and the way the chemical is used.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Model codes of practice, available from the Safe Work Australia website, provide information on how to manage the risks of hazardous chemicals in the workplace, prepare a Safety Data Sheet and label containers of hazardous chemicals. Your Work Health and Safety (WHS) regulator should be contacted for information on WHS laws and relevant Codes of Practice in your jurisdiction.

Conclusions

The conclusions of this evaluation are based on the information described in this Evaluation Statement.

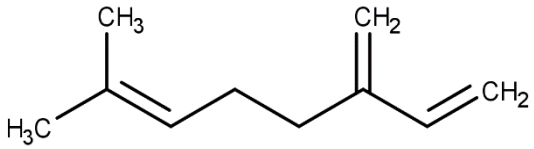
Considering the proposed means of managing risks, the Executive Director is satisfied that the identified human health risks can be managed within existing risk management frameworks. This is provided that all requirements are met under environmental, workplace health and safety, and poisons legislation as adopted by the relevant state or territory, and the proposed means of managing the risks identified during this evaluation are implemented.

Note: Obligations to report additional information about hazards under *Section 100 of the Industrial Chemicals Act 2019* apply.

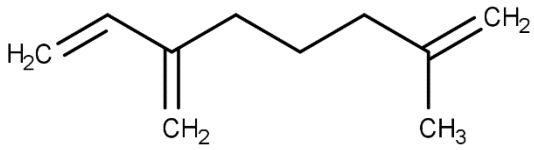
Supporting Information

Chemical Identity

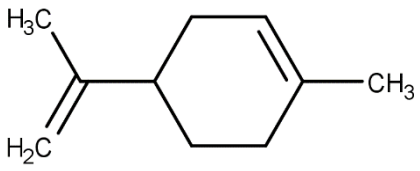
Myrcene exists in two isomeric forms, namely β-myrcene (3-methylene-7-methyl-1,6-octadiene) and α-myrcene (2-methyl-6-methylene-2,7-octadiene). β-Myrcene is the most common isomer which contains an isopropylidene group and is referred to as 'myrcene' in the literature. β-Myrcene contains three carbon-carbon double bonds and a gem-dimethyl terminal (Api et al. 2020; EFSA 2015; Government of Canada 2020; IARC 2019; NTP 2010; REACH).

Chemical name	1,6-octadiene, 7-methyl-3 methylene-
CAS No.	123-35-3
Synonyms	myrcene β-myrcene 2-methyl-6-methylene-2,7-octadiene 3-methylene-7-methyl-1,6-octadiene β-geraniolene
Structural formula	
Molecular formula	C10H16
Molecular weight (g/mol)	136.2
SMILES	CC(=CCCC(=C)C=C)C

Chemical identity information for related chemicals

Chemical name	1,7-octadiene, 2-methyl-6-methylene-
CAS No.	1686-30-2
Synonyms	α-myrcene
Structural formula	
Molecular formula	C10H16
Molecular weight (g/mol)	136.2

SMILES	C=CC(=C)CCCC(=C)C

Chemical name	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (R)-
CAS No.	5989-27-5
Synonyms	d-limonene
Structural formula	
Molecular formula	C10H16
Molecular weight (g/mol)	136.2
SMILES	C=C(C)C1CC=C(C)CC1

Relevant Physical and Chemical Properties

The chemical is a yellow oily liquid with a characteristic pleasant terpene odour and citrus-like taste. The chemical is reported to be practically insoluble in water with a reported log Kow value of 4.8 (REACH). In general monoterpenes have moderate to high vapour pressures. The chemical has reported vapour pressure of 1.7–2.4 mmHg at 25°C. No kinematic viscosity data are available for myrcene. However, the structurally similar chemical limonene has a kinematic viscosity of 0.9–1.1 mm^2 /s at 25°C (ECHA 2019).

Introduction and use

Australia

The chemical has reported industrial and professional use as a cleaning product.

International

The chemical is a monoterpene found in more than 200 plant species. It is a major component of many essential oils including hops, lemongrass, verbena oil, galbanum oil and bay oils.

The chemical is listed in the cosmetic ingredient identification database and COSING database with reported functions of perfuming, flavouring agents; skin protectants and skin conditioning agent (EC; Personal Care Products Council). The chemical is listed on the IFRA

transparency list (IFRA). The 95th percentile concentration in hydroalcoholics is reported as 0.021% (Api et al. 2020)

The chemical has reported domestic use in a range of domestic products, including:

- air freshener products
- cleaning products
- polishes and waxes
- paints, inks and toners.

A North American database identifies the chemical in a range of products including laundry detergents, cleaning liquids and sprays and air fresheners. Concentrations up to 5% were reported (DeLima Associates).

The chemical is used as an intermediate in the production of:

- a range of terpene alcohols such as menthol, geraniol, neriol, linalool, and isophytol
- terpene polymers, terpene-phenol resins and terpene-maleate resins.

The chemical has reported non-industrial use, including as a flavouring additive in food and beverages (NTP 2010).

Existing Australian Regulatory controls

AICIS

No specific controls are currently available for the chemical. The chemical is listed on the Australian Industrial Chemicals Introduction Scheme (AICIS) − List of chemicals with high hazards for categorisation.

Public

No restrictions for industrial use have been identified for the chemical in Australia.

Myrcene has restrictions for its non-industrial use in the *Therapeutic Goods (Permissible Ingredients) Determination No. 1 of 2022* as excipients in medicines (TGA 2022) at certain concentrations depending on its use as a flavour or a fragrance:

‘Permitted for use only in combination with other permitted ingredients as a flavour or a fragrance.

- If used in a flavour, the total flavour concentration in a medicine must be no more than 5%.
- If used in a fragrance, the total fragrance concentration in a medicine must be no more than 1%.’

Workers

The chemical is not listed on the Hazardous Chemical Information System (HCIS) and no specific exposure standards are available in Australia (Safe Work Australia).

International Regulatory Status

United States of America (USA)

Myrcene is prohibited from use as an added synthetic flavour substance in the USA under the 1958 Delaney Clause that bans all carcinogens from use as food additives (Felter 2020). This does not apply to essential oils containing myrcene.

Health Hazard Information

Toxicokinetics

The chemical is readily absorbed via the oral and dermal routes of exposure. In rats and rabbits the chemical was well absorbed after oral administration. In a study in male rabbits receiving the chemical via gavage at 670 mg/kg bw/day for 2 days, approximately 25% of the chemical was recovered in urine during the 3 days following treatment. In female rats receiving a single dose of the chemical via gavage at 1000 mg/kg bw, the chemical was detected in blood at 14.1 µg/L 60 minutes after treatment. A study in rats indicated absorption through intact skin (IARC 2019). No information is available on inhalation absorption of the chemical. The structurally similar chemical d-limonene is reported to have lower dermal absorption than inhalation absorption (NICNAS 2002).

In female rats, the chemical was mainly distributed in the adipose tissue, and several organs like the brain, liver, kidneys and testes (Adams et al. 2011; IARC 2019; NTP 2010).

In a study in male albino rabbits, more than 70% of the oral dose were excreted as diol metabolites in the urine (IARC 2019; NTP 2010). In studies in rats and rabbits, the main urinary metabolites of myrcene were 10-hydroxylinalool and 7-methyl-3-methylene-oct-6-ene-1,2-diol (IARC 2019; NTP 2010). Epoxidation of carbon–carbon double bonds were observed in both rats and rabbits (IARC 2019). In humans, the chemical was metabolised by CYP-450 mediated epoxidation via epoxide hydrolase to yield epoxides and diols, which in turn conjugated with glucuronic acid and were excreted in the urine (Adams et al. 2011).

Urine was the predominant route of excretion of conjugated myrcene glycol and diol metabolites in rats and rabbits (IARC 2019).

Myrcene shares similarities in epoxide formation and autoxidation with the structurally similar chemical d-limonene (NICNAS 2002; NTP 2010).

Acute Toxicity

Oral

Based on the available data, the chemical has low acute oral toxicity.

In a non-guideline acute oral toxicity study, Swiss mice (groups of 1 or 3/sex/dose) were administered single oral doses of 1000, 1500, 2250, 3380, 5060, 7560 or 11390 mg/kg bw of β-myrcene in corn oil. All animals survived at doses of 3380 mg/kg bw and below. Mortality was recorded in 2/3 males and 3/3 females at 5060 mg/kg bw; 3/3 males and 2/3 females at 7560 mg/kg bw; and 1/1 male and 1/1 female at 11390 mg/kg bw. Necropsy showed vacuolisation of hepatic cells and accumulation of lipids and hyperkeratosis in the

non-glandular part of the stomach of the surviving animals. Reported sub-lethal signs of toxicity at doses 5060 mg/kg bw and above included palpebral ptosis (drooping of the upper eyelid), hypoactivity and ataxia. The reported LD50 was >2000 mg/kg bw (REACH; Adams et al. 2011).

In a non-guideline study, Wistar rats (1–2/sex/dose) were treated with single oral doses of 670, 1000, 1500, 2250, 3380, 5060, 7590 or 11390 mg/kg bw β-myrcene in corn oil. No mortality or signs of toxicity were observed at any treatment doses. An oral LD50 of >5000 mg/kg bw in rats was reported in the study (Adams et al. 2011; REACH).

In an OECD TG 401 acute toxicity study, male Wistar rats (n=10) were treated with a single dose of 5000 mg/kg bw of undiluted β-myrcene by gavage. One animal died after the treatment and all treated animals showed lethargy and urinary incontinence. The oral LD50 was reported to be >5000 mg/kg bw in the study (Adams et al. 2011; REACH).

Dermal

Based on the available data, the chemical has low acute dermal toxicity.

In an OECD TG 402 study, New Zealand White (NZW) rabbits (n=10) were treated with β-myrcene (5000 mg/kg bw) via an occlusive dressing for 24 hours. No mortality or signs of systemic toxicity were observed. All treated animals showed slight to moderate erythema and oedema during the initial days of treatment, but these were completely reversed 7 days post treatment. An acute dermal LD50 of >5000 mg/kg bw was reported in the study (Adams et al. 2011; REACH).

Inhalation

No data are available for the chemical.

Corrosion/Irritation

Skin Irritation

Based on the available data, the chemical is likely to be irritating to skin and warrants classification. Although the in vitro test does not distinguish between irritation and corrosion, based on the lack of severe effects in the acute dermal toxicity study, corrosive effects are not expected.

In a Good Laboratory Practice (GLP) compliant in vitro skin irritation study similar to OECD TG 439 (in vitro reconstructed human epidermis (RHE) test method for skin irritation), the chemical was applied to RhE for an exposure period of 15 minutes, followed by an observation period of 42 hours. A mean tissue viability value of 25.9 ± 2.6% was reported in this study, and the chemical was determined to be irritating to the skin. Interpretation of results obtained from OECD TG 439 studies do not allow for distinction between irritation and corrosion (REACH).

In a non-guideline study the chemical was applied to abraded or intact rabbit skin for 24 hours under occlusion. The chemical was reported to be moderately irritating to rabbit skin (Surendran et al. 2021). In the acute dermal toxicity study (see **Acute toxicity** section) slight to moderate erythema and oedema were observed during the initial days of treatment, which were completely reversed 7 days post-treatment.

The structurally similar chemical limonene is classified as a skin irritant.

In silico

Myrcene is part of the training set in the OASIS TIMES skin irritation model where the chemical is reported to be irritating to skin based on experimental data. The chemical was also predicted to be irritating to skin (in domain) with an alert for conjugated hydrocarbons.

Eye Irritation

Based on the available data, the chemical is an eye irritant and warrants hazard classification.

In an eye irritation study conducted according to OECD TG 405, the chemical (undiluted) was instilled in the conjunctival sac of one eye of each NZW rabbit (n= 3 males). The reported mean Draize score (24, 48 and 72 hours) for conjunctival redness was 2 for 2 out of 3 animals. The score after 7 days was 1 in all animals but redness had fully revered by day 8. The reported mean score for chemosis was 2 for one animal and one for 2 out of 3 animals. The mean scores for corneal opacity and iritis were 0 in all animals. No adverse effects were observed in the eye of the control animal (REACH).

Sensitisation

Skin Sensitisation

Based on the available data, the chemical is not considered to cause skin sensitisation. However, autooxidised products of the chemical have the potential to be skin sensitisers.

In a local lymph node assay (LLNA) performed in accordance with OECD TG 429, female CBA/J mice (4/group) were treated with 25 µL of 2.5, 5, 10, 25 or 50% chemical in acetone/olive oil (4:1 v/v) for 3 days. The reported stimulation indices (SI) were 0.66, 0.96, 0.76, 2.59 and 1.78, respectively. The estimated concentration producing a 3 fold increase in lymphocyte proliferation (EC3) was; therefore, >25% indicating absence of or only weak sensitisation potential. No mortality or clinical signs were observed during the study (REACH; Api et al. 2019).

In silico data

No structural alerts for skin sensitisation were present for the chemical using OECD QSAR Toolbox v4.2. However, when autoxidation or skin metabolism was simulated there were mechanistic alerts for protein binding and skin sensitisation.

Observations in humans

In a study in 1511 dermatitis patients, 1 patient had a reaction to oxidised myrcene (3% in petrolatum) (Matura 2005).

Repeat Dose Toxicity

Oral

Based on the available data, the chemical is potentially harmful following repeated oral exposure to high doses of the chemical. Effects in the kidneys were reported in several rat studies at doses ≥250 mg/kg bw/day. In mice the liver was affected at doses >250 mg/kg bw/day.

In sub-chronic and chronic studies in rats complex renal pathology including both α2u-globulin nephropathy, and an unusual nephrosis involving the outer stripe of outer medulla (OSOM) was observed (Cesta 2013). Alpha2u-globulin nephropathy is specific to male rats and not relevant to humans. The main effect in female rats was renal tubule necrosis. Nephrotoxicity was not observed in mice.

In a 90 day repeat dose oral toxicity study conducted according to OECD TG 408, F344/N rats (10/sex/dose) were administered the chemical in corn oil by gavage at doses of 0, 250, 500,1000, 2000 or 4000 mg/kg bw/day, 5 times per week. Mortality and reduction in mean bodyweight were reported at doses ≥500 mg/kg bw/day. Absolute and relative liver and kidney weights were increased in most treated animals. The incidence of renal tubule necrosis was significantly increased in all treated groups. Hyaline droplet accumulation was observed in males at all doses except at 2000 mg/kg bw/day. Hyaline droplets were not observed in female rats. The formation of hyaline droplets is related to α2u-globulin nephropathy (commonly observed in male rats) and is regarded as not relevant to humans (Hard et al. 1993). In both male and female rats from the 1000 mg/kg dose groups, nephrosis in the outermost region of the OSOM was reported with early indication of nephrosis observed at 500 mg/kg bw/day. Other clinical effects observed included: significant decrease in creatinine levels and increased incidence of porphyrin pigmentation in the Harderian glands in males (500 mg/kg bw/day); significantly increased incidence of chronic inflammation (1000 and 2000 mg/kg bw/day in both sexes); increased severity and incidence of olfactory epithelium degeneration (2000 mg/kg bw/day in both sexes); splenic atrophy (2000 mg/kg bw/day in both sexes); increased incidence of mesenteric lymph node atrophy (2000 mg/kg bw/day in males; and 1000 and 2000 mg/kg bw/day in females); and acute inflammation of the forestomach (in 4/10 females at 2000 mg/kg bw/day). A lowest observed adverse effect level (LOAEL) of 250 mg/kg bw/day was reported based on increased liver and kidney weights. An NOAEL was not established in this study (Api et al. 2019; Cesta 2013; REACH; Government of Canada 2020; NTP 2010).

In a 90 day study, conducted according to OECD TG 408, Sprague Dawley (SD) rats (n=10/sex/dose) were fed myrcene in diet at 0, 20.4, 58.8 or 115.2 mg/kg bw/day in males and 24.2, 70, or 135.9 mg/kg bw/day in females. No adverse effects were observed. NOAELs of 115 and 136 mg/kg bw/day for males and females, respectively, were established by the study authors (Bastaki et al. 2018).

In an OECD 408 compliant 90 day study, SD rats (n=10/sex/dose) were fed diet containing myrcene at 0, 700, 2100, or 4200 mg/kg feed. Based on stability data, weekly diet refreshment, and measured dietary intake, adjusted calculated mean daily intakes of 8.0, 40, and 44 mg/kg bw/day for males, and 9.6, 48 and 53 mg/kg bw/day for females for 90 days were calculated. No adverse effects including changes in macroscopic or microscopic histopathology, or organ weight changes were observed. The NOAEL was 44 mg/kg bw/day for males and 53 mg/kg bw/day for females (EFSA 2015).

In a 2 year carcinogenicity study, F344 rats (n=50/sex/dose) were administered the chemical in corn oil by gavage, 5 days a week for 105 weeks at doses 250, 500 or 1000 mg/kg

bw/day. All treated groups showed significant increase in nephropathy. The frequency of renal tubule nephrosis was significantly increased in all treatment groups except in females at 250 mg/kg bw/day dose group. Linear papillary mineralisation (indicative of α2u-globulin nephropathy) was observed in male rats exposed to 250 and 500 mg/kg bw/day. It was not observed in male rats treated with 1000 mg/kg bw/day or in all treated female rats. Although chronic progressive nephropathy (CPN) was observed, end stage CPN was not observed in any kidney. Incidences of focal suppurative inflammation and chronic active inflammation of the nose occurred in 250 and 500 mg/kg bw/day groups, respectively (Cesta et al. 2013; REACH; Government of Canada 2020; IARC 2019; NTP 2010).

In a 90 day repeat dose oral toxicity study conducted according to OECD TG 408, B6C3F1 mice (10/sex/dose) were administered the chemical in corn oil at doses of 0, 250, 500,1000, 2000 or 4000 mg/kg bw/day, 5 times per week, via oral gavage. Mortality was reported at doses ≥2000 mg/kg bw/day. Significantly reduced bodyweight gain was reported at 500 mg/kg bw/day in females and 1000 mg/kg bw/day in males. Relative liver weights were increased at all doses in males, but the increase of absolute liver weights was only significant at 250 mg/kg bw/day. Relative and absolute liver weights were increased in females receiving 500 or 1000 mg/kg bw/day. Relative kidney weights were increased in all dosed female groups, but absolute kidney weights were only significantly increased at 1000 mg/kg bw/day. No kidney weights were reported for males. A NOAEL of 250 mg/kg bw/day was established in females and 500 mg/kg bw/day in males (Government of Canada 2020; NTP 2010; REACH).

In a 2 year carcinogenicity study, B6C3F1 mice (n=50/sex/dose) were administered the chemical in corn oil by gavage, 5 days a week for 105 weeks at doses 250, 500 or 1000 mg/kg bw/day. The frequency of liver neoplasms was significantly increased at 250 mg/kg bw/day (both sexes) and 500 mg/kg bw/day (males only) doses. A significant increase in hepatocellular hypertrophy was observed in both sexes at 500 mg/kg bw/day, with increased frequencies of mixed cell foci in females. In addition, significant increase in bone marrow atrophy, lymph node follicle atrophy in spleen, and epithelial hyperplasia in forestomach were observed in females at 500 mg/kg bw/day (REACH; Government of Canada 2020; IARC 2019; NTP 2010).

Dermal

No data are available for the chemical.

Inhalation

No data are available for the chemical.

Genotoxicity

Based on the available data, the chemical is not considered to be genotoxic.

In vitro

The chemical was reported to be negative in two bacterial reverse mutation assays conducted according to OECD TG 471 using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and *Escherichia coli* strain WP2 uvrA at concentrations up to 10000 µg/plate, with and without metabolic activation (Adams et al. 2011; Api et al. 2019; REACH).

The chemical was reported to be negative in an in vitro mammalian chromosome aberration assay conducted according to OECD TG 473 (in human lymphocytes) at concentrations up to 1000 µg/mL for up to a 24 hour exposure period, with and without metabolic activation (Adams et al. 2011; Api et al. 2019; REACH).

In an in vitro mammalian cell gene mutation assay conducted according to OECD TG 476 in (Chinese hamster ovary (CHO) cells), the chemical was reported to be negative at concentrations up to 1000 µg/mL, with and without metabolic activation (Adams et al. 2011; Api et al. 2019; REACH).

In vivo

The chemical was reported to be negative in a mammalian erythrocyte micronucleus assay (equivalent to OECD TG 474), in peripheral blood samples from B6C3F1 mice (5/sex/dose) at concentrations up to 2000 mg/kg bw (Adams et al. 2011; Api et al. 2019; REACH).

Carcinogenicity

The available data are insufficient to determine whether the chemical has carcinogenic potential relevant to humans.

The National Toxicology Program (NTP 2010) reported that the chemical causes increased incidences of combined malignant and benign kidney tumours in male rats and malignant and combined malignant and benign liver tumours in male mice. There was equivocal evidence of carcinogenicity in female rats and mice (IARC 2019; NTP 2010).

The International Agency for Research on Cancer (IARC) has classified the chemical as ‘Possibly carcinogenic to humans’ (Group 2B), based on inadequate evidence for carcinogenicity in humans, but sufficient evidence in experimental animals (IARC 2019).

In a study similar to OECD TG 451, F344 rats (n=50/sex/dose) were administered the chemical in corn oil by gavage, 5 days a week for 105 weeks at doses of 250, 500 or 1000 mg/kg bw/day. Mortality was reported for all male rats in 1000 mg/kg bw/day dose group due to renal toxicity before the end of the study. In male rats, a statistically significant increase in renal tubule adenoma was observed at 500 mg/kg/day and a significant increase in combined incidence of renal tubule adenoma or carcinoma at 250 mg/kg bw/day (14%) and 500 mg/kg bw/day (18%). No malignant tumours of the kidney occurred in the treated groups of female rats. An equivocal finding of renal tubule adenoma were reported. The 4% incidence was higher than historical values. The incidence of renal tubule nephrosis was increased in all groups of treated male and female rats (IARC 2019; NTP 2010).

Kidney effects observed in studies with the structurally similar chemical d-limonene were considered specific to male rats and not relevant to humans. No kidney effects of d-limonene were observed in female rats (NICNAS 2002).

Three of the seven criteria for concluding that a chemical induces tumours of the kidney by an α2u-globulin nephropathy have been met for myrcene. However not all criteria have been demonstrated including male rat specificity for nephropathy. Histopathological analysis (see **Repeat dose toxicity oral**) indicate that although the tumours at lower doses are likely to be associated with α2u-globulin induced nephropathy, tumours at higher doses (including in females) may be due to nephrosis. The absence of end stage CPN indicates that this may not be involved in the development of tumours. Due to the high response in male rats

attributed to the specific nephropathy, it is not clear the extent to which nephrosis contributes to tumour formation (Cesta 2013; IARC 2019; NTP 2010)

In an OECD TG 451 study, B6C3F1 mice (n=50/sex/dose) were administered the chemical in corn oil by gavage at 250, 500 or 1000 mg/kg bw/day, 5 days/week for 104 weeks for females and 105 weeks for males. High mortality was observed at 1000 mg/kg bw/day for both sexes. The incidences of liver neoplasms were significantly increased in males at 250 and 500 mg/kg bw/day, and in females at 250 mg/kg bw/day only. Mean body weights in 1000 mg/kg bw/day dose group males and 500 mg/kg bw/day dose group females were less than the control group in weeks 11 and 17 of the study, respectively. Increased incidence of hepatocellular hypertrophy was observed at 500 mg/kg bw/day. The incidence of lymphoid follicle atrophy in the spleen was significantly increased and incidences of inflammation and epithelial hyperplasia of the forestomach were increased in females at 500 mg/kg bw/day dose group. (REACH; Government of Canada 2020; IARC 2019; NTP 2010). Male B6C3F1 rats are known as a strain with a particularly high background incidence of liver tumours (Goldsworthy and Fransson-Steen 2002).

Reproductive and Development Toxicity

Based on the available information, the chemical is not considered to have specific reproductive and developmental toxicity. The chemical caused an increase in pup mortality and affected developmental land marks at 500 mg/kg bw/day in a non-GLP compliant peri- and postnatal developmental toxicity study. In this study maternal toxicity was not observed at 500 mg/kg bw/day. However, in a detailed GLP compliant repeat dose toxicity study (see **Repeat dose toxicity** section), mortality and reduced body weights were reported at doses ≥500 mg/kg bw/day, and kidney weights and the incidence of renal tubule necrosis were increased at doses ≥250 mg/kg bw/day. In the other developmental studies adverse effects was only observed at maternally toxic doses. Therefore, it is likely the effects seen in the peri- and postnatal study are secondary to maternal toxicity.

In a non-GLP compliant peri- and postnatal developmental toxicity study, pregnant Wistar rats (12–20/dose) were administered myrcene via oral gavage at dose of 0, 250, 500, 1000 or 1500 mg/kg bw/day from gestational day (GD) 15 through parturition and lactation up to weaning (postnatal day [PND] 21). The reproductive capacity of the F1 generation was evaluated after reaching maturity (120 days after birth) by mating males and females from the same treatment group but from different litters. Mortality was reported in 5 pregnant females (parental generation) at 1500 mg/kg bw/day. Body weights were reduced on GD20 in females receiving ≥1000 mg/kg bw/day. The incidence of still births was increased at 1000 mg/kg bw/day. Increased labour duration was reported at 500 and 1000 mg/kg bw/day. Pup mortality (F1) was significantly increased and body weight decreased at ≥500 mg/kg bw/day. Delayed appearance of developmental landmarks such hair growth (≥500 mg/kg bw/day) ear unfolding and eye opening (≥1000 mg/kg bw/day) were reported. F1 females had a statistically significant decrease in fertility at doses ≥1000 mg/kg bw/day. The NOAEL for maternal toxicity was considered to be 500 mg/kg bw/day based on decreased body weights at 1000 mg/kg bw/day. The NOAEL for developmental toxicity was considered to be 250 mg/kg bw/day based on decreased pup body weights, increased pup mortality, parturition disturbance, and the delayed appearance of developmental landmarks at ≥500 mg/kg bw/day. The NOAEL for reproductive toxicity was considered to be 500 mg/kg bw/day based on impaired fertility in F1 females at ≥1000 mg/kg bw/day (Api et al. 2020).

In a one-generation reproductive toxicity non-GLP compliant similar to OECD TG 415, Wistar rats (n=45 females, 15 males/dose/group) were exposed to the chemical in peanut oil via gavage at concentrations of 0,100, 300 or 500 mg/kg bw/day. Animals were administered the chemical once daily, 7 days a week for 91 days. Males were treated for 10 consecutive

weeks prior to mating and thereafter. Females were treated for 2 consecutive weeks prior to mating and thereafter. Developmental and reproductive toxicity parameters were assessed. No mortality or other signs of toxicity were observed in the treated animals. No signs of maternal toxicity, effects on mating or pregnancy indices, and postnatal weight gains were observed. At 500 mg/kg bw/day a significant increase in resorption rate, decrease in number of live foetuses, and increase in frequency of fused zygomatic, dislocated sternum and lumbar extra ribs were reported. NOAEL values of 300 mg/kg bw/day and toxicity and 500 mg/kg bw/day were determined for reproductive and parental toxicity, respectively (Api et al. 2019; REACH; Government of Canada 2020; NTP 2010).

In a developmental toxicity study conducted similar to OECD TG 414, female Wistar rats (16 females/group in the low and mid-dose groups and 29 females in the high dose group) were treated with the chemical in corn oil at doses of 250, 500 or 1200 mg/kg bw/day during gestation days 6 to 15. All treated females were euthanised on gestation day 20, and developmental and reproductive toxicity parameters were assessed. Mortality was reported in one dam on gestation day 11 at 1200 mg/kg bw/day dose group. A statistically significant decrease in maternal weight gain and reduced gravid uterus weight was reported in high dosed dams. No adverse effects were observed for the 250 and 500 mg/kg bw/day dose groups. The following clinical effects were observed at 1200 mg/kg bw/day (the highest dose tested), compared with controls:

- statistically significant reduction in number of implantation sites, live foetuses, and individual foetal weights
- higher rate of irregularly positioned hind paws
- significantly higher incidences of delayed ossification; skull bones (9.6%); caudal vertebrae (37.8%); metacarpus (9.1%) and metatarsus (29.2%).

Based on the available data, a maternal NOAEL of 500 mg/kg bw/day was established based on the mortality and decreased maternal weight gain at 1200 mg/kg bw/day. A NOAEL for developmental toxicity was determined to be 500 mg/kg bw/day, based on increased incidence of skeletal malformations reported at 1200 mg/kg bw/day (Api et al. 2019; Government of Canada 2020; NTP 2010; REACH).

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