Alpha-pinene: Human health tier II assessment

29 June 2018

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-	80-56-8
Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (1S)-	7785-26-4
Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (1R)-	7785-70-8
Terpenes and terpenoids, turpentine oil, .alpha.-pinene fraction	65996-96-5

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit:**www.nicnas.gov.au**

Disclaimer

ACRONYMS & ABBREVIATIONS

Grouping Rationale

This group assessment contains chemicals related to alpha-pinene. Three of the chemicals in this group are: alpha-pinene (unspecified isomer) (CAS No. 80-56-8), the (1S,5S)- or (-)-alpha-pinene (CAS No. 785-26-4) isomer and the (1R,5R)- or (+)-alpha-pinene isomer (CAS No. 7785-70-8). They are closely structurally-related and are expected to have similar toxicological properties. The chemicals are naturally-occurring and the racemic mixture of both enantiomers does not occur in nature. In this assessment, 'alpha-pinene', refers to the unspecified isomer, unless stated otherwise.

This assessment also includes the chemical 'oil of turpentine, alpha-pinene fraction' (CAS No. 65996-96-5). This chemical is the distillation fraction of turpentine oil containing >80 % alpha-pinene . While this fraction is expected to also contain small amounts of the other terpene hydrocarbons in turpentine (beta-pinene, delta-3-carene, camphene, terpinolene, carene and limonene), its toxicological profile is expected to be closely related to that of alpha-pinene (CAS No. 80-56-8).

Import, Manufacture and Use

Australian

The chemicals have reported cosmetic use as fragrance compounds in Australia.

The chemicals are components of essential oils within Australia (Essentially Australia).

The chemical, alpha-pinene (unspecified isomer) (CAS No. 80-56-8) has reported domestic uses in automotive aftermarket products including car wash soaps, boat wash soaps, polishes, and rubbing compounds.

International

The following international uses have been identified through the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers, Galleria Chemica, the Substances and Preparations in Nordic countries (SPIN) database, the European Commission Cosmetic Ingredients and Substances (CosIng) database, the United States (US) Personal Care Products Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary, the US Household Products database, the US National Library of Medicine's Hazardous Substances Data Bank (HSDB), and various international assessments (Cachao et al., 1986; Lear et al., 1996).

The chemicals have reported cosmetic use as fragrance compounds (at reported concentrations between 0.1–1.0 %).

The chemicals have reported domestic uses, including in:

- cleaning/washing products;
- paints, lacquers and varnishes; and
- car-care products.

The chemicals have reported commercial uses, including in:

- fuel additives;
- surface treatment products;
- lubricants and additives;
- degreasers (cold degreasing, de-waxing, de-polishing);
- wood impregnation agents, wood preserving agents; and
- solvents (as a constituent of turpentine oil).

The chemicals have reported non-industrial use as food additives and are found in therapeutic products.

Restrictions

Australian

The chemicals are not separately listed in the *Poisons Standard—the Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP). Turpentine oil, of which alpha-pinene is a major component, is listed in Schedule 5 (SUSMP, 2018).

Schedule 5:

TURPENTINE OIL except in preparations containing 25 per cent or less of turpentine oil.

Schedule 5 chemicals are described as 'Substances with a low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with simple warnings and safety directions on the label.' Schedule 5 chemicals are labelled with 'Caution' (SUSMP, 2018).

The chemical alpha-pinene has restrictions for its non-industrial use as an excipient in listed medicines (TGA, 2017) at certain concentrations depending on its use as a flavour or a fragrance additive:

- 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 %.

International

The chemicals are covered by a listing on the EU Cosmetic Directive 76/768/EEC Annex III Part 1: List of Substances which Cosmetic Products must not contain except subject to the restrictions and conditions laid down (peroxide value must be less than 10 mmoles/L) (Galleria Chemica).

Pinacea derivatives, including the chemicals in this group, are included in the International Fragrance Association (IFRA) Standards: Essential oils and isolates derived from the *Pinacea* family, should only be used when the level of peroxides is kept to the lowest practicable level, for instance by adding antioxidants at the time of production. Such products should have a peroxide value of less than 10 mM peroxide per litre (IFRA, 2009).

Existing Worker Health and Safety Controls

Hazard Classification

The chemicals are not listed on the Hazardous Chemical Information System (HCIS) (Safe Work Australia).

Exposure Standards

Australian

No specific exposure standards are available.

International

The following exposure standards were identified (Galleria Chemica):

The chemicals are covered under exposure limits for monoterpenes which are between 112–150 mg/m^3 (20–25 ppm) time weighted average in different countries such as Canada, Estonia and Spain and short-term exposure limit of 167–300 mg/m^3 (30–50 ppm) in Canada and Estonia.

Health Hazard Information

The alpha-pinenes are organic compounds which are found in various species of plants, including in coniferous trees. They are typically the main ingredient in pine and turpentine oil, which also often contains beta-pinene, delta-3-carene, camphene, terpinolene, carene and limonene in lesser amounts. alpha-Pinene is typically produced in nature as a multi-constituent substance, containing mixtures of the (–) and (+) enantiomers. Where required, data will be read-across between the chemicals in this group as they are expected to have similar toxicological profiles.

Toxicokinetics

A toxicokinetic study was conducted wherein 8 male human volunteers were exposed to 450 mg/m^3 turpentine by inhalation for 2 hours in an exposure chamber. Inhalational exposure resulted in a 62 % uptake of alpha-pinene. The mean blood clearance 21 hours after exposure was 0.8 L.kg-1.hr-1. The mean half-life of the slow phase of alpha-pinene elimination was 32 hours (Filipsson, 1996).

In a metabolism study, albino rats were orally administered (+)-, (–)-, and (+/–)-alpha−pinenes. The main urinary metabolite recovered in urine 3 days after administration was (–)-trans-verbenol (REACHa).

A study in humans found that alpha-pinene was metabolised to cis-verbenol, trans-verbenol, myrtenic acid and myrtenol following oral administration. Two unknown metabolites, whose predicted structures were 4-hydroxymyrtenic acid and dihydromyrtenic acid, were also identified. Metabolism of the chemicals occurred quickly and the metabolites were almost entirely cleared 10 hours after exposure (REACHa).

Dermal absorption of alpha-pinene was assessed in an ex vivo study using human skin. The chemical was absorbed rapidly to the stratum corneum layer, and also to viable epidermis and dermis. However, it did not permeate across the skin to the receptor medium (REACHa).

In a case of intentional self-poisoning following ingestion of 400-500 mL pine oil (consisting of 57 % alpha-pinene, 8 % beta-pinene, 26 % delta-3-carene, 6 % limonene and 3 % other hydrocarbons), gastrointestinal absorption of the monoterpenes was found to be low (Köppel et al., 1981; REACHa).

Acute Toxicity

Oral

Based on test results, the chemicals have moderate acute oral toxicity in rats. Hazard classification is recommended based on the available animal data (see **Recommendation** section). Human data support this classification (see **Acute Toxicity: Observation in Humans** section).

The chemical alpha-pinene was assessed for acute oral toxicity according to the Organisation for Economic Cooperation and Development (OECD) Test Guideline (TG) 423 (acute oral toxicity). Female Sprague Dawley (SD) rats were administered the chemical by oral gavage at 300 or 2000 mg/kg body weight (bw). The low and high doses were administered to 6 and 3 animals, respectively. All 3 animals in the high dose group died 24 hours after dosing. No mortalities occurred in the low dose animals and no treatment-related effects were observed at necropsy. The high dose animals exhibited reduced activity 30 minutes after dosing and post-mortem histopathological examination revealed thinning of their corpora callosa. The results indicate an oral median lethal dose (LD50) higher than 300 mg/kg bw and lower than 2000 mg/kg bw for the test chemical. In accordance with the test guideline, the LD50 cut-off of the test substance may be considered to be 500 mg/kg body weight by oral route in the rat (REACHa).

The chemicals or mixtures containing the chemicals could have the potential to cause chemical pneumonitis if aspirated. This would be dependent on the viscosity of the chemical as introduced (NICNASa). The threshold viscosity value for classification as an aspiration hazard is 20.5 mm^2/s at 40 °C (Safe Work Australia).

Dermal

The chemicals are expected to have low acute toxicity based on a result from an animal test following dermal exposure. The LD50 in rats is >2000 mg/kg bw.

In a study conducted according to OECD TG 402 (acute dermal toxicity), SD rats (5 animals/sex) were topically administered alpha-pinene at 2000 mg/kg bw, under semi-occlusive conditions. No mortality was reported during the study. No systemic signs of toxicity were observed, body weight gain was not affected and no macroscopic organ pathology was observed upon necropsy. Local cutaneous reactions (erythema and dryness) were observed in all 10 animals but were fully reversed by day 13. On the basis of these findings, the chemical is not considered to be acutely toxic via the dermal route (REACHa).

Inhalation

The chemicals are expected to have low acute toxicity based on results from an animal test following inhalation exposure.

A non-guideline acute inhalational toxicity study was conducted to assess alpha-pinene. Five female CF1 mice were exposed to aerosolised test material (concentration not reported) for 1 minute in a 2.6 L chamber. No mortalities occurred, no adverse clinical signs were observed and no respiratory effects were reported (FFHPVC, 2006).

Observation in humans

Suicides or suicide attempts by ingesting pine oil (the major constituent of which is alpha-pinene) are scarcely reported in the literature. Accidental intoxications in children are more common. The lethal dose of pine oil is expected to be in the range of 60-120 g for adults. Common signs of intoxication by pine oil include impaired consciousness, psychomotoric excitation, delirium, headache, nausea, ataxia, paresis, gastroenteritis, tachycardia and renal failure (Köppel et al., 1981).

A man who attempted suicide by ingesting 400-500 mL of pine oil was admitted to hospital for treatment. The pine oil was assessed by gas chromatography/mass spectrometry and was found to be comprised of 57 % alpha-pinene, 8 % beta-pinene, 26 % delta-3-carene, 6 % limonene and 3 % other hydrocarbons. The patient exhibited signs of toxicity including psychomotor excitation, erythema of the mouth, flushing of the face, ataxia and spontaneous hyperventilation. Death was prevented as a result of medical intervention (Köppel et al., 1981).

Corrosion / Irritation

Respiratory Irritation

No classification is warranted for respiratory irritation; however, studies in mice suggest that turpentine, of which alpha-pinene is a major constituent, is a sensory irritant (NICNASa). Sensory irritation is the result of the chemical stimulating the trigeminal nerve endings in the cornea and nasal mucosa, which evokes a stinging or burning sensation in the eyes and upper respiratory tract. This is a receptor mediated mode of action and occurs at relatively low concentrations. Sensory irritation is different to eye and skin irritation used for hazard classification and also does not involve cytotoxicity. Normally, irritation is a result of physical damage to the cells, whereas sensory irritation is a nerve response (NICNASb). Sensory irritation is not considered to be specific target organ toxicity under GHS and; therefore, is not classifiable.

The concentration of turpentine that elicits a respiratory rate decrease of 50 % (RD50) was determined using a mouse bioassay measuring a decrease in breathing rate due to stimulation of the trigeminal nerve endings in the nasal mucosa. In the study, Oncin France 1 and National Institute of Health Swiss mice were exposed to turpentine (53 % delta-3-carene, 15 % beta-pinene, 14 % alpha-pinene and 2 % limonene) (8/strain), in a glass tube (head only exposure). The concentration in the chamber was regulated by airflow and the actual concentration in the chamber was continuously monitored by infrared spectroscopy. Control mice (n=8) were exposed to room air only. The maximum response generally occurred after 30 minutes. At higher concentrations, body movements slowed down and slight sedation or drowsiness was observed. Recovery was rapid and no macroscopic effects were seen 1 hour or 7 days after the end of the exposure. An RD50 of 1173 ppm (6.5 mg/L) was reported (NICNASa).

Skin Irritation

Based on test results, the chemicals are expected to be mildly irritating to the skin (see **Recommendation** section) and hazard classification is warranted.

An in vitro study was conducted using EpiSkin (reconstructed epidermis from normal human keratinocytes). alpha-Pinene (10 µL) was applied directly to the reconstructed tissue for 15 minutes (with 3 sample replicates). An MTT conversion assay was performed to access the percentage of viable cells in the tissue. EpiSkin viability was found to be ≤50 %. On the basis of this finding, the investigators reported that the chemical is considered to be a dermal irritant, warranting classification under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (REACHa).

In a study conducted according to OECD TG 402 (see **Dermal Acute Toxicity** section), SD rats of both sexes (5 animals/sex) were topically administered a single application of alpha-pinene at 2000 mg/kg bw, under semi-occlusive conditions. Local cutaneous reactions (erythema and dryness) were observed in all 10 animals, and were fully reversed by day 13 (REACHa).

Hartley guinea pigs (6 animals per dose) were topically administered alpha-pinene (20 µL) on shaved intact skin, at 0, 0.8, 4, 20 and 100 % (in acetone:olive oil (v/v 4:1)). At 24 hours after administration, 0/6, 0/6, 1/6, 6/6 and 6/6 animals showed erythema in the 0, 0.8, 4, 20 and 100 % groups, respectively. The chemical was considered to be a dermal irritant on the basis of these results (Wei et al., 2006).

Eye Irritation

The available data do not support classification for eye irritation.

An eye irritation study was conducted according to OECD TG 492 (Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage). alpha-Pinene (undiluted) (50 µL) was applied to the reconstructed tissue for 30 minutes. The percentage of tissue viability was reported to be 80.39 %. On the basis of this finding, the investigators reported that the test item does not require classification as an eye irritant under the GHS (REACHa).

Sensitisation

Skin Sensitisation

Based on the available human data (see **Sensitisation**: **Observation in Humans**), the chemicals are considered to be skin sensitisers warranting hazard classification.

A popliteal lymph node (PLN) assay was conducted to assess the sensitisation potential of (–)-alpha-pinene. Female Wistar rats were treated by subcutaneous injection with 50 µL of (–)-alpha-pinene (0.5, 2.5 or 5 mg) into the right hind footpad while the contralateral footpad was injected with the vehicle (DMSO) alone. Weight and cellularity indices (WI and CI) for draining PLNs were determined 7 days after treatment. The chemical was positive ($WI \geq 2$ and $CI \leq 5$) under these test conditions. In a secondary PLN assay (T-cell priming test), animals whose immune systems had been primed to the chemical (5 mg), were re-administered the chemical via subcutaneous injection (0.5 mg). Assessment of WI and CI were conducted 5 and 7 days following the second injection. The chemical was negative in the second test. The chemical induced a clear immuno-stimulatory response due to its irritant properties but it was not considered to be a sensitising agent (Friedrich et al., 2007).

The chemical alpha-pinene was assessed for its potential to produce skin sensitisation in a guinea pig maximisation test (GPMT). Female Hartley guinea pigs (6/dose group) were induced with 0.1 mL of the chemical at 4 % (in acetone: olive oil (v/v 4:1)). After 7 days, an occluded patch was applied to the shaved skin with 0.15 mL of the chemical. After 14 days, animals were exposed to a challenge dose (0.8 %) in the same vehicle as the induction dose. The chemical was negative for sensitisation under these test conditions (Wei et al., 2006).

A study was conducted to assess the sensitisation potential of the components of the Myoga (Zingiber Myoga Roscoe) plant, one of which is alpha-pinene. In a Local Lymph Node Assay (LLNA), alpha-pinene was topically-administered to the dorsum of both ears of 4 CBN/J mice. The chemical was applied at concentrations of 0, 1, 25 and 100 %. No EC3 value (the estimated concentration needed to produce a 3-fold increase in lymphocyte proliferation) could be determined up to 100 %, indicating that it was not a skin sensitiser under these test conditions (Wei et al., 2010).

The Quantitative Structure–Activity Relationship (QSAR) modelling for skin sensitisation using the OECD QSAR Toolbox (version 3.4) indicated that there were protein binding alerts for the predicted auto-oxidation metabolic products of alpha-pinene.

The chemical alpha-pinene is predicted to be a skin sensitiser using the VEGA skin sensitisation model (CAESAR). The chemical falls within the applicability domain of the model.

The chemical (–)- alpha-pinene is predicted to be a moderate skin sensitiser (predicted LLNA EC3%: 9.6) using Derek Nexus v5.0.2. The prediction is based on the triggered structured alert for terpenoids. The prediction strength is 'equivocal' (REACHb).

In the EU, the ECHA Guidance on the Application of the CLP Criteria (2013), the decision logic was used to determine the skin sensitising potential of alpha-pinene multiconstituent.

The chemicals (–)-beta-pinene and alpha-pinene are structurally-similar (both are bicyclic monounsaturated terpenes and are positional isomers). They also share very similar physico-chemical properties. (-)-beta-pinene has been classified as a Category1B skin sensitiser (based on an EC3 value of 29 %, obtained in a Local Lymph Node Assay) and is present in alpha-pinene multiconstituent above the generic concentration limit of 1 % for classification as skin sensitiser (REACHa).

It is expected that alpha-pinene multiconstituent will have the same weak potential for skin sensitisation. This, coupled with the available data (see **Sensitisation**: **Observation in Humans** section), suggest classification of alpha-pinene for sensitisation is warranted.

Turpentine oil (CAS No. 8006-64-2), of which alpha-pinene is the primary constituent, is classified as hazardous with hazard category ‘Skin sensitisation – category 1’ and hazard statement ‘May cause an allergic skin reaction' (H317) in the Hazardous Chemical Information System (HCIS) (Safe Work Australia). Data for the mixture (including a positive Guinea Pig Maximisation test) support this classification (NICNASa).

Observation in humans

Human studies indicate that alpha-pinene is a skin sensitiser (Cachao et al., 1986; Lear, et al., 1996; Dharmagunawardena et al., 2002; REACHa).

Patch tests were conducted with 6 terpenes on 22 patients in Portugal. Seventeen people were found to be allergic to alpha-pinene (Cachao et al., 1986).

A study which looked at the incidence of sensitisation in workers using oil of turpentine was conducted. Investigators reported 24 cases of hand dermatitis in pottery workers involved in ceramic decoration, painters, liners, gilders, enamellers and a fine china painter, seen in a 6-month period. Fourteen of which were found to be sensitised to turpentine, 8 to alpha-pinene, 4 to delta-3-carene and 2 to turpentine peroxides (Lear, et al., 1996)

A study was conducted in men (aged 18–50) to assess the potential for turpentine to produce contact sensitisation. For the induction phase, 25 healthy adult males of African descent were dermally exposed 5 times to turpentine at a concentration of 50 %, for a period of 48 hours under occlusive conditions. A challenge dose (at 20 %) was then dermally administered to the volunteers. Specific timings of dosings were not reported. Erythema was considered to be the minimum criteria for determining a positive response. A total of 18/25 volunteers were determined to have become sensitised to the chemical. The investigators concluded that turpentine was a strong sensitiser under these conditions. No specific information relating to alpha-pinene was reported (REACHa).

Repeated Dose Toxicity

Oral

No data are available.

Dermal

No data are available.

Inhalation

Based on the results of 90-day repeated dose inhalation toxicity studies in Fischer 344 (F344) rats and B6C3F1 mice, the chemicals are expected to cause toxicity via repeated inhalational exposure. Therfore, hazard classification is warranted (see **Recommendation** section).

A repeated dose inhalation toxicity study was conducted in rats using alpha-pinene according to OECD TG 413 (subchronic inhalation toxicity: 90-day study). Rats (F344) were inhalationally exposed to the chemical in whole body chambers, for 6 hours a day, 5 days per week, for 14 weeks. Animals of both sexes (10/sex/dose) were exposed at 25, 50, 100, 200 or 400 ppm. Given the nature of whole-body inhalational chambers, investigators noted that exposure was likely to be significantly higher than intended as a result of oral exposure from grooming. Females appeared to be more sensitive than males with 6 mortalities from the highest dose group during the study. Animals in the high dose group showed significantly reduced weight gain compared with controls. Males in the highest dose exhibited renal lesions including granular casts and hyaline droplets; however, these were indicative of alpha-2µ-globulin nephropathy and are not relevant to humans. Males from the 2 highest dose groups had significantly decreased sperm counts in the cauda epididymis tissue when compared with controls. This finding was subject to some doubt as to biological significance, as the samples were fixed at a relatively high temperature (65 °C); there were no supporting histopathological findings and all other sperm parameters assessed were unchanged. A No Observed Adverse Effect Concentration (NOAEC) of 100 ppm was reported for males on the basis of sperm effects. The NOAEC was 200 ppm for females, on the basis of mortalities and reduced rate of weight gain (REACHa).

A preliminary range-finding experiment was conducted prior to the study above. Inhalational exposure of F344 rats (for 6 hours a day, 5 days a week for 2 weeks) in the same system as above, to 800 and 1600 ppm was overtly toxic and resulted in mortality. Significant weight changes and liver weight increases were noted. Ataxia, tremors and abnormal breathing were observed in animals prior to death (REACHa).

A repeated dose inhalation toxicity study in mice was conducted using alpha-pinene according to OECD TG 413. The experiment was conducted with identical conditions to the first study described in this section. B6C3F1 mice were inhalationally exposed to the chemical in whole body chambers, for 6 hours a day, 5 days a week for 14 weeks. Males and females in the 100 ppm dose group exhibited minimal to moderate hyperplasia in the transitional epithelium of the urinary bladder. Decreased numbers of sperm per mg of cauda epididymis tissue were observed in the 200 and 400 ppm males, and decreased caudal sperm counts were observed in the 100, 200, and 400 ppm males. Again, the relevance of these findings was questioned as the samples were fixed at 65 °C and there were no supporting histopathological findings. All other sperm parameters assessed were unchanged. The investigators also noted the same limitation resulting from the expected oral exposure from grooming. The NOAEC for female mice was 50 ppm based on epithelial changes in the urinary bladder. The NOAEC for males was reported to be 50 ppm, based on epithelial hyperplasia, and significantly decreased caudal sperm counts in the 100 ppm groups (REACHa).

A preliminary range-finding experiment was conducted prior to the study above. Inhalational exposure (for 6 hours a day, 5 days a week for 2 weeks) to B6C3F1 mice in the same system, to 800 and 1600 ppm was overtly toxic and lead to death. Significant body weight changes, kidney and liver weight increases were noted. Ataxia, tremors and abnormal breathing were observed in animals prior to death (REACHa).

Genotoxicity

No evidence of alpha-pinene producing genotoxicity was reported in several in vitro studies and in an in vivo study.

In vitro

The chemical alpha-pinene (as a multiconstituent) was assessed for genotoxicity in a study conducted according to OECD TG 471 (bacterial reverse mutation assay). The bacterial strains *Salmonella typhimurium* TA 1535, TA 1537, TA 98, TA 100 and *Escherichia coli* WP2 were incubated with the chemical at concentrations ranging up to 5000 µg/plate, in the absence or presence of metabolic activation with S9 liver extract. The chemical did not produce a statistically significant increase in the number revertant bacterial colonies in any of the strains tested, at any concentration, either in the presence or absence of metabolic activation. The chemical was not considered to be genotoxic under these experimental conditions (REACHa).

The chemical alpha-pinene (as a multiconstituent) was assessed for genotoxicity in a study conducted according to OECD TG 476 (in vitro mammalian cell gene mutation test). Chinese Hamster ovary cells were incubated with the chemical at concentrations up to 2000 µg/mL, in the presence or absence of metabolic activation with S9 liver extract. The chemical did not produce a statistically significant increase in the number of mutant cells at any concentration tested, in the presence or absence of metabolic activation. The chemical was non-genotoxic under these test conditions (REACHa).

The chemical alpha-pinene (as a multiconstituent) was assessed for genotoxicity in a study according to OECD TG 487 (in vitro mammalian cell micronucleus test). Human lymphocytes were incubated with the test chemical at concentrations up to 2000 µg/mL, in the presence or absence of S9 metabolic activation. The chemical did not produce any statistically significant increase

in the frequency of micronuclei at any concentration. Therefore, the chemical was non-genotoxic under these conditions (REACHa).

In vivo

The chemical alpha-pinene was assessed for genotoxicity according to OECD TG 474 (mammalian erythrocyte micronucleus test). Male and female B6C3F1 mice were inhalationally exposed to the chemical at 0, 25, 50, 100, 200 or 400 ppm, 5 days a week, for 13 weeks. Peripheral blood erythrocytes were harvested from animals 24 hours after their final exposure and assessed for the frequency of micronucleus formation. There was no statistically significant increase in the frequency of micronucleated peripheral blood cells in male or female B6C3F1 mice. The chemical was not considered to be genotoxic under these test conditions (REACHa).

Carcinogenicity

No animal data are available. The human data are insufficient to warrant hazard classification.

Observation in humans

Two epidemiological studies have assessed the association between occupational exposure to turpentine or terpene, and cancer outcomes. A study in Finnish woodworkers found a weak association between exposure to terpenes (primarily alpha-pinene and delta-3-carene) lasting longer than 1 month, and the incidence of respiratory cancers. Another study found an association between paternal exposure to turpentine and the incidence of neuroblastoma in their offspring (NTP, 2016).

Reproductive and Developmental Toxicity

A reproduction toxicity study in SD rats is currently ongoing according to TG OECD 421 (reproduction/developmental toxicity screening test) (REACHa).

Questionable reproductive toxicity findings (reduction in caudal sperm) were reported in repeated dose inhalational toxicity studies in rats and mice (see **Repeated Dose Toxicity: Inhalation** section for further information).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include systemic acute toxicity from oral exposure, local effects (irritation and skin sensitisation) and sensory irritation. The chemicals can also cause harmful effects following repeated exposure through inhalation. The chemicals or mixtures containing the chemicals could also have the potential to cause chemical pneumonitis if aspirated depending on the viscosity as introduced.

The distillation fraction 'oil of turpentine, alpha-pinene fraction' (CAS No. 65996-96-5), which contains >80 % alpha-pinene is expected to have the same toxicological profile as the other chemicals in this assessment, based on the toxicological profile of turpentine (NICNASa).

Public Risk Characterisation

Considering the range of domestic, cosmetic and personal care products that may contain the chemicals, the main route of public exposure is expected to be through the skin, inhalation from products applied as aerosols, and potential oral exposure from lip and oral hygiene products.

In Europe, the chemicals are restricted in cosmetics and can only be used if the peroxide levels are below 10mM (CosIng). The distribution of purified alpha-pinene for fragrance purposes is expected to be controlled by members of IFRA. The restriction of

the chemicals under the IFRA Standard is expected to sufficiently address the public risks associated with exposure to the chemicals through fragrances (e.g. concentration limits of peroxide levels in the product of 10mM) (IFRA, 2009).

Consumer products containing the chemicals could oxidise over time; however, the concentration of the chemical in domestic and cosmetic products is expected to be low, hence peroxide levels are expected to very low. The risk to public health is not considered to be unreasonable and further risk management is not considered necessary for public safety.

Occupational Risk Characterisation

Given the critical local health effects, the chemicals could pose an unreasonable risk to workers unless adequate control measures to minimise dermal, oral and inhalation exposure are implemented. The chemicals should be appropriately classified and labelled to ensure that a person conducting a business or undertaking at a workplace (such as an employer) has adequate information to determine the appropriate controls.

The data available support an amendment to the hazard classification in the HCIS (Safe Work Australia) (see **Recommendation** section).

Sensory irritation and respiratory symptoms are reported in humans following exposure to mixed monoterpenes. Exposure standards to minimise the potential for these effects have been established overseas. An exposure standard encompassing the total level of monoterpenes may be beneficial to mitigate the risk of adverse effects.

NICNAS Recommendation

Assessment of the chemicals is considered to be sufficient provided that risk management recommendations are implemented and all requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

It is recommended that Safe Work Australia consider whether introduction of exposure controls for cumulative monoterpene hydrocarbon levels are required to adequately minimise the risk to workers. A Tier III assessment may be necessary to provide further information about whether exposure controls are needed to offer adequate protection to workers.

Regulatory Control

Public Health

Products containing the chemicals should be labelled in accordance with state and territory legislation (SUSMP, 2018).

Work Health and Safety

The chemicals are recommended for classification and labelling aligned with the GHS as below. This does not consider classification of physical hazards and environmental hazards.

For mixtures containing the chemicals, the aspiration hazard classification should only be applied if the kinematic viscosity criteria for aspiration classification in the GHS is met.

From 1 January 2017, under the model Work Health and Safety Regulations, chemicals are no longer to be classified under the Approved Criteria for Classifying Hazardous Substances system.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Not Applicable	Harmful if swallowed - Cat. 4 (H302) May be fatal if swallowed and enters airways - Aspi. Cat. 1 (H304)
Irritation / Corrosivity	Not Applicable	Causes skin irritation - Cat. 2 (H315)
Sensitisation	Not Applicable	May cause an allergic skin reaction - Cat. 1B (H317)
Repeat Dose Toxicity	Not Applicable	May cause damage to organs through prolonged or repeated exposure - Cat. 2 (H373)

[a] Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

[b] Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

[*] Existing Hazard Classification. No change recommended to this classification

Advice for consumers

Products containing the chemicals should be used according to the instructions on the label.

Advice for industry

Control measures

Control measures to minimise the risk from oral, dermal and inhalation exposure to the chemicals should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using hazardous chemicals depend on the physical form and the manner in which the chemicals are used. Examples of control measures that could minimise the risk include, but are not limited to:

- using closed systems or isolating operations;
- using local exhaust ventilation to prevent the chemicals from entering the breathing zone of any worker;
- health monitoring for any worker who is at risk of exposure to the chemicals, if valid techniques are available to monitor the effect on the worker's health;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemicals.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

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.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemicals are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of the chemicals has not been undertaken as part of this assessment.

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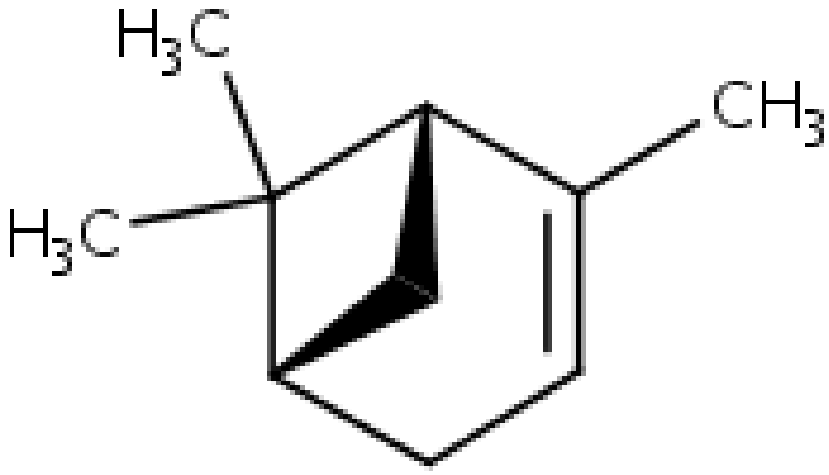
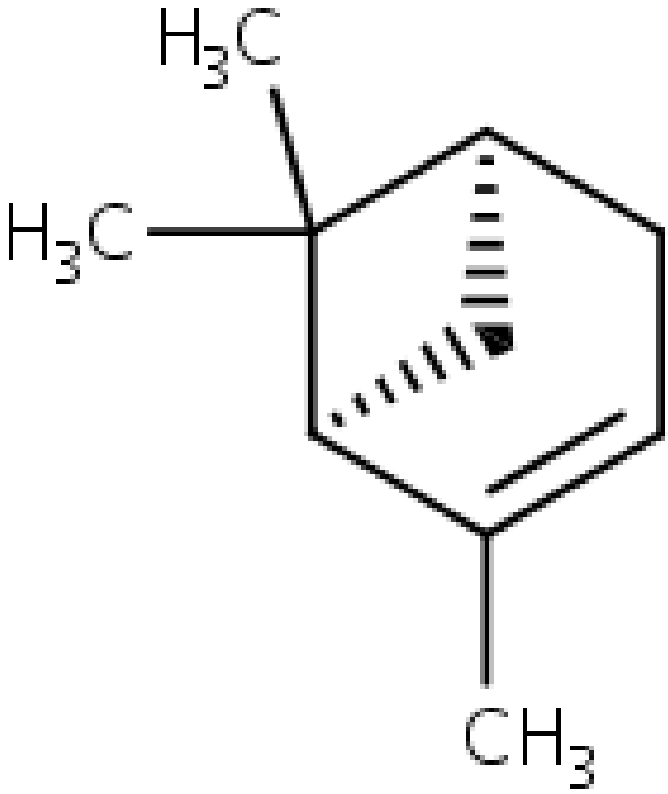
Last Update 29 June 2018

Chemical Identities

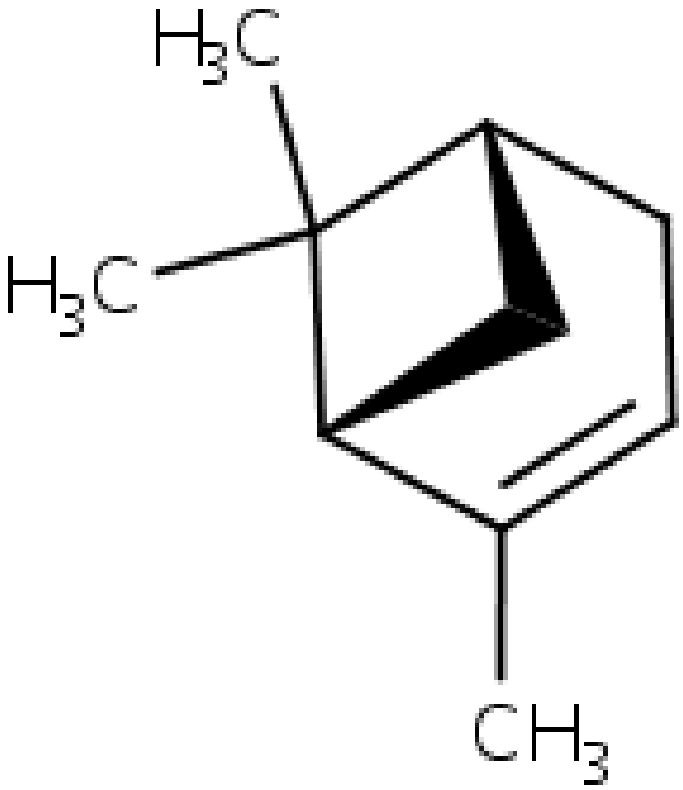
Chemical Name in the Inventory and Synonyms	**Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-** pin-2(3)-ene bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (.+-.)- 2,6,6-trimethylbicyclo(3.1.1)-2-hept-2-ene alpha-pinene
CAS Number	80-56-8
Structural Formula	

	
Molecular Formula	C10H16
Molecular Weight	136.24

Chemical Name in the Inventory and Synonyms	**Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (1S)-** (-)-alpha-pinene (-)-2-pinene 2-pinene, (1S,5S)-(-)-
CAS Number	7785-26-4
Structural Formula	

	
Molecular Formula	C10H16
Molecular Weight	136.24

Chemical Name in the Inventory and Synonyms	**Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (1R)-** (+)-alpha-pinene (+)-pin-2(3)-ene 2-pinene, (1R,5R)-(+)-
CAS Number	7785-70-8
Structural Formula	

	
Molecular Formula	C10H16
Molecular Weight	136.24

Chemical Name in the Inventory and Synonyms	**Terpenes and terpenoids, turpentine oil, .alpha.-pinene fraction** oil of turpentine, alpha-pinene fraction
CAS Number	65996-96-5
Structural Formula	**No Structural Diagram Available**

Molecular Formula	Unspecified
Molecular Weight	Unspecified

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