Terpinene, terpinolene and phellandrene: Human health tier II assessment

26 October 2018

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-	99-83-2
1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	99-85-4
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	99-86-5
Cyclohexene, 3-methylene-6-(1-methylethyl)-	555-10-2
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	586-62-9
1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-, (R)-	4221-98-1
Terpinene	8013-00-1

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**

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ACRONYMS & ABBREVIATIONS

Grouping Rationale

The chemicals in this group are structurally similar monocyclic terpene hydrocarbons. They consist of a cyclohexane ring with one methyl and one isopropyl group in para positions. Each structure also includes 2 double bonds at varying positons, both conjugated and unconjugated. The presence of the double bonds makes the chemicals susceptible to autoxidation, which is critical for hazard properties of these chemicals. Therefore, these chemicals are expected to have a similar toxicity profiles and they also have common uses as fragrances in perfumes, personal care and household products.

CAS No. 8013-00-1 refers to terpinenes in general, which includes alpha-terpinene, gamma-terpinene and terpinolene (CAS Nos. 99-85-4, 99-86-5 and 586-65-9) (ChemIDPlus).

Import, Manufacture and Use

Australian

No specific Australian use, import, or manufacturing information has been identified for the majority of the chemicals in this group. However, 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- (CAS No 99-85-4) has reported domestic use in automotive aftermarket products including car wash soaps, boat wash soaps, polishes and rubbing compounds.

International

The following international uses were identified through Galleria Chemica; the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers; Substances and Preparations in the Nordic countries (SPIN) database; US Environmental Protection Agency (EPA) Chemical and Product Categories (CPCat); the United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary and Cosmetic Ingredients; Government of Canada; and Cosmetic Ingredients and Substances (CosIng) database.

The chemicals have reported cosmetic uses as fragrances in perfumes and personal care products.

The chemicals (except CAS No. 4221-98-1) are listed on the IFRA transparency list of fragrance materials (IFRA, 2017).

Terpinene (CAS No. 8013-00-1) is listed on the Government of Canada revised in commerce list (ICL).

Some of the chemicals have reported domestic uses in:

- cleaning products (all chemicals);
- air fresheners (all chemicals except CAS No. 8013-00-1);
- surface treatments (all chemicals);
- paint lacquers and varnishes (CAS Nos. 586-62-9, 99-85-4, 99-83-2 and 4221-98-1);
- laundry products (CAS Nos. 99-86-5, and 99-85-4);
- polishing agents (CAS Nos. 99-86-5, and 99-85-4);
- absorbents and adsorbents (CAS No. 586-62-9); and
- odour agents (CAS No. 586-62-9).

The chemicals gamma-terpinene (CAS No. 99-85-4) and terpinolene (CAS No. 99-86-5) are listed in the US household product database. The products for which the chemicals were identified as ingredients were largely cleaning products. Concentrations where listed were up to 2.5 % (Household Products Database, US Department of Health and Human Services).

Some of the chemicals have reported commercial uses in the manufacture of furniture (CAS Nos. 586-62-9, 99-86-5 and 99-85-4) and in car care products (CAS No. 586-62-9).

Some of the chemicals have reported site-limited uses in:

- binding agents (CAS Nos. 586-62-9, 99-86-5 and 99-85-4);
- extraction of crude petroleum and natural gas (CAS Nos. 586-62-9 and 99-86-5);
- solvents (CAS Nos. 586-62-9, 99-86-5 and 99-85-4); and
- manufacture of motor vehicles, trailers and semi-trailers (CAS No. 586-62-9).

Terpinolene (CAS No. 586-62-9) and alpha-phellandrene (CAS No. 99-83-2) have reported uses as cigarette additives.

Some of the chemicals have reported non-industrial uses in:

- biocides (CAS Nos. 99-86-5, 586-62-9 and 4221-98-1);
- pesticides (CAS Nos. 586-62-9 and 99-85-4);
- flavouring of food (all chemicals).

Restrictions

Australian

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

The chemicals (CAS Nos. 99-85-4, 99-86-5, 586-62-9 and 99-83-2) have restrictions for their non-industrial uses in the Therapeutic Goods (Permissible Ingredients) Determination No. 1 of 2018 as excipients in medicines (TGA, 2018) at certain concentrations depending on their 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 %.

International

The chemicals gamma-terpinene, alpha-terpinene and terpinolene (CAS Nos. 99-85-4, 99-86-5 and 586-62-9) are subject to the EU Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Annex III: List of Substances which cosmetic products must not contain except subject to the restrictions laid down. Peroxide levels must be less than 10 mM. This limit applies to the substance and not to the finished cosmetic product (CosIng).

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

No specific exposure standards are available.

Health Hazard Information

The chemicals in this group are found in nature as flavours or fragrances in plants, flowers and fruit. They are also components of many essential oils such as tea tree, angelica fruit, tangerine and cajeput oil.

The chemicals are susceptible to oxidation in contact with air, light or the skin. The oxidised products readily form adducts with skin proteins and this is responsible for their skin sensitising properties (Bergstrom et al., 2006; SCCS, 2016).

Data for d-limonene (CAS No. 5989-27-5) or the limonene multi-constituent containing dl-limonene (CAS No. 138-86-3), alpha- and gamma-terpinenes and other minor components was used to support the conclusions of the hazard assessment. Limonene is structurally related to the chemicals in this assessment, with the only difference being in the positions of the double bonds.

Toxicokinetics

There are limited toxicokinetic data available for the chemicals

The chemicals are small lipophilic substances with log Kow values below 5. Therefore, the chemicals are expected to be orally and dermally bioavailable (Lipinski et al., 2001). Metabolites of the chemicals are expected to be excreted in the urine (NICNAS, 2002).

Following exposure to air or light or on contact with skin, the chemicals are expected to rapidly degrade forming oxidation products (Bergstrom, et al., 2006; SCCS, 2016). Limited information is available on the rate or relevant conditions of auto-oxidation. The limited experimental data suggest that alpha-terpinene (CAS No. 99-86-5), alpha-phellandrene (CAS No. 99-83-2) and beta-phellandrene (CAS No. 555-10-2) are oxidised rapidly (Bergstrom et al., 2006; SCCS, 2016).

Acute Toxicity

Oral

The chemicals are expected to have low acute toxicity based on reported median lethal dose (LD50) values >2000 mg/kg for terpinolene, gamma-terpinene and alpha-phellandrene.

In an oral acute toxicity study conducted similarly to Organisation of Economic Co-operation and Development (OECD) test guideline (TG) 401, rats (10/sex/dose; strain not specified) were orally treated (method not specified) with 3, 3.5, 4 or 5 mL/kg bw of terpinolene (CAS No. 586-62-9) and observed for 14 days. Mortality was observed at 3.5 mL/kg and above. An oral LD50 of 4.38 mL/kg bw corresponding to approximately 3740 mg/kg bw was reported (REACHa).

The following LD50 values were reported for the chemicals in rats:

- 3652 mg/kg bw for gamma-terpinene (Opdyke, 1976a);
- 1683 mg/kg bw for alpha-terpinene (Opdyke, 1976b; CLH, 2018);
- 5170 mg/kg bw for alpha-phellandrene (CAS No. 99-83-2) (Opdyke, 1978); and
- 5700 mg/kg bw for alpha-phellandrene (CAS No. 4221-98-1) (REACHb).

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. The threshold viscosity value for classification as an aspiration hazard is 20.5 mm^2/s at 40 °C.

Dermal

The chemicals are expected to have low acute dermal toxicity based on the dermal LD50 values of >4000 mg/kg bw in rabbits.

The reported LD50 values for gamma-terpinene are >5000 mg/kg (Opdyke, 1976a), 4300–5000 mg/kg for terpinolene (Opdyke, 1976c; REACHa) and >5000 mg/kg bw for alpha-phellandrene (CAS No. 4221-98-1) (REACHb). No study details are available.

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

Based on in vitro, in vivo and irritation studies in humans (refer to ***Observation in humans*** section) the chemicals may be slightly irritating to skin at high doses. The effects are not sufficient to warrant hazard classification.

In two in vitro studies performed according to OECD TG 439, 10 µL of terpinolene (CAS No. 586-62-9) or alpha-phellandrene (CAS No. 4221-98-1) was applied on reconstructed human epidermis for 15 min. The mean tissue viability was 84.2 % for terpinolene and 94.2 % for alpha-phellandrene. Substances that reduce viability by less than 50 % are not classified as irritants. Therefore, the chemicals are not considered skin irritants (REACHa).

In a study conducted similarly to OECD TG 404, New Zealand White rabbits (NZW) (3/sex) received topical applications of 0.5 mL of 2 % alpha-terpinene in repellent solution (composition not provided) to intact and abraded skin for 4 h under occlusion. The average erythema and oedema scores were $\leq$ 2 out of 4 at 24, 48 and 72 h (individual scores were not available). Any effects observed were fully reversible (REACHc). No further information is available.

In studies in rabbits, gamma-terpinene (neat) and alpha-phellandrene (neat) was applied to intact or abraded rabbit skin for 24 h under occlusion. The chemicals were moderately irritating (Opdyke, 1978). Terpinolene (neat) was not irritating in a similar study (Opdyke, 1976c).

Eye Irritation

The chemicals may be slightly to moderately irritating to eyes. The effects are not sufficient to warrant hazard classification.

In an OECD TG 405 eye irritation study, 0.1 mL of terpinolene was applied to one eye of 4 rabbits (strain and sex not specified) while the other eye served as the control. The irritation scores at the 3 different time-points (24, 48 and 72 h) were combined and averaged. The average irritation scores for the 4 individual rabbits were 0, 0, 0, 0 for corneal opacity; 0, 0, 0.33, 0 for iris irritation; 1.33, 0.33, 0.33, 1 for conjunctival redness and 1, 0.33, 0.67, 1 for chemosis. After 7 days, irritation had completely resolved (REACHa).

In an OECD TG 405 eye irritation study, 0.1 mL of alpha-phellandrene (CAS No. 4221-98-1) was applied to one eye of 4 female specific pathogen free (SPF) albino rabbits while the other eye served as the control. The irritation scores at the 3 different time-points (24, 48 and 72 h) were combined and averaged. The average irritation scores for the 4 individual rabbits were 0, 0, 0, 0 for corneal opacity; 0, 0, 0.33, 0 for iris irritation; 1, 0.67, 0.67, 1 for conjunctival redness and 0.67, 0.33, 0.33, 0.33 for chemosis. After 7 days, irritation had completely resolved (REACHb).

In a study conducted according to Korean Food and Drug Administration (KFDA) guidelines, 0.1 mL of 2 % alpha-terpinene in repellent solution (further details on composition is not available) was applied to the eyes of 6 NZW rabbits. The eyes were examined for irritation scores at 1, 2, 3, 4, 7, 10 and 13 days after application. The average scores (of 6 rabbits) at 24, 48 and 72 h after exposure were 2.6, 1.6, 1.3 for corneal opacity; 0, 0, 0 for iris irritation; 2.3, 2.16, 1.3 for conjunctival redness and 2.3, 1.5, 0.5 for chemosis. After 10 days, irritation had completely resolved (REACHc). No further information is available.

Observation in humans

Terpinolene (20 %), alpha-terpinene (5 %), gamma-terpinene (5 %) and alpha-phellandrene (8 %) produced no irritation after 48 h in closed patch tests in human subjects (Opdyke, 1976a–c; Opdyke, 1978).

Sensitisation

Skin Sensitisation

The chemicals in their original form (non-oxidised chemicals) are not expected to be skin sensitisers. However, the chemicals can form sensitising hydroperoxides or epoxides in contact with oxygen, light or skin. The chemicals are likely to oxidise over time, increasing their skin sensitising potency.

Based on the available animal and human data (refer to ***Observation in humans*** section), terpinolene (CAS No. 586-62-9 and 4221-98-1), alpha-phellandrene (CAS No. 99-83-2) and alpha-terpinene (CAS No. 99-86-5) are moderate sensitisers and warrant hazard classification as category 1B skin sensitisers (refer to ***Recommendation*** section).

Alpha-terpinene may be a strong sensitiser when oxidised; however, this has only been demonstrated by oxidation under artificial laboratory conditions (Rudback et al., 2012). Limited information is available on the speed and the level of the autoxidation of the chemicals in formulated products. Therefore, classification as category 1B skin sensitiser is considered sufficient. beta-Phellandrene may be a moderate sensitiser; however, the available data are insufficient to support classification. No sensitisation data are available for gamma-terpinene.

In a local lymph node assay (LLNA) performed in accordance with OECD TG 429 female CBA/J mice (5/dose) received topical applications of 2.5, 5.0, 10, 25 or 50 % (v/v) terpinolene in acetone/olive oil on three consecutive days. The reported stimulation indices (SI) were 1.24, 2.14, 3.43, 14.44 and 20.51 for concentrations of 2.5, 5.0, 10, 25 and 50 % respectively. The reported concentration producing a three-fold increase in lymphocyte proliferation (EC3) was 8 %, indicating moderate sensitisation potential (REACHa).

In an LLNA performed in accordance with OECD TG 442B (BrdU ELISA), female CBA mice (5/dose) received topical applications of 10, 25 or 100 % (v/v) alpha-phellandrene (CAS No. 4421-98-1) in acetone/olive oil on three consecutive days. The reported stimulation indices (SI) were 1.48, 2.86 and 3.68 for 10, 25 and 100 % respectively. In the BrdU ELISA LLNA, the cut-off for skin sensitisation is SI 1.6. The reported concentration producing a 1.6 fold increase in lymphocyte proliferation (EC1.6) was 15 %, indicating weak sensitisation potential (REACHb).

In a non-guideline study, a series of terpene hydrocarbons including alpha-terpinene, beta-terpinene, alpha-phellandrene, and beta-phellandrene were screened for their sensitising capacity by LLNA. Female CBA/Ca mice (3–4/dose) received topical applications of the test substances in acetone:olive oil (4:1 v/v) on three consecutive days. The reported for stimulation indices (SI) were:

- 1.1, 1.5, 3.4 and 8.9 for 1, 5, 10, 15 and 25 % alpha-terpinene;
- 1.4, 1.3, and 2.1 for 1, 10 and 25 % beta-terpinene;
- 1.1, 5, and 28 for 1, 10 and 25 % alpha-phellandrene; and
- 1.1, 4.8 and 23 for 1, 10 and 20 % beta-phellandrene.

The reported EC3 values from the study were 8.9 % for alpha-terpinene, 5.4 % for alpha-phellandrene and 5.6 % for beta-phellandrene (Bergstrom, 2006; CLH, 2018).

In another non-guideline LLNA, female CBA/Ca mice (3/dose) received topical applications of alpha-terpinene (air-exposed for 3 weeks) at 0.1–25 % (v/v) in acetone/olive oil or alpha-terpinene (air-exposed for 7 weeks) at 1–30 % (v/v) in acetone/olive oil. The chemical was oxidised by exposure to daylight and intermittent stirring for a total of 4 h/day. For air-exposed alpha-terpinene (3 weeks) the reported SI were 0.8, 3.2, 13, 17 or 12 for concentrations of 0.1, 1, 5, 10 or 25 %, respectively. The reported EC3 value was 0.9 % indicating strong sensitisation potential. For air-exposed alpha-terpinene (7 weeks) the reported SI were 3, 10, 13, 8.2 and 9.4 for concentrations of 1, 5, 10, 15 or 30 %, respectively. The reported EC3 value was 1 % indicating strong sensitisation potential. The levels of autoxidation of these chemicals are likely to be higher than in consumer

products because the chemicals were autoxidised in the laboratory in an artificial manner (by stirring and exposure to light) (CLH, 2018; Rudback et al., 2012).

(Q)SAR predictions

Skin sensitisation was predicted using OASIS–TIMES (tissue metabolism simulator) software (version 2.27.19). The chemicals were predicted to be non-sensitisers. However, several auto-oxidised metabolites of the chemicals were predicted to be weak or strong sensitisers. This was supported by mechanistic alerts for hydroperoxide free radical formation, protein alkylation, Michael type and nucleophilic additions.

No structural alerts for skin sensitisation were present for any of the chemicals (OECD QSAR Toolbox v3.4). However, when auto-oxidation or skin metabolism were simulated, mechanistic alerts, including alerts for protein binding and skin sensitisation, were present for metabolites of the chemicals.

Observation in humans

The components of tea tree oil, including alpha-terpinene, alpha-phellandrene and terpinolene, are known to rapidly oxidise and form hydroperoxides or epoxides in contact with air. Allergic reactions to tea tree oil are frequently reported (de Groot et al., 2016). In human patients sensitive to tea tree oil, 7/11 had a positive reaction to alpha-terpinene (5 %), 4/11 to alpha-phellandrene (5 %) and 11/11 to terpinolene (10 %) (Hausen et al., 1999).

Terpinolene and alpha-terpinene were recognised as fragrance allergens in the Scientific Committee on Consumer Safety (SCCS) Opinion on fragrances allergens in cosmetic products (SCCS, 2012).

Repeated Dose Toxicity

Oral

Based on available information, the chemicals are not expected to cause serious damage to health from repeated oral exposure.

In a combined repeated dose toxicity study with reproduction / developmental toxicity screening test, conducted in accordance with OECD TG 422, Sprague Dawley (SD) rats (10/sex/dose) received 0, 800, 2500 or 5000 ppm terpinolene in the diet for 49 days (males) or 56 days (females). An additional group of animals receiving the highest dose (6/sex) were kept for observation two weeks after the final day of dosing. No treatment-related mortalities, clinical effects or adverse macroscopic findings were observed. Reduced body weight gain was reported in males and females receiving 5000 ppm in diet probably due to statistically significant treatment-related reduction in food intake. Absolute and relative liver weights were increased in males receiving 2500 or 5000 ppm. These findings were associated with hepatocellular hypertrophy which was partly reversible following a two week recovery phase without dosing. Therefore, the liver effects were considered adaptive in nature. Minimal to marked multifocal tubular degeneration in the kidney was observed in all male treatment groups. At the highest dose the tubular findings were associated with hyaline droplets. These effects related to alpha 2µ-globulin nephropathy which are commonly observed in male rats and are not thought to be relevant to humans (Hard et al., 1993). Based on significant reduction in food intake in females at the highest dose, no observed adverse effect level (NOAEL) values of 2500 ppm (161.5 mg/kg bw/day) and 5000 ppm (294.6 mg/kg bw/day) were reported for females and males, respectively (REACHa).

In a combined repeated dose toxicity study with a reproduction / developmental toxicity screening test, conducted in accordance with OECD TG 422, SD rats (12/sex/dose) received 0, 25, 75 or 200 mg/kg bw/day alpha-phellandrene (CAS No. 4221-98-1) via the diet for 49 days (males) or 62 days (females). An additional group of animals receiving the highest dose (6/sex) were kept for observation two weeks after the final day of dosing. All animals survived the study and no clinical signs of toxicity were observed. Females receiving 200 mg/kg bw/day displayed reduced food intake and decreased body weight gain. There were no treatment-related effects on urinalysis, haematology, clinical chemistry or thyroid hormone. Increases in absolute or relative liver weights were observed in both sexes at the two highest doses. Hepatocellular hypertrophy was observed in males at 75 mg/kg bw/day and in both sexes at 200 mg/kg bw/day. Two weeks after the end of dosing the hepatocellular hypertrophy was resolved in animals receiving 200 mg/kg bw/day. Therefore, liver effects were considered adaptive in nature. The reported NOAEL for

males was 200 mg/kg bw/day and NOAEL for females was 75 mg/kg bw/day based on reduced food intake and body weight gain at 200 mg/kg bw/day (REACHb).

In a study in SD rats (OECD TG 422), limonene multi-constituent containing dl-limonene (CAS No. 138-86-3), alpha- and gamma-terpinenes and other minor components was administered to rats in the diet at 0, 800, 2500 or 5000 ppm bw/day for 42 days (males) or 63 days (females). An additional group of animals (mated males and un-mated females) receiving the highest dose (5/sex) were kept for observation two weeks after the final day of dosing (recovery phase). One mortality occurred on day 8 after the end of dosing. Increased incidence of vocalisation was noted in females receiving 5000 ppm bw/day. Overall body weight gain and weekly mean food consumption data for the dosing period were generally low for animals receiving 2500 or 5000 ppm but it only reached statistical significance in un-mated females receiving the highest dose. This was associated with reduced food intake. However, during the recovery phase, food intake and body weight gain increased, indicating that the reduced body weight gains were due to decreased food palatability. In males receiving 2500 or 5000 ppm bw/day alpha 2µ-globulin nephropathy was observed. This effect is specific to male rats and is not relevant for humans (Hard et al., 1993). An NOAEL of 5000 ppm (equivalent to 298 mg/kg bw/day in males and 316 mg/kg bw/day in females) was reported (REACHd).

Similar effects in the kidneys in male rats were also seen in studies with d-limonene. Other than in male rats, the critical organ in animals is the liver, with effects having been observed in mice, rats and dogs. Exposure affects the amount and activity of liver enzymes, liver weight, cholesterol levels and bile flow. However, no microscopic changes have been reported in the liver at these or higher levels. The liver effects in animals were considered to be due to physiological adaptation (NICNAS, 2002).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

Based on the available in vitro data for terpinolene (REACHa), alpha-terpinene (REACHc), alpha-phellandrene (EFSA, 2009; REACHb), gamma-terpinene (EFSA, 2009), limonene (NICNAS, 2002; REACHe) and a terpene mix (REACHd), the chemicals are not expected to be genotoxic.

In vitro

Terpinolene was negative in:

- point mutation studies in *Salmonella typhimurium* strains TA97, TA98 TA100, TA102 and TA1535 at concentrations up to 5000 µg/plate, with and without metabolic activation; and
- chromosome aberration assay in human lymphocytes at concentrations up to100 µg/mL for 3 h with and without metabolic activation or 20 h without metabolic activation.

alpha-Terpinene was negative in

- *S. typhimurium* strains TA97a, TA98, TA100 and TA1535 at concentrations up to 5000 µg/plate, with and without metabolic activation.

alpha-Phellandrene (CAS No. 4221-98-1) was negative in:

- *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 at concentrations up to 5000 µg/plate, with and without metabolic activation.
- point mutation studies in Chinese hamster lung fibroblasts (V79) at concentrations up to 30 µg/mL without metabolic activation and up to 1364 µg/mL with metabolic activation.

- chromosome aberration assay in human lymphocytes at concentrations up to 1364 µg/mL for 4 h with and without metabolic activation and up to 500 µg/mL for 20 h without metabolic activation.
- a sister chromatid exchange assay in Chinese hamster ovary cells at concentrations up to 136.2 µg/mL (CAS No. not reported).

gamma-Terpinene was negative in:

- point mutation studies in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 at concentrations up to 5000 µg/plate with and without metabolic activation; and
- in an unscheduled DNA synthesis study in rat hepatocytes at concentrations up to 30 µg/mL.

A mix of the terpenes including limonene, terpinolene, alpha- and gamma-terpinene (proportions not specified) was negative in:

- point mutation studies in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 at concentrations up to 5000 µg/plate with and without metabolic activation;
- point mutation studies in Chinese hamster lung fibroblasts (V79) cells at concentrations up to 5000 µg/mL with and without metabolic activation; and
- chromosome aberration assay in human lymphocytes at concentrations up to 800 µg/mL for 3 h with and without metabolic activation or 24 h without metabolic activation.

In vivo

The analogue of the chemicals, dl-limonene, was negative in an in vivo comet assay in kidney cells from male SD rats, after oral exposure 0, 1000 or 2000 mg/kg bw limonene for 3–6 or 22–26 h.

No evidence of mutagenicity was reported in an in vivo spot test with mice, involving the intraperitoneal injection of limonene at 215 mg/kg bw/day on days 9−11 during gestation.

Carcinogenicity

No data are available for the chemicals. However, data from the structurally related analogue limonene suggest that the chemicals are not likely to be carcinogenic.

In a 103 week study conducted similarly to OECD TG 451, Fisher (F344/N) rats (50/sex/dose) received d-limonene (CAS No. 5989-27-5) in corn oil orally by gavage at 0, 75 or 150 mg/kg bw/day (males) or 0, 300 or 600 mg/kg bw/day (females) for 103 weeks (5 days/week). No treatment-related clinical signs were observed during the study. No neoplastic lesions were found in female rats. In males, there were increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney (NICNAS, 2002). The mechanism of nephro-carcinogenicity is thought to be specific to male rats and is not relevant to humans (Hard et al., 1993).

In a 103 week study conducted similarly to OECD TG 451, B6C3F1 mice (50/sex/dose) received d-limonene in corn oil orally by gavage at 0, 250 or 500 mg/kg bw/day (males) or 0, 500 or 1000 mg/kg bw/day (females) for 103 weeks (5 days/week). No treatment-related clinical signs were observed during the study. There was no evidence of carcinogenic activity of d-limonene in male or female rats (NICNAS, 2002).

Reproductive and Developmental Toxicity

Based on the information available, the chemicals are not considered to be specific reproductive or developmental toxicants. The available studies for terpinolene, alpha-phellandrene and limonene multi-constituent show no effects on reproduction. Adverse effects on development occurred only at maternally toxic doses.

In a combined repeated dose toxicity study with reproduction / developmental toxicity screening (OECD TG 422) (refer to ***Repeated dose toxicity*** section) SD rats (10/sex/dose) received 0, 800, 2500 or 5000 ppm terpinolene in the diet for 42 days (males) or 56 days (females) including a three week maturation phase, pairing, gestation and early lactation. No treatment-

related effects were detected on mating performance, fertility or gestation lengths. Body weight gain and food consumption were reduced during gestation and lactation in females receiving 5000 ppm in diet. There were no treatment-related clinical signs of toxicity or adverse macroscopic findings in the offspring. Litters from females receiving 5000 ppm in diet had reduced weights at day 7 after birth. This effect was considered to be related to the reduced food intake of the mother at 5000 ppm (equivalent to 294.6 mg/kg bw/day). The reported NOAEL for maternal and developmental toxicity was 2500 ppm (equivalent to 161.5 mg/kg bw/day). The NOAEL for reproductive toxicity was 294.6 mg/kg bw/day (REACHa).

In a combined repeated dose toxicity study with reproduction / developmental toxicity screening (OECD TG 422) (refer to ***Repeated dose toxicity*** section) SD rats (12/sex/dose) received 0, 25, 75 or 200 mg/kg bw/day of alpha-phellandrene via the diet for 42 days (males) or 62 days (females) including a three week maturation phase, pairing, gestation and early lactation. Two females receiving 200 mg/kg bw/day and one female receiving 25 mg/kg bw/day lost their litters. No treatment-related adverse effects were observed including effects on the oestrous cycle, mating period, mating index, gestation period, male and female fertility indexes, gestation index, mean litter size and sex ratio of pups. Females receiving 200 mg/kg bw/day displayed reduced food intake and decreased body weight gain during gestation and lactation. Litters from females at 200 mg/kg bw/day showed reduced body weights at birth and 4 and 13 days after birth. The reported NOAEL for maternal and developmental toxicity was 75 mg/kg bw/day. The NOAEL for reproductive toxicity was 200 mg/kg bw/day (REACHb).

In a combined repeated dose toxicity study with reproduction / developmental toxicity screening (OECD TG 422) (refer to ***Repeated dose toxicity*** section) SD rats (10/sex/dose) received 0, 800, 2500 or 5000 ppm limonene multi-constituent in the diet for 42 days (males) or 63 days (females) including a three week maturation phase, pairing, gestation and early lactation. The overall mean achieved doses were 50, 148 and 298 mg/kg bw/day in males and 69, 216 and 393 mg/kg bw/day in females. No treatment-related effects were detected in mating performance, fertility or gestation lengths. There were no treatment-related clinical signs of toxicity or adverse macroscopic findings in the offspring. During gestation body weight gain and mean food consumption for all treated female groups were similar to control values; however, they were significantly reduced during the first 4 days of lactation in females at the highest dose. Litters from these females also showed reduced body weight gain during days 4–7 of lactation. The reported NOAEL for maternal toxicity was 2500 ppm (equivalent to 216 mg/kg bw/day). The NOAEL for reproductive toxicity was 5000 ppm, equivalent to 298 and 393 mg/kg bw/day for males and females, respectively (REACHd).

In a non-guideline developmental toxicity study, female Wistar rats (15–28/dose) were orally treated (gavage) at 0, 30, 60, 125 or 250 mg/kg bw/day of alpha-terpinene in corn oil from day 6 to 15 of gestation. The two highest doses were maternally toxic as indicated by a reduction in maternal weight gain (minus uterus weight). No effects were reported on number of corpora lutea, implantation sites per litter, resorptions per implantations or live foetuses per litter. Significant reduction of pregnancies from sperm-positive females was reported at the highest dose. If the effect was on ability to get pregnant or on implantation, it would have been unrelated to treatment starting on day 6 of gestation. Treatment related effects would only be expected on the development of the embryo or foetuses. However, no information was available on implantations or the resorptions in the sperm positive females not falling pregnant. Significant reduction in litter weights was also reported at highest dose. At doses of 60 mg/kg bw day and above there was a significant dose-dependent increase in foetuses with delayed ossification and skeletal abnormalities. Delayed (or incomplete) ossification of developing foetal bones is one of the most common skeletal variations reported in developmental toxicity studies. These variations are often repairable via postnatal skeletal remodelling, are not mechanistically linked to malformation, and often seen in the presence of maternal or foetal toxicity (Carney et al., 2007). Relative and absolute kidney weights were also increased in the offspring at doses above 60 mg/kg bw day; however, this change was not clearly dose-dependent. An NOAEL for maternal toxicity of 125 mg/kg bw/day was reported (Arajuo et al., 1996; CLH, 2018).

Risk Characterisation

Critical Health Effects

The critical health effect is skin sensitisation from oxidised products of the chemicals.

Public Risk Characterisation

Based on the international uses identified, the substances may be used in cosmetic and domestic products in Australia. The chemical, 1,4-cyclohexadiene, 1-methyl-4-(1-methylethyl)- (CAS No 99-85-4) was reported to be used in domestic products

including car wash soaps, boat wash soaps, polishes and rubbing compounds, in Australia. The general public could be exposed to the chemicals when using cosmetics or domestic products containing the chemicals.

Consumer products containing the chemicals can oxidise over time. 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. In Europe, gamma-terpinene, alpha-terpinene and terpinolene are restricted in cosmetics and can only be used if the peroxide levels are below 10mM (CosIng). The concentration of the individual chemicals in domestic and cosmetic products is expected to be low, hence peroxide levels are expected to be very low. Therefore, the risk to public health is not considered to be unreasonable and further risk management is not considered necessary for public safety.

These conclusions do not apply when the chemicals are components of essential oils. Essential oils containing these chemicals will be assessed separately.

Occupational Risk Characterisation

During product formulation, dermal, ocular and inhalation exposure may 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 chemicals at lower concentrations could also occur while using formulated products containing the chemicals. The level and route of exposure will vary depending on the method of application and work practices employed. Good hygiene practices to minimise oral exposure are expected to be in place.

Given the critical systemic acute and local health effects, the chemicals could pose an unreasonable risk to workers unless adequate control measures to minimise dermal 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) (refer to ***Recommendation*** section).

NICNAS Recommendation

Assessment of these chemicals are considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Regulatory Control

Work Health and Safety

Terpinolene (CAS No. 586-62-9 and 4221-98-1), alpha-phellandrene (CAS No. 99-83-2) and alpha-terpinene (CAS No. 99-86-5) are recommended for classification and labelling aligned with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as below. This does not consider classification of physical hazards and environmental hazards.

The remaining chemicals in this group should be classified for aspiration hazard (H304) only.

For mixtures containing any of 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	May be fatal if swallowed and enters airways - Aspi. Cat. 1 (H304)
Sensitisation	Not Applicable	May cause an allergic skin reaction - Cat. 1B (H317)

[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 to minimise the risk from dermal 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 a hazardous chemical 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:

- 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 these chemicals has not been undertaken as part of this assessment.

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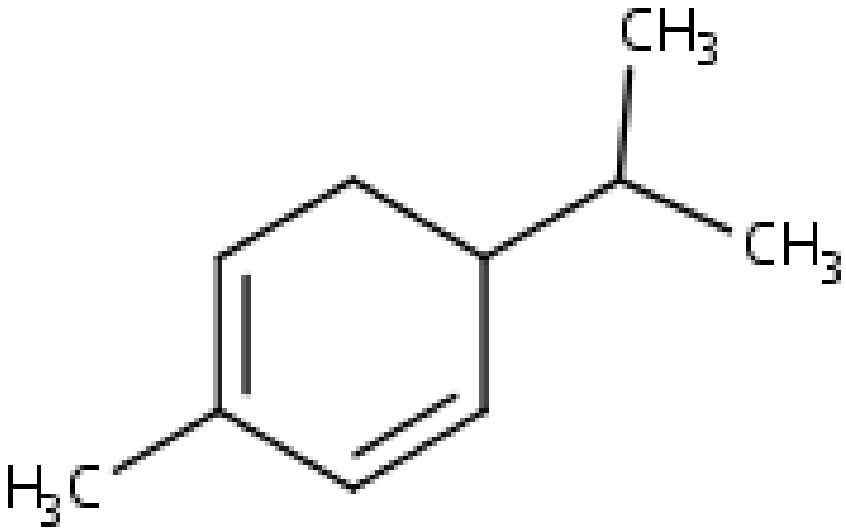
Last Update 26 October 2018

Chemical Identities

Chemical Name in the Inventory and Synonyms	**1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-** p-mentha-1,5-diene alpha-phellandrene
CAS Number	99-83-2

Structural Formula	
Molecular Formula	C10H16
Molecular Weight	136.20

Chemical Name in the Inventory and Synonyms	**1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-** .gamma.-terpinene p-mentha-1,4-diene moslene crithmene
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	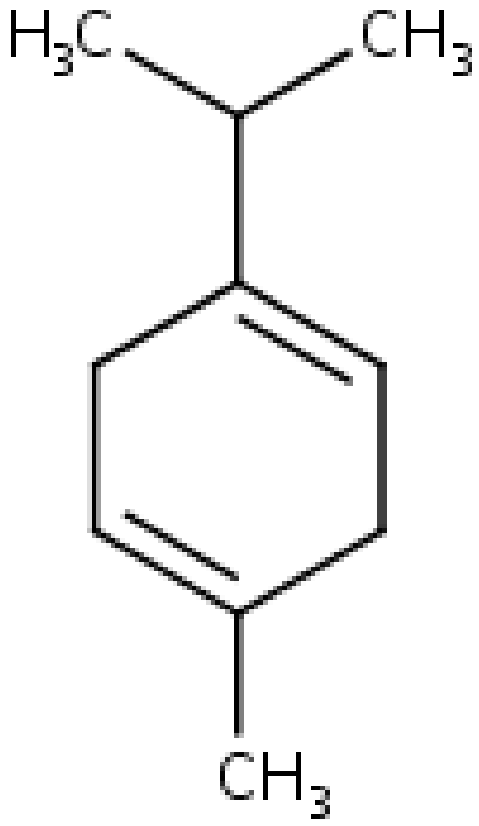
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	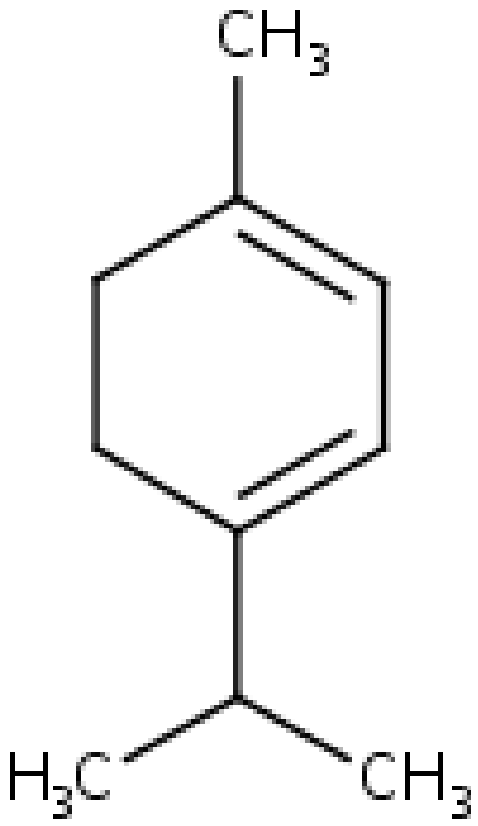
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	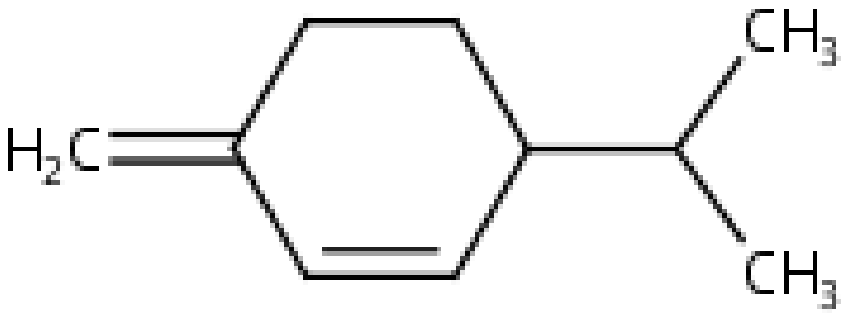
Molecular Formula	C10H16
Molecular Weight	136.20

Chemical Name in the Inventory and Synonyms	**Cyclohexene, 1-methyl-4-(1-methylethylidene)-** terpinolene 1,4(8)-p-menthadiene isoterpinene
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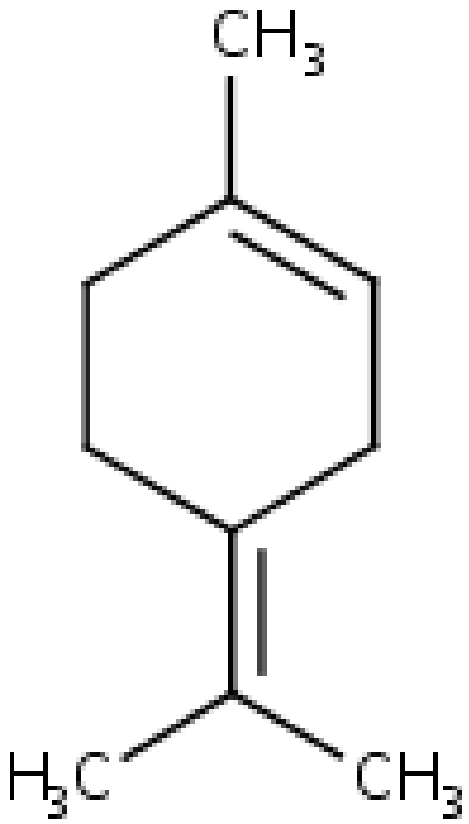
Structural Formula	

	
Molecular Formula	C10H16
Molecular Weight	136.20

Chemical Name in the Inventory and Synonyms	**1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-, (R)-** p-mentha,1,5-diene (-)-a-phellandrene alpha-phellandrene
CAS Number	4221-98-1
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	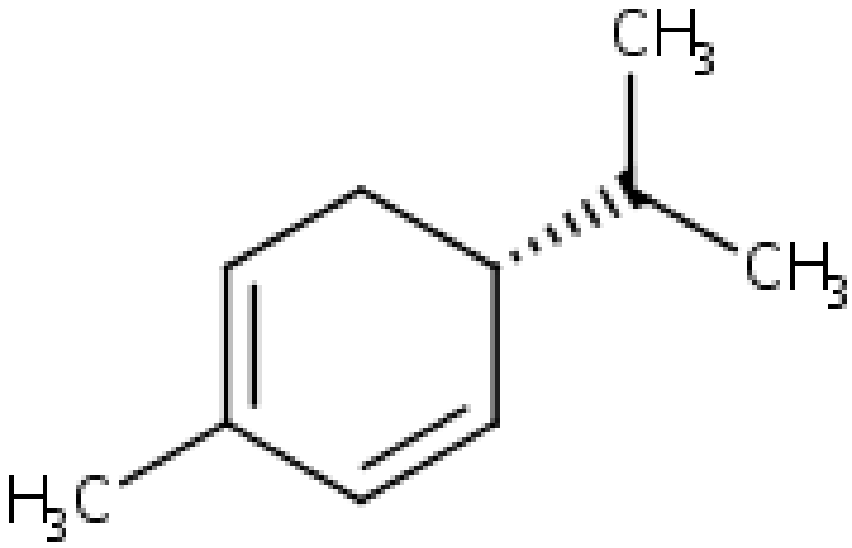
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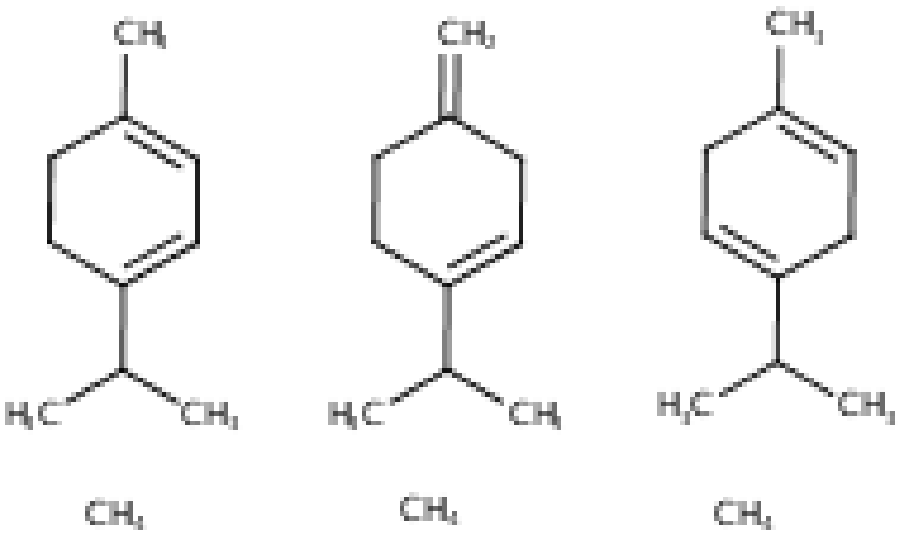
Structural Formula	

	
Molecular Formula	C10H16
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