Isoeugenol and its constituent isomers: Human health tier II assessment

26 October 2018

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Phenol, 2-methoxy-4-(1-propenyl)-	97-54-1
Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-	5912-86-7
Phenol, 2-methoxy-4-(1-propenyl)-, (E)-	5932-68-3

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

Isoeugenol (CAS No. 97-54-1) is a mixture of two isomers based on the configuration of the carbon-carbon double bond, phenol, 2-methoxy-4-(1-propenyl)-, (Z)- (cis-isoeugenol) and phenol, 2- methoxy-4-(1-propenyl)-, (E)- (trans-isoeugenol). Isoeugenol was originally assessed and published in Tranche 14. Additional information on the chemical regarding respiratory irritation has become available, warranting a further review. This updated assessment includes new data and consequent amended classification for respiratory irritation, and expands the assessment to cover the cis- and trans-isomers of isoeugenol.

Sensitisation is a key endpoint and the double bond configuration that differs between the two isomers is not expected to be relevant for the activation step prior to protein binding in the development of sensitisation (ECHA, 2016) or for other endpoints. Therefore, hazard data obtained for isoeugenol are considered relevant for the individual isomers.

Import, Manufacture and Use

Australian

No specific Australian industrial use, import, or manufacturing information has been identified.

The chemicals have reported non-industrial uses, including in pharmaceuticals (Pasay et al., 2010).

International

The following international uses have been identified through 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 Product Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; the Compilation of Ingredients Used in Cosmetics in the US (CIUCUS) (Personal Care Products Council, 2011); the US National Library of Medicine's Hazardous Substances Data Bank (HSDB); the International Fragrance Association (IFRA); and various international assessments (NTP, 2010; SCCS, 2011).

The chemicals have reported cosmetic use as a perfuming agent. Isoeugenol and trans-isoeugenol are listed on the IFRA Transparency List.

The chemicals have reported domestic uses including in:

- washing and cleaning agents such as machine washing liquids and detergents;
- air fresheners;
- polishes and waxes;
- surface treatment products; and
- paints, lacquers and varnishes.

Isoeugenol has reported commercial uses in adsorbents and absorbents.

The chemicals have reported non-industrial uses including in veterinary and human pharmaceuticals and as a flavouring agent.

Restrictions

Australian

The chemicals are listed in the *Poisons Standard—the Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP) in Schedules 5 and 6 (SUSMP, 2018). These restrictions reflect changes made to the Schedule following scheduling recommendations for isoeugenol in the previous IMAP assessment published in Tranche 14.

Schedule 6:

'ISOEUGENOL except:

(a) when included in Schedule 5; or

(b) in preparations not intended for skin contact containing 10 per cent or less of isoeugenol; or

(c) in preparations intended for skin contact containing 0.02 per cent or less of isoeugenol.’

Schedule 5:

'ISOEUGENOL in preparations not intended for skin contact containing 25 per cent or less of isoeugenol except in preparations containing 10 per cent or less of isoeugenol.'

Schedule 6 chemicals are described as 'Substances with a moderate potential for causing harm, the extent of which can be reduced through the use of distinctive packaging with strong warnings and safety directions on the label'. Schedule 6 chemicals are labelled with 'Poison' (SUSMP, 2018).

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).

International

Isoeugenol and trans-isoeugenol are listed in the European Union (EU) Cosmetics Regulation 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—maximum authorised concentration in the finished cosmetic product: 0.02 %.

European Union (EU): Using isoeugenol or trans-isoeugenol in cosmetics in the European Union is subject to the restrictions described in EU Regulation Annex III (Cosing). The chemicals may be used in cosmetics and personal care products at a maximum authorised concentration in the finished cosmetic product: 0.02 %. 'The presence of the substance must be indicated in the list of ingredients referred to in Article 19(1)(g) when its concentration exceeds: - 0.001% in leave-on products - 0.01 % in rinse-off products.'

Isoeugenol is also listed on the New Zealand Cosmetic Products Group Standard—Schedule 5—Table 1: Components cosmetic products must not contain except subject to the restrictions and conditions laid down (Galleria Chemica).

Existing Worker Health and Safety Controls

Hazard Classification

Isoeugenol is classified as hazardous, with the following hazard categories and hazard statements for human health in the Hazardous Chemicals Information System (HCIS) (Safe Work Australia). This classification is based on the recommended amendment to the hazard classification in the Hazardous Substances Information System (HSIS) (the Safe Work Australia online classification database at the time) from the IMAP assessment published in Tranche 14 :

- Acute Toxicity – Category 4; H302 (Harmful if swallowed)
- Acute Toxicity – Category 4; H312 (Harmful in contact with skin)
- Skin Irritation – Category 2; H315 (Causes skin irritation)
- Eye Irritation – Category 2A; H319 (Causes serious eye irritation)
- Skin Sensitisation – Category 1; H317 (May cause an allergic skin reaction)
- Carcinogenicity – Category 2; H351 (Suspected of causing cancer)

There are no specific hazard classifications for the individual isomers.

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific exposure standards are available.

Health Hazard Information

The chemical 2-methoxy-4-(1-propenyl)-phenol (CAS No. 97-54-1), also referred to as isoeugenol, is a member of the propenylhydroxybenzenes category (EFSA, 2012). It is found in the essential oils of many plants including clove, nutmeg, sandalwood, dill seed, gardenia and petunia and is used primarily as a fragrance compound in a range of applications (NTP, 2010; EFSA, 2012).

Hazard information in this assessment is based on isoeugenol unless specified otherwise. Limited data are available for the trans-isomer of isoeugenol, which is likely to be the main constituent of the trade product (82–88%) (SCCS, 2011). This data is reported, where available. Otherwise, data are read across from the mixture.

Toxicokinetics

The toxicokinetics of isoeugenol were assessed in Fischer 344 (F344) rats and B6C3F1 mice. Following administration by gavage, rapid absorption of the chemical (as determined by plasma concentrations) occurred within 20 minutes in both sexes and in both species used in this study. Absolute bioavailability of the chemical was reported to be higher in female rats than in the males of either species. An apparent dose-response relationship between the administered dose and plasma concentrations was also noted in both experiments. Isoeugenol was extensively distributed to extravascular tissues following intravenous administration in both rats and mice. The chemical was eliminated from the circulation rapidly and extensively (NTP, 2010).

The metabolism of isoeugenol has been hypothesised to progress via the following (NTP, 2010) :

- hydroxylation of the terminal methyl group to form the corresponding substituted 3-hydroxy-1-phenylpropene;
- oxidation of the propenyl double bond to form the corresponding 1,2-oxide;
- O-dealkylation; and
- glucuronide or sulfonate conjugation.

Male F344 rats administered radiolabelled isoeugenol orally (at 156 mg/kg body weight (bw)) absorbed more than 85 % of the dose within 72 hours. Excretion occurred for the most part via the urine. Approximately 10 % of the dose was excreted in the faeces and less than 0.1 % was recovered as CO_2.

Following intravenous administration in F344 rats, isoeugenol was eliminated rapidly with a half-life of 12 minutes and a systemic clearance rate of 1.19 L/minute per kg. After 72 hours, total radioactivity remaining was less than 0.25 % in both routes of exposure (Badger, 2002).

Human data

Radiolabelled isoeugenol has been demonstrated to be absorbed through human skin in studies using cadaveric skin specimens. Final penetration through the skin ranged between 0.29 and 11 %, depending on the vehicle used (Jimbo et al., 1983).

Acute Toxicity

Oral

Isoeugenol is classified as hazardous with hazard category ‘Acute Toxicity—Category 4’ and hazard statement ‘Harmful if swallowed’ (H302) in the HCIS (Safe Work Australia). The available data (median lethal dose—LD50 — ranging from 1290–1880 mg/kg bw) support this classification.

In an oral acute toxicity study, different quantities of undiluted isoeugenol were administered by intubation to Osborne-Mendel rats (five/sex/group). Although few experimental details were provided, an oral LD50 of 1560 mg/kg bw was determined. Sub-lethal effects included failure to groom and coma (HERA, 2005; EMA, 2011).

A review of the literature by the US National Toxicology Program (NTP) (2010) found that oral LD50 values for isoeugenol ranged from 1290 to 1880 mg/kg bw for rats and 1130 to 1780 mg/kg bw for guinea pigs.

In a pre-guideline study similar to OECD Test Guideline (TG) 401, the LD50 value for trans-isoeugenol in mice (1/sex/dose) was reported as 541.5 mg/kg bw (REACH).

Dermal

Isoeugenol is classified as hazardous with hazard category ‘Acute Toxicity—Category 4’ and hazard statement ‘Harmful in contact with skin’ (H312) in the HCIS (Safe Work Australia). The available data support this classification. Isoeugenol has moderate acute toxicity based on results from animal tests following dermal exposure. The LD50 in rabbits is 1910 mg/kg bw.

In a dermal acute toxicity study, undiluted isoeugenol was applied to the intact skin of three male and three female rabbits under occlusive patches at 860, 1350, 1700, 3400 or 5000 mg/kg bw respectively for 24 hours. Mortalities occurred in these groups at 0/6, 1/6, 2/6, 4/6 and 6/6 respectively. Animals in the 1350 mg/kg group exhibited erythema and skin haemorrhage, which healed in surviving animals during the observation period. Dose-dependent effects observed in the higher dose groups included pulmonary congestion and internal haemorrhage at the severe end of the spectrum. On the basis of these findings, an LD50 of 1910 mg/kg bw was calculated. No systemic or local effects were observed in animals administered the chemical at 860 mg/kg bw (HERA, 2005).

Inhalation

No data are available.

Corrosion / Irritation

Respiratory Irritation

There is evidence to support classification of the chemicals as hazardous with hazard category ‘Respiratory Irritation—Category 3’ and hazard statement ‘May cause respiratory irritation’ (H335) (see **Recommendation** section).

In a subacute inhalation toxicity study in SD rats, exposure to isoeugenol five days per week for two weeks caused dose-related epithelial inflammation and degeneration of the nasal cavity in all treatment groups and both sexes. All nasal cavity findings were minimal to mild in severity and expected to be reversible following removal of the irritant (REACH, see **Repeated Dose: Inhalation** section).

Skin Irritation

Isoeugenol is classified as hazardous with hazard category ‘Skin Irritation—Category 2' and hazard statement ‘Causes skin irritation’ (H315) in the HCIS (Safe Work Australia). The available data are limited but support this classification. No conventional skin irritation studies have been conducted on 100 % isoeugenol.

The potential for isoeugenol to irritate the skin has been assessed in a number of animal studies.

In a non-guideline study, undiluted isoeugenol was applied to the dorsal skin of albino Angora rabbits and guinea pigs under occlusion for 24 hours. Patches were removed and a second application was made 30 minutes later. Macro and microscopic examination of excised skin revealed evidence of severe irritation (HERA, 2005).

In another study, isoeugenol (0.5 mL of a 1 % solution) was applied topically under occlusion to three albino rabbits. No evidence of irritation was observed on intact or abraded skin (HERA, 2005). Occlusive patch testing in miniature swine with undiluted isoeugenol did not show evidence of irritation (HERA, 2005).

Skin irritation was observed in acute dermal (see **Acute toxicity: dermal**) and sensitisation (see **Skin sensitisation**) studies.

Skin irritation has been reported in humans (see **Skin irritation: Observation in humans**). No evidence of dermal corrosion has been reported.

In a study with trans-isoeugenol performed according to OECD TG 430 in vitro skin corrosion: transcutaneous electrical resistance test method (TER) reported to be in compliance with good laboratory practice (GLP), the chemical was considered

unlikely to have the potential to cause corrosion in vivo following application to the pelt of a female Wistar rat for 24 hours (REACH).

Eye Irritation

Isoeugenol is classified as hazardous with hazard category ‘Eye Irritation—Category 2A’ and hazard statement ‘Causes serious eye irritation (H319).

Isoeugenol has been reported to irritate the eyes. Ocular effects were not reversible within 72 hours. Although no conventional studies have been conducted, the chemical caused irritation even at a concentration of 1 %.

Two eye irritation studies (each on three albino rabbits) tested the irritating potential of isoeugenol at 1% and 1.25 % in denatured alcohol (0.1 mL applied without rinsing). The chemical caused mild conjunctival irritation at 1 % and severe conjunctival irritation with chemosis and discharge was observed at 1.25 % in both studies. Ocular effects resolved within four and seven days for the 1 % and 1.25 % groups, respectively (HERA, 2005).

An in vitro eye irritation study performed with trans-isoeugenol using fresh bovine cornea according to OECD TG 437 and was reported to be in compliance with GLP. However, no prediction could be made regarding the potential for eye irritation because the results were outside the OECD decision criteria for this endpoint (REACH).

Observation in humans

Isoeugenol (at 32 %) was found to be moderately irritating when applied to the skin of 50 adult male volunteers under occlusive patches and left for 48 hours. When applied at a concentration of 2 % under occlusion, the chemical caused mild irritation in one of 30 subjects (assessed at 72 hours after the patch was removed).

Human skin irritation data are also derived from preliminary sensitisation studies. Data from 11 separate screening studies for human maximisation tests showed that occluded isoeugenol was not irritating at 8 % in petrolatum on a total of 323 volunteers. When 8 % isoeugenol was mixed with an equal amount of eugenol (CAS No. 97-53-0) in petrolatum, irritant effects were observed in 22 subjects using the same study design. An occluded dose of 8 % isoeugenol (approximately 4 mg/cm^2) in petrolatum has been reported consistently as being tolerated by humans in clinical studies (HERA, 2005).

Sensitisation

Skin Sensitisation

Isoeugenol is classified as hazardous with the hazard category ‘Skin sensitisation—category 1’ and hazard statement ‘May cause an allergic skin reaction’ (H317) in the HCIS (Safe Work Australia). The data are sufficient to support amending this classification (see **Recommendation** section). The chemicals are considered to be skin sensitisers based on human data, positive results seen in guinea pig maximisation tests (GPMT) and local lymph node assays (LLNA) obtained from testing isoeugenol.

The estimated concentration to produce a three-fold increase in lymphocyte proliferation (EC3) was determined from over 40 separate LLNA tests carried out on the chemical. The weighted mean EC3 value of 2 % was reported from the combined data (HERA, 2005). Lower EC3 values have been reported elsewhere in the literature, including as low as 0.54 % (SCCS, 2011)

In a GPMT with isoeugenol, animals were intradermally induced at 0.15 % followed by topical induction at 25 %. A challenge phase was conducted seven days later with topical application of a 5 % solution. Responses were seen in 100 % of animals (HERA, 2005).

Positive responses were observed when the chemical was tested in Freund’s complete adjuvant (FCA) tests (HERA, 2005). In one test, guinea pigs (10/group) were intradermally induced at 1, 3 or 10 % followed by a challenge using a topical application of the chemical at the same respective concentrations. Responses were seen in 5/10, 9/10 and 10/10 animals in the 1, 3 and 10 %

induction and challenge groups, respectively. In another FCA test, eight guinea pigs were intradermally induced at 5 %, followed by a challenge by topical application at the same concentration. Responses were seen in all eight animals.

A study found that inhibition of the liver enzyme cytochrome P450 1A decreased the degree of the allergic responses to the chemical in LLNA tests. This suggested that the enzyme plays an important role in sensitisation from isoeugenol (Scholes et al., 1994).

The Human and Environmental Risk Assessment on ingredients of household cleaning products (HERA) estimated a no expected sensitisation level (NESL) of 250 µg/cm^2 using a 'weight of evidence' approach from a large number of predictive tests (HERA, 2005).

Observation in humans

Human maximisation tests have been conducted in over 650 volunteers (across 25 separate tests). The majority of these tested the sensitising effect of isoeugenol at a concentration of 8 %. In total, over 100 of 484 test subjects exhibited evidence of sensitisation (HERA, 2005).

Human repeat insult patch tests (HRIPT) have also been used extensively to assess the potency and possible induction thresholds of isoeugenol (under 24-hour occlusion). Across 10 separate tests, negative reactions were obtained when induction and challenge concentrations were 0.5 % (equivalent to 260 µg/cm^2). Positive results were generally obtained for induction and challenge concentrations at 1 % (equivalent to 800 µg/cm^2) or higher. The majority of these tests had over 40 volunteers and the chemical was typically administered under semi-occlusive patches, for 24 hours (induction phase). The vehicle in these tests was typically ethanol (HERA, 2005).

Repeated Dose Toxicity

Oral

The available data suggest that the chemicals have low repeated dose toxicity, based on results from animal tests following oral exposure.

Rats (strain not specified) were fed a diet containing isoeugenol at 1 % (approximately 800 mg/kg bw/day) for 16 weeks, or at 0.1 % for 28 weeks. No microscopic or gross pathological changes were observed. In another study, the chemical was administered to rats (strain and number not specified) at 1 % in diet for 16 weeks. No treatment-related effects were reported. A similar study on Osborne-Mendel rats (five/sex) was conducted where animals were given 0.1 % isoeugenol in diet. No effects on growth, haematology or any macroscopic or microscopic tissue changes were observed (Hagan et al., 1967; HERA, 2005).

In another study, F344 rats (10/sex/dose) were administered isoeugenol by gavage at 0, 37.5, 75, 150, 300 or 600 mg/kg bw/day, five days per week for 14 weeks. No treatment-related mortality was reported. Mean body weights were decreased in the highest dose group compared with controls. Liver weights were significantly increased in females in the 300 and 600 mg/kg bw/day groups, and minimal to mild periportal hepatocellular cytoplasmic alteration was reported. The highest dose females showed atrophy of the olfactory nerve bundles (NTP, 2010; EMA, 2011).

In a similar experimental design, the same doses were administered to B6C3F1 mice by gavage. No treatment-related mortality occurred. In the 600 mg/kg bw/day dose group, the mean body weights of males were significantly decreased compared with controls. Liver weights were increased compared with controls for 300 and 600 mg/kg bw/day males. Minimal atrophy of the olfactory epithelial tissue and nerve bundles was also observed in mice administered 600 mg/kg bw (NTP, 2010; EMA, 2011).

Developmental toxicity studies have provided information on the effects of repeated dosing of isoeugenol (See **Reproductive and developmental** t**oxicity** section). In a study where the chemical was administered by oral gavage to rats on gestation days (GD) 6–19, reduced body weights and gestational weight gains were observed, indicating maternal toxicity.

Dermal

No data are available.

Inhalation

Very limited data are available. Based on limited data, trans-isoeugenol was found to be irritating and have low systemic toxicity following inhalation exposure.

Rats (CD) (10/sex/group) were exposed to isoeugenol in a subacute inhalation toxicity study performed according to OECD TG 412. The chemical was administered by inhalation as an aerosol at concentrations of 1, 10 or 100 mg/m^3 (0.15, 1.5, and 14.9 ppm respectively) with mass median aerodynamic diameter (MMAD) of 1.4, 1.3 and 2.2 μm respectively, for six hours per day, five days per week for two weeks (10 total exposures). Portal of entry effects suggestive of irritation were present in the nasal cavity of males and females exposed to the test substance. Epithelial inflammation and degeneration of the nasal cavity were observed in all treatment groups and sexes. A dose-response relationship was demonstrated given that increased incidence and severity of degeneration and subacute inflammation were observed at the higher doses compared with effects observed at the lowest dose. All nasal cavity findings were minimal to mild in severity and expected to be reversible following removal of the irritant. Histologic changes indicative of localised irritation were observed in the nasal cavity at all exposure levels. Therefore, a no observed effects level (NOEL) could not be determined for this study and the lowest observed adverse effect level (LOAEL) based on localised irritation was established at 1 mg/m^3 (REACH).

Genotoxicity

Based on the weight of evidence from the available in vitro and in vivo genotoxicity studies, the chemicals are not considered to be genotoxic. Some in vitro genotoxicity tests indicated weakly positive results, but all in vivo tests were negative.

In vitro

Isoeugenol was tested as negative for mutagenicity in *Salmonella typhimurium* strains TA 97, TA 98, TA 100, TA 102, TA 1535, TA 1537 and TA 1538, in the absence or presence of metabolic activation when tested up to concentrations of 600 µg/mL (limit of cytotoxicity). The chemical was also negative for mutagenicity in *Escherichia coli* strain WP2 uvrA trp, with and without metabolic activation (HERA, 2005; NTP, 2010; EMA, 2011).

In an unscheduled DNA synthesis assay, hepatocytes were harvested from F344 rats and were incubated with isoeugenol at concentrations ranging up to 1.0 mM. The chemical did not cause any mutagenicity in this assay (Burkey et al., 2000).

The chemical gave positive results in a sister chromatid exchange (SCE) assay using human lymphocytes when tested at 0.5 mM (82 mg/L), where the numbers of SCE per treated cells were 10.3 at 0.25 mM and 14.0 at 0.5 mM. Isoeugenol produced no significant increase in the frequency of chromatid breaks and SCEs in Chinese hamster ovary cells at lower concentrations. The effects in human lymphocytes were reported at concentrations which produced high levels of cytotoxicity (HERA, 2005; NTP, 2010; EMA, 2011).

In vivo

In a mammalian bone marrow erythrocyte micronucleus study, male mice were treated with isoeugenol at doses up to 2000 mg/kg bw. There was no significant increase in the number of micronucleated polychromatic erythrocytes in harvested cells. A second bone marrow erythrocyte micronucleus study found that the chemical induced a 3.2-fold increase in the frequency of micronucleated cells in female mice at the highest dose of 600 mg/kg bw. A third bone marrow erythrocyte micronucleus study found that the chemical did not produce any evidence of genotoxicity in mice, when dosed with up to 2000 mg/kg bw and 1500 mg/kg bw in males and females, respectively (EMA, 2011).

Carcinogenicity

Isoeugenol is classified as hazardous with the hazard category ‘Carcinogenicity—Category 2’ and hazard statement ‘Suspected of causing cancer (H351) in the HCIS (Safe Work Australia). The available data support this classification.

In a two-year carcinogenicity study, F344 rats (50/sex/group) were dosed with isoeugenol by oral gavage at 0, 75, 150, or 300 mg/kg bw, five days per week for 105 weeks. Survival rates of the exposed animals were comparable to controls. Mean body weights of males in the high dose group were increased compared with controls. Two males in the high dose group developed thymomas, while two other males in this group developed mammary gland carcinomas. Some animals in the mid and high dose groups developed mild atrophy of the olfactory nerves and olfactory epithelial metaplasia (NTP, 2010; EMA, 2011).

A similar experiment was conducted in B6C3F1 mice (50/sex/group) where animals were dosed with isoeugenol by oral gavage at 0, 75, 150 or 300 mg/kg bw, five days per week for 104 weeks (females) and 105 weeks (males). Survival was decreased in males in the high dose group and body weights were reduced in both males and females in this group. In all groups, males exhibited increased incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatocellular carcinoma and adenoma (combined). Incidence of hepatic clear cell foci were also increased in the male mice that received 75 or 150 mg/kg bw/day. There was also a significant increase in histiocytic sarcomas (at multiple tissue sites) in females across all groups. Olfactory epithelial metaplasia was observed in all exposed groups. Bowman's gland hyperplasia was also significantly increased in all exposed groups. Mild renal papillary necrosis and renal tubule necrosis were significantly increased in the high dose group females. There were dose-dependent increases in the incidences of forestomach squamous hyperplasia, inflammation (statistically significant in high dose males and females) and (for males only) ulceration (NTP, 2010; EMA, 2011).

Isoeugenol has not been considered by the International Agency for Research on Cancer.

Reproductive and Developmental Toxicity

Isoeugenol does not appear to cause specific reproductive or developmental toxicity. The reproductive and developmental effects seen were secondary to maternal toxicity.

In a developmental toxicity study, pregnant SD outbred albino rats were dosed with isoeugenol at 0, 250, 500 or 1000 mg/kg bw/day by gavage on GD 6–19. There was no treatment-related maternal mortality. Dose-related evidence of sedation and aversion to treatment (rooting behaviour) in all dosed groups, as well as an increased incidence of piloerection in the 500 and 1000 mg/kg bw/day groups were observed. Dose-dependent reductions were observed in maternal body weight, weight gain and gestational weight gain. Gravid uterine weight was significantly decreased in the mid and high dose groups. Prenatal mortality was not affected by treatment. Foetal morphological abnormalities were similar when compared with controls, except for unossified sternebrae in the high dose group. A LOAEL for maternal toxicity was determined to be 250 mg/kg bw/day. Based on intra-uterine growth retardation and delayed skeletal ossification, a no observed adverse effect level (NOAEL) for developmental toxicity was determined to be 500 mg/kg bw/day (George et al., 2001).

In a developmental toxicity study, time-mated CD rats were orally administered isoeugenol at 0, 250, 500 or 1000 mg/kg bw/day by gavage on GD 6–19. Maternal liver weights were increased in the low dose group. In the mid and high dose groups, the incidence of piloerection and lethargy was increased and maternal body weight and gravid uterine weight were significantly decreased. No morphological abnormalities were observed in foetal skeletons in the mid or low dose group. At the 1000 mg/kg bw/day dose there was a significant increase in the incidence of skeletal variations with 14/179 foetuses showing unossified sternebrae (HERA, 2005; EMA, 2011).

In a three-generation continuous breeding study, isoeugenol was administered (at 0, 70, 230 or 700 mg/kg bw/day) to 20 adult male and female SD rats (F0 generation) and dosing was continued after post-natal day 21 to males and females of the subsequent F1 generation. There was a dose-related decrease in mean bodyweights of the mid and high dose males and high-dose females in the F0 and F1 generations. There was a reduction in the mean number of live male pups born to the F0 generation. Decreased pup weights were reported across male and female F2 pups. General signs of toxicity (including hyperkeratosis, hyperplasia in stomach mucosa and decreased body weight) were observed in the F0 mid and high dose groups. Mild reproductive toxicity at 700 mg/kg bw was indicated by decreased male and female pup weights and by a decreased number of male pups per litter (HERA, 2005; NTP, 2010).

Other Health Effects

Neurotoxicity

In a neurotoxicity study, isoeugenol did not have an effect on spontaneous motor activity or catatonia in mice, when administered at 100 mg/kg bw or 200 mg/kg bw (HERA, 2005).

In a separate study, Wistar rats were administered the chemical at 10, 40, 80 or 160 mg/kg bw. The chemical had no effects on rope climbing performance in male Wistar rats when dosed at 10, 40 or 80 mg/kg bw (via injection, no details provided). Non-specific effects including severe depression and paralysis of the hindquarters were observed at 160 mg/kg bw (HERA, 2005).

Endocrine Disruption

Isoeugenol did not give any indication of potential endocrine-receptor binding potential in two in vitro studies conducted in *Saccharomyces cerevisiae*, for which the genome had been modified by the incorporation of the DNA sequence of the human oestrogen receptor and expression plasmids. In a competitive binding assay with methyltrienolone for a recombinant rat androgen receptor, the IC50 (half maximal inhibitory concentration) value for isoeugenol was found to be 0.0002 and the relative binding affinity was 0.0015 %, indicating that the chemical is a weak binder (HERA, 2005).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include systemic effects that are long-term (carcinogenicity) or acute (skin sensitisation). The chemical is harmful following ingestion or skin contact and can also cause skin, eye and respiratory irritation.

Public Risk Characterisation

The chemical is currently listed on Schedules 5 and 6 of the *Poisons Standard—the Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP, 2018) for preparations containing the chemical (see **Restrictions - Australia** section) at concentrations greater than those specified in the SUSMP, a number of warning statements, first aid instructions and safety directions relating to the chemical apply.

Although the uses in cosmetic and the domestic products in Australia are not known, the chemical is widely used as a fragrance compound in cosmetic and/or domestic products overseas at concentrations up to 0.02 %. The primary risk to consumers using products containing the chemical is skin sensitisation following exposure to cosmetics or consumer products containing the chemicals. The SUSMP limits the concentrations of isoeugenol and its isomers in consumer products and provides warning labels, depending on whether skin contact is expected. These controls are considered adequate to minimise the risk to public health posed by domestic and cosmetic products containing the chemicals; therefore, the chemicals are not considered to pose an unreasonable risk to public health.

Occupational Risk Characterisation

Given the critical systemic long-term, systemic acute, and local health effects, the chemical could pose an unreasonable risk to workers unless adequate control measures to minimise dermal, ocular and respiratory exposure are implemented. The chemical should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) 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).

NICNAS Recommendation

Assessment of the chemicals is 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

Public Health

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

Work Health and Safety

The chemicals in this group are recommended for classification and labelling aligned with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as below.

This assessment does not consider classification of physical hazards and environmental hazards. These are outside the scope of this assessment and any existing HCIS classifications in those categories are not recommended to be changed.

The recommended classification is based on read across principles (see **Grouping rationale** section) and should be used as a default for all members of the group. If empirical data become available for any member of the group indicating that a lower (or higher) classification is appropriate for a specific chemical, these may be used to amend the default classification for that chemical.

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.

The HSIS was an online database intended for use with the 1994 Hazardous Substances Regulatory Framework. In 2012 the Hazardous Substances Regulatory Framework was replaced by the model work health and safety legislation. This legislation references classification in accordance with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). As such, HSIS was replaced by the Hazardous Chemical Information System (HCIS), which contains classification information in accordance with the GHS.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Not Applicable	Harmful if swallowed - Cat. 4 (H302)* Harmful in contact with skin - Cat. 4 (H312)*
Irritation / Corrosivity	Not Applicable	Causes serious eye irritation - Cat. 2A (H319)* Causes skin irritation - Cat. 2 (H315)* May cause respiratory irritation - Specific target organ tox, single exp Cat. 3 (H335)
Sensitisation	Not Applicable	May cause an allergic skin reaction - Cat. 1A (H317)
Carcinogenicity	Not Applicable	Suspected of causing cancer - Cat. 2 (H351)*

[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, ocular 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 a hazardous chemical depend on the physical form and the manner in which the chemical is used. Examples of control measures which could minimise the risk include, but are not limited to:

- using closed systems or isolating operations;
- health monitoring for any worker who is at risk of exposure to the chemical, 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 chemical.

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 chemical 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 have not been undertaken as part of this assessment.

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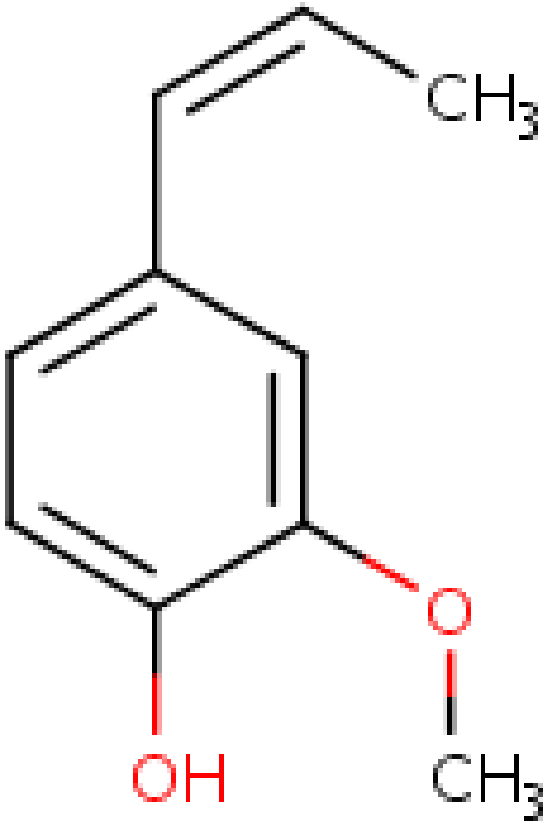
Last Update 26 October 2018

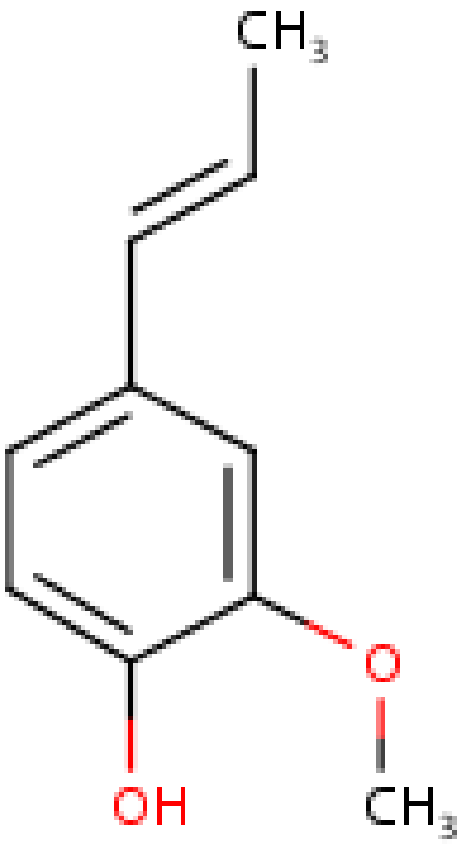
Chemical Identities

Chemical Name in the Inventory and Synonyms	**Phenol, 2-methoxy-4-(1-propenyl)-** isoeugenol 1-(3-methoxy-4-hydroxyphenyl)-1-propene 3-methoxy-4-hydroxy-1-propen-1-ylbenzene 1-hydroxy-2-methoxy-4-propen-1-ylbenzene
CAS Number	97-54-1
Structural Formula	
Molecular Formula	C10H12O2
Molecular Weight	164.20

Chemical Name in the Inventory and Synonyms	**Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-** isoeugenol, cis- 2-methoxy-4-propenylphenol, cis- 4-propenylguaiacol, cis-
CAS Number	5912-86-7
Structural Formula	

	
Molecular Formula	C10H12O2
Molecular Weight	164.20

Chemical Name in the Inventory and Synonyms	**Phenol, 2-methoxy-4-(1-propenyl)-, (E)-** isoeugenol, trans- p-propenylguaiacol, trans-
CAS Number	5932-68-3
Structural Formula	

	
Molecular Formula	C10H12O2
Molecular Weight	164.20

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