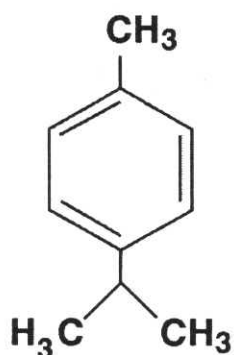# *P*-CYMENE

Chemical formula

$C_{10}H_{14}$

Synonyms

p-Cymene; *para*-isopropyltoluene; 4-isopropyl-1-methylbenzene; p-cymol.

CAS Registry number

99-87-6

Official classification

CE: List B (CE, 1992); upper level: 5 mg/kg (beverages and food)
   except condiments, seasonings: 100 mg/kg (CE, 1992).

Natural occurrence

Widely distributed in plant oils, e.g. terpentine and citrus oils.
Raspberry: 1.3 mg/ml; other fruits: up to 0.3 mg/ml; carrot: trace-21.6 mg/ml; celery
leaves: 0.3 mg/ml; pepper: 40-1100 mg/ml (CE, 1992).

Metabolism

p-Cymene is readily absorbed through the skin (Wepierre, 1963; Wepierre *et al.*, 1968), gastro-intestinal tract (Bakke & Scheline, 1970; Ishida *et al.*, 1981; Southwell *et al.*, 1980) and lungs (Gerarde, 1960) in a variety of mammalian species. The majority of the absorbed fraction is metabolised in the liver and excreted in the urine (Ishida *et al.*, 1981; Southwell *et al.*, 1980; Walde *et al.*, 1983). Early studies in dog, sheep, brushtail possum and koala indicated that the major pathway was oxidation of the methyl group to give cumic acid, followed by its conjugation with glycine to give cuminuric acid. However, more recent studies identified more than 20 metabolites in the urine of rabbit, rat and guinea-pig, and qualitative as well as quantitative differences have been reported between the three species. The first evidence that p-cymene can undergo aromatic hydroxylation, with the formation of carvacrol, was obtained in guinea pig (Scheline, 1991).

TOXICOLOGICAL DATA

Local effects

Skin Irritation.
    Human.- The neat material has been reported to be a mild skin irritant (CHRIS, 1991; Gerarde, 1960) [presumably in man, although the basis for this statement is not clear]. No irritation was seen in 25 volunteers given 48-hr covered patch tests with 4% in petrolatum (Opdyke, 1974) or in ten volunteers given ten daily 24-hr covered applications of up to 4% in petrolatum or diethyl phthalate (Opdyke, 1974). Ointment containing 30% cymene [apparently *para*-isopropyltoluene] has been used as a local analgesic, and thus was presumably non-irritant at this concentration (Martindale, 1989).
    Non-human.- p-Cymene was moderately irritating when applied neat under cover for 24 hr to intact or abraded rabbit skin (Opdyke, 1974) and when applied to guinea-pigs (Stelmakh *et al.*, 1983). Multiple applications of the neat material caused swelling, hair loss and some bleeding, in rats (Stelmakh *et al.*, 1986).

Eye Irritation

    Human.- A brief and unsubstantiated comment indicates that contact with p-cymene causes mild eye irritation (CHRIS, 1991).

Other Local Effects

    Human.- p-Cymene has been described as irritant to the mucous membranes [presumably in man, although experimental support for this statement was not given] (Lee, 1987).

Ointment containing 30% cymene [apparently *para*-isopropyltoluene] produced local analgesia when applied to the skin (Martindale, 1989).

Non-human.- Slight local irritation was seen when rats were exposed by inhalation to 27-55 g/m³ for four consecutive periods of 20-50 minutes, with time to recover between exposures (Furnas & Hine, 1958).

Sensitisation and intolerance

Human.- In an unsuccessful attempt to induce sensitisation (following the maximisation procedure) none of 25 subjects displayed local reactions when given patch tests with 4% p-cymene in petrolatum, followed by a challenge with the same concentration (Opdyke, 1974). The maximisation procedure involves an induction phase of five 48-hr closed patch tests over a 15-day period followed after a 2-wk rest by a final 48-hr closed challenge patch (Kligman & Epstein, 1975). p-Cymene is included in a list of potential sensitisers with a concentration of 1% in petrolatum recommended for diagnostic [24/48-hr covered] patch tests (Fisher, 1975). [The basis for its inclusion is not given.]

Non-human.- A study indicated that p-cymene caused sensitisation in guinea-pigs (Stelmakh *et al.*, 1983).

Acute data.

Non-human.- *Oral.*- Rat LD_{50}: 4.75 g/kg bw (Jenner *et al.*, 1964). When given by stomach tube, the average lethal dose in rats was 5.11 g/kg bw and in mice was 2.2 g/kg bw (Stelmakh *et al.*, 1983), while in another study a dose of 4.3 g/kg bw killed nine out of ten rats (Gerarde, 1960). Rats and mice given near lethal doses displayed various central nervous system (CNS) effects, diarrhoea, bloody tears, shallow breathing and a scrawny appearance (Jenner, 1964; Stelmakh *et al.*, 1983).

Dermal.- Rabbit LD_{50}: >5g/kg bw [24-hr covered contact] (Opdyke, 1974). Rats given an apparently single application of 0.5 g/kg bw had changes in the cellular fraction of the blood after 6 days (Stelmakh *et al.*, 1986). Rats died following immersion of the tail for 4 hr in neat p-cymene or in a 50% dilution [in an unspecified oil], whilst concentrations of 25% or greater caused tissue abnormalities (Stelmakh *et al.*, 1986). [It was not possible to estimate the doses involved in this study.]

Inhalation.- Rat. Breathing difficulties, twitching, quivering, salivating, depression of the CNS, failure of muscular co-ordination, respiratory arrest and death occurred when rats were exposed to 27-55 g/m³ for four consecutive periods of 20 to 50 minutes, with time to recover between exposures. No tissue changes were seen in the brain, spinal cord or sciatic nerve (Furnas & Hine, 1958). Exposure to 5 g/m³ for an unspecified period was fatal (WSC, 1960), whereas undefined high exposures, probably for 2-3 hr, produced tremors, convulsions, lethargy, shallow breathing and virtual coma (Stelmakh *et al.*, 1983). Rats exposed to 172 mg/m³ or greater for 4 hr showed behavioural, reflex

and body temperature changes, which were not seen at 70 mg/m^3 (Stelmakh *et al.*, 1986).

Mouse. The average lethal concentration was claimed to be 24 g/m^3 [exposure period and temperature unspecified] (Stelmakh *et al.*, 1983).

Mice exposed to [undefined] high concentrations, apparently for 2-3 hr, had tremors and convulsions, followed by lethargy, shallow breathing, and virtual coma (Stelmakh et al., 1983). No overt toxicity was seen in mice exposed to an atmosphere saturated with p-cymene [for an unspecified period] (Gerarde, 1960). Saturated air at 17.3°C contains about 7 g p-cymene/m^3 (Gerarde, 1960). Exposure to 282 mg/m^3 or greater for 2 hr produced effects upon mobility, body temperature, and certain reflex and behavioural responses, while 162 mg/m^3 caused none of these effects nor changes in unspecified blood or biochemical [probably urinary] parameters (Stelmakh *et al.*, 1986).

Intraperitoneal.- The average lethal dose in mice was 1.44 g/kg bw (Stelmakh *et al.*, 1983). Deaths occurred when 2.16 g/kg was injected into guinea-pigs (Chassevant & Garnier, 1903).

Intravenous.- Rats given 324 mg/kg bw, over a period of 1 hr, displayed disturbances in a reflex reaction (Tham *et al.*, 1984).

Subacute data

Human.- Oral.- Daily administration of 3-4 g [approximately 43-57 mg/kg bw/day] for 2-3 days caused nausea, headache and vomiting (Zeigler, 1873).

Non-human.- Oral: According to an unclear report, in a study mice were given 0.22 g p-cymene / kg bw [probably] daily for 4 days by stomach tube. This dose was apparently increased by a factor of 1.5 every 4 days for a total of 24-28 days [giving a final maximum dose apparently of about 1.65 g/kg bw daily], when reduced mobility was reported (Stelmakh *et al.*, 1983) [only overt effects were recorded in this study].

Rats given approximately 0.5 g/kg bw/day or greater for 60 days by stomach tube suffered unspecified toxic effects, whilst doses of 1 g/kg bw/day did not cause deaths. Higher doses [probably around 2.5 g/kg bw/day] affected the nervous system, liver (increased weight and enzyme activity), kidneys (increases in weight and the excretion of protein and urea) and blood cells (Stelmakh *et al.*, 1986).

Dogs given 2 g/day [approximately 0.2 g/kg bw/day, apparently orally] experienced no overt adverse effects apart from diarrhoea (Gerarde, 1960).

Dermal.- Twenty-one applications [probably daily] caused decreases in body temperature, reflex responses and blood haemoglobin levels in rats (Stelmakh *et al.*, 1986) [it was not possible to estimate the doses administered].

Inhalation.- Male rats were exposed to 50 or 250 ppm *p*-cymene 6 hr/day, 5 days/wk, for a period of four weeks, followed by an exposure-free period of eight weeks. Yield of synaptosomal proteins, isolated from whole brain minus cerebellum, was statistically significantly reduced; synaptosomal noradrenaline and dopamine concentrations and acethylcholinesterase, butyrylcholinesterase, and lactate dehydrogenase activities were statistically significantly increased when expressed relative to synaptosomal proteins (Lam *et al.*, 1996).

Injection.- Subcutaneous injection at 1.7 g/day [approximately 0.85 g/kg bw/day] for 2 days produced some changes in the white blood cells and bone marrow in rabbits (Woronow, 1929).

Chronic data

Human.- Inhalation.- A worker in the sulphite paper pulp industry exposed for 20 yr to vapours from a mixture containing 0.034% p-cymene (and unspecified concentrations of other chemicals) developed, over a period of about 20 months, weakness, breathing difficulty, nose bleeds, severe anaemia, and an abnormality of the bone marrow followed by death from cerebral haemorrhage. The worker constantly handled and frequently chewed pieces of sulphite pulp. Although the investigator suggested that the fatal symptoms were possibly due to the p-cymene exposure (Carlson, 1946), others have pointed out that this association has not been convincingly established (Gerarde, 1960; Lee, 1987). According to a brief citation, exposure to about 1.1-2.7 g p-cymene / m^3 caused dizziness, headaches and nausea (OHMTADS, 1991) [no further details were provided].

Genotoxicity and mutagenicity

There was no mutagenic activity in tests with p-cymene in the bacterium *Escherichia coli*, in the absence of a metabolic activation system (Szybalski, 1958) or with isopropyltoluene [unspecified isomer] in a fairly limited Ames test using the bacterium *Salmonella typhimurium* in the presence of a liver metabolic activation system (Rockwell & Raw, 1978).

An old report indicated that isopropyltoluene [unspecified isomer] had no effect on the appearance of the chromosomes of the yeast *Saccharomyces cerevisiae*, in the absence of an added metabolic activating system (Levan, 1947).

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DECANAL

Chemical formula

$C_{10}H_{20}O$

$$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CHO$$

Synonyms

1-Decanal; aldehyde C10, capraldehyde, caprinic aldehyde, decaldehyde, decyl aldehyde.

Chemical abstracts registry number

112-31-2

Official classification

Reported in EPA TSCA inventory of chemicals (Lewis, 1996) and in FEMA/FDA GRAS list (Ash & Ash, 1995).

C.E.: list A.

Usage level: 2.3ppm (non alcoholic beverages)
4.1ppm (ice cream, ices)
5.7ppm (candy)
6.6ppm (baked goods)
3ppm (gelatins, puddings)
0.6ppm (chewing gum) (Ash and Ash, 1995).

The total daily intake per person of all linear saturated alcohols, aldehydes and acids combined from use as flavor ingredients is estimated to be <2mg/kg (NAS, 1987).

Decanal has been found in over 50 sources including citrus oil, orange oil, citronella and lemongrass (Lewis, 1996; Martindale, 1996).

Natural occurrence

In Cassia, Neroli and other oils.

Metabolism

Aldehydes are chemically reactive compounds that undergo various reactions *in vivo*. The main detoxification pathway for these substances probably consist of oxidation to the corresponding acids (Williams, 1959). Aldehydes readily interact with various nucleophilic entities, e.g. amino- and thiol- groups (Schauenstein *et al.*, 1977). Human cytosolic aldehyde dehydrogenase (ALDH-1) has a K_m 2.9±0.4nM for decanal, that is 8 times lower than that of the mitochondrial isozyme (ALDH-2), which has a K_m 22±3nM (Klyosov, 1996).

Lipid peroxidation, induced by several substances, produces a great variety of stable, diffusible aldehydes including alkanals (Luo *et al.*, 1997; Toyokuni *et al.*, 1997). These aldehydes are extremely active and can act as "second cytotoxic messengers" (Luo *et al*, 1997).

The role of microsomal membrane-bound aldehyde dehydrogenase in the detoxication of aldehydic products of lipid peroxidation has been studied: aldehyde dehydrogenase purified from adult male Wistar rat microsomes showed a K_m 2.6µM (Antonenkov, 1987).

TOXICOLOGICAL DATA

Local effects

Skin irritation.
    Non-human.- Severe irritation in rabbit in a 24-hrs open-patch test with 14,372 µg (Lewis, 1996).
In another study, mild irritation was observed in rabbits after 24-hrs closed patch with 500mg (Lewis, 1996).

Acute toxicity

Moderately toxic by ingestion, mildly toxic by skin contact (Ash & Ash,1995).
    Non-human.- <u>Oral</u>.- LD₅₀ rat: 3730 mg/kg (Lewis, 1996); 1050 mg/kg/day treatment in mice caused a reduction of food consumption (Schafer and Bowles, 1985); in birds estimated LD₅₀ is > 111 mg/kg, based on food consumption data over a 18 hour period (Schafer *et al.*, 1983).
<u>Dermal</u>.- LD₅₀ rabbit: 5040 mg/kg (Lewis, 1996); in a 14 day study the LC₅₀ of decanal to fish was 20.4 µmoles/l (Deneer, 1988).

In vitro toxicity

A 20-hr exposure to both decanal and N-amino-N'-1-octylguanidine (AOG) at 200μM causes a synergistic bactericidal activity against *Escherichia coli* J96 (Rideout, 1986). Decanal and AOG, combined at 28μM each, cause human erythrocyte lysis within 80 minutes, but this effect was not observed when either AOG or decanal were used alone (Rideout, 1986). Decanal and AOG also exhibit a synergistic cytotoxic activity against HeLa cells, as evaluated by trypan blue exclusion (Rideout, 1986).

In ascites sarcoma BP8 cells a 48-hr treatment with 1-0.1-0.01mM decanal inhibited cell growth by 87-60-0% respectively (Pilotti *et al.*, 1975).

In isolated hamster brown fat cells, decanal inhibited noradrenaline induced respiration by 100 and 61% at 1 and 0.1 mM respectively (Pettersson *et al.*, 1980).

5mM decanal caused complete ciliostasis within 3 min in chicken tracheal organ cultures (Pettersson *et al.*, 1982).

30min treatment with 25mM decanal induced 76% [^{3}H]uridine leakage, used as a marker of membrane damage (Thelestam *et al.*, 1980).

In a comparative study, four *in vitro* short term tests have been used: cell growth of Ascites sarcoma BP8 cells, oxidative metabolism of isolated brown fat cells from adult hamster, ciliary activity of chick embryo trachea and membrane damage of human diploid embryonic lung fibroblasts; decanal resulted highly active in all the four tests, in a similar way as evaluated by an arbitrary scale (Curvall *et al.*, 1984).

Carcinogenicity

Non-human.- 0.45 and 2.15 g/kg bw decanal (17 i.p. injections over a 8-week period, 24-week duration of the experiment) were considered not carcinogenic in the A mouse pulmonary tumor system (Stoner *et al.*, 1973).

Genotoxicity and mutagenicity

Mammalian cells in vitro.- In a mutagenicity screening of food additives, based on a chromosomal aberration test with CHL Chinese hamster fibroblast cell line, decanal gave negative results (Ishidate *et al.*, 1984).

Bacterial assays.- In the Ames' test on *Salmonella typhimurium* tester strains TA 98, TA 100, with and without metabolic activation, decanal was found to be not mutagenic at non-toxic doses, up to 3μmol/plate (Florin *et al.*, 1980). In a mutagenicity screening of food additives, based on reverse mutation assay with *Salmonella typhimurium* (Ames test), decanal gave negative results (Ishidate *et al.*, 1984). DNA repair test: 5mg/disc, *Bacillus subtilis* tester strain (Lewis, 1996).

Structure-activity relationship

A study has been performed in which four classes of chemicals have been defined. To each of these classes a QSAR-derived method is assigned to enable the estimation of the

acute aquatic toxicities of chemicals falling into it, calculating either an expected effect concentration, such as the LC_{50}, or an expected range of possible effect concentrations, from a compound's octanol-water partition coefficient, P_{ow} (Verhaar et al., 1992).

The acute toxicity, expressed as 14 day LC_{50}, of decanal to the guppy is described satisfactorily by a QSAR employing hydrophobicity (P_{ow}) as the only parameter (Deneer, 1988).

A significant correlation was found between NR_{50} values, determined in the neutral red assay on goldfish scale GFS cells, and P_{ow}, as well as between cells NR_{50} and guppies LC_{50} (Saito et al., 1993).

Also in the *Tetrahymena pyriformis* population-growth impairment assay the toxicity of bioreactive aldehydes can be modeled adequately by simple P_{ow}-dependent QSARs (Schultz et al., 1994).

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EUGENOL

Chemical formula

$C_{10}H_{12}O_2$
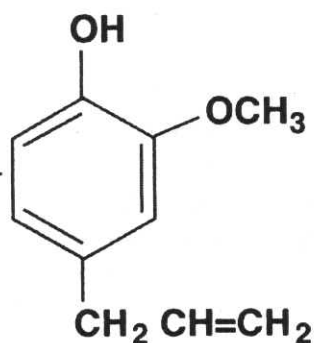
Synonyms

Phenol, 2-methoxy-4-(2-propenyl); 4-allyl-2-methoxyphenol; caryophyllic acid; eugenic acid; 2-methoxy-1-hydroxy-4-allylbenzene; allyl guaiacol.

CAS registry number

97-53-0

Official classification

CE: List A (CE, 1992).
FAO/WHO Expert Committee on Food Additives. ADI: 2.5 mg/kg b.w. The daily per capita comsumption is estimated at 0.6 mg (CSA, 1988; IARC, 1985; Nagababu and Lakshmaiah, 1992).
IARC: Group 3 (IARC, 1987).

Natural occurrence

Very widespread occurrence in essential oils. Major component of clove bud oil, clove leaf oil, cinnamon leaf oil, and oil of basil.
Present in cigarette and wood smoke (Hawthorne *et al.*, 1988; Jansson *et al.*, 1988). Detected in untreated effluent from a paper mill and municipal wastewater (Turoski *et al.*, 1983; Ellis *et al.*, 1982).

Metabolism

In mammals, the major organ responsible for metabolism is the liver, where microsomal mixed function oxidases affect some side chain oxidation accompanied by some O-demethylation (IARC, 1985); formation of sulphate and glucuronide conjugates is the major route of metabolism and the formation rate of the two conjugates is related to the dose (Sutton *et al.*, 1985). Reduction of the double bond is effected in rats and mice by gut microflora but is more extensive in the rat. This does not appear to occur in humans (Richardson, 1992). Oral rats 203 mg/kg resulted in 50% of the dose being eliminated as conjugates in urine and 13 % as oxidation products (dihydrodiols) (Delaforge *et al.*, 1980).
The pharmacokinetics of eugenol have been assessed in humans receiving oral doses of 50 mg. Rapidly absorbed and metabolised, almost completely excreted in the urine in 24 hr. Unmetabolised compound accounted for < 0.1% dose. Mayor urine metabolites were phenolic conjugates, 50% of the conjugated metabolites were eugenol-glucuronide and sulphate (Fischer *et al.*, 1990).

TOXICOLOGICAL DATA

Local effects

Eugenol can be an irritant to lungs (LaVoie, 1986); in human patch tests eugenol was moderatly irritating (Motoyoshi, 1979). In irritation test using guinea pigs 10% eugenol caused irritant reactions. The frequency of allergic-type positive reactions in cosmetics dermatitis patients to 5% eugenol was 2.6% (Itoh, 1982).

Sensitisation and intolerance

Studies have produced mixed results but sensitisation has been reported in guinea pig, mouse and humans (Motoyoshi *et al.*, 1979; Richardson, 1992).

Acute toxicity

Oral LD_{50}: 2-3 g/kg in rats, guinea pig, mouse (Jenner *et al.*, 1964).

Intraperitoneal LD_{50}: 2 g/kg in rats (Sober, 1950), 0.5 g/kg in mice (Caujolle and Meynier, 1960).

Dermal LD_{50}: 1.2 g/kg in rabbits (Beroza et al., 1975).

Acute mammalian toxicity is associated with drop in body temperature, muscle weakness, loss of righting reflex, cardiovascular an respiratory effects, and tissue irritation (Caujolle et al., 1960; Beroza et al., 1975; Dallmeier & Carlini, 1981; Lauber & Hollander, 1950; Richardson, 1992; Sober, 1950; LaVoie et al., 1986).

Sub-acute toxicity

Rats receiving <= 6000 ppm in diet for 13 wk showed no adverse effects, 12000 ppm caused weight loss (NTPRTD, 1992). Oral rats (34 day) unspecified concentration caused damage to liver and gastrointestinal tract, particularly the forestomach (Hagan et al., 1965).

In vitro **toxicity**

ID_{50} (evaluated with Neutral Red uptake): 0.26±0.016 mM on the HepG2 cells (Thompson et al., 1990).

In HepG2 cells eugenol is oxidised by a 3-methylcholantrene-inducible isoenzyme of cytP-450 to a reactive intermediate that binds to cellular proteins and forms conjugates with glutatione, resulting into the depletion of intracellular glutathione (Thompson et al., 1990).

Cytotoxicity of eugenol occurs at mM concentrations in HaCaT (human keratinocytes) cell line (Neutral Red Uptake assay) and is manifested by early changes in cell cytoskeleton (α actin); IC_{50} (4hr) = 2.38mM; IC_{50} (24hr) = 1.73mM (Audabram et al., 1997a). Eugenol do not redox cycle to any appreciable degree and exhibit rather an anti-oxidant activity (*cis*-Parinaric acid and DiChloroFluorescein Diacetate assays; Audabram et al., 1997a). A change in mitochondrial transmembrane potential and an increase in cytosolic free calcium concentration are early events in the toxicity of eugenol (Audabram et al., 1997a).

The electrophilic arylation of cellular nucleophiles is a key mechanism by which eugenol is cytotoxic in human keratinocytes, as it is shown by depletion of intracellular glutathione and covalent binding of reactive intermediate(s) from eugenol to cellular proteins (Audabram et al., 1997b). Eugenol needs to be activated metabolically into the skin to form protein reactive species, and protein sulphydryl groups, as well as amine groups, are cellular targets for these metabolites; however, there is not clear evidence that cytochrome P450 (at least 1A1 isoenzyme) play a major role in this cell type (Audabram et al., 1997b).

250 µM eugenol inhibits the generation of reactive oxygen species (Pulla Reddy & Lokesh, 1994), as demonstrated by the prevention of the oxidation of Fe^{2+} in Fentons reaction and by the inhibition of: superoxide anion generation in xanthine-xanthine oxidase system (50%); hydroxyl radicals generation, as measured by deoxyribose degradation (70%) and by the hydroxylation of salicylate to 2,3-dihydroxy benzoate

(46%). These data support the role of eugenol as effective radical quencher and antioxidant which helps in controlling radical mediated pathologies.

The activation of eugenol to form DNA adducts and oxidative base damage has been investigated (Bodell et al., 1998). Eugenol treatment of HL-60 cells produced a dose-dependent formation of three DNA adducts. Since the combination of 100 µM eugenol and 100 µM H_2O_2 increased the levels of DNA adducts by 14-fold, peroxidase activation seems to be involved in adduct formation. Indeed, in vitro activation of eugenol with either horseradish peroxidase or myeloperoxidase and H_2O_2 produced the same DNA adducts identified in HL-60 cells; this was inhibited by the addition of either ascorbic acid or glutathione. Peroxidase activation of eugenol produced the same UV spectrum as eugenol quinone methide, the putative reactive intermediate. Reaction of quinone methide with DNA produced two principal adducts, one of which has been identified also in HL-60 cells.

To improve the safety of eugenol application in dentistry, the reactivity of dimerized eugenol has been studied using ESR spectroscopy (Satoh et al., 1998). Eugenol produced radicals in alkaline solutions, while its dimer was inactive. Eugenol was more effective than its dimer in scavenging the superoxide anion (O^{2-}) generated by hypoxanthine and xanthine oxidase reaction.

Comparative kinetic analyses of the mechanisms of toxicity of eugenol and its putative toxic metabolite quinone methide have been carried out in cultured rat liver cells (Clone 9, ATCC) using a variety of vital fluorescence bioassays (Thompson et al., 1998). 10 to 100 µM eugenol depleted intracellular GSH, inhibited the gap junction-mediated intercellular communication and the generation of ROS and had a modest effect on the mitochondrial membrane potential. At high concentrations (1000 µM) eugenol also affected $[Ca^{2+}]_i$, plasma membrane potential and pH. Effects of eugenol quinone methide were seen at lower concentrations (1 to 10 µM). Coadministration of glutathione ethyl ester completely protected cells from cell death caused by eugenol and its quinone methide. The authors hypothesize that eugenol mediates its hepatotoxic effects primarily through depletion of cytoprotective thiols and interference in thiol-dependent processes such as gap junction-mediated intercellular communication.

The effect of eugenol on xanthine oxidase (XO) xanthine(X)-Fe^{3+}-ADP mediated lipid peroxidation has been studied in liver microsomal lipid liposomes (Nagababu & Lakshmaiah, 1997). Eugenol inhibited the lipid peroxidation in a dose-dependent manner. The authors conclude that eugenol inhibits XO-X- Fe^{3+}-ADP mediated peroxidation by inhibiting the XO activity per se in addition to quenching various radical species.

The neuroprotective efficacy of eugenol against NMDA-, oxygen-glucose deprivation-, and xanthine/xanthine oxidase-induced neurotoxicity in primary murine cortical cultures has been examined (Wie et al., 1997). Eugenol (100-300 µM) attenuated NMDA-induced acute neurotoxicity (by 20-60 %) and elevation in neuronal $^{45}Ca^{2+}$ uptake (by 10-30%). In the oxygen-glucose deprivation neurotoxicity, eugenol prevented acute neuronal swelling and reduced neuronal death by 45-60% in a concentration-dependent fashion. Oxidative neuronal injury induced by xanthine/xanthine oxidase was also significantly reduced (75-90%) by eugenol addition. The authors hypothesise that

eugenol may play a protective role against ischemic injury by modulating both NMDA receptor and superoxide radical.

Carcinogenicity

Rats receiving 10000 ppm for 42 days showed a negative result in tumour initiation and promotion test (Ward *et al.*, 1989). Rats receiving eugenol after a single dose of diethyl nitrosamine, for several weeks in an assay based on the number and area of induced glutatione s-transferase placental positive foci in liver, showed a positive result at dietary dose of 10000 ppm (Allavena *et al.*, 1992). In a 1 yr feeding study in mice, no evidence of tumorigenicity was detected (Miller, 1983). National Toxicology program investigated eugenol in a 2 yr feeding study in rats and mice. Results with male and female rats were negative for carcinogenicity and equivocal for male and female mice. Male mice showed an increase in adenomas and carcinomas but only at the lowest dose, while in female equivocal signs of hepatocellular neoplasm were observed (Nagababu & Lakshmaiah, 1992). Dermal treatment of mice with 10 mg / 3 wk resulted in a positive anti-tumourigenic action (Van Duuren, 1976).

Genotoxicity and mutagenicity

Mammalian cells in vitro.- Chromosomal aberrations in Chinese hamster ovary cells with metabolic activation (Ishidate *et al.*, 1984); sister chromatid exchanges in Chinese hamster ovary cells +/- metabolic activation at concentrations which caused severe cell cycle delay (Galloway, 1987). Unscheduled DNA synthesis in rat liver cells was negative; the compound was cytotoxic at higher doses (Ito & Imaida, 1992; Danneman *et al.*, 1983; Richardson, 1992).

Bacterial assays.- Negative in *Salmonella typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537, +/- metabolic activation (Gerberick *et al.*,1992).

Ecotoxicity

LC_{50} (1-96 hr) fathed minnows 24 mg/l (Mattson *et al.*, 1976).

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2-HEXENAL

Chemical formula

$C_6H_{10}O$

$$CH_3CH_2CH_2CH = CHCHO$$
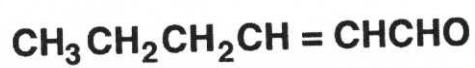
Synonyms

3-Propylacrolein.

CAS registry number

505-57-7

Official classification

CE: List A (CE, 1992).
WHO: Permitted for food use under Sec 121.1164 of the code of federal regulations.

Natural occurrence

Constituent of many foods, hornbeam leaves, and of scent glands of many bugs, e.g. *Pternistria bispina*; alarm pheromone of e.g. *Cimex lectularius*.
Banana: 18-76 mg/kg; other fruits: up to 15 mg/kg; tomato: 0.01-10 mg/kg; cucumber: 0.3-3 mg/kg; tea: 1.6-25 mg/kg (CE, 1992).

TOXICOLOGICAL DATA

Acute toxicity

Oral.- LD50: 780-1130mg/kg in rats, 1550-1570 mg/kg in mice
Intraperitoneal.- LD50: 100-200 mg/kg in both species (Gaunt *et al.*, 1971).

Sub-acute data

Oral.- 90-day rat study: NEL 80 mg/kg b.w./day (Opdyke, 1975).

Genotoxicity and mutagenicity

Bacterial assays.- S. Tiphymurium TA 97-98-100-102-104 +/- metabolic activation in liquid and gas phases: negative (Andersen *et al.*, 1994).

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LINALOOL

Chemical formula

$C_{10}H_{18}O$
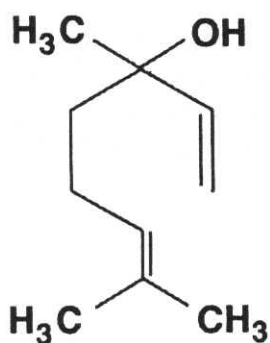
Synonyms

1,6-octadien-3-ol, 3,7-di-methyl; allo-ocimenol; linalyl alcohol; 2,6-dimethyl-2,7-octadien-6-ol; 3,7-dimethyl-1,6-octadien-3-ol.

CAS registry number

78-70-6

Official classification

CE: List A (CE, 1992).
JEFCA.ADI: an ADI group of 0-0.5 mg/kg bw was allocated for citral, geranyl acetate, citronellol and linalyl acetate; however, it has been recommended that at least one member of this group of compounds should be studied for effects of long-term exposure (JECFA, 1980).

Natural occurrence

Linalool is an optically active molecule and both forms (d- and l-) naturally occur in many essential oils, in varying proportions. D-linalool is the major component of oil of *Mentha arvensis*, and occurs in many essential oils, including rose, neroli and lavender. L-linalool is the major component of oil of *Cryptocarya moschata* and *C. aschersoniana*, and occurs in coriander oil.

Metabolism

The metabolism of radioactively-labelled linalool has been investigated in the rat. Following a dose of 500 mg/kg bw by stomach tube 93% was excreted within 72 hr in the urine (55%), faeces (23%) and expired air (15%). The remaining radioactivity was located mainly in the liver (0.5%), gut (0.6%), skin (0.8%) and skeletal muscle (1.2%) (Parke *et al.*, 1974a).

TOXICOLOGICAL DATA

Local effects

Skin irritation.
        Human.- Mild irritation resulted from the 48-hr covered application of a 32% solution in acetone (Motoyoshi *et al.*, 1979). Up to 30% in petrolatum has been recommended in patch tests [generally involving 24/48-hr covered contact] to test individuals for sensitisation to linalool, and this concentration would thus be expected to be non-irritant to most healthy individuals (Nater & De Groot, 1985). Linalool was non-irritant to the skin of an unspecified number of volunteers when tested at 20% in petrolatum in a 48-hr covered patch test (Opdyke, 1976), and when applied in covered patch tests [duration unspecified] to 28 volunteers at 20% in vaseline or ointment (Richardson, 1992). No irritation was reported in several other patch test studies using levels up to 8% (Richardson, 1992; Opdyke, 1976).
        Non-human.- The neat material applied to intact or abraded rabbit skin for 24 hr under cover caused mild to moderate irritation (Opdyke, 1976). A regime in which the neat material was applied to the uncovered skin of rabbits, followed 24 hr later by a second application and then a third 24 hr after that, was described as severely irritating (Motoyoshi *et al.*, 1979). Similar regimes in the rat and guinea-pig produced moderate irritation (Motoyoshi *et al.*, 1979).
In three separate experiments, the neat material applied under a semi-occluded patch to the intact skin of groups of three or four rabbits for 4 hr produced, at worst, only slight reddening and swelling (ECETOC, 1995).
A 48-hr covered patch was not irritant to the skin of six miniature swine (Motoyoshi *et al.*, 1979).

Other local effects

Non-human.- Daily application of a 20% solution in ethanol to guinea-pig skin for 8-10 days caused some thickening of the epidermis (Schaaf, 1961).

Sensitisation and intolerance

Human.- Linalool was identified using patch tests [generally 24/48-hr covered contact] as a causative allergen in three of 75 patients with allergic contact dermatitis to cosmetics (De Groot, 1987). In another series of 119 patients with contact dermatitis caused by cosmetics, one reacted to 10% linalool in petrolatum when it was applied as a covered patch for 48 hr (De Groot *et al.*, 1988) [there may have been some overlap between the two groups of patients]. A man developed allergic contact dermatitis and responded to a patch test [probably involving 24/48-hr covered contact] with 30% linalool in petrolatum (De Groot & Liem, 1983).

In an unsuccessful attempt to induce sensitisation (following the maximisation procedure), 25 volunteers were given five consecutive 48-hr covered patch tests with 8% [presumably in petrolatum], alternated with treatment with a mild irritant, over a 15-day period, followed 10 days later by a 1-hr covered challenge patch with 10% and then a 48-hr covered patch with 8%. None of the volunteers developed a reaction indicative of sensitisation (Grief, 1967). Other attempts to sensitise groups of 25 individuals in maximisation tests were also unsuccessful in regimes involving concentrations of up to 20% (where specified, in petrolatum) (Ishihara *et al.*, 1986; Opdyke, 1976).

Non-human.- Sensitisation was not induced in a guinea-pig maximisation test in which 10% [presumably in petrolatum] was used as the induction and challenge concentrations (Ishihara *et al.*, 1986). The maximisation test generally involves several intradermal injections of the test substance and an adjuvant to increase the immune response, followed 1 wk later, by a single 48-hr covered skin application of the test substance. Three weeks after the start of the induction phase, a challenge 24-hr covered skin application is made.

Acute toxicity

Non-human.- Oral.- LD_{50} rat: 2.8-4.2 g/kg bw (Jenner *et al.*, 1964; Levenstein, 1973). Rats given lethal doses developed muscle incoordination and died within 4-18 hr (Jenner *et al.*, 1964).

Inhalation.- Decreased movement was observed in mice inhaling linalool vapour for 30-60 minutes. The concentration of linalool was estimated as 3.2 mg/l in one study (Buchbauer *et al.*, 1991; Buchbauer *et al.*, 1993).

Dermal.- LD_{50} rabbit: >5 g/kg bw (Opdyke, 1976) [exposure conditions unspecified].

Subacute toxicity.

Non-human.- Rat: Increased liver weight and an induction of liver peroxisomal enzymes was observed in rats given 1.5 g/kg bw/day for 5 days, by stomach tube (Roffey *et al.*, 1990).

An increase in the level of cytochrome P450 within liver microsomes was observed in rats given 0.6 g/kg bw/day for 3 days by stomach tube, although the level had returned to normal after 6 days' treatment (Chadha & Madyastha, 1984).

Decreased liver weight and changes in the activity of certain liver enzymes (including cytochrome P450) occurred in rats given 0.5 g/kg bw/day for up to 64 days (Parke *et al.*, 1974b).

Mouse: There were no effects on body weight or spleen and thymus weight, and no overt signs of toxicity when 30 mice were given up to 375 mg/kg bw/day for 5 days by stomach tube. Immune function appeared normal; there was no increase in mortality in a host-resistance assay in which a group of twenty of the treated mice were challenged with *Listeria monocytogenes* bacteria, and there were no change in the antibody plaque-forming cell response to sheep red blood cells in the remaining ten treated mice (Gaworski *et al.*, 1994).

Subchronic toxicity

Human.- Over a 3-month period, linalool was not associated with any increased symptoms among 200 unexposed controls and 496 workers occupationally exposed to flea control products, including linalool (Ames *et al.*, 1989). [No further details were given.]

Carcinogenicity

Non-human.- Rat: A group of 50 rats was fed a diet containing 1% linalool [approximately 0.5 g/kg bw/day] for 2 wk, treated with a carcinogen known to induce mammary tumours, and maintained on the linalool diet for further 18 wk. There was no change in the latency period to tumour development or in the number of tumours induced by the carcinogen (Russin *et al.*, 1989).

Mouse: No increase in the number of lung tumours was observed when groups of 15 male and 15 female mice, of a strain susceptible to tumours at this site, were given 125 or 25 mg/kg bw, three times/wk for 8 wk by intraperitoneal injection and observed for a further 16 wk. Only a limited range of tissues were examined (Stoner *et al.*, 1973).

Linalool was described as eliciting a weak tumour-promoting response on the skin when it was applied as a 20% solution in acetone to mice. Details of the study are unclear, but probably involved an initial single application of a known skin carcinogen followed three weeks later by a weekly application of 0.25 ml [approximately 1.5 g/kg bw] for 33 wk (Roe & Field, 1965) [no further details were given].

Modern regulatory guidelines recommend that groups of 50 rodents of each sex are exposed for a minimum of 2 yr (rats) or 1½ yr (mice) to one of several doses and that a comprehensive range of tissues is examined microscopically.

Genotoxicity and mutagenicity

Mammalian cells in vitro.- Mutations were induced in mouse lymphoma cells treated in the presence but not absence of a liver metabolic activation system (Heck *et al.*, 1989).
Chromosomal aberrations were not observed in a Chinese hamster fibroblast cell line in the absence of a liver metabolic activation system (Ishidate *et al.*, 1984).
*Bacterial assays.-* Linalool was not mutagenic in Ames tests using *Salmonella typhimurium* bacteria both in the presence (Eder *et al.*, 1980; Heck *et al.*, 1989; Rockwell & Raw, 1978 Ishidate M. *et al.*, 1984) and absence (Eder *et al.*, 1980; Heck *et al.*, 1989) of a liver metabolic activation system.
It was not mutagenic to *Escherichia coli*, but has given both positive (Yoo, 1985) and negative (Haley, 1982) results in rec-type assays, which provide indirect evidence of DNA damage, with *Bacillus subtilis*.

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THYMOL

Chemical formula

$C_{10}H_{14}O$
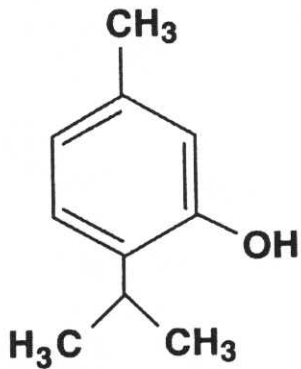
Synonyms

5-Methyl-2-(1-methylethyl)-phenol; p-mentha-1,3,5-trien-3-ol, p-cymen-3-ol; 6-isopropyl-m-cresol; 3-hydroxy-4-isopropyltoluene; 3-hydroxy-p-cymene; thymianic camphor.

CAS registry number

89-83-8

Official classification

CE: List B (CE, 1992).

Natural occurrence

Thymol occurs in many essential oils. Especially found in the Labiateae. Rich sources are *Thymus vulgaris* L. and *Monarda puctatata* oils.
Mandarin oil: 1,800 mg/kg; bilberry: 0.001 mg/kg; cranberry: trace (CE, 1992).

Metabolism

Thymol administered to male albino rats by gavage, at dose 1 mmol/Kg was excreted mainly in the urine after 24 hrs. Large quantities of the compound were excreted unchanged or as glucuronide and sulphate conjugates. Extensive oxidation, mainly at the methyl group, also occurred, giving rise to derivatives of benzyl alcohol, 2-phenyl propanol and their corresponding carboxylic acid. Ring hydroxylation produced only a minor metabolite (Austgulen *et al.*, 1987).
Absorbed from the intestinal tract and excreted unchanged and as glucuronide (species unspecified) (Martindale, 1993).

Toxicological data

Local effects

Irritating to gastric mucosa. Fats and alcohol increase absorption and aggravate symptoms (species unspecified) (Martindale, 1993).

Sensitisation and intolerance

A conctact allergy developed when thymol reacted with the degradation products of a triazine derivative, both present as preservatives in a heparinoid cream.

Acute toxicity

Oral.- LD_{50}: 980 mg/kg in rat (Jenner, 1964), 640 mg/kg in mouse (Richardson, 1992), 250 mg/kg in cat (Saunders, 1959), 750 mg/kg in rabbit (Saunders, 1959).
Subcutaneous.- LD_{50}: 800 mg/kg in mouse (Saunders, 1959), 1600 mg/kg in rat (Richardson, 1992).
Intravenous.- LD_{50}: 60 mg/kg in rabbit (Saunders, 1959), 100 mg/kg in mouse (Richardson, 1992), 150 mg/kg in dog (Richardson, 1992).

Sub-acute toxicity

Oral.- 19-weeks rat study: NEL 500 mg/Kg b.w. day (Hagan *et al.*, 1967).

In vitro toxicity

Growth of V79 cells was completely inhibited by 30 µg/l for 24-48 hrs. Cells exposed to 30-300 µg/l for 2 hrs showed concentration dependent inhibition of DNA, RNA and protein synthesis (Richardson, 1992).

Thymol antioxidant activity was investigated using human aortic endothelial cells (HAEC) to mediate the oxidation of low-density lipoprotein (LDL) (Pearson *et al.*, 1997). Thymol (1.25-10µM) inhibited LDL oxidation in this culture system, in a dose-dependent manner.

The concentration of structurally diverse isoprenoids required to inhibit the increase in a population of murine B16(F10) melanoma cells during a 48-h incubation by 50% has been determined (He *et al.*, 1997). IC_{50} value for thymol was 120µM.

Genotoxicity and mutagenicity

Thymol induced sister chromatid exchanges in syrian hamster embryo cells (Richardson, 1992). Laboratory animals treated with thymol for 24 hrs showed morphological cell transformation initiated by DNA synthesis (Richardson, 1992).

S. Typhimurium TA 97 +/- metabolic activation (S9): negative (Azizan and Blevins, 1995).

Embryotoxicity and teratogenicity

Immature female rats were administered 1 mg/rat/day for 4 days with 10 immunising units of gonadotrophic hormone on day 2nd by subcutaneous injection. Animals were sacrificed on day 5 and showed no statistically significant increase in ovarian weight but a significant increase in uterine weight (Richardson, 1992).

Ecotoxicity

LC_{50}: 7.5 mg/l in salmon (estimated; duration unspecified).

EC_{50}: 3 mg/l in Photobacterium phosphoreum (Heil & Lindsay, 1989; Richardson, 1992).

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Abbreviations:

ADI: Acceptable Daily Intake
CE: Council of Europe
    List A: Flavouring substances which may be used in foodstuffs.
    List B: Flavouring substances for which further information is required before the Committee of Experts is able to offer a firm opinion on their safety-in-use.
EPA: U.S. Environmental Protection Agency
FAO: Food and Agriculture Organization
FDA: Food and Drug Administration
FEMA: Flavouring Extract Manufacturers' Association
GRAS: Generally Recognised As Safe
IARC: Intenational Agency for Research on Cancer
    Group 1:   The agent is carcinogenic to humans
    Group 2A: The agent is probabily carcinogenic to humans
    Group 2B: The agent is possibly carcinogenic to humans
    Group 3:   The agent is not classificable as to its carcinogenicity to humans
    Group 4:   The agent is probabily not carcinogenic to humans
NEL: No Effect Level
QSAR: Quantitative Structure-Activity Relationship
TSCA: Toxic Substance Control Act
WHO: World Health Organization