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Up to date review of toxicological data of some plant volatiles with antifungal activity

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1999, 63 p. Rapporti ISTISAN 99/14

Toxicological cards of the essential oil caraway and of ten volatiles compounds components of essential oils, all with fungicide activity, are presented: carvacrol, D-carvone, cinnamaldehyde, 1,8-cineole, p-cymene, decanal, eugenol, 2-hexenal, linalool and thymol. The cards have been prepared in the context of the EU project FAIR1-CT95-0722, which has the aim of implementing the use of natural pesticides for plant protection during cultivation and storage. Literature data have been collected from 1980, screening different archives (BIOSIS, CAB, CHEMABS, RTECS, MEDLINE) and Council of Europe classifications.

Key words: Antifungal activity, essential oils, plants volatiles compounds.

Istituto Superiore di Sanità
Rassegna aggiornata di dati tossicologici di alcune sostanze volatili con attività fungicida.
Paola Bonsi, Massimo De Vincenzi, Anna Stammati e Flavia Zucco
1999, 63 p. Rapporti ISTISAN 99/14 (in inglese)

Contiene schede tossicologiche di un olio essenziale (caraway oil) e di dieci sostanze volatili, componenti di olii essenziali e tutti con attività fungicida: carvacrol, D-carvone, cinnamaldehyde, 1,8-cineole, p-cymene, decanal, eugenol, 2-hexenal, linalool e thymol. Le schede sono state preparate nell'ambito del progetto europeo FAIR1-CT95-0722, che ha lo scopo di sviluppare l'uso di fungicidi naturali per la protezione delle piante durante la coltivazione e lo stoccaggio. Sono stati raccolti i dati della letteratura dal 1980 ad oggi, consultando diversi archivi (BIOSIS, CAB, CHEMABS, RTECS, MEDLINE) e le classificazioni del Consiglio d'Europa.

Parole chiave: Olii essenziali, fungicidi naturali, sostanze volatili delle piante.

The toxicological cards included in this report have been prepared in the context of the European Project FAIR1-CT95-0722 "Production, processing and practical application of natural antifungal crop protectants".
Italian Group Leader: Dott.ssa Annalaura Stammati.

CONTENTS

INTRODUCTION

The curative and flavouring capacity of some plants was known since the ancient times when Hippocrates recommended: "Let your food be your medicine...Let your medicine be your food". The empiric use of some plants in medicine has then been clarified after a more precise knowledge of the active ingredients and their biological activities. Nevertheless, after the II world war, with the explosion of the chemical industry, this type of natural approach has been abandoned by most people, also because of the faster results obtained with the synthetic compounds.

Near the threshold of 2000 year it seems to assist to a slow change in the course, due to a better knowledge of the side effects of drugs on human health and to the feeling that natural products are "good". Even if most of spices are on the Food and Drug Administration's GRAS (Generally Recognised as Safe) list, it is very well known that some herbs and medicinal plants are dangerous, thus rendering very important the knowledge of their biological activity. Moreover, it must be taken into account that all herbs and spices vary in their composition and that they contain hundred of compounds with different effects (1). In order to face this problem, an European project, NETTOX, (coordinated by a Danish laboratory) has been carried out from 1995 to 1997 in order to: a) work out a structure and organisation of a future European database on critically assessed compositional and toxicological information for inherent food plant toxicants; b) establish a network of scientists with special interest in inherent food plant toxicants; c) initiate risk assessments on the most important European food plant toxicants and d) discuss the possible reduction of risk by modern breeding and processing methods, taking into consideration agronomic and organoleptic aspects. Two people from the ISS Toxicology and Ecotoxicology laboratory took part in this project which was divided in three research groups: toxicological evaluation/risk assessment, plant/food composition/human exposure, information system/quality criteria. The results of the project are reported in three position papers of the groups (2).

Beside the pharmacological, also the flavouring properties of herbs, spices and plants are known since many years together with the antioxidative and antimicrobial ones (3).

More recent is the application of natural products to the protection of plants from pests, in particular from insects, weeds and diseases, the latter mainly due to viruses, bacteria and fungi (4). In fact, due to the growing concern for the environment and to the growing resistance of pests to the synthetic pesticides (5) used in the last 40-50 years, a large number of natural products have been screened for their potentiality against different plant diseases, also because these natural products are at least more biodegradable than synthetic ones (6).

Among them, essential oils extracted from plants as a mixture of different compounds have been shown to exhibit a range of biological activities, including antibacterial and antifungal activity (3). Aldehydes and to a lesser extent ketones, appear to be the most potent natural fungicides, the degree of the antifungal activity being probably related to the chain lenght of aliphatic volatiles and to the presence, position and nature of the functional groups in the molecule (7). Many plant volatiles have been shown to effectively inhibit the growth of fungal storage pathogen both *in*

vivo and *in vitro* on various crops (8,9,10). Moreover, due to their volatility, they have the advantage to cause less risk for human health for which concerns the presence in the treated plants of residues which could be assumed through the food. Nevertheless, while it is clear that the environment is more protected by the use of natural compounds, it is worthwhile to carefully check for their potential toxicity in the light of the growing exposure of human beings, due to the increasing application of such compounds.

Problems related to pesticides are followed with great attention by OECD and in 1994 a three year poject has been established that includes projects on data requirements for biological pesticides, test guidelines, hazard assessment, re-registration and risk reduction. During the 1996 Pesticide Forum the development of new environmentally friendly pest control products was encouraged and at the February 1998 Pesticide Forum it was agreed that OECD countries should work together on the harmonisation of data requirements for micro-organisms and pheromones.

An European project (FAIR1-CT95-0722)(BIOSIS, CAB, CHEMABS, RTECS, MEDLINE) started on January 1996 (ending on December 1999) with the aim of implementing natural, antifungal agents for the effective control of fungal pests of seeds, grains, tubers, roots, bulbs and plantlets during storage and cultivation. Agents derived either from plants, or from micro-organisms are under examination. The project coordinated by a group from The Netherlands includes 7 european laboratories, 3 from industry: Luxan (NL), Prophyta (D) and Kemira (NL) and 4 public Institutions: Agrotechnological Research Institute (NL), Institut fur Lebensmitteltechnologie (D), VTT Technical Research Centre (F) and Istituto Superiore di Sanità (I).

The italian group which is the only one with toxicological competences, has the following tasks: 1) to investigate the literature and collect the available toxicological data concerning the green chemicals under study; 2) to perform preliminary *in vitro* toxicity testing of the green chemicals selected on the base of their efficacy and of the formulated preparations.

A paper has been prepared with the first cytotoxicity and genotoxicity data on four plant volatiles (cinnamaldehyde, carvacrol, thymol and carvone) obtained by the ISS group in collaboration with two groups from Finland and The Netherlands and is now in press in Food and Chemical Toxicology (11).

All the available toxicological data present in the literature on .the plant volatiles of interest for a possible application (caraway oil, carvacrol, d-carvone, cinnamaldehyde,p-cymene, decanal, eugenol, E-2-hexenal, linalool, thymol) have been collected and will be the object of this report. The literature has been screened since 1980 by consulting different files (BIOSIS, CAB, CHEMABS, RTECS, MEDLINE). Moreover, the classifications of the Council of Europe of the selected compounds have also been consulted, when availble.

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CARAWAY OIL

Synonyms

Kümmelöl; Oleum cari; Oleum Carui; Oleum Carvi

Official classification

ADI: an acceptable daily intake of up to 1mg per kg bw was set for (+)-carvone. No ADI was allocated for (–)-carvone (FAO/WHO, 1991);
CE: list N1 (1970);
FDA: GRAS list (CFR, 1984).

Natural occurrence

Several data about caraway oil have been reviewed by Lawrence in 1996. Putievsky and coll. (1994) compared the chemical composition of caraway oil produced from seed of various origins; the main components of caraway oil were limonene (33.8-46%) and carvone (46.7-62.3%). Ravid and coll. (1992) determined that the enantiomeric distribution of carvone (51.1-82.4%) in caraway seeds and oil was >99% (S)-(+)-carvone and trace (R)-(–)-carvone. More recently, Bouwmeester and coll. (1995) determined the enantiomeric ratio of limonene, carvone, cis-carveol, and trans-carveol in a number of samples of caraway seed oil and extract. Bourrel and coll. (1995) reported the results of the analysis of a sample of caraway oil, used in an antimicrobial screen study, by GC/MS and GC.

Metabolism

Carvone and limonene induce the detoxifying enzyme glutathione S-transferase in several mouse target tissues (Zheng *et al.*, 1992).

Toxicological Data

Toxicological data about caraway oil itself are very old; for recent toxicological information refer to caraway oil components.

Local effects

Skin irritation.

Non-human.- Undiluted caraway oil applied to the backs of hairless mice produced no irritating effects (Opdyke, 1973).

Caraway oil applied full strength to intact or abraded rabbit skin was irritating (Opdyke, 1973).

Tested at a concentration of 4% in petrolatum, it produced no irritation in a 48-hr closed-patch test in 25 human subjects (Opdyke, 1973).

Other local effects

Non-human.- Low-level phototoxic effect have been reported for caraway oil, but these are not considered significant (Opdyke, 1973).

Sensitisation and intolerance

Human.- A maximisation test (Kligman, 1966) was carried out on 25 volunteers. The material was tested at a concentration of 4% in petrolatum and produced no sensitisation reactions (Opdyke, 1973).

Acute toxicity

Non-human.- Oral.- LD_{50} rat: 3.5 ml/kg bw (Opdyke, 1973);
Dermal.- LD_{50} rabbit: 1.78 ml/kg bw (Opdyke, 1973).

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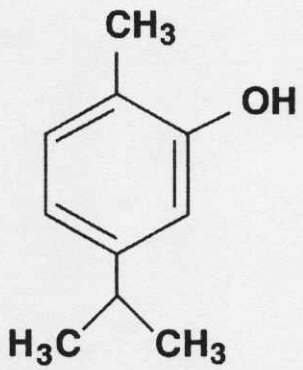
CARVACROL

Chemical formula

$C_{10}H_{14}O$



Synonyms

Antioxine; o-cresol; o-thymol; isothymol; phenol, 3-isopropyl-6-methyl; 2-methyl-5-isopropylphenol; 5-isopropyl-p-cymene; 2-hydroxy-p-cymene.

CAS registry number

499-75-2

Official classification

CE: List B (CE, 1992).

Natural occurrence

Cranberry: trace; beer: 0.2-2 mg/kg (CE, 1992).

Metabolism

Carvacrol administered to male albino rats by gavage, at dose 1 mmol/Kg was excreted mainly in the urine after 24 hrs. Large quantities of 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). Carvacrol appears to be slowly absorbed from intestine in rabbit, since 22 hr after administration of 1.5g some 30% was still in gastrointestinal tract, about 25 % of dose having been excreted in that time in urine (William, 1959). Carvacrol applied to intact shaved abdominal skin of mouse was not absorbed within 2 hrs (Meyer & Meyer, 1959).

TOXICOLOGICAL DATA

Acute toxicity

Oral.- LD 50: 100 mg/kg in rabbits (Budavari, 1989). Depression within 10 min. and coma within 1 hr in rats: 1640 mg/kg (Jenner, 1964).

In vitro toxicity

Moderate cytotoxic effects in HeLa cells (Stoichev *et al.*, 1967). In Bovine seminal vescicles microsomes, at 4.1 mg/ml, carvacrol inhibits by 50% the formation of prostaglandines E1. Papaverine-like antispasmodic action on the isolated mouse small intestine (Burstein *et al.*, 1975).

Carvacrol antioxidant activity was investigated using human aortic endothelial cells (HAEC) to mediate the oxidation of low-density lipoprotein (LDL) (Pearson *et al.*, 1997). Carvacrol (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 carvacrol was 120μM. 50μM carvacrol with 75μM β-ionone, another isoprenoid, has less effect than the sum of the individual effects.

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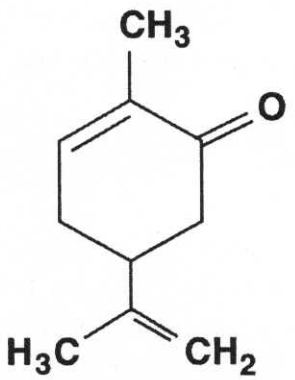
D-CARVONE

Chemical formula

$C_{10}H_{14}O$



Synonyms

D-*p*-mentha-6,8,(9)-dien-2-one;2-methyl-5-(1-methylethenyl)-2-cyclohexen -1-one; 5-isopropenyl-2-methyl-2-cyclohexen-1-one.

CAS registry number

99-49-0

Official classification

CE: List A (CE, 1992).
WHO.ADI: 1 mg/Kg b.w. (NTPRTD, 1992).

Natural occurrence

Constituent of caraway (*Carum carvi*) seed and dill (*Anethum graveolens*) seed oils. Also in oils of *Lippia carviodora*, *Orthodon carvoriferum* and *Artemisia* spp.

Metabolism

Carvone is metabolised by rabbits to carbinol and 1,5-dimethyl-1,5 -hexadien-1,6-dicarboxylic acid (Opdyke, 1973).

TOXICOLOGICAL DATA

Acute toxicity

Oral.- LD 50: 1640 mg/kg in rats. The death time was 1 hr-3 days (Jenner, 1964).

Sub-acute and sub-chronic toxicity

Sixteen-day study 1,600 or 3,500 mg/kg in mice: death within 7 days with increase of relative liver weight for dosed male mice and decrease of relative thymus weights for dosed female mice. No compound-related lesions were observed (Hagan *et al.*, 1967).
Thirteen-week study in male mice and 9/10 female mice: top dose 1,500 mg/kg: death before the end of the study. No compound-related histopathologic changes were observed (Hagan *et al.*, 1967).

Chronic toxicity

Sixteen weeks studies at 10000 ppm in rats: growth retardation and testicular atrophy.
Twenty-six/twenty-seven weeks and one year study in rats at 2500 ppm and at 1000 ppm: no effect (Hagan *et al.*, 1967).
Two years study at 375 or 750 mg/kg in corn oil, 5 day/wk in rats: survival of 37/50, 42/50, 36/50 (Hagan *et al.*, 1967).
d-Carvone is considered to have insecticidal properties (Lichtenstein *et al.*, 1974). When used at sublethal dosages, d-carvone increased the toxicity of carbaryl, carbofuran, and parathion to insects and is therefore considered synergstic for carbamate and organophosphorus insecticides (Fuhremann *et al.*, 1978).

Carcinogenicity

No neoplastic lesions attributed to d-carvone dosing were observed in mice (NTPRTD, 1992).

Genotoxicity and mutagenicity

Carvone shows no genotoxic activity on *Drosophila melanogaster*, in the concentration range of its insecticidal activity. Both toxic and genotoxic activities of aromatic plant essential oils are not in accordance with those of their major constituents, due to synergistic/antagonistic phenomena (Franzios *et al.*, 1997).

Mammalian cells in vitro.- SCE and chromosomal aberrations in chinese hamster ovary cells +/- metabolic activation (S9): positive (Galloway *et al.*, 1985).

Bacterial assays.- Salmonella typhimurium TA 98-100-1535-1537 +/- metabolic activation (S9): negative (Haworth, 1983).

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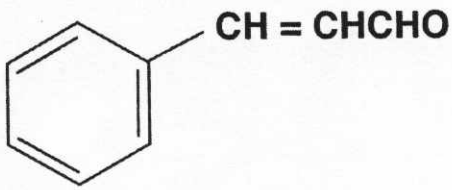
CINNAMALDEHYDE

Chemical formula

C_9H_8O



Synonyms

Cinnamic aldehyde, 3-phenylacrolein.

CAS registry number

104-55-2

Official classification

ADI: for total cinnamic compounds: 1.25 mg/kg (CE, 1992).
CE: List A (CE, 1992).
WHO. ADI: 0.7 mg/kg bw/day (WHO, 1984).
FDA: approved for food use (Opdyke, 1979).

Natural occurrence

Constituent of cinnamon and cassia oils.
Cranberry: 0.001mg/kg; bilberry: 0.0002mg/kg (CE, 1992).

Metabolism

At the dose of 2 and 250 mg/kg bw by i.p. injection (male and female Fisher 344 rats and CD1 mice) and at 250 mg/kg by oral gavage in male rats and mice, 94% of the administered dose was recovered in the execrete after 72 hrs in both species, 75-81% present in the urine, mainly as hippuric acid, 3-hydroxy-3-phenylpropionic acid, benzoic acid and benzoyl glucuronide (Peters, 1994).
In Fisher 344 rats, (i.v. administration), a large fraction of C. was oxidized to cinnamic acid (biological half-life: 1,7 hr). After administration by gavage at 200-500 mg/kg bw using corn oil as vehicle, the maximal blood concentrations were in order of 1 µg/ml and were maintained over a 24 hr period. The majority of C. administered orally was escreted in urine as hippuric acid within 24 hrs (Yuan, 1992).

TOXICOLOGICAL DATA

Sensitisation and intolerance

A panel of 86 volunteers has been tested for their capacity to develop non-immunologic contact urticaria using chemicals of different structural type and urticant ability, including 125 mM cinnamaldehyde in petrolatum (Coverly *et al.*, 1998).

Acute toxicity

Oral.- LD_{50}: 2220 mg/kg in rats; causes depression, diarrhoea and scrawny appearance (Jenner, 1964).

Sub-acute toxicity

At 10000 ppm in rats (16 days study): slight hepatic cell swelling and slight hyperkeratosis in the squamous portion of the stomach. No effect at 2500 and 1000 ppm (Hagan *et al.*, 1967).
Rats and mice of both sexes received C. (0-3000 mg/ kg b.w. for rats and 0-10000 mg /kg b.w. for mice) by daily oil gavage for 2 wks, or in micro-encapsulated form (0-10% C.) in feed (2 wks for rats, 3 wks for mice). Gavage doses of 2620 mg/kg/day and above in mice and 940 mg/kg/day and above in rats produced nearly 100% mortality; there is no deaths in animals receiving microencapsulated C. The use of microencapsulation allows the delivery of higher net doses of chemicals, with a lower toxicity, moreover, this kind of exposition is more close to that of humans (Hebert, 1994).

In vitro toxicity

50% growth inhibition of L1210 mouse leukemia cells at 4.8 µg/ml. Inhibition of DNA, RNA and protein syntesis (Moon, 1983).

ID_{50} of cinnamic acid on KB cells at 72 hrs: 19.50 µg/ml (Mochida, 1988).
Cinnamaldehyde has been shown to inactivate glutathione reductase (Jagt *et al.*, 1997). 500 µM cinnamaldehyde inactivated glutathione reductase activity by 50% in 20' in the presence of NADPH. In the absence of NADPH cinnamaldehyde produced a slow inactivation of glutathione reductase. Thus, there are NADPH-dependent and –independent pathways of inactivation of glutathione reductase by cinnamaldehyde and other α,β-unsaturated aldehydes considered in this study.
The growth-inhibiting activity of *Cinnamomun cassia* bark-derived materials toward five human intestinal bacteria has been examined using an impregnated paper disk method and compared with that of four commercially available compounds (Lee and Ahn, 1998). The biologically active component of C. cassia bark was characterised as cinnamaldehyde by spectral analysis. At 1 and 0.5 mg/disc, cinnamaldehyde revealed potent inhibition against *Clostridium perfrigens* and *Bacteroides fragilis*, also growth of *Bifidobacterium bifidum* was significantly inhibited, whereas weak or no inhibitory activity was obtained against *Bifidobacterium longum* or *Lactobacillus acidophilus*. Little or no inhibition was observed after treatment with eugenol.

Genotoxicity and mutagenicity

Data on genotoxicity and mutagenicity of cinnamaldehyde are controversial. In fact cinnamaldehyde has been reported to be mutagenic in several bacterial, insect, and mammalian test systems (Galloway *et al.*, 1987; Ishidate *et al.*, 1984; Palmer, 1984; Woodruff *et al.*, 1985; Galli *et al.*, 1992; Neudecher, 1992) but also non-mutagenic or even antimutagenic (Generoso *et al.*, 1986; Prival *et al.*, 1982; Azizan & Blevins, 1995; Galli *et al.*, 1992; Ishidate *et al.*, 1984; Neudecher, 1992; Marnett *et al.*, 1985; Ohta *et al.*, 1983). Some of the studies are summarized in Table 1.
The effects of dietary bioantimutagens on spontaneous and heterocyclic amine-induced micronucleus frequencies have been studied in metabolically competent human hepatoma Hep-G2 cells (Sanyal *et al.*, 1997). 500 µg/ml cinnamaldehyde caused a moderate increase of micronuclei numbers in Hep-G2 cells. 5 µg/ml cinnamaldehyde caused only a moderate reduction of heterocyclic amine-induced micronucleus frequencies (50%) and in this case the inhibitory effect increased with the exposure concentration
In rats and in mice micronucleus test was positive in the liver with high doses of C. while the increase of micronucleated cells was minimum in rats and absent in mice forestomach mucosa (Mereto *et al.*, 1994). Micronucleus test on mice: negative (Hayashi *et al.*, 1988).

Embryotoxicity and teratogenicity

Cinnamaldehyde is toxic in rat embryos at doses which do not affect the mother (Abramovici & Rachmuth-Roizman, 1983); teratogenic effect in the chick embryo (Mantovani *et al.*, 1989).

Table 1. – Genotoxicity and mutagenicity studies on cinnamaldehyde

Test	Strain	Metabolic activation	Result	Reference
Ames	*S.typhimurium* TA 97, TA 98, TA 100	+/- (S9)	-	Azizan & Blevins, 1995
Ames	*S.typhimurium* TA 98, TA 100	+/-	-	Galli *et al.*, 1992
Ames	*S.typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538	+/- (S9)	-	Galli *et al.*, 1992
Ames (modified)	*S.typhimurium* TA 104	+/- (S9)	-	Galli *et al.*, 1992
Ames (modified)	*S.typhimurium* TA 100	+/- (S9)	-	Galli *et al.*, 1992
Liquid preincubation assay	*Saccaromyces cerevisiae*		-	Galli *et al.*, 1992
Reversion test	*Drosophila*		-	Galli *et al.*, 1992
DNA repair test (Rec assay)	*Drosophila*		+	Galli *et al.*, 1992
UV-induced gene conversion and point mutation test	Chinese hamster fibroblasts		-	Galli *et al.*, 1992
Drosophila sex-linked recessive lethal mutation test	V 79		+	Ishidate *et al.*, 1984
Drosophila reciprocal translocation test	L 5178Y/TK+/- cells		-	Ishidate *et al.*, 1984
Chromosomal aberration			+	Ishidate *et al.*, 1984
HGPRT test			-	Neudecher, 1992
Trifluorothymidine-resistant colonies			+ (chromosomes 11 and 14)	Neudecher, 1992

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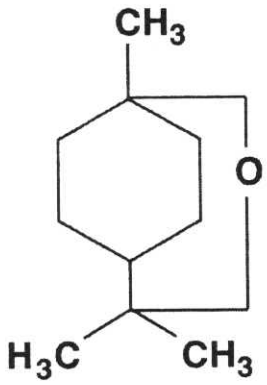
1,8-CINEOLE

Chemical formula

$C_{10}H_{18}O$



Synonyms

Eucalyptol, Limonene oxide; 1,8-epoxy-p-menthane.

CAS registry number

470-82-6

Official classification

CE: List B (CE, 1992);
upper level (CE, 1992): 0.1 mg/kg (beverages), 5 mg/kg (food); except: candy, confectionery (15 mg/kg); alcoholic beverages (50 mg/kg).
FACC (UK): Upper level of 20 ppm in food (FACC, 1976).
FDA: 1,8-cineole is safe for a variety of purposes, but lacks evidence of effectiveness (FDA, 1976, 1982a,b, 1987 & 1990).

Natural occurrence

1,8-Cineole occurs in eucalyptus, lavender and many other oils.
Grapefruit juice: 0.09 mg/kg; black currant: 0.1mg/kg; cranberry: trace; bilberry: trace 0.005mg /kg; nutmeg oil: 20,000-40,000 mg/kg (CE, 1992).

Metabolism

Eucalyptol undergoes oxidation *in vivo* with the formation of hydroxycineole, which is excreted as hydroxycineoleglucuronic acid (Williams, 1959).

TOXICOLOGICAL DATA

Local effects

Skin Irritation.

Human.- No irritation occurred in 25 subjects given a 48-hr covered application of 16% in petrolatum (Opdyke, 1975).
Pure 1,8-cineole in concentrations of 3.8, 8.0, 12.0, 16.0, 19.9, 24.0 and 28.1% in soft white paraffin did not produce skin irritancy when tested by occlusive patch on 25 human subjects; similarly, eight tea tree oil preparations containing 1.5, 3.1, 5.7, 10.4, 15.0, 18.4, 24.4 and 28.8% cineole did not produce skin irritancy when tested as 25% formulations in soft white paraffin on 25 human subjects (Southwell *et al.*, 1996).

Non-human.- When applied uncovered to the shaved skin of four guinea-pigs for 24 hr, the highest concentration causing no irritation was reported to be a 50% solution (Sharp, 1978). Undiluted eucalyptol was not irritant to intact or abraded rabbit skin when applied under cover for 24 hr (Opdyke, 1975).
There were no signs of eye irritation in 63 subjects exposed for 8 hr to the vapour from a mixture containing 1.7 % eucalyptol placed in a hot steam vaporiser (FDA, 1976).

Other local effects

Human.- Nasal irritation was observed in four of nine infants after the accidental nasal instillation of eucalyptol drops (Melis *et al.*, 1989). However, no nasal or pharyngeal mucosal irritation was noted in 63 subjects exposed for 8 hr to the vapour from a mixture containing 1.7% eucalyptol placed in a hot steam vaporiser (FDA, 1976).

Non-human.- When 5% eucalyptol in liquid petrolatum was sprayed on to the nasal mucosa of rabbits, daily for up to 9 months, tissue changes in the nose and lungs have been recorded (Fox, 1930).
Intradermal injection of a 0.25% solution into four guinea-pigs produced slight irritation 24 hr later (Sharp, 1978).

Sensitisation and intolerance

Human.- Of 20 people allergic to turpentine, four also reacted to 10% eucalyptol in oil (Michailov *et al.*, 1970). Application of 16% in petrolatum in 25 normal volunteers, provoked no local reactions indicative of sensitisation (Opdyke, 1975). [The maximisation test procedure used involved an initial induction phase of five 48-hr covered patch tests, followed 10-14 days later by a 48-hr covered challenge patch (Kligman, 1966; Kligman & Epstein, 1975.)]

Contact dermatitis reactions to tea tree oil have been reported. In one of these investigations, De Groot and Weyland (1992) claimed that the allergen for a man who had ingested tea tree oil was eucalyptol. Knight and Hausen (1994), as well as Southwell and colleagues (1996) described several patients allergic to tea tree oil, none of whom gave positive reactions to eucalyptol.

Non-human.- Sensitisation was not induced in ten guinea-pigs treated by intradermal injection followed 14 days later by a further injection and skin application (Sharp, 1978).

Acute toxicity

Human.- *Inhalation.*- After the accidental nasal instillation of eucalyptol drops one of nine infants developed a rapid heart beat (Melis *et al.*, 1989). A baby whose face was sprayed with a medicated aerosol exhibited coughing, rapid breathing and heart rate, and pneumonitis, that cleared after 3 days. The aerosol apparently contained eucalyptol, camphor, menthol, thymol and naphthalene [all at unspecified concentrations], and the role of the different constituents was not elucidated (Krueger, 1967).

No effects on blood cell count or urine analysis were detected in 63 subjects exposed for 8 hr to the vapour from a mixture containing 1.7% eucalyptol placed in a hot steam vaporiser, and liver and kidney function tests in 25 of the subjects revealed no deleterious effects (FDA, 1976).

Some improvements in respiratory function occurred in 24 adults with colds who inhaled for 20 or 60 min eucalyptol concentrations of 18.2 or 18.9 mg/m^3, respectively, in a mixture with other fragrant vapours (Cohen & Dressler, 1982).

Non-human.- *Oral.*- Rat LD_{50}: 2480 mg/kg bw (Jenner *et al.*, 1964).

A lethal dose in rats caused rapid cyanosis and stupor followed by irregular breathing, extreme sensitivity to noise, convulsions, and death from respiratory failure (Brownlee, 1940). Rats developed depression of the central nervous system and coma after a high dose and appeared scrawny for 3-4 days, but recovery from non-fatal doses occurred within 7 days (Jenner *et al.*, 1964). In rats, 462 mg / kg bw eucalyptol reduced the activity of a liver enzyme involved in cholesterol biosynthesis (Clegg *et al.*, 1980; Clegg *et al.*, 1982), and decreased the rate of sterol formation in the liver (Clegg *et al.*, 1980). Bile secretion was increased in rats given 40.1 g/kg bw (Mörsdorf, 1966).

Mice given 500 mg/kg bw displayed an increase in liver enzyme activity as indicated by a decreased pentobarbital sleeping time (Noble *et al.*, 1982).

Inhalation.- The activity of a liver enzyme was found increased when rats were exposed to an aerosol of eucalyptol (nebulized at 150 mg/min) for 5 min and then killed up to 32 days later (Hohenwallner & Klima, 1971).

Rabbits exposed to 15.4-77.1 mg vaporised eucalyptol for 80 min showed an improvement in lung compliance (a measure of inspiration volume). Electron microscopy revealed no morphological damage to the cilia of the respiratory passages (Zänker *et al.*, 1980). The lung defence systems (in particular, the rates of lung bacterial transport, inactivation and phagocytic ingestion) were unaffected in rats and mice exposed for 4 or 8 hr to vaporised eucalyptol at peak concentrations in the range 0.6-3.0 mg/m^3 (Goldstein *et al.*, 1976).

Injection.- In mice, guinea-pigs and dogs treated by the subcutaneous and intramuscular routes, LD_{50} values in the range of 1-3.5 g/kg bw have been reported (Dzhumagalieva, 1955; Northover & Verghese, 1962).

In rats treated subcutaneously with 250 or 500 mg /kg bw, liver enzyme activity was increased (as indicated by a decreased pentobarbital sleeping time and accelerated metabolism of various chemicals) and bile flow was stimulated (Jori *et al.*, 1969; Jori *et al.*, 1972a). Mice treated intraperitoneally with 300 mg/kg bw showed increased liver enzyme activity (Jarosch *et al.*, 1977) whereas 30 mg/kg bw had a sedating effect which was not seen at 15 mg/kg bw (Ortiz de Urbina *et al*, 1989).

Skin.- The acute dermal LD_{50} value in rabbits exceeded 5 g/kg (Moreno, 1972).

Subacute toxicity

Human.- Oral.- Ten patients with chronic respiratory obstruction who were given a formulation providing a total of 300 mg eucalyptol in four divided doses, daily for seven days, showed an improvement in their condition (increased mucociliary clearance) (Von Dorow *et al.*, 1987).

Inhalation.- In four of five subjects exposed to an aerosol of 0.4 ml eucalyptol for 10 min a day for 10 days, the disappearance of another chemical from the bloodstream was increased, suggesting an enhancement of liver enzyme activity (Jori *et al.*, 1970).

Skin.- Two infants developed convulsions after application of an ointment containing 0.8% eucalyptol and other substances to the skin. One was given several applications on the chest over 48 hr, receiving altogether about 0.1g eucalyptol, and the other received two applications on the waist 21 hr apart. The ointment also contained 5% guaiacol, 3% terebinth oil, 0.8% terpinol, 0.4% pine oil and 0.2% mint oil, and the role of the different constituents was not elucidated (Castot *et al.*, 1980).

Non-human.- Oral.- Rat. When rats (six of each sex per group) were given eucalyptol (either by stomach tube on 5 days/wk or in microencapsulated form in the diet) for 28 days at the average doses of 150-3516 mg/kg bw/day, male body weight gain was reduced at 600 mg/kg bw/day or more. A comprehensive tissue examination showed some evidence of damage to the liver, kidneys and parotid salivary gland in the males at doses of 381 mg/kg bw/day or more (particularly when the compound was

administered in the diet), but not at 300 mg/kg bw/day. Liver, kidney, hearth, brain, thymus, lung and testicle weights were unaffected. No adverse effects were seen in females (Wolff *et al.*, 1987a).

Three rats given 460 mg/kg bw/ day by stomach tube gradually lost weight, and died after 11-19 days, but 160 mg/kg bw/day for 34 days caused only a slight loss of weight, and no organ damage was evident at gross examination 2 wk after the end of treatment (Brownlee, 1940).

Mouse. When groups of six males and six females were fed microencapsulated eucalyptol for 28 days at average daily doses of 600, 1322, 2448 or 5607 mg/kg bw to males and 705, 1532, 3152 or 6777 mg/kg bw to females, liver weight in males was increased at all but the lowest level, and at the top dose there was an increase in brain weight in females. Kidney, heart, thymus, lung and testicle weights were unaffected. An extensive microscopic examination revealed only a minimal enlargement of liver cells in one female at 705 mg/kg bw, one male at 1322 mg/kg bw, and 4-6 animals of each sex at the two highest dose levels.

In groups of six male and six female mice given eucalyptol by stomach tube at doses of 150-1200 mg/kg bw/day, 5 days/wk for 28 days, there were no statistically significant effects on organ weights or tissue structure, apart from oesophageal and stomach lesions that were attributed to the stomach tube procedure (Wolff *et al.*, 1987b).

Cat. Repeated doses of 153 or 1530 mg/kg by stomach tube caused an immediate increase in respiratory volume and rate, a gradual decrease in blood pressure and pulse rate, followed by respiratory depression and death (Brownlee, 1940).

Inhalation.- Liver enzyme activity was enhanced (as judged by an increase in metabolism of various chemicals and a decrease in pentobarbital sleeping time) and bile flow was increased in rats exposed to an aerosol of eucalyptol for a total of about 30-90 min or more over 4 days. Industrial exposures were for periods of between 5 and 30 min, and the eucalyptol was nebulized at a rate of 50 mg/min (Jori *et al.*, 1969; Jori *et al.*, 1970; Jori *et al.*, 1972a).

In rats exposed to an aerosol of eucalyptol (nebulized at 100 mg/min) for 5 min/day for 2 days, and for 10 min/day for the next 3-6 days, a liver enzyme activity was increased, but no changes in the liver tissues were discerned following examination by electron microscope (Hohenwallner & Klima, 1971). Liver enzyme activity was also increased in rats exposed for 3-9 days to eucalyptol vapour at 1.542 mg/litre/hr (Madhava Madyastha & Chadha, 1986).

Injection.- Subcutaneous injection of 500 mg/kg bw/day for 3 or 4 days induced liver enzyme activity, increased liver weight and increased bile flow in rats (Hohenwallner & Klima, 1971; Jori & Briatico, 1973; Jori *et al.*, 1972a,b).

Subchronic toxicity

Human.- *Oral.*- Hereditary hepatic porphyria was exacerbated in a woman who over several months drank substantial quantities of a mouthwash containing less than 25% of eucalyptol [precise amount unspecified]. A further investigation suggested that eucalyptol's ability to stimulate liver enzyme activity was the major cause (Bickers *et al.*, 1975).

Chronic toxicity

Non-human.- *Oral.*- Mouse. Male mice were given eucalyptol by stomach tube at 8 or 32 mg/kg bw/day, 6 days/wk for 80 wk, and observed for a further 16-24 wk. No treatment-related effects were observed on body weight, food consumption, survival, weights of adrenals, kidneys, liver, lungs or spleen, or the microscopic appearance of the brain, lungs, liver or kidneys (Roe *et al.*, 1979).

In vitro toxicity

Concentration-dependent cytotoxicity (as measured by trypan blue exclusion) has been observed after 24 hr treatment of African Green Monkey kidney (Vero) cells with 1:500-1:4000 1,8-cineole (Sivropoulou *et al.*, 1997).

Reproductive toxicity

Non-human.-*Oral.*- A preparation containing 2% eucalyptol was given to rats on days 9 to 14 of pregnancy at dose levels providing 3, 15 or 30 mg eucalyptol/kg bw/day. Autopsy on day 20 revealed decreased placental, foetal and new-born pup weights and an apparent increase in the number of foetuses with a skeletal anomaly at the highest dose level, which also produced maternal weight loss. At the two lower levels there were no adverse effects on foetal growth and development or pup survival. The preparation also contained 32% *l*-menthol, 17% pinene, 6% menthone, 5% borneol, 5% *d*-camphene, 0.1% rheochrysidin and olive oil, but it is not clear whether the observed effects can be ascribed to a particular chemical component (Hasegawa & Toda, 1978).
Injection.- When rats were given eucalyptol at 500 mg/kg bw/day by subcutaneous injection on days 10-14 of pregnancy, or during the last 4 days of pregnancy, liver enzyme activity was increased in foetuses and offspring. Treatment of lactating mothers on days 2-6 after delivery did not significantly increase activity of either enzyme in the offspring (Jori & Briatico, 1973).

Carcinogenicity

Non-human.- *Oral.*- No increase in tumour incidence was found in groups of 52 male mice given 8 or 32 mg/kg bw/day by stomach tube, 6 days/wk, for 80 wk, and observed for a further 16-24 wk. However, microscopic examination was limited to the

brain, lungs, liver and kidney, plus all macroscopically observed tumours and abnormal growths (Roe *et al.*,1979).

Current regulatory guidelines recommend that groups of at least 50 male and 50 female mice are treated at one of several dose levels throughout their life (about 2 yr) and that a comprehensive tissue examination is undertaken.

Eucalyptol fed to 52 rats at 1% in the diet [about 500 mg/kg bw/day] for 20 wk did not significantly affect the incidence of mammary tumours induced by a known carcinogen (Russin *et al.*, 1989).

Dermal.- No skin tumours developed in 55 mice when their skin was painted with a mixture of seven cyclic terpenes including eucalyptol [level unspecified] twice weekly for 4.5 or 6 months. When 55 mice were alternately painted with the terpene mixture and a known skin carcinogen both twice weekly for 4.5 or 6 months, the potency of the carcinogen was diminished (Benko *et al.*, 1963).

Genotoxicity and mutagenicity

Mammalian cells in vitro.- In Chinese hamster ovary cells eucalyptol induced chromosomal effects (sister chromatid exchanges) only in the absence of metabolic activation, at doses that caused cell cycle delay. [It is possible that these effects may have been secondary to a general toxic action.] Eucalyptol did not induce chromosome damage, with or without a liver metabolic activation system (Galloway *et al.*, 1987). In another study in Chinese hamster ovary cells, it did not increase the incidence of sister chromatid exchanges induced by prior treatment with a known mutagen (Sasaki *et al.*, 1989).

Bacterial assays.- Eucalyptol gave no evidence of activity, both in the presence and absence of a liver metabolic activation system, in Ames mutagenicity tests with *Salmonella typhimurium* (Haworth *et al.*, 1983; Haley, 1982) and in a rec assay for DNA damage in *Bacillus subtilis* (Haley, 1982; Yoo, 1985).

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