METHYL CINNAMATE

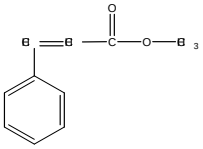
SYNONYMS

Cinnamic acid, methyl ester
Methyl 3-phenyl propenoate
Methyl cinnamylate
Methyl 3-phenyl-2-propenoate
Methyl 3-phenylacrylate
Methyl cinnamate

CHEMICAL FORMULA

$C_6H_{10}O_2$

CHEMICAL STRUCTURE



IDENTIFIER DETAILS

CAS Number	:	103-26-4
CoE Number	:	333
FEMA	:	2698
EINECS Number	:	203-093-8
E Number	:	-

SPECIFICATIONS

Melting Point: 34-38^{0}C
Boiling point: 261-262^{0}C

PURPOSE

Flavouring substance.

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
2	10	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
Acceptable	JECFA	2000	No safety concern at current levels of intake when used as a flavouring agent.

FDA Status:[CFR21]

Section Number	Comments
172.515	Synthetic flavouring substances and adjuvants permitted for direct addition to food for human consumption.

HUMAN EXPOSURE

Natural Occurrence: Methyl cinnamate is reportedly found in the oil from rhizomes of *Alpinia malaccensis* ; in the oil from leaves of *Ocimum canum* Sims, in the oil of *Narcissus jonquilla* L and in the oil from rhizomes of *Gastrochilus panduratum* of which two isomers (*cis- and trans-*) are known [Fenaroli, 1995] . Also reported to be found in cranberry, guava, pineapple, strawberry fruit and jam, cinnamon leaf, C amembert che eses, cocoa, avocado, plum, prun e, cloudberry, starfruit, plum brandy, rhubarb, beli (*Agele marmelos Correa*), loquat and bourbon vanilla [Fenaroli 2005].

Reported Uses: Methyl cinnamate is reportedly used in baked goods at 12.22 ppm, frozen dairy at 7.61 ppm, meat products at 5.0 ppm, soft candy at 11.39 ppm, gelatin pudding at 7.39 ppm, non-alcoholic beverages at 2.09 ppm, alcoholic beverages at 2.55 ppm, hard candy at 0.16 ppm, and chewing gum at 34.18 ppm [Fenaroli, 2005].

Sources other than foods: Cinnamyl compounds are fundamental to plant biochemistry, being required for the formation of lignin in plants, and are consequently ubiquitous throughout the plant kingdom [Goodwin *et al.,* 1972].

TOXICITY DATA

Carmines (2002), Rustemeier *et al* ., (2002), Roemer *et al* ., (2002) and Vanscheeuwijck *et al.,* (2002) reported on a testing program designed to evaluate the potential effects of 3 33 ingredients added to typical commercial blended test cigarettes on selected biol ogical and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], determin ation of smoke chemical constituents and a 90-day rat inhalation study. Based on the findings of these studies, the authors concluded that the addition of the combined ingredients, including methyl cinnamate at levels up to 1 ppm, "did not increase the overall toxicity of cigarette smoke" [Carmines, 2002].

In Vivo Toxicity Status

LD_{50}	Rat (oral)	2610 mg/kg	[Lewis 2000].
LD_{50}	Rabbit (skin)	>5gm/kg	[ToxNet, 2010]

Methyl cinnamate has been shown to be rapidly absorbed in the gut of rats dosed at 240 mg/kg/bw. At no time did the authors find more than 5% of the dose detected in the lower gut of rats and rabbits. No more than 9% of the total dose was recovered as cinnamic acid [the hydrolysis product of methyl cinnamate] in the stomach and 40% in the gut. The rate of absorption of cinnamic acid and methyl cinnamate was found to be similar. No ester was found in the peripheral blood of either rats or rabbits. However, cinnamic acid and menthol were de tected in the circulating blood of both dosed rats and rabbits. In both rats and mice dosed at 50 mg/kg/ bw 66% of the methyl cinnamate was excreted as he benzoic acid glycine conjugate [Fahelbum *et al.,* 1977]. Methyl cinnamate has been shown to conjugat e with glutathione to form mercapturic acid metabolites in female rats [Delbressin *et al.,* 1982],

being excreted primarily in the urine and to a minor extent in the faeces [JECFA 2001].

Pollitt (1974), administered radio labelled 3-phenyl-propionoic aci d to a single healthy human volunteer, the author reported that 110% of the dose was recovered as the benzoic acid from the urine within 100 minutes.

A single administration of 50 mg/kg/bw of methyl cinnamate to a healthy human was found to be excreted 66 % as hippuric acid and 5% as benzoylglucoronide [Fahelbum *et al.,* 1977].

Methyl cinnamate administered to rats at 25 mg/kg bw as part of a flavourings mixture containing predominantly cinnamaldehyde [900 mg/kg] and other derivatives was fed in the diet fo r 12 Weeks. There was found to be a statistically significant decrease in food consumption for both males and females when compared with controls. There were no limited organ weight differences between the treated group and the controls [liver kidney and b rain] with no macroscopic findings. Microscopic findings were not reported [Trubeck Laboratories, 1958].

Male and female Sprague-Dawley albino rats (5/dose) with initial body weights of 150–250 g were dosed via gavage with methyl cinnamate at dose levels up to 6.0 g/kg/bodyweight. Methyl cinnamate was administered as a 50% solution or in corn oil. Observations were made 1 – 4 h rs post dose and once daily thereafter for 14 days. Gross necropsy was conducted on all animals. Clinical signs observed during th e study included a decrease in respiration at a dose of 3.16 g/kg and higher. Necropsy revealed fluid filled stomachs of the animals who died 24 h after dosing. Gross observations at necropsy were normal for all other animals. The LD_{50} was calculated to be 2.61 g/kg (95% C.I. 2.00 – 3.41 g/kg [Bhatia *et al.,* 2007].

Carcinogenicity and Mutagenicity

Methyl cinnamate derivatives have been shown to enhance UV-induced DNA damage; enhancing mutagenesis by the inhibition of DNA excision repair enzymes in *Escherichia coli* [B/r strain], however, methyl cinnamate it self has been reported as having no mutagenic activity [Shimoi *et al.,* 1985].

JECFA (2001), conclude that cinnamyl alcohol and related compounds lack direct mutagenic or genotoxic activity as indicated by the negative results obtained in bacterial test systems. The mixed results for the DNA repair assays and various antimutagenicity studies were associated with cytotoxicity as noted by Sekizawa *et al.,* [1982]. The evidence of genotoxic activity found in isolated mammalian cells, with the cinnamyl compounds causing chromosomal aberrations and/or mutations with or without metabolic activation was not seen *in vivo*. JECFA (2001) considered that there was no *in vivo* evidence of any mutation, clastogenic or genotoxic activity [JECFA, 2001].

Dermal Toxicity

Methyl cinnamate has been reported to be negative in attempts to sensitise the skin of 19 guinea pigs using both patch tests and sensitisation tests [Hausen *et al.,* 1992].

The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 0/4 deaths at that dose. Methyl cinnamate was administered as a 50% solution or the test material was suspended in corn oil. Four male and female New Zealand white rabbits weighing 2.5 –3.0 kg, received a single dermal application of methyl cinnamate at a dose of 5.0 g/kg/bw. The dose site, approximately 240 cm^2 (about 10% of the body surface) was clipped and the skin was abraded in one-half of the animals and intact in the other half of the animals. The test area was covered for 24 h with a non-absorbent binder. Observations were made for 14 days. Gross necropsy was conducted on all animals. No clinical effects were observed during the study. Gross observations at necropsy were normal for all animals [Bhatia *et al.,* 2007].

In a human maximization study, no irritation was observed after a 48-h closed patch test with 10% methyl cinnamate in petrolatum on the forearms of five healthy, male and female volunteers [Bhatia *et al.,* 2007].

Methyl cinnamate was evaluated for irritation, at several dose levels, during the induction phase of an open epicutaneous test. A 0.1 ml aliquot of methyl cinnamate applied to an area measuring 8 cm^2 on the clipped flank of 6–8 male and female out bred Himalayan white-spotted guinea pigs. The application site was left uncovered and reactions were read after 24 h. A total of 21 daily applications were made. The minimal irritating concentration was found to be 3% (vehicle not specified) [Bhatia *et al.,* 2007].

In an eye irritation test, a 0.1 ml aliquot of neat methyl cinnamate was instilled into one eye of each of six New Zealand white rabbits with no further treatment. The untreated eyes served as controls. Observations were made at 1, 4, 24, 48, 72 and 96 h and daily thereafter for a total of 7 days. Conjunctival irritation was observed in 1/6 rabbits for 24-h. Under the conditions of this test, methyl cinnamate was considered to be non-irritating [Bhatia *et al.,* 2007].

A maximization test was carried out with 2% (1380 lg/cm^2) methyl cinnamate in petrolatum on 25 healthy, male volunteers. Application was under an occluded patch to the same site on the forearms of all subjects for five alternate-day 48-hr periods. Patch sites were pre-treated with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a ten-day rest period, challenge patches were applied to fresh sites on the back for 48 h under occlusion. The challenge site was pre-treated for 1 hr with 10% SLS. The challenge site was read at 48 and 72 hr. No sensitization reactions were observed. Using the same above method described above, another maximization test was carried out with 10% (6900 lg/cm^2) methyl cinnamate in petrolatum on 25 healthy, male and female volunteers. No sensitization reactions were observed [Bhatia *et al.,* 2007].

Reproductive and Developmental Toxicity

Administration of cinnamaldehyde [a structurally related compound] to pregnant rats at 5, 25 or 250 mg/kg bw on days 7-17 of gestation lead to poor cranial ossification at all doses. An increased incidence of dilated pelvis, reduced renal papillae, dilated ureters at the low and intermediate dose and an increased number of foetuses with two or more abnormal sternebrae at the intermediate dose. The authors concluded that as the changes were not dosage-related they may be related to the decreased maternal weight gain for the two higher dose groups [Mantovani *et al.,* 1989].

Inhalation Toxicity

A recent study investigated the effect of cigarettes, containing various additives in three combinations, in a 90 day nose-only s moke inhalation study in rats [Vanscheeuwijck *et al.* , 2002]. These ingredients included methyl cinamate at up to 1 ppm, a level described as a multiple of its typical use in a US cigarette. The data from this study along with that from a number of other biological and chemical studies indicate that the addition of the combined ingredients "did not increase the inhalation toxicity of the smoke, even at the exaggerated levels used" [Vanscheeuwijck *et al.*, 2002].

The addition of methyl cinnamate at 23 ppm to reference cigarettes, used in a 90 day-sub-chronic inhalation exposure in rats, led to a series of pathological changes to smoke exposure that were indistinguishable from those changes caused by the control cigarettes. This indicated that addition of me thyl cinnamate to a reference cigarette had no discernable effect upon the type or severity of the treatment related pathological changes associated with tobacco smoke exposure [Baker *et al.,* 2004].

Other Relevant Studies

Female white New Zealand rabbit s, weighing 3–4 kg, rec eived a single oral dose of 500 mg methyl cinnamate suspended in warm water. Urine was collected for 24 h r. Metabolites were isolated and examined by paper thin layer chromatography (TLC) and gas–liquid chromatography GLC) . The following metabolites were identified as a percentage of the dose: hippuric acid (56.0%) and glucosiduronic ac id (8%). Using the same method, Wistar rats were dosed with 50 mg of methyl cinnamate. The following metabolites were identified as a percentage of the dose: hippuric acid (67.0%) and glucosiduronic acid (3%) [Bhatia *et al.,* 2007].

Behavioural data

No data identified.

***In Vitro* Toxicity Status**

Roemer *et al.,* (2002) reported on a study in which cigarettes containing various additives in three differe nt combinations were produced. Smoke condensates prepared from these cigarettes were then tested in two different *in vitro* assays. The mutagenicity of the smoke condensate was assayed in

the *Salmonella* plate incorporation [Ames] assay with tester strains TA98, 100, 102, 1535 and 1537 (+/- S-9). The cytotoxicity of the gas/vapour phase and the particulate phase was determined in the neutral red uptake assay with mouse embryo BALB/c 3T3 cells. The authors concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients which included methyl cinnamate at levels up to 1 ppm (a multiple of its typical use in a US cigarette) [Roemer *et al*., 2002].

Baker *et al.,* [2004]; examined the effects of the addition of 482 tobacco ingredients upon the biological activity and chemistry of mainstream smoke. The ingredients, essentially different groups of flavourings and casings, were added in different combinations to reference cigarettes. The addition of methyl cinnamate at 23 ppm was determined not to have affected the mutagenicity of the total particulate matter (TPM) of the smoke in either the Ames, *in vitro* micronucleus assay or the neutral red assay when compared with that of the control cigarettes [Baker *et al.,* 2004].

Methyl cinnamate was shown to be negative in a DNA repair assay using *B. subtilis* M45 (rec) and H17 (rec) at 20 g/disc without metabolic activation [Oda *et al.,* 1979].

It was reported however to be positive in the sister chromatid exchange assay in Chinese hamster ovary cells, without metabolic activation at 33.3 mol/l only when cells were pre-treated with mitomycin C which is a known mutagen [Sasski *et al.,* 1989].

Methyl cinnamate has been reported to mildly inhibit the adhesion of aortic smooth muscle cells to collagen coated dishes [Shimokado *et al.,* 1994].

Additional information concerning the *in vitro* mutagenicity of this material may be found in "An Interim report on data originating from Imperial Tobacco Limited's Genotoxicity testing programme September 2003" or "An updated report on data originating from Imperial Tobacco Limited's external Genotoxicity testing programme – Round 2 August 2007".

The mutagenicity of the smoke condensate was assayed in the *Salmonella* plate incorporation [Ames] assay with the tester strain TA98 in the presence of an S9 metabolic activation system. The cytotoxicity of the cigarette condensate was determined in the neutral red uptake assay and the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt assay (MTS assay) with the human hepatocellular liver carcinoma cell line, HEP-G2. It was concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was/was not increased by the addition of the ingredients, which included methyl cinnamate at levels up to 127 ppm.

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of methyl cinnamate is detailed in the Report on Thermochemical Properties of Ingredients document. In the aforementioned document, the term 'pyrolysis' means the heating of an ingredient in isolation under controlled conditions in an analytical device to examine its degradation potential. The expression 'transfer data' on the other hand is used to describe the fate of an ingredient in qualitative and quantitative terms following the smoking of a tobacco product to which it has been applied.

A 2004 study by Baker and Bishop analysed the pyrolytic breakdown of 291 tobacco ingredients usin g combustion conditions that simulate cigarette combustion. Due to the combustion conditions the results likely predict the natural behaviour of these compounds during combustion on the cigarette, and allow estimation of the degree of intact transfer into the mainstream smoke. Under pyrolysis methyl cinnamate was found to transfer 98.4% intact, other breakdown product included ethyl cinnamate (0.7%), benzaldehyde (0.6%), styrene (0.3%).

REACH Statement

This ingredient has been registered under REACH. Under REACH, registrants have an obligation to provide information on substances they manufacture or import. This information includes data on hazardous properties (covering various toxicological endpoints), guidance on safe use and classification and labelling. The European Chemicals Agency (ECHA) makes this information publicly available on its website: http://echa.europa.eu/.

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