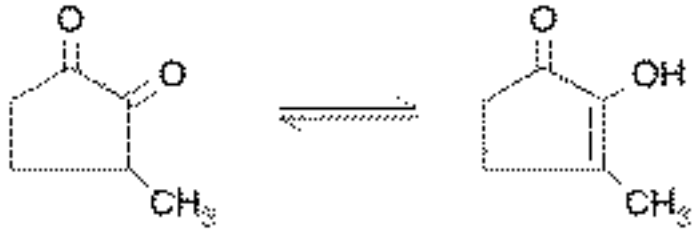
METHYL CYCLOPENTENOLONE

SYNONYMS

2-Hydroxy-3-methyl cyclopent-2-en-1-one
2-Hydroxy-3-methyl-2-cyclopenten-1-one
3-Methyl-2-cyclopentene-2-ol-one
3-Methyl-1,2-cyclopentanedione
Corilone
Corylone
Cyclotene
Maple lactone

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_6H_8O_2$

IDENTIFIER DETAILS

CAS Number	:	80-71-7 or 765-70-8
CoE Number	:	758
FEMA	:	2700
EINECS Number	:	201-303-2
E Number	:	-

SPECIFICATIONS

Melting Point: 107°C
Boiling point:

PURPOSE

Flavouring substance

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
15	50	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
Acceptable	JECFA	1998	No safety concerns at current levels of intake when used as a food additive.

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 cyclopentenolone is reportedly formed during the dry distillation of wood; found also in the corresponding tar oil; and has been identified in fenugreek [Fenaroli, 1995].

Reported Uses: Methyl cyclopentenolone is reportedly used in baked goods at 26.40 ppm, breakfast cereals at 100.0 ppm, frozen dairy at 16.58 ppm, meat products at 3.70 ppm, soft candy at 25.57 ppm, sweet sauce at 15.75 ppm, gelatin pudding at 8.68 ppm, non-alcoholic beverages at 5.66 ppm, alcoholic beverages at 8.75 ppm, hard candy at 16.92 ppm, and chewin g gum at 7.59 ppm [Fenaroli, 2005].

TOXICITY DATA

Carmines (2002), Rustemeier *et al* . (2002), Roemer *et al* . (2002) and Vanscheeuwijck *et al.* (20 02) reported on a testing program designed to evaluate the potential effects of 333 ingredients added to typical commercial blended test cigarettes on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity scre en [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], determination 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 ingre dients, including methyl cyclopentenolone at levels up to 124 ppm, "did not increase the overall toxicity of cigarette smoke" [Carmines *et al.,* 2002].

Renne *et* al., (2006) evaluated the effects of tobacco flavouring and casing ingredients on both mutagenicity, and a number of physiological parameters in Sprague-Dawley (SD) rats. Test cigarettes containing a mixture of either 165 low-uses or eight high-use flavouring ingredients which included methyl cyclopentenolone at 26 ppm, were compared to a typical c ommercial tobacco blend without flavouring ingredients. The Ames assay (TA 98, 100,102, 1535 and 1537 ±S9) did not show any increase in Mutagenicity from "low" or "high" cigarette smoke condensate compared to the control. SD rats were exposed by nose-onl y inhalation for 1h/day, 5 days/wk for 13 weeks to smoke at concentrations of 0.06, 0.2 or 0.8mg/L from the test or reference cigarettes, or to air only. Plasma nicotine, COHb and respiratory parameters were measured periodically. Rats were necropsied af ter 13wk of exposure or following 13 wk of recovery from smoke exposure. Biological endpoints assessed included; clinical appearance, body weight, organ weights, and lesions (both gross and microscopic). The results of these studies did not indicate any consistent differences in toxicological effects between smoke from cigarettes containing the flavouring or casing ingredients and reference cigarettes.

***In Vivo* Toxicity Status**

Test	Species	Route	Reported Dosage
LD_{50}	Mouse	Gavage	1350 mg/kg
LD_{50}	Mouse	Oral	1350 mg/kg
LD_{50}	Rat	Oral	1850 mg/kg
LD_{50}	Guinea Pig	Gavage	1400 mg/kg

(JECFA, 2000; Moreno, 1976; Dow Chemical. 1953; Givaudan Corp, 1952; Leberco, 1952)

Groups of 15 male and female rats [strain unspecified] were exposed to a diet containing 0 or 1 % Methylcyclopentenolone for 6 months, which equated to 0 or 500 mg/kg/day. At the end of the study there was found to be no statistically significant differences between the control and treated group in any of the parameters examined [Dow Chemical Co. 1953]. Therefore the no observed effect level [NOEL] was set at 500 mg/kg/day, which is more than 30,000 the daily *per capita* intake [eaters only] of 15 and 12 g/kg bw from its use as a flavouring agent in Europe and the United States , respectively [JECFA, 1999].

The administration of 3-ethyl –2-hydroxy-2-cycolpenten-1-one [a compound with a very similar structure to that of 2-hydroxy-3-methyl-2-cyclopenten-1-one], to groups of 10 male and 10 female Charles River CD rats at 0, 100, 200 or 400 mg/kg/day was performed for 13 Weeks. There was found to be no adverse affects seen in any of the parameters investigated with a NOEL set at 400 mg/kg [King *et al.,* 1979, as cited in JECFA 1999].

A mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including methyl cyclopentenolone at 23 ppm. The authors concluded that the study "did not indicate any substantive effect of these ingredients on the tumorigenicity of cigarette smoke condensate " [Gaworski *et al.* , 1999]. [It should be noted that the cigarettes contained a typical American blend humectant and sugar component (*i.e.* glycerine 20,000 ppm, propylene glycol at 24,000 ppm, and brown invert sugar at 24,000 ppm)] [Gaworski *et al.*, 1999].

Reproductive and Developmental Toxicity

In a combined carcinogenicity and reproductive study with 3-ethyl –2-hydroxy-2 cycolpenten-1-one, three successive generations of Charles River CD-Cobs rats were dosed with 3-ethyl –2-hydroxy-2 cycolpenten-1-one in the diet at 0, 30, 80 or 200 mg/kg/day. The subsequent generations after mating the F0 generation lead to the F1 generation, which led to the F2 generation and at a later stage the F1 generation was mated again to form the F3 generation. Slightly depressed growth was reported for the high dose females in the F1 generation. However this was not found to be statistically significant when it was compared to the vehicle control group. There was no effect of treatment upon any of the fertility or reproductive parameters measured. The incidence of malignant and benign tumours in treated animals was similar to those reported in the controls. The NOEL was 200 mg/kg/day [King *et al.,* 1979, as cited in JECFA 1999].

Four groups of 10 rats were orally administered Methyl cyclopentenolone at 0, 50, 250 and 500 mg/kg/day 7 days prior to cohabitation, through cohabitation (maximum 7 days), gestation, delivery and 4 days postpartuition. Those rats that did not deliver a litte r were necropsied on Day 25, with delivered pups being sacrificed on day 4 postpartum. The NOAEL for maternal toxicity was < 50 mg/kg/day and developmental offspring effects was 500 mg/kg/day [Vollmuth *et al.,* 1990].

Inhalation Toxicity

A recent study in vestigated the effect of cigarettes, containing various additives in three combinations, in a 90 day nose-only smoke inhalation study in rats (Vanscheeuwijck *et al.* , 2002). These ingredients included methyl cyclopentenolone at 124 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 smok e, even at the exaggerated levels used" [Vanscheeuwijck *et al.*, 2002].

When tested at 23 ppm in cigarettes, in a 13-week inhalation study, the presence of methyl cyclopentenolone "…had no discernible effect on the character of extent of the biologic respo nses normally associated with inhalation of mainstream cigarette smoke in rats. "[Gaworski *et al.* , 1998]. [However, it should be noted that the cigarettes had been spiked with a number of flavour ingredients in combination prior to smoking, and they contained a typical American blend humectant and sugar component (*i.e.* glycerine 20,000 ppm, propylene glycol at 24,000 ppm, and brown invert sugar at 24,000 ppm)] [Gaworski *et al.*, 1998].

The addition of methyl cyclopentenolone at 53 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 methyl cyclopen tenolone 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

Administration of -diketones are rap idly absorbed from the gastro-intestinal tract of rats and mice [Gabriel *et al.,* 1972]. It is anticipated that in humans they will metabolise the aliphatic acyclic -diketone by -hydroxylation of the tertiary methyl group to yield the corresponding ketoc arboxylic acid. The acid may then undergo decarboxylation to yield CO_2 and a simple carboxylic acid. This carboxylic acid may then be completely metabolised by the fatty acid and citric acid cycles. It has also been reported that at high concentrations an other pathway may predominate, this involves reduction to the diol and subsequent conjugation with glucuronic acid [Williams, 1959; Gabriel *et al.,* 1972 & Otsuka *et al.,* 1996].

In an immunotoxicity assay methyl cyclopentenolone failed to modulate the cell mediated or humoral immune response of female CD $_1$ mice, when it was administered intragastrically for five days at doses up to 500 mg/kg/day [Gaworski *et al.*, 1994].

Behavioural Data

No data identified.

***In Vitro* Toxicity Status**

Methylcyclopentenolone was found to be negative in the Ames mutagenicity assay with the following strains TA1535, TA1537, TA1538, TA98 and TA100 at 10,000 g/plate both with and without metabolic activation (Heck *et al.,* 1989). It has also been reported to be negative when test ed at 0.002-200 mol/plate in strains TA98, TA100 and TA102 both with and without metabolic activation [Aeschbacher *et al.,* 1989].

Methylcyclopentenolone was found to be negative in the Unscheduled DNA synthesis assay using rat hepatocytes with metabolic activation at concentrations up to 500 g/plate [Heck *et al.,* 1989].

Methylcyclopentenolone has been reported to be positive in the sister chromatid exchange assay with human lymphocytes with a dose response at concentrations between 0 – 3 mM [Jansson *et al.,* 1986].

Roemer *et al.,* (2002) reported on a study in which cigarettes containing various additives in three different 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, TA100, TA102, TA1535 and TA1537 in the presence and absence of an S9 metabolic activation system. 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 cyclopentenolone at levels up to 124 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 cyclopentenolone at 53 ppm was determined not to hav e 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].

A total of 95 ingredients were tested individually through addition at different concentrations to the tobacco of experimental cigarettes. Mainstream cigarette

smoke chemistry analysis, bacterial mutagenicity testing, and cytotoxicity testing were conducted. The authors concluded that these ingredients, which included methyl cyclopentenolone applied at levels up to 3500 ppm on cigarettes produced minimal changes in the overall toxicity profile of mainstream cigarette smoke, and in some cases, the addition of high levels of an ingredient caused a small reduction in toxicity findings, probably due to displacement of burning tobacco [Gaworski *et al*., 2011].

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of methyl cyclopentenolone 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.

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