Methyl cyclopentenolone

Ingredient synonym names

2-Cyclopenten-1-one, 2-hydroxy-3-methyl- 2-Hydroxy-3-methylcyclopent-2-enone 3-Methylcyclopentane-1,2-dione hydrate Cyclotene
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

IDENTIFIER DETAILS

Ingredient chemical structure

CAS Number	FEMA Number	Additive Number
80-71-7, 765-70-8	2700	-

Ingredient EC Number	FL Number	CoE Number
201-303-2	07.056	758

Chemical formula C6H8O2

Ingredient CLP Classification

Ingredient REACH Registration Number

-

Acute Oral Toxicity	Eye Damage/Irritation	Carcinogenity
0	0	0

Acute Dermal Toxicity	Respiratory Sensitisation	Reproductive Toxicity
0	0	0

Acute Inhalation Toxicity	Skin Sensitisation	Aspiration Toxicity
0	0	0

Skin Corrosive/Irritant	Mutagenicity/ Genotoxicity	Specific Target Organ Toxicity
0	0	0

Methyl cyclopentenolone

SPECIFICATIONS

Melting Point	107°C	Boiling Point	-

STATUS IN FOOD AND DRUG LAWS

Acceptable Daily Intake (ADI, mg/kg)	Acceptable (JECFA,1998)
Acceptable Daily Intake (ADI) comments	No safety concerns at current levels of intake when used as a food additive.
FDA Status	[CFR21]: 172.515 - Synthetic flavouring substances and adjuvants permitted for direct addition to food for human consumption.

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

HUMAN EXPOSURE

Ingredient Natural Occurence (if applicable)

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].

References - Ingredient Natural Occurence

Ingredient 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 chewing gum at 7.59 ppm [Fenaroli, 2005].

References - Ingredient Reported Uses

Fenaroli, (2005). Fenaroli's Handbook of Flavour Ingredients, 5th Edition. CRC Press, Boca Raton, USA.

TOXICITY DATA

In Vivo Data

Acute Toxicity Data

LD50 – Mouse - Gavage - 1350 mg/kg
LD50 - Mouse - Oral - 1350 mg/kg
LD50 - Rat – Oral - 1850 mg/kg
LD50 - Guinea Pig - Gavage - 1400 mg/kg

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

Dow Chemical Co. (1953). Toxicity of cyclotene – Summary Unpublished report from Biochemical Research Department Midland Michigan. Submitted to WHO by the Flavour and Extract Manufacturers Association of the United States, Washington D.C. United States.

Givaudan Corp. (1952). Acute oral toxicity of methylcyclopentenolone in mice. Unpublished report to the Research Institute of Fragrance Materials. Submitted to WHO by the Flavour and Extract Manufacturers Association of the United States, Washington D.C. United States

JECFA (1999). Safety evaluation of certain food additives. Prepared by the fifty-first meeting of the joint FAO/WHO Expert committee on food additives. 42: IPCS Geneva. 353-379.

Leberco Labs. (1952). Acute toxicity of methylcyclopentenolone. Unpublished report. Submitted to WHO by the Flavour and Extract Manufacturers Association of the United States, Washington D.C. United States.

Moreno (1976). Acute toxicity Studies in rats mice rabbits and guinea pigs. Unpublished report to the Research Institute of Fragrance Materials. Submitted to WHO by the Flavour and Extract Manufacturers Association of the United States, Washington D.C. United States.

In Vivo Carcinogenicity/Mutagenicity

No data identified.

References - In Vivo Carcinogenicity/Mutagenicity

No data identified.

Dermal Toxicity

No data identified.

References - Dermal Toxicity

No data identified.

Reproductive/ 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 litter 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].

References - Reproductive/ Developmental Toxicity

JECFA (1999). Safety evaluation of certain food additives. Prepared by the fifty-first meeting of the joint

FAO/WHO Expert committee on food additives. 42: IPCS Geneva. 353-379.

King et al., (1979). Three generation and chronic toxicity study in rats. Unpublished report from Pfizer Central Research. Submitted to WHO by the Flavour and Extract Manufacturers Association of the United States, Washington D.C. United States

Vollmuth TA, Bennett MB et al,. (1990) An evaluation of food flavouring ingredients using an in vivo reproductive and developmental toxicity screening test. Teratology 41(5) 597.

Inhalation Toxicity

No data identified.

References - Inhalation Toxicity

No data identified.

Cardiac Toxicity

No data identified.

References - Cardiac Toxicity

No data identified.

Addictive Data

No data identified.

References - Addictive Data

No data identified.

Behavioral data

No data identified.

References - Behavioral data

No data identified.

In Vivo - Other Relevant Studies

Administration of α-diketones are rapidly 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 α-diketone by α-hydroxylation of the tertiary methyl group to yield the corresponding ketocarboxylic acid. The acid may then undergo decarboxylation to yield CO2 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 another 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 CD1 mice, when it was administered intragastrically for five days at doses up to 500 mg/kg/day [Gaworski et al., 1994].

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].

References - In Vivo - Other Relevant Studies

Gabriel et al., (1972). Metabolism of acetoin in mammalian liver slices an extracts. Biochemical Journal. 124: 793-800.

Gaworski et al., (2011). An evaluation of the toxicity of 95 ingredients added individually to experimental cigarettes: approach and methods. Inhalation Toxicology: 1-12.

JECFA (1999). Safety evaluation of certain food additives. Prepared by the fifty-first meeting of the joint FAO/WHO Expert committee on food additives. 42: IPCS Geneva. 353-379.

King et al., (1979). Three generation and chronic toxicity study in rats. Unpublished report from Pfizer Central Research. Submitted to WHO by the Flavour and Extract Manufacturers Association of the United States, Washington D.C. United States

Otsuka et al., (1996). A detoxification route for acetaldehyde: Metabolism of diacetyl, acetoin, and 2-3 butane diol in liver homogenate and perfused liver of rats Journal of Biochemistry. 119: 246-251.

Williams (1959) Detoxification mechanisms: In The Metabolism and Detoxification of Drugs Chapman and Hall. London pp 62, 78.

In Vitro Data

In Vitro Carcinogenicity/Mutagenicity

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

References - In Vitro Carcinogenicity/Mutagenicity

Aeschbacher et al., (1989). Contribution of coffee aroma constituents to the mutagenicity of coffee. Food & Chemical Toxicology. 27(4): 227-232.

In Vitro - Other Relevant Studies

Methylcyclopentenolone was found to be negative in the Unscheduled DNA synthesis assay using rat hepatocytes

with metabolic activation at concentrations up to 500 μg/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].

The cytotoxicity of e-liquids exposed to HepG2 cells was determined using the neutral red uptake assay (NRU). The mutagenicity of e-liquids was assayed in Ames with tester strains TA98, TA100, TA102, TA135, TA1537 in the presence and absence of an S9 metabolic activation system. The genotoxic potential of e-liquids was determined in the in vitro micronucleus assay using V79 hamster lung fibroblasts in the presence of an S9 metabolic activation system. It was concluded that the in vitro cytotoxicity, mutagenicity and genotoxicity of e-liquids was not increased by the addition of ingredients, which included Methyl Cyclopentenolone.

References - In Vitro - Other Relevant Studies

Heck et al., (1989). An evaluation of the food flavouring ingredients in a genetic toxicity screening battery. Toxicologist 9: 257.

In vitro toxicity testing of e-liquids (Internal Document Reports)

Jansson et al., (1986). In vitro studies of biological effects of smoke condensate. II Induction of sister chromatid exchanges in human lymphocytes by weakly acidic semivolatile constituents. Mutation Research. 169: 129-139.

Emissions and Associated Toxicity Data

A recent study investigated 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 smoke, 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 responses 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 cyclopentenolone 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].

Roemer (2014) and Schramke (2014) reported on a testing program designed to evaluate the potential effects of 350 ingredients added to an experimental kretek cigarette on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], Mouse Lymphoma assay, determination of smoke chemical constituents, a 4-day in vivo micronucleus assay and a 90-day rat inhalation study. Based on the results of these studies, the authors concluded that the addition of ingredients commonly used in the manufacture of kretek cigarettes, including methyl cyclopentenolone at levels up to 108 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Methyl cyclopentenolone

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 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].

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].

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 333 ingredients added to typical commercial blended test cigarettes on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [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 ingredients, 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 commercial 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-only 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 after 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.

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].

Methyl cyclopentenolone

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.

References - Emissions and Associated Toxicity Data

Baker RR, et al., (2004). An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. Food & Chemical Toxicology. 42 Suppl: S53-83.

Carmines (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results. Food & Chemical Toxicology. 40: 77-91

Gaworski et al., (2011). An evaluation of the toxicity of 95 ingredients added individually to experimental cigarettes: approach and methods. Inhalation Toxicology: 1-12.

ITL internal report titled: Report on the Thermochemical Properties of Ingredients.

Renne, R.A., Yoshimura, H., Yoshino, K., Lulham, G., Minamisawa, S., Tribukait. Dietz, D.D., Lee, K.M., Westerberg, R.B. (2006). Effects of flavouring and casing ingredients on the toxicity of mainstream cigarette smoke in rats. Inhalation Toxicology. 18:685-706.

Roemer et al. (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 3: In vitro genotoxicity and cytotoxicity. Food & Chemical Toxicology. 40: 105-111.

Roemer (2014) Toxicological assessment of kretek cigarettes: Part 1: background, assessment approach, and summary of findings. Regul Toxicol Pharmacol.; 70 Suppl 1: 2-14.

Roemer (2014) Toxicological assessment of kretek cigarettes Part 6: the impact of ingredients added to kretek cigarettes on smoke chemistry and in vitro toxicity. Regul Toxicol Pharmacol.; 70 Suppl 1: 66-80.

Rustemeier et al., (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke. Food & Chemical Toxicology. 40: 93-104

Schramke (2014) Toxicological assessment of kretek cigarettes. Part 7: the impact of ingredients added to kretek cigarettes on inhalation toxicity. Regul Toxicol Pharmacol; 70 Suppl 1: 81-9.

Vanscheeuwijck et al., (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity. Food & Chemical Toxicology. 40: 113-131.