Menthol

Botanical Source

Synonyms

IUPAC Name

CAS Reference 89-78-1; 2216-51-5; 15356-60-2 (D-); 1490-04-6

E Number

Food Legislation

Council of Europe (CoE)	
Number	Comment
63	Listed by the Council of Europe as acceptable for use in food.

US Food and Drug Administration	
Number	Comment
172.515	Approved by the US FDA. FDA 21 CFR 172.515

Joint FAO/WHO Expert Committee on Food Additives (JECFA)		
Number	ADI	Comment
427	18000	ADI 0-4 mg/kg bw

FEMA	
FEMA No.	Comment
2665	Generally recognised as safe as a flavour ingredient:GRAS List Number 3

Natural Occurrence and Use in Food
Found in peppermint plant, honey, mint, rum, cocoa, eggs, guava, raspberry, rice, spearmint; used in candy, mouthwash.

Estimated Intake from Food and Drink	
Daily Intake mg/kg/day	FEMA Possible Average Daily Intake mg
0.1694	13.419

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Tobacco Product Related Chemical and Biological Studies for Ingredients Added in a Mixture

Smoke Chemistry		
Published Source	Level Tested %	Comment
BAT	2.00000	At maximum application level this ingredient is not associated with significant increases in levels of Hoffmann analytes in smoke.
Philip Morris	1.80000	An overall assessment of the data suggests that this ingredient did not add to the toxicity of smoke.

Ames Activity		
Published Source	Level Tested %	Comment
BAT	2.00000	Within the sensitivity and specificity of the system the Ames activity of the cigarette smoke condensate was not increased by the addition of the ingredient.
Philip Morris	1.80000	Within the sensitivity and specificity of the system the Ames activity of the cigarette smoke was not increased by the addition of the ingredient.

Micronucleus		
Published Source	Level Tested %	Comment
BAT	2.00000	Within the sensitivity of the in vitro micronucleus assay the activity of the cigarette smoke condensate was not increased by the addition of the ingredient.

Neutral Red		
Published Source	Level Tested %	Comment
BAT	2.00000	Within the sensitivity of the test system the in vitro cytotoxicity of the cigarette smoke condensate was not increased by the addition of the ingredient.
Philip Morris	1.8000	Within the sensitivity of the test system the in vitro cytotoxicity of the cigarette smoke was not increased by the addition of the ingredient.

Inhalation		
Published Source	Level Tested %	Comment
BAT	2.00000	The results indicate that the addition of the ingredient had no discernible effect on the inhalation toxicity of mainstream smoke.
Lorillard	0.03530	The results indicate that the addition of the ingredient had no discernible effect on the inhalation toxicity of mainstream smoke.
Philip Morris	1.80000	The data indicate that the addition of the ingredient, when added with one of three groups, did not increase the inhalation toxicity of the smoke.

Mouse Skin Painting		
Published Source	Level Tested %	Comment
Lorillard	0.50000	None of the changes appeared to be substantial enough to conclude that the tumour promotion capacity of the condensate was discernibly different between condensate produced from cigarettes with the ingredient in comparison with condensate from cigarettes without the ingredient.

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Tobacco Product Related Chemical and Biological Studies for Ingredients Tested Singly

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Toxicological Data on the Unburnt Ingredient

[+ve, positive; -ve, negative; ?, equivocal
with, with metabolic activation; without, without metabolic activation]

In vivo

Species	Test conditions	Endpoint	Results	References
Groups of 5-15 rats	Administration of up to 145 mg l-menthol/kg bw/day for 1 or 5 days by gavage. Bone marrow cells examined for aberrations.	Chromosome damage	-ve probably a well-conducted study	LBI, 1973
Rats (no further details given in expert report)	Up to 3 g l-menthol/kg bw (probably oral, but not specified in expert report). Bone marrow examined for aberrations.	Chromosome damage	-ve a good quality study	LBI, 1975, cited in JECFA, 1999
Mouse (7-8 males/group)	Four chromosome aberration tests with dl-menthol, performed over 5 years. Animals given single intraperitoneal doses of 0, 225, 450 or 900 mg/kg bw. Bone marrow	Chromosome damage	Equivocal Weak +ve in one 17-hr and one 36-hr study, -ve in the two others. Good quality studies.	NTP, 1989, 1991, 1992, 1993

	harvested 17 hr (two studies) or 36 hr after treatment.			
Rats (no further details given in expert report)	Up to 3 g l-menthol/kg bw (probably oral, but not specified in expert report). Dominant lethal assay (i.e. presumably early foetal deaths monitored when treated males were mated with untreated females).	Germ cell mutations and/or chromosome damage	-ve probably a good quality study	LBI, 1975, cited in JECFA, 1999
Mice (groups of 5-6 males)	dl-Menthol, given at 0, 0.25, 0.5 or 1 g/kg bw/day for 3 days by i.p. injection. Bone marrow cells assessed for micronuclei. Top dose killed 3/6 mice.	Chromosome damage	-ve Good quality study	NTP, 1988; Shelby et al. 1993
Mice (3-5 males/group)	Micronucleus assay. Animals given intraperitoneal injections of dl-menthol at 0, 0.25, 0.5	Chromosome damage	-ve Good quality study	NTP, 1988

	or 1 g/kg bw/day for 3 days. Peripheral blood examined.			
Mouse (4 males/group)	Sister chromatid exchange test with dl-menthol. Animals given single intraperitoneal doses of 0, 225, 450 or 900 mg/kg bw. Bone marrow examined 23 hr after treatment.	Chromosome effects	-ve Good quality study.	NTP, 1987
Mice (group of 4)	Gavage administration of dl-menthol at 3 g/kg bw, one mouse killed at each timepoint (0, 3, 8 and 24 hours). DNA damage assessed (using the sensitive Comet assay) in stomach, colon, liver, kidney, bladder, lung, brain and bone marrow.	DNA damage	-ve a good quality study	Sasaki et al. 2000
Drosophila melano-	Sex-linked recessive	Germ cell mutation	-ve	NTP, undated

gaster	lethal assays. Feeding of adults for 3 days at 50,000 ppm, or injection into adults of concentrations of 10 or 150 ppm. Three broods were produced and monitored.		Good quality study.	

In vitro

Test system	Test conditions	Endpoint	Activation status	Results	References
Under the US National Toxicology Program, a number of good quality in vitro genotoxicity tests with dl-menthol (Ames test for bacterial mutation, mouse lymphoma test for mammalian cell mutation, cytogenetics assays in Chinese hamster ovary cells for chromosome damage and chromosome effects in mammalian cells) have been carried out.				-ve in all tests	NTP, 1984-1985
Human peripheral blood lymphocytes	Chromosome aberration and sister chromatid exchange assay. L-Menthol tested up to 10 mmol/l [1.56 mg/ml].	Chromosome damage and chromosome effects	With and without S9	-ve	Murthy et al. 1991 (cited in JECFA, 1999)
TK6 Human lymphoblasts	Cells incubated with menthol (unspecified isomeric form) at up to 1.2 mmol/l [0.19 mg/ml] for 3 hr, harvested after	Chromosome damage	Without	+ve	Hilliard et al. 1998

	20-38 hr, and assessed for chromosome aberrations.				
Hamster ovary and lung cells, and human WI-38 embryonic lung cells	Various studies on dl- and l-menthol, at up to 10 mg/ml. Cells assessed for chromosome aberrations and SCE.	Chromosome damage and chromosome effects	Apparently without	-ve Possible weak +ve for dl-form in the absence of S9 (cited in Bibra, 1990 for Sofuni et al. 1985)	Genotoxicity Database, undated; JECFA, 1999 (citing 5 studies: Ishidate et al. 1984; Litton Bionetics Inc., 1975; Sofuni et al. 1985; Tennant et al. 1987)
Human blood cells and hamster V79 lung cells	d-Menthol tested at up to 2 mmol/l [0.31 mg/ml]. Cells examined for DNA damage.	DNA damage	Not specified in expert report, apparently without	-ve	JECFA, 1999 (citing 1 study: Hartmann & Speit, 1997)
Chinese hamster ovary (CHO) cells	Chromosome aberration assay. Cells treated with up to 0.23 mg/ml (top concentration was toxic) dl-menthol for 3 hr and evaluated at 20 hr.	Chromosome damage	Not known	+ve	Galloway et al. 1998
Chinese hamster ovary (CHO) cells	Chromosome aberration assay. Cells treated with up to 0.25 mg/ml dl-menthol for 8 hr and	Chromosome damage	With and without S9	-ve Possible weak +ve in the absence of S9 (cited in	Ivett et al. 1989

	evaluated at 10.5 hr (without S9) or treated for 2 hr and evaluated at 12.5 hr (with S9).			Bibra, 1990)	
Hamster ovary cells	Incubated with dl-menthol (CCRIS (2006a) reports that unspecified isomeric form was tested) at up to 1.8 mmol/l [0.28 mg/ml] for 3 hr, harvested at 20 hr and assessed for chromosome aberrations. Toxic at 1.2 mmol/l [0.19 mg/ml] and above.	Chromosome damage	With and without S9 CCRIS (2006a) reports without.	-ve at non-toxic concs, weak +ve at toxic concs	Hilliard et al. 1998
Mouse lymphoma cells	Two studies on dl-menthol, up to 0.2 mg/ml.	Somatic cell mutations	Not specified in expert report, apparently without	-ve	JECFA, 1999 (citing 2 studies: Myhr & Caspary, 1991; Tennant et al. 1987)
Chinese hamster ovary cells	Comet assay. Cells were exposed to dl-menthol, various conditions. [Only brief	DNA damage	Not specified	-ve [reported as "inactive" in abstract but as +ve at	Kiffe et al. 2003

	abstract available.]			0.5 mg/ml in RTECS record for dl-menthol, CAS 15356-70-4 (RTECS, 2003)]	
Rat hepatocytes	d-Menthol tested at up to 1.3 mmol/l [0.20 mg/ml]. Cells examined for DNA damage.	DNA damage	Not applicable	+ve	JECFA, 1999 (citing 1 study: Storer et al. 1996)
Salmonella typhimurium, strain TA1535/ pSK1002	Umu assay. dl-Menthol (CAS 15356-70-4) tested up to 500 µg/ml.	Mutation	With and without S9	-ve	Yasunaga et al. 2004
Salmonella typhimurium	Host-mediated assay. Mice (10/group) given l-menthol orally at up to 145 mg/kg bw/day for 1 or 5 days.	Mutation	No data	Equivocal Possibly weak +ve	LBI, 1973 (cited in Bibra, 1990)
Salmonella typhimurium strains TA92, TA94, TA98, TA100, TA1535, TA1537, TA2637, G46 and *Escherichia coli*, strain WP2 uvrA	Various studies on dl- and l-menthol, at up to 5 mg/plate.	Mutation	With and without S9	-ve [included good quality studies]	Genotoxicity Database, undated; JECFA, 1999 (citing 7 studies: Andersen & Jennies, 1984; Ishidate et al. 1984; Litton Bionetics Inc., 1975; Nohmi et al. 1985; Tennant et al.

					1987; Yoo, 1986)
Salmonella typhimurium strains TA97, TA98, TA100, TA1535	Ames assay. dl-Menthol tested up to 666 µg/plate.	Mutation	With and without S9	-ve	Zeiger et al. 1988
Bacillus subtilis, strains M45 and H17	Rec assay measuring differential killing. 10 mg l-menthol/disk. Paper in Japanese.	DNA damage [indicative test]	Probably without	+ve	Yoo, 1985
Bacillus subtilis	Gene mutation assay. L-Menthol tested up to 20 mg/plate.	Mutation	Probably without	-ve	Oda et al. 1978 (cited in JECFA, 1999)
Bacillus subtilis	DNA repair assay. L-Menthol tested up to 10 mg/disk.	DNA damage [indicative test]	Probably without	-ve	Yoo, 1986 (cited in JECFA, 1999)
Saccharomyces cerevisiae, strain D3	Gene mutation assay. L-Menthol tested up to 0.2 ml/plate.	Mutation	Probably without	Equivocal	Litton Bionetics Inc., 1975 (cited in JECFA, 1999)

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