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Opinion of the Scientific Committee on Food on Capsaicin

(adopted on 26 February 2002)

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Terms of reference

The Committee is asked to advise the Commission on substances used as flavouring substances or present in flavourings or present in other food ingredients with flavouring properties for which existing toxicological data indicate that restrictions of use or presence might be necessary to ensure safety for human health.

In particular, the Committee is asked to advise the Commission on the implications for human health of capsaicin in the diet.

Introduction

Previous evaluations

The Committee of Experts on Flavouring Substances of the Council of Europe evaluated the capsaicinoids in Capsicum preparations used as flavourings. A TDI of 0-0.2 mg/kg bw, expressed as total capsaicinoids, was numerically derived from the results of a population based case-control study conducted in Mexico-City, where chilli pepper consumers were at high risk for gastric cancer compared with non-consumers. The daily intake of the chilli pepper consumers was estimated to be 4 mg capsaicinoids/kg bw and a safety factor of 20 was applied. In addition, general limits of 5 ppm for foods and beverages, 10 ppm for hot foods and beverages, 20 ppm for hot ketchup and 50 ppm for tabasco, harissa, hot pimento oils and similar preparations expressed as total capsaicinoids were suggested (Council of Europe, 2001).

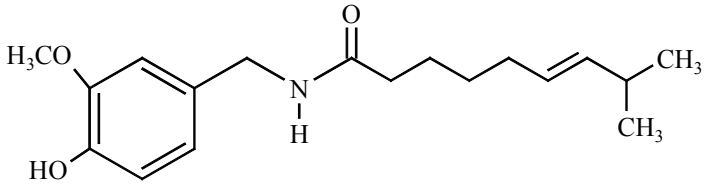
Current regulatory status

Capsaicin is listed in the register of chemically defined flavouring substances laid down in Commission Decision 1999/217/EC (EC, 1999), as last amended by Commission Decision 2002/113/EC (EC, 2002).

Chemical characterisation

Name:	Capsaicin (N-(4-hydroxy-3-methoxybenzyl)-8-methyl-trans-6-nonenamide)

Synonyms:	8-Methylnon-6-enoyl-4-hydroxy-3-methoxybenzylamide; trans-8-methyl-N-vanillyl-6-nonenamide; Isodecenoic acid vanillylamide
FL No:	16.014
CAS No:	404-86-4
FEMA No:	3404
CoE No:	2299
EINECS:	206-969-8
Structure:	



Capsaicin in Capsicum preparations is always accompanied by other capsaicinoids: mainly dihydrocapsaicin, but also small amounts of nordihydro-, homo-, homodihydro-, nor-, and nornorcapsaicin. The capsaicinoids present in the Capsicum fruit are predominantly capsaicin and dihydrocapsaicin, making up 80 to 90%. The ratio of capsaicin to dihydrocapsaicin is generally around 1:1 and 2:1 (Govindarajan and Sathyanarayana, 1991).

Exposure assessment

Capsaicinoids are mainly ingested as naturally occurring pungency-producing components of capsicum spices (chilli, cayenne pepper, red pepper). They typically range from 0.1 mg/g in chilli pepper to 2.5 mg/g in red pepper and 60 mg/g in oleoresin red pepper (Parrish, 1996). Pepper varieties from *Capsicum frutescens, annuum* and *chinense* were found to contain 0.22 - 20 mg total capsaicinoids/g of dry weight (Thomas *et al.*, 1998). Cayenne pepper samples had mean capsaicin and dihydrocapsaicin contents of 1.32 and 0.83 mg/g dry weight, respectively (Lopez-Hernandez, 1996).

The consumption of capsicum spices was reported to be 2.5 g/person/day in India, 5 g/person/day in Thailand (Monsereenusorn, 1983) and 20 g/person (one chilli pepper) per day in Mexico (Lopez-Carrillo, 1994). Assuming a content of capsaicinoids in these spices of about 1%, the daily intake of capsaicinoids in these countries has been estimated to be 25 - 200 mg/person/day or in the case of a person with 50 kg body weight 0.5 – 4 mg/kg bw/day (Council of Europe, 2001).

The maximum daily intake of capsaicin in the U.S. and Europe from mild chillies and paprika was roughly estimated to be 0.025 mg/kg bw (Govindarajan and Sathyanarayana, 1991), equivalent to 1.5 mg/person/day. According to a recent estimation, the mean and maximum intake of capsaicin from industrially prepared food products containing the recommended general limit of 5 ppm would be 0.77 and 2.64 mg/day, respectively (CREDOC/OCA, 1998).

Hazard identification/characterisation

Capsaicin and other members of the group of capsaicinoids produce a large number of physiological and pharmacological effects such as effects on the gast**r**ointestinal tract, the cardiovascular and respiratory system as well as the sensory and thermoregulation system. These effects result principally from the specific action of capsaicinoids on primary afferent neurons of the C-fiber type. This provides the rationale for their use to treat some peripheral painful states, such as rheumatoid arthritis (Surh and Lee,1995)

In addition, capsaicinoids are powerful irritants, causing burn and pain at low concentrations on the skin and mucous membranes. Given orally, they induce an increase of salivation and gastric secretion, a rapid change of sensation, warm to intolerable burning, and gastrointestinal disorders depending on the dose (Govindarajan and Sathyanarayana, 1991).

Absorption, distribution, metabolism and excretion

Capsaicinoids, when administered to rats intragastrically are readily absorbed and metabolized to a great extent in the liver before reaching the general circulation and extra hepatic organs (Donnerer *et al.*, 1990). In vitro and in vivo studies have shown that capsaicinoids are metabolized by different pathways: (1) hydrolysis of the acid-amide-bond and oxidative deamination of the formed vanillylamine, (2) hydroxylation of the vanillyl ring, possibly via epoxidation, (3) one electron oxidation of the ring hydroxyl forming phenoxy radicals and capsaicinoid dimers, (4) oxidation at the terminal carbon of the side chain (Surh and Lee, 1995). Within 48 hrs after oral administration of dihydrocapsaicin to male rats, 8.7% of the dose were excreted unchanged in urine and 10% in faeces. Metabolites found in urine were vanillylamine (4.7%), vanillin (4.6%) vanillyl alcohol (37.6%) and vanillic acid (19.2%) in free form or as glucuronides (Kawada and Iwai, 1985).
Based on results of Miller *et al.* (1983), who demonstrated the covalent binding of dihydrocapsaicin to hepatic microsomal proteins, the formation of electrophilic intermediates (arene epoxides, phenoxy radicals or quinone type derivatives formed after O-demethylation) and subsequent covalent binding to cellular macromolecules is discussed to play a role in the etiology of capsaicin-induced toxicity including mutagenicity and carcinogenicity (Surh and Lee, 1995).

Acute toxicity

The acute toxicity of capsaicin shows a large variation depending on the route of administration. In male mice, the LD_{50} varies from 0.56 mg/kg bw (i.v.) to 60 – 75 mg/kg bw (in ethanol) and 190 (122 – 294) mg/kg bw (in dimethyl sulfoxide), following intragastric intubation. The possible cause of death was considered to be due to respiratory paralysis

(Glinsukon *et al.* 1980). Intraduodenal and intragastric administration of 10% Capsicum as well as 0.014% capsaicin in 0.85% saline to male rats produced morphological damages in the duodenal mucosa (Nopanitaya and Nye, 1974).

Subacute/subchronic toxicity

A 4-week feeding study with groups of 5 male B6C3F1 mice with 0, 0.5, 1.0, 2.5, 5.0, 7.5, and 10% ground red chilli *(Capsicum annuum*) in the diet showed slight glycogen depletion and anisocytosis of hepatocytes in the 10% group. Other organs did not reveal any lesions. General health, body weight and food intake were not adversely affected (Jang *et al.*, 1992).

Groups of 10 – 14 rats were fed by stomach tube with 50 mg/kg bw/day capsaicin or 0.5 g/kg bw/day Capsicum extract for 10 - 60 days. There were significant reductions of growth, plasma urea, glucose, phospholipids, triglycerides, total cholesterol, free fatty acids, glutamic pyruvic transaminase, and alkaline phosphatase in both groups with a tendency for Capsicum treated animals to show more adverse effects. No gross pathological changes and no differences in organ weights from control values were observed at autopsy, only a slight hyperemia in the livers and reddening with increasing mucous materials in the gastric mucosa. The organs, however, were not examined histopathologically (Monsereenusorn, 1983).

BALB/c mice received an alcoholic chilli extract in drinking water 5 days a week till 16 months of age (27 males, 25 µg capsaicin/week, equivalent to about 0.125 mg/kg bw/d) or on the tongue 2 days a week for 14 months (22 males without and 19 males with 1% atropin solution prior to application, 50 µg capsaicin/week, equivalent to about 0.25 mg/kg bw/d). Compared with 40 untreated mice, the treated animals showed increased mortality and histopathological changes in liver, kidneys, stomach and tongue. The lesions in the liver observed in all treated mice were in the form of focal necrosis with inflammatory cells around, fatty changes and fibrosis (Agrawal and Bhide, 1987).

36 male Syrian hamsters received 20 µl alcoholic chilli extract with 50 µg capsaicin (equivalent to about 0.5 mg/kg bw/d) 5 days a week by cheek pouch application for 14 months. 30 untreated animals and 17 hamsters treated with 20 µl alcohol were used as controls. The animals treated with chilli extract had increased mortality and histopathological lesions in liver, kidneys, stomach and cheek pouch. The main lesions were liver cirrhosis, observed in 49 % of examined livers from exposed hamsters compared to 8 and 17 % in the control groups and glomeruli degeneration in 50% of examined kidneys of exposed animals compared to 8 and 0 % in the control groups, respectively (Agrawal and Bhide, 1988).

Capsaicin, administered intraperitoneally to adult male mice at doses of 0.4, 0.8 or 1.6 mg/kg bw/day on 5 consecutive days, did not induce significant alterations in epididymal weights, caudal sperm counts, testicular weights or testicular histology. In the sperm morphology

assay, sperms at 1, 3, 5 and 7 weeks did not reveal any treatment-related increase in the incidence of sperm-head abnormalities (Muralidhara and Narasimhamurthy, 1988).

In a 13-week study performed to determine the maximum tolerated dose, groups of 10 male and 10 female B6C3F1 mice received a mixture of 64.5% capsaicin and 32.6% dihydrocapsaicin at those levels of 0, 0.0625, 0.125, 0.25, 0.5, and 1% in the diet. Significant reduction of food intake and body weight gain in all dose groups, especially in treated females, and significantly increased liver/body weight ratios of both sexes and renal toxicity (focal tubular dilatation) in the 1% treated males were observed (Akagi *et al.*, 1998).

Capsaicin (purum) was administered at concentrations of 0.0625, 0.125, 0.25, 0.5 and 1% in the diet of groups of 4 male and 4 female Swiss albino mice for 35 days. When the animals died at an age of 62 – 126 weeks, one adenocarcinoma of the duodenum had developed at each dose level, except for the highest dose, while no such tumours occurred in a historical control group of 100 males and 100 females. There was no concurrent control group and the observed tumour incidence was not dose-related (Toth *et al.*, 1984).

Chronic toxicity/carcinogenicity

15 out of 26 rats fed for seven months with 10% chillies in a semisynthetic diet containing ardein, a purified protein of the ground nut, developed neoplastic changes in the liver (hepatomas, multiple cystic cholangiomas, solid adenomas or adenocarcinomas of the bile duct). Although no tumour developed in rats fed the basic diet without chillies, the authors stress, that it cannot be said whether chillies have a specific carcinogenic effect or whether a deficiency in the diet aggravated by a non-specific irritant caused the tumours (Hoch-Ligeti, 1951).

Capsaicin (capsaicin 65%, dihydro- 31%, nordihydro- 0.9%, homo- 1%, homodihydro- 0.6%, nor- 0.5%, nornor- 0.3%), administered in a semisynthetic diet at 0.03125% to 50 male and 50 female Swiss albino mice for their life span from 6 weeks of age, induced benign polypoid adenomas of the caecum in 22% of females ($p < 0.05$) and 14% of males, compared to 8% in the untreated female and male controls (incidence of historical controls not given). The survival rate was not substantially altered (Toth and Gannet, 1992).

Groups of 50 male and 50 female B6C3F1 mice were given 0, 0.025, 0.083, and 0.25 % capsaicinoid mixture (64% capsaicin and 32.6% dihydrocapsaicin) in the diet for 79 weeks, equivalent to daily doses of up to 220 and 200 mg/kg bw in males and females, respectively. In all dose groups, food intake was significantly reduced, in females also the body weight gain and in males the liver/body weight ratio. No evidence of carcinogenicity was found. Renal cell adenomas developed only in one male mouse of the 0.025 and 0.25% groups. (Akagi *et al.*, 1998).

A number of studies have shown that capsaicin or chilli extract can act as tumour promoters (Surh and Lee, 1995 and 1996). Thus, capsaicin (0.002% in drinking water for 6 weeks) has been reported to act as a promoter for the development of diethylnitrosamine-initiated enzyme-altered foci in the liver of male rats (Jang and Kim, 1988). Chilli extract has also been shown to have a promoting effect on the development of stomach and liver tumours in BALB/c mice initiated by methyl-acetoxy methylnitrosamine and benzene hexachloride, respectively (Agrawal et.al., 1986). In another study, rats fed diets containing hot chilli pepper showed slightly higher incidence of N-methyl-N-nitrosoguanidine-induced gastric cancer (Kim *et al.*, 1985).

On the other side, capsaicin has been suggested to exert chemoprotective effects through modulation of metabolism of carcinogens and their interaction with target cell DNA (Surh and Lee, 1995 and 1996).

Genotoxicity

Capsaicin (purum) was found to be mutagenic in *Salmonella typhimurium* strain TA 98 in the presence of Aroclor induced rat liver S9 fraction (Toth *et al.*, 1984), whereas another study in which S9 from phenobarbital-induced rats was used for metabolic activation was negative (Buchanan *et al.*, 1981). Capsaicin containing 20% dihydrocapsaicin exhibited mutagenicity in Salmonella strains TA 98, TA 100 and TA 1535 in the presence of S9 mixture from Aroclor-induced rats. An alcoholic chilli extract was mutagenic only in strain TA 98 (Nagabhushan and Bhide, 1985 and 1986). Capsicum pepper oleoresin was reported to have mutagenic activity in Salmonella strains SD 1018 and SD 7823 without metabolic activation (Damhoeri *et al.*, 1985). In addition, also a modified SOS microplate assay indicated a genotoxic activity of capsaicin (Venkat *et al.*, 1995).

Capsaicin containing 20% dihydrocapsaicin and alcoholic chilli extract failed to induce 8-azaguanine resistant mutants in Chinese hamster V79 cells with and without metabolic activation by S9 from Aroclor-induced rats (Nagabhushan and Bhide, 1985). On the other side, synthetic capsaicin, dihydrocapsaicin and a crude mixture of capsaicinoides from *Capsicum frutescens* activated with hamster hepatocytes were mutagenic in the V 79 assay measured by resistance to ouabain and 6-thioguanine (Lawson and Gannet, 1989).

The results of the comet assay and a DNA fragmentation assay show that capsaicin is able to induce DNA damage in human neuroblastoma cells (Richeux *et al.*, 1999). Capsaicin also induced DNA strand breakage with calf thymus and plasmid DNA in the presence of Cu (II) (Singh *et al.*, 2001).

Capsaicin containing 20% dihydrocapsaicin induced micronuclei in polychromatic erythrocytes in the mouse-bone-marrow assay at 7.5 mg/kg i.p. (Nagabhushan and Bhide, 1985). It also produced a significant increase of micronucleated normochromatic erythrocytes

in the peripheral blood and SCEs in bone marrow cells of male mice at 1.46 and 1.94 mg/kg i.p. (Diaz Barriga Arceo, 1995). A fraction of an alcoholic extract from the fruits of *Capsicum frutescens*, containing 3-acetamido-2-methyltetradecane as major component, has been found positive in the mouse-bone-marrow micronucleus assay after i.p. administration (Villasenor and Ocampo, 1994 and 1995). Dominant-lethal mutations in mice were not induced by capsaicin (Muralidhara and Narasimhamurthy, 1988).

Furthermore, capsaicin inhibited DNA biosynthesis in the testes of Swiss mice injected intraperitoneally (Nagabhushan and Bhide, 1985).

Some other studies cannot be evaluated, because important experimental details have not been published.

Reproductive and developmental toxicity

No data available

Human data

In a case-control study in Mexico-City which included 220 cases of gastric cancer and 752 controls randomly selected from the general population, chilli pepper consumers were at a 5.5 fold greater risk for gastric cancer than non-consumers. Persons who rated themselves as heavy consumers of chilli peppers were even at a 17 fold greater risk. However, when chilli pepper consumption was measured as frequency per day, a significant dose response relationship was not observed (Lopez-Carrillo *et al.*, 1994).

In another case-control study in India, red chilli powder was found to be a risk factor for cancer of the oral cavity, pharynx, esophagus, and larynx (2- to 3-fold risk with a dose-response relationship) compared with population controls, but not with hospital controls (Notani and Jayant, 1987).

In an Italian case-control study, chilli was briefly mentioned as being protective against stomach cancer (Buiatti *et al.*, 1989). Chilli peppers, however, are not heavily consumed in Northern Italy, where this study was conducted, and it is possible that chilli consumption was correlated with other protecting spices such as onions and garlic that are more heavily consumed in Italy (comment from Lopez-Carrillo *et al.*, 1994).

Summary of hazard identification/characterisation

Capsaicin, capsaicinoid mixtures, chillies and chilli extracts have been tested toxicologically by oral administration to mice, rats and hamsters. Some of these studies indicated a carcinogenic potential of capsaicin. These studies are regarded, however, as limited. A more recent carcinogenicity study did not show carcinogenic effects in mice. In humans, however, high consumption of chillies has been reported to be a risk factor for cancer of the upper gastrointestinal tract, possibly due to the irritating effect of capsaicinoids.

Genotoxic effects of capsaicin and capsaicinoid mixtures have been shown *in vitro* and *in vivo*.

Risk characterisation

The Committee concluded that the available data did not allow it to establish a safe exposure level for capsaicinoids in food.

The human intake of capsaicinoids in India, Thailand and Mexico, where capsicum spices are heavily consumed, has been estimated to be 25 – 200 mg/day. The high consumption of chillies in Mexico and India was reported to be associated with cancer of the upper digestive tract. In contrast, the maximum daily intake from mild chillies and paprika in Europe was roughly estimated to be 1.5 mg/day. In the one study conducted in Europe, no increase in the incidence of gastric cancer was found in association with occasional and lower intakes of chillies.

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