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Final Report on the Safety Assessment of Capsicum Annuum Extract, Capsicum Annuum Fruit Extract, Capsicum Annuum Resin, Capsicum Annuum Fruit Powder, Capsicum Frutescens Fruit, Capsicum Frutescens Fruit Extract, Capsicum Frutescens Resin, and Capsaicin[1]

Capsicum-derived ingredients **function as skin-conditioning agents—miscellaneous, external analgesics, flavoring agents, or fragrance components in cosmetics. These ingredients are used in 19 cosmetic products at concentrations as high as 5%. Cosmetic-grade material may be extracted using hexane, ethanol, or vegetable oil and contain the full range of phytocompounds that are found in the Capsicum annuum or Capsicum frutescens plant (aka red chiles), including Capsaicin. Aflatoxin and *N*-nitroso compounds (*N*-nitrosodimethylamine and *N*-nitrosopyrrolidine) have been detected as contaminants. The ultraviolet (UV) absorption spectrum for Capsicum Annuum Fruit Extract indicates a small peak at approximately 275 nm, and a gradual increase in absorbance, beginning at approximately 400 nm. Capsicum and paprika are generally recognized as safe by the U.S. Food and Drug Administration for use in food. Hexane, chloroform, and ethyl acetate extracts of Capsicum Frutescens Fruit at 200 mg/kg resulted in death of all mice. In a short-term inhalation toxicity study using rats, no difference was found between vehicle control and a 7% Capsicum Oleoresin solution. In a 4-week feeding study, red chilli (Capsicum annuum) in the diet at concentrations up to 10% was relatively nontoxic in groups of male mice. In an 8-week feeding study using rats, intestinal exfoliation, cytoplasmic fatty vacuolation and centrilobular necrosis of hepatocytes, and aggregation of lymphocytes in the portal areas were seen at 10% Capsicum Frutescens Fruit, but not 2%. Rats fed 0.5 g/kg day^{-1} crude Capsicum Fruit Extract for 60 days exhibited no significant gross pathology at necropsy, but slight hyperemia of the liver and reddening of the gastric mucosa were observed. Weanling rats fed basal diets supplemented with whole red pepper at concentrations up to 5.0% for up to 8 weeks had no pathology of the large intestines, livers, and kidneys, but destruction of the taste buds and keratinization and erosion of the gastrointestinal (GI) tract were noted in groups fed 0.5% to 5.0% red pepper. The results of 9- and 12-month extension of this study showed normal large intestines and kidneys. In rabbits fed Capsicum Annuum Powder at 5 mg/kg day^{-1} in the diet daily for 12 months damage to the liver and spleen was noted. A rabbit skin irritation test of Capsicum Annuum Fruit Extract at concentrations ranging from 0.1% to 1.0% produced no irritation, but Capsicum Frutescens Fruit Extract induced concentration-dependent (at 25 to 500 μg/ml) cytotoxicity in a human buccal mucosa fibroblast cell line. An ethanol extract of red chili was mutagenic in *Salmonella typhimurium* TA98, but not in TA100, or in *Escherichia coli*. Other genotoxicity assays gave a similar pattern of mixed results. Adenocarcinoma of the abdomen was observed in 7/20 mice fed 100 mg red chilies per day for 12 months; no tumors were seen in control animals. Neoplastic changes in the liver and intestinal tumors were observed in rats fed red chili powder at 80 mg/kg day^{-1} for 30 days, intestinal and colon tumors were seen in rats fed red chili powder and 1,2-dimethyl hydrazine, but no tumors were observed in controls. In another study in rats, however, red chile pepper in the diet at the same dose decreased the number of tumors seen with 1,2-dimethylhydrazine. Other feeding studies evaluated the effect of red chili peppers on the incidence of stomach tumors produced by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, finding that red pepper had a promoting effect. Capsicum Frutescens Fruit Extract promoted the carcinogenic effect of methyl(acetoxymethyl)nitrosamine (carcinogen) or benzene hexachloride (hepatocarcinogen) in inbred male and female Balb/c mice dosed orally (tongue application). Clinical findings include symptoms of cough, sneezing, and runny nose in chili factory workers. Human respiratory responses to Capsicum Oleoresin spray include burning of the throat, wheezing, dry cough, shortness of breath, gagging, gasping, inability to breathe or speak, and, rarely, cyanosis, apnea, and respiratory arrest. A trade name mixture containing 1% to 5% Capsicum Frutescens Fruit Extract induced very slight erythema in 1 of 10 volunteers patch tested for 48 h. Capsicum Frutescens Fruit Extract at 0.025% in a repeated-insult patch test using 103 subjects resulted in no clinically meaningful irritation or allergic contact dermatitis. One epidemiological study indicated that chili pepper consumption may be a strong risk factor for gastric cancer in populations with high intakes of chili pepper; however, other studies did not find this association. *Capsaicin* functions as an external analgesic, a fragrance ingredient, and as a skin-conditioning agent—miscellaneous in cosmetic products, but is not in current use. Capsaicin is not generally recognized as safe and effective by the U.S. Food and Drug Administration for fever blister and cold sore treatment, but is considered to be safe and effective as an external analgesic counterirritant. Ingested Capsaicin is rapidly absorbed from the stomach and small intestine in animal studies. Subcutaneous injection of Capsaicin in rats resulted in a rise in the blood concentration, reaching a maximum at 5 h; the highest tissue concentrations were in the kidney and lowest in the liver. In vitro percutaneous absorption of Capsaicin has been demonstrated in human, rat, mouse, rabbit, and pig skin. Enhancement of the skin permeation of naproxen (nonsteroidal anti-inflammatory agent) in the presence of Capsaicin has also been**

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel.

demonstrated. Pharmacological and physiological studies demonstrated that Capsaicin, which contains a vanillyl moiety, produces its sensory effects by activating a Ca^{2+}-permeable ion channel on sensory neurons. Capsaicin is a known activator of vanilloid receptor 1. Capsaicin-induced stimulation of prostaglandin biosynthesis has been shown using bull seminal vesicles and rheumatoid arthritis synoviocytes. Capsaicin inhibits protein synthesis in Vero kidney cells and human neuroblastoma SHSY-5Y cells in vitro, and inhibits growth of *E. coli*, *Pseudomonas solanacearum*, and *Bacillus subtilis* bacterial cultures, but not *Saccharomyces cerevisiae*. Oral LD_{50} values as low as 161.2 mg/kg (rats) and 118.8 mg/kg (mice) have been reported for Capsaicin in acute oral toxicity studies, with hemorrhage of the gastric fundus observed in some of the animals that died. Intravenous, intraperitoneal, and subcutaneous LD_{50} values were lower. In subchronic oral toxicity studies using mice, Capsaicin produced statistically significant differences in the growth rate and liver/body weight increases. Capsaicin is an ocular irritant in mice, rats, and rabbits. Dose-related edema was observed in animals receiving Capsaicin injections into the hindpaw (rats) or application to the ear (mice). In guinea pigs, dinitrochlorobenzene contact dermatitis was enhanced in the presence of Capsaicin, injected subcutaneously, whereas dermal application inhibited sensitization in mice. Immune system effects have been observed in neonatal rats injected subcutaneously with Capsaicin. Capsaicin produced mixed results in *S. typhimurium* micronucleus and sister-chromatid exchange genotoxicity assays. Positive results for Capsaicin were reported in DNA damage assays. Carcinogenic, cocarcinogenic, anticarcinogenic, antitumorigenic, tumor promotion, and anti–tumor promotion effects of Capsaicin have been reported in animal studies. Except for a significant reduction in crown-rump length in day 18 rats injected subcutaneously with Capsaicin (50 mg/kg) on gestation days 14, 16, 18, or 20, no reproductive or developmental toxicity was noted. In pregnant mice dosed subcutaneously with Capsaicin, depletion of substance P in the spinal cord and peripheral nerves of pregnant females and fetuses was noted. In clinical tests, nerve degeneration of intracutaneous nerve fibers and a decrease in pain sensation induced by heat and mechanical stimuli were evident in subjects injected intradermally with Capsaicin. An increase in mean inspiratory flow was reported for eight normal subjects who inhaled nebulized 10^{-7} M Capsaicin. The results of provocative and predictive tests involving human subjects indicated that Capsaicin is a skin irritant. Overall, studies suggested that these ingredients can be irritating at low concentrations. Although the genotoxicity, carcinogenicity, and tumor promotion potential of Capsaicin have been demonstrated, so have opposite effects. Skin irritation and other tumor-promoting effects of Capsaicin appear to be mediated through interaction with the same vanilloid receptor. Given this mechanism of action and the observation that many tumor promoters are irritating to the skin, the Panel considered it likely that a potent tumor promoter may also be a moderate to severe skin irritant. Thus, a limitation on Capsaicin content that would significantly reduce its skin irritation potential is expected to, in effect, lessen any concerns relating to tumor promotion potential. Because Capsaicin enhanced the penetration of an anti-inflammatory agent through human skin, the Panel recommends that care should be exercised in using ingredients that contain Capsaicin in cosmetic products. The Panel advised industry that the total polychlorinated biphenyl (PCB)/pesticide contamination should be limited to not more than 40 ppm, with not more than 10 ppm for any specific residue, and agreed on the following limitations for other impurities: arsenic (3 mg/kg max), heavy metals (0.002% max), and lead (5 mg/kg max). Industry was also advised that aflatoxin should not be present in these ingredients (the Panel adopted $\leq$15 ppb as corresponding to "negative" aflatoxin content), and that ingredients derived from Capsicum annuum and Capsicum Frutescens Plant species should not be used in products where *N*-nitroso compounds may be formed. The Cosmetic Ingredient Review (CIR) Expert Panel concluded that Capsaicin and Capsicum Annuum Extract, Capsicum Annuum Fruit Extract, Capsicum Annuum Resin, Capsicum Annuum Fruit Powder, Capsicum Frutescens Fruit, Capsicum Frutescens Fruit Extract, and Capsicum Frutescens Resin are safe as cosmetics in the practices of use and concentration as described in this safety assessment, when formulated not to be irritating.

INTRODUCTION

Derivatives of several Capsicum species are listed in the *International Cosmetic Ingredient Dictionary and Handbook* as cosmetic ingredients (Pepe et al. 2002). Capsaicin is the pungent component of these Capsicum-derived ingredients and itself is listed as a cosmetic ingredient.

This report considers the available data that are relevant to the safety assessment of Capsicum-derived cosmetic ingredients and Capsaicin in cosmetic formulations. In order to facilitate the consideration of a large volume of information, the report is presented in two parts.

Part 1 addresses Capsicum Annuum Extract, Capsicum Annuum Fruit Extract, Capsicum Annuum Resin, Capsicum Annuum Fruit Powder, Capsicum Frutescens Fruit, Capsicum Frutescens Fruit Extract, and Capsicum Frutescens Resin.

Part 2 addresses Capsaicin. Numerous studies on Capsaicin/red pepper have been identified in the published literature, and these data may be useful in terms of evaluating the safety of botanical ingredients that are derived from the Capsicum plant.

A combined summary is provided, along with a discussion and conclusion regarding the safety of these ingredients in cosmetic products.

PART 1: CAPSICUM-DERIVED INGREDIENTS

CHEMISTRY

Definition and Structure

According to the *United States Pharmacopeia*, Capsicum is defined as the dried ripe fruit of *Capsicum frutescens* Linné (known in commerce as African Chilies), or of *Capsicum annuum* Linné var. *connoides* Irish (known in commerce as Tabasco Pepper), or *Capsicum annuum* var. *longum* Sendt (known in commerce as Louisiana Long Pepper, or a hybrid between the Honka variety of Japanese Capsicum and the Old Louisiana Sport Capsicum, known in commerce as Louisiana Sport Pepper [Fam. Solanaceae]) (Committee of Revision of the United States Pharmacopeial Convention 2000).

Capsicum is listed in the *International Cosmetic Ingredient Dictionary and Handbook* as another name for Capsicum Annuum Resin and Capsicum Frutescens Fruit (Pepe et al. 2002).

Capsicum (a.k.a. Cayenne Pepper, Chili, and Pepper Red) has also been defined as the unripe fruits of *Capsicum fastigiatum*, *Capsicum annuum*, and other Solanaceae (Grant 1972) and

referred to as Tincture of Tabasco Pepper (alcoholic solution of capsicum) (Sax 1979).

The pungent compounds of the *Capsicum* fruit are called capsaicinoids (Capsaicin and its analogs), and the following five naturally occurring capsaicinoids have been reported: Capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin. These capsaicinoids are the major components of most *Capsicum* species, constituting approximately 95% or more of the total capsaicinoid content (Krajewska and Powers 1987). Using mass spectroscopy, the following components of capsaicinoids in *Capsicum annuum* were determined: Capsaicin (69%), dihydrocapsaicin (22%), nordihydrocapsaicin (7%), homocapsaicin (1%), and homodihydrocapsaicin (1%) (Bennett and Kirby 1968).

The definitions of the cosmetic ingredients (along with other names) that are included in this safety assessment are given below:

Capsicum Annuum Extract: Capsicum Annuum Extract (CAS no. 84625-29-6) is defined as an extract of the plant *Capsicum annuum*. Other names for this ingredient include Capsicum Annuum (EU) and Extract of Capsicum Annuum (Pepe et al. 2002).

Capsicum Annuum Fruit Extract: This ingredient is defined as an extract of the fruit of *Capsicum annuum*. Other names include Capsicum Annuum (EU), Extract of Capsicum Annuum Fruit, and Tougarasi Ekisu (Japan) (Pepe et al. 2002).

Capsicum Annuum Resin: Capsicum Annuum Resin is defined as a resinous material that is obtained from *Capsicum annuum*. Other names for this ingredient are as follows: Capsicum, Capsicum Annuum (EU); Capsicum Annuum Oleoresin; Capsicum Oleoresin; Oleoresin, Capsicum Annuum; Resin, Capsicum; and Resin, Capsicum Annuum. Capsicum Annuum Resin is marketed under the trade name, Paprika Oleoresin (Pepe et al. 2002). The definition of Paprika Oleoresin relating to its use as a color additive (for food), along with its status as a food additive, are included in the section "Noncosmetic Use" later in this report.

Capsicum Annuum Fruit Powder: This ingredient is defined as a powder of the dried fruit of *Capsicum annuum*. Other names include Capsicum Annum (EU) and Powdered Capsicum Annuum. Capsicum Annuum Fruit Powder is marketed under the trade name, Capsicum Powder (CTFA 2003a).

Capsicum Frutescens Fruit: Capsicum Frutescens Fruit is defined as a plant material that is derived from the dried ripe fruit of the capsicum, *Capsicum frutescens*. Other names for this ingredient are as follows: Capsicum, Capsicum Frutescens (EU), Cayenne Pepper, and Red Pepper (Pepe et al. 2002). According to another source, it is the dried ripe fruit of *Capsicum frutescens* Linné, *Solonaceae* (Gennaro 1990).

Capsicum Frutescens Fruit Extract: This ingredient is defined as an extract of the dried fruit of the capsicum, *Capsicum frutescens*. Other ingredient names are as follows: Capsicum Extract, Capsicum Frutescens (EU), Capsicum Tincture, Extract of Capsicum, and Extract of Capsicum Frutescens (Pepe et al. 2002).

Capsicum Frutescens Resin: Capsicum Frutescens Resin (CAS no. 8023-77-6) is defined as a resinous material that is obtained from *Capsicum frutescens*. Other names for this ingredient are as follows: Capsicum (Capsicum Frutescens) Oleoresin; Capsicum Frutescens (EU); Capsicum Oleoresin; Oleoresin Capsicum Africanus; Resin Capsicum; and Resin, Capsicum Frutescens. Capsicum Frutescens Resin is marketed under the trade name AEC Capsicum Oleoresin and under the trade name mixture, Paprika-Extract, Oil Soluble Extra Conc. (Pepe et al. 2002).

Chemical and Physical Properties

Chemical descriptions, provided by the cosmetics industry, on Capsicum Annuum Fruit Extract, Capsicum Frutescens Fruit Extract, trade name mixtures containing Capsicum Frutescens Fruit Extract, and Capsicum Frutescens Resin are included in Table 1. Additional data from other sources are summarized in the text.

Capsicum Oleoresin from *Capsicum annuum* Linnaeus or *Capsicum frutescens* Linnaeus is a clear red to dark red, somewhat viscous liquid that is of a characteristic odor and flavor. It is partly soluble in alcohol (with oily separation and/or sediment) and is soluble in most fixed oils (National Academy of Sciences 1996).

In addition to the information in Table 1, Capsicum (*Capsicum frutescens*) Oleoresin has also been described as a dark red, extremely acrid and pungent liquid (Rietschel and Fowler 1995).

According to the *United States Pharmacopoeia,* Capsicum Oleoresin is an alcoholic extract of the dried ripe fruits of *Capsicum annuum var. minimum* and small fruited varieties of *C. fruiscons* (Solanaceae). Capsicum Oleoresin contains not less than 8% of total capsaicins (capsaicin [$C_{18}H_{27}NO_3$], dihydrocapsaicin [$C_{18}H_{29}NO_3$], and nordihydrocapsaicin [$C_{17}H_{27}NO_3$]) (Committee of Revision of the United States Pharmacopeial Convention 2000).

Properties of Capsicum Oleoresin (from *Capsicum annuum*) are included in Table 2.

Composition/Impurities

Capsicum Annuum

Azizan and Blevins (1995) confirmed the presence of Capsaicin in *Capsicum annuum* by thin-layer chromatography (TLC) and gas chromatography (GC). Using TLC, it was determined that Capsaicin constitutes 0.03% of the total weight of fresh *Capsicum annuum*. However, a higher value of 1.27% of the total weight was determined using gas chromatography.

The existence of other capsaicinoids in *Capsicum annuum* such as homocapsaicin, norhydrocapsaicin, dihydrocapsaicin, and homodihydrocapsaicin reported by Suzuki (1980) was

TABLE 1
Cosmetic ingredient chemical descriptions, properties, and methods of production

Ingredient	Chemical composition	Properties	Method of production
Capsicum Annuum Fruit Extract (from CTFA 2002a)	Standardized to 0.5% Capsaicin. The full spectrum extract contains the full range of phytocompounds that is found in native plant material, i.e., fruit pods [capsaicinoids (amids of the vanillyl amine)]	Reddish-orange to reddish-brown, fine powder, soluble or dispersible in water; mildly hygroscopic with a pungent odor	Full spectrum, water extraction process; spray dried extract powder is carrier free
Capsicum Annuum Fruit Extract trade name mixture (from CTFA 2002a)	Very low concentration of Capsaicin (content has not been measured), 20% paprika oleoresin, 44% Polysorbate 80, 18% propylene glycol, 16% sorbitan oleate, and 2% natural tocopherol	Dark orange-red, viscous liquid, soluble in water	Hexane extraction of the pods of paprika (*Capsicum annuum*) fruit; solvent is removed as steam, and the extract is solubilized in emulsifiers and propylene glycol
Capsicum Frutescens Fruit Extract (from CTFA 2003b)	Contains 5% Capsaicin and unstated concentration of carotenoids	Viscous, very dark red liquid with a characteristic odor and specific gravity (0.905 to 0.970 at 20°C)	Produced via an extraction procedure, the details of which were not provided
Capsicum Frutescens Fruit Extract (from CTFA 2003b)	Standardized to 0.5% Capsaicin, also contains phytocompounds found in native plant material	Reddish-orange to reddish-brown fine powder, soluble or dispersible in water; mildly hydroscopic with a pungent odor	Full spectrum water extraction process; spray dried extract powder is carrier free
Capsicum Frutescens Fruit Extract trade name mixture (from CTFA 2003b)	Capsicum Frutescens Fruit Extract (10% to 25%), propylene glycol (75% to 90%), 0.1% to 1% phenoxyethanol, methylparaben, ethylparaben, butylparaben, and propylparaben preservatives, 1 ppm maximum heavy metals, traces of Capsaicin, essential oil, capsaicinoids, carotenoids, vitamins, flavonoids, saponins, organic acids, sugars, proteins, traces of solanine, soladinine, and 2-methoxy-3-isobutylpyrazine.	Clear, orange-colored liquid, soluble in water, with a pH range of 4.5 to 5.5, refractive index of 1.420 to 1.440 at 20°C) and density of 1.030 to 1.050 (at 20°C)	Dried plant material is mixed with propylene glycol (10 parts) and agitated at room temperature for 48 hours, after which the solid particles are separated by centrifugation, and filtered (for sterilization) under 5 atmospheres of pressure
Capsicum Frutescens Fruit Extract trade name mixture (from CTFA 2003b)	Capsicum Frutescens Fruit Extract (5% to 15%), Capsaicin (1%), denatured alcohol (70% to 85%), PEG-60 hydrogenated castor oil (5% to 15%), and carotenoids (concentration not stated)	Transparent dark amber to dark red liquid with a characteristic odor, with a pH range of 6.0 to 7.0 and specific gravity of 0.83 to 0.9 (at 20°C)	Produced via an extraction procedure, the details of which were not provided
Capsicum Frutescens Fruit Extract trade name mixture (from CTFA 2003b)	Capsaicin (unknown concentration), dry matter (4% to 6%), water/propylene glycol (40%), and a preservative (0.25%, name not stated)	Yellowish to reddish-brown liquid with a characteristic smell and a density of 1.040 to 1.056 (at 20°C) and a pH range of 5.0 to 6.5	Cold maceration and percolation

Capsicum Frutescens Fruit Extract trade name mixture (from CTFA 2003b)	Actives in the plant source include Capsaicin; dihydrocapsaicin; nordihydrocapsaicin; omocapsaicins I and II; octyl-, nonyl-, and decylvanillamide, capsanthin; kryptxanthin; zeaxanthin; capsorubin; lutein; α-, β-, and neo-β-carotene; β-carotene epoxide; ascorbic acid; provitamins A, B1, B2, C, E, and PP; nicotinic acid amide; tocopherols; cynaroside; quercetin; apiin; luteolin-7-monoglucoside; capsicoside; citric and malonic acid; fructose; galactose; sucrose; planteose; asparagine; serine; solanine; soladinine traces; 2-methoxy-3-isobutylpyrazine; anguin; oleoresin; citrozanthin; violaxanthin; and capsorubin	Yellowish to reddish-brown liquid with a characteristic smell, density of 1.040 to 1.056 (at 20°C), and a pH range of 5.0 to 6.5	Produced via cold maceration and percolaton
Capsicum Frutescens Fruit Extract trade name mixture (from CTFA 2003b)	10% to 20% Capsicum Frutescens Fruit Extract, 80% to 90% vegetable oil, 0.62% to 10% total Capsaicins (including nordihydrocapsaicin, homocapsaicin, Capsaicin, and dihydrocapsaicin), $\leq$25 ppm heavy metals, and $\leq$15 ppm heavy metals	Sallow yellow, viscous fluid that is caustic and has a pungent aroma. It is soluble in ethanol, and has a specific gravity of 0.91 to 0.93 and a refractive index of 1.45 to 1.49. Scoville unit = $\geq$ 100,000	Produced by an extraction procedure (details not provided) using ethanol and vegetable oil as solvents
Capsicum Frutescens Fruit Extract supplied as 0.2% Capsicum	0.2% Capsicum with unknown Capsaicin concentration	Slight color, soluble in water	Ground Cayenne Pepper, water, propylene glycol, and a preservative are blended in a tank, aged for 5 to 7 days, filtered, and packaged
Capsicum Frutescens Fruit Extract trade name (Vegetol Capsicum LC 481Huileux) mixture (from CTFA 2002b; Gattefossé s.a. 1998)	1% to 5% Capsicum Frutescens Fruit Extract, 10% to 25% Prunus Armeniaca (Apricot) Kernel Oil, and 70% to 89% mineral oil	Oily liquid with characteristic odor, color of a 50% solution is 9 to 12 on gardner scale, soluble in vegetable and mineral oils, insoluble in water, with a specific gravity of 0.830 to 0.865 (at 20°C), a refractive index of 1.455 to 1.475 (at 20°C); a viscosity of 14 to 26 mPascal-s (at 20°C), and a total aerobic organisms count of <100/g	Extracted by prolonged maceration of *Capsicum frutescens* in vegetable oil–mineral oil mixture; materials used in production are exclusively from vegetable and petrochemical origin

(Continued on next page)

TABLE 1
Cosmetic ingredient chemical descriptions, properties, and methods of production *(Continued)*

Ingredient	Chemical composition	Properties	Method of production
Capsicum Frutescens Resin (from CTFA 2002c)	Standardized oleoresins with capsicum content described as consisting mainly of pungent principles (Capsaicin, dihydrocapsaicin, nordihydrocapsaicin, and others). Other constituents include capsanthin, carotene, lutein, fats (17%), proteins (15%), and vitamins A, C, and others plus a small amount of volatile oil	Not available	Oils and oleoresins blended to manufacture a standardized product relative to heat, taste, and color; definite specification for each preparation is difficult to provide, but, as a guide, if the specification is 20% Capsaicin content, the remaining composition (80%) would be as follows: fats (47%), proteins (40%), volatile oil (10%), and vitamins and pigments (2% to 3%).
Capsicum Frutescens Resin (from CTFA 2002c; Scoville 1912)	6.2% to 6.7% *w/w* Capsaicin (by UV method). Solvent residue level = 25 ppm maximum	Red/brown viscous liquid with a pungent odor, a taste in bland medium at 1 ppm, and a Scoville heat value of 1,000,000 to 1,200,000	Dried *Capsicum frutescens* is subjected to hexane/methanol extraction, yielding a natural flavoring, followed by solvent removal and adjustment with vegetable oil in order to standardize the Capsaicin content, color, and Scoville heat value
	9.5% to 10.5% *w/w* Capsaicin, determined by UV absorption	Red/brown viscous liquid with a pungent odor, a taste in bland medium at 1 ppm, and a Scoville heat value of 1,500,000 to 1,800,000.	
	19% to 21% *w/w* Capsaicin determined by UV absorption	Red/brown viscous liquid with a pungent odor, a taste in bland medium at 0.25 ppm, and Scoville heat value of 3,200,000 to 3,500,000.	

TABLE 2
Properties of Capsicum Oleoresin (from *Capsicum annuum*)

Property	Value	References
Form	Dark red viscous liquid	Sandia National Labs 1995
	Dark-red colored thick paste	Siris Impex 2002
Odor	spicy odor; odor threshold limit $\approx$ 10 ppm	Sandia National Labs 1995
Taste	Pungent	Siris Impex 2002
Molecular weight	305.4 g/mole	Siris Impex 2002
Specific gravity	1.0073 to 1.073 at 25°C	Siris Impex 2002
Solubility	Fully dispersible in water; soluble in benzene, alcohol, ketone, ether, and paraffin oils	Siris Impex 2002
Melting point	< -60°C	Siris Impex 2002
Boiling point	$>$187°C	Siris Impex 2002
Flash point	215°F	Siris Impex 2002

considered the most important factor in explaining this discrepancy (Azizan and Blevins 1995).

The isolation of fungi from and the production of aflatoxins by species of *Aspergillus* in samples of red pepper of the genus *Capsicum* have been reported (Silva et al. 1990).

A low concentration of aflatoxin B_1 (0.8 μg/kg) was detected in one of two samples of red pepper collected in Osaka, Japan. The samples analyzed in this study were imported foods collected from retail stores in Osaka prefecture on an arbitrary basis from 1988 to1992. TLC with a fluorescence TLC scanner was the analytical method that was used (Tacuchi et al. 1995).

Using TLC, aflatoxin was not detected in any of 10 samples of red pepper (*Capsicum annuum*) purchased in Benin City, Nigeria (Ibeh et al. 1991).

In another study, the following aflatoxins were detected in 2 of 10 red peppers collected in Tokyo, Japan: aflatoxin B_1 (2.6 to 9.1 ppb; average = 5.9 ppb), aflatoxin B_2 (0 to 0.4 ppb; average = 0.2 ppb), and aflatoxin G_1 (0 to 1.9 ppb; average = 1.0 ppb). The samples analyzed in this study were commercially available market basket samples of foods and foodstuffs collected in Tokyo during the years 1986 to 1990. High-performance thin-layer chromatography was the analytical method (Tabata et al. 1993).

Using TLC, aflatoxin in Shiro and ground red pepper (both *Capsicum annuum*) ranged from 100 to 500 ppb and 250 to 525 ppb, respectively. Pepper samples were collected from government owned goods stores, retail shops, and open markets in Addis Ababa, Ethiopia (Fufa and Urga 1996).

In an analysis performed by Adegoke et al. (1996), ordinary mature red pepper (*Capsicum annuum*) contained 75.7% to 78.2% moisture and, on a dry weight basis, vitamin C (36.1 to 38.5 mg/100 g), crude protein (2.4% to 2.8%), total soluble solids (9.3% to 9.9%), and fungi (fungal counts of log 3.32 to 3.39/g). Samples of sundried, matured red pepper contained 12.7% to 26.8% moisture and, on a dry weight basis, vitamin C (5.0 to 6.4 mg/100 g), crude protein (0.8% to 1.2%), total soluble solids (3.3% to 4.1%), and fungi (fungal counts of log 3.32 to 3.39/g). Dominant fungi isolated from pepper samples consisted of *Rhizopus oryzaze*, *Aspergillus niger*, *Aspergillus flavus*, *Geotrichum candidum*, and *Saccharomyces* species. Aflatoxin B_1 values varied from nondetectable to 2.2 μg/kg.

The U.S. Department of Agriculture determined that negative aflatoxin content means 15 parts per billion (ppb) or less for peanuts that have been certified as meeting edible quality grade standards (7 CFR 996.11).

Roychowdhury and Sukul (1997) reported that in India the insecticide fluvalinate (a synthetic pyrethroid) has been used during cultivation of *Capsicum annuum* vegetable crops. Chili foliage, fruit, and cropped-soil samples from each replication were drawn separately at 0 (2 h after spraying), 1, 3, 5, 7, and 10 days after fluvinate application and detected using gas-liquid chromatography. After spraying levels were 1.11 ppm (chili foliage), 0.35 ppm (fruit), and 1.06 ppm (cropped-soil). These residues had dissipated to a nondetectable level by day 10 after pesticide application. Half-life values in foliage, fruit, and soil were in the range of 1 to 1.7 days.

Twenty chili samples (from different suppliers) obtained from shops and market traders in West Germany were analyzed for volatile *N*-nitroso compounds using mineral oil vacuum distillation of alkaline slurries. Over 75% of both dried, whole chilies and chili powder samples analyzed contained *N*-nitrosodimethylamine (0.66 to 16.5 μg/kg) and *N*-nitrosopyrrolidine (0.48 to 6.0 μg/kg) (Tricker et al. 1988).

Cremer and Eichner (2000) reported hexanal; limonene; 6-methyl-5-hepten-2-one; tetramethylpyrazine (tentatively); 2-methoxy-3-isobutylpyrazine; phenylacetaldehyde; methylsalicylate; α-ionone (tentatively); and β-ionone peaks in a gas chromatogram of the volatiles of a spice, paprika powder (*Capsicum annuum*), heated at a temperature of 90°C for 60 min. The authors concluded that the formation of 6-methyl-5-hepten-2-one confirms that *Capsicum annuum* fruits contain lycopene.

Capsicum Annuum Resin

Siris Impex (2002) reported the following specifications for Capsicum Annuum Resin: loss on drying (not more than 5% *w/w*); Capsaicin content, on dry basis using high-performance thin-layer chromatography (HPTLC) (not less than 6% *w/w*); lead (not more than 10 ppm); arsenic (not more than 2 ppm); heavy metals (not more than 20 ppm); total bacterial count (not more than 10,000 colony-forming units [CFU]/g; yeast and molds (not more than 100 CFU/g); *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium* (absent); aflatoxins B_1+ B_2+ G_1+ G_2 (not more than 5 ppb); and pesticide traces (absent).

Capsicum Frutescens Fruit

Capsicum Frutescens Fruit (a.k.a. Capsicum or Cayenne Pepper) contains capsaicin (crystalline pungent alkaloid), flavonoids, fatty acids, dihydrocapsaicin and related alkaloids, carotene, capsanthin, and vitamins A and C (D'Amelio 1999). According to an earlier publication, it also contains: 0.42% natural Capsaicin, cryptoxanthin, capsanthin, capsorubin (0.4%), carotene (0.06%), vitamin C, citric acid, tartaric acid, malic acid, tannic acid, thiamine, ascorbutic acid, fatty oil (15% to 20%), some volatile oil, starch (0.8% to 1.4%), pentosans (8.75%), and pectin (2.33%) (Villaseñor et al. 1995).

According to the *United States Pharmacopeia*, specifications for Capsicum Frutescens Fruit include not more than 1.25% acid-insoluble ash and not more than 1% foreign organic matter, other than stems and calyces, the proportion of which does not exceed 3% (Committee of Revision of the United States Pharmacopeial Convention 2000).

Sun-dried, matured red *Capsicum frutescens* contains 9.4% to 18.7% moisture and the following other components (dry weight basis): vitamin C (5.8 to 6.3 mg/100 g), crude protein (0.8% to 1.1%), total soluble solids (0.9% to 2.6%), and fungi (fungal counts of log 3.2 to 3.4/g). Dominant fungi isolated from pepper samples consisted of *Rhizopus oryzaze*, *Aspergillus niger*, *Aspergillus flavus*, *Geotrichum candidum*, and *Saccharomyces* spp. No aflatoxins were detected (Adegoke et al. 1996).

Using thin-layer chromatography, aflatoxins (8 to 35 μg/kg) were found in 10 samples of red pepper (*Capsicum frutescens* Linnaeus) collected at Assiut Governorate in Egypt (El-Kady et al. 1995).

Methods of Production

Capsicum Annuum Resin

Capsicum Oleoresin (from *Capsicum annuum* Linnaeus or *Capsicum frutescens* Linnaeus) is obtained by solvent extraction of dried pods of *Capsicum frutescens* Linnaeus or *Capsicum annuum*. It is clear to dark red and may be decolorized, in keeping with a good manufacturing practice (National Academy of Sciences 1996).

According to another source, Capsicum (*Capsicum frutescens*) Oleoresin is extracted from cayenne pepper (a.k.a. Capsicum Frutescens Fruit) pods with alcohol or ethyl ether (Rietschel and Fowler 1995).

Capsicum Frutescens Resin

After grinding dried ripe fruits of *Capsicum frutescens* L. (chili peppers) into a fine powder, the oleoresin may be obtained by distillation of the powder in an appropriate solvent. Evaporation of the solvent yields the liquid oleoresin and associated fatty matter, which is removed by decanting or filtration (Environmental Protection Agency [EPA] 1992).

The manufacturing process for obtaining Capsaicin powder and oleoresin that is used by a primary supplier (Kalsec, Inc.) has been described as follows: the peppers are ground and extracted with food grade hexane, and the resulting extract is filtered with diatomaceous earth. After distillation to remove the hexane (level will not exceed 25 ppm), the raw extract is analyzed for color intensity and capsaicinoid content (EPA 1992).

UV Absorption

Capsicum Annuum Fruit Extract

Ultraviolet (UV) absorption spectra on two trade name chemicals (Capsicum Tincture and Capsicum Tincture SH) under which Capsicum Annuum Fruit Extract is marketed were provided. For Capsicum Tincture, absorbance at wavelengths ranging from 250 to 450 nm was observed. A small peak was noted at approximately 275 nm, and a gradual increase in absorbance, beginning at approximately 400 nm, was also observed. For Capsicum Tincture SH, absorbance over the same range of wavelengths (250 to 450 nm) was noted, with an absorption maximum occurring at approximately 275 nm. A gradual increase in absorbance, beginning at approximately 400 nm, was also observed (Maruzen Pharmaceuticals Co., Ltd. 2002).

Stability/Reactivity

Capsicum Annuum Fruit Extract

Capsicum Annuum Fruit Extract is a non-reactive, stable powder (CTFA 2002a).

Capsicum Frutescens Fruit Extract

Capsicum Frutescens Fruit Extract (standardized to 0.5% Capsicum) is described as a non-reactive, stable powder (CTFA 2002b).

Capsicum

The exposure of Tincture of Tabasco Pepper (another name for Capsicum) to heat constitutes a fire hazard (Sax 1979).

USE

Purpose in Cosmetics

In cosmetic products, Capsicum Annuum Extract, Capsicum Annuum Resin, Capsicum Annuum Fruit Powder, and Capsicum Frutescens Fruit Extract each function as a skin-conditioning agent—miscellaneous, whereas the function of

Capsicum Frutescens Fruit in cosmetics is unknown (Pepe et al. 2002; CTFA 2003b).

Capsicum Annuum Fruit Extract functions as an external analgesic, flavoring agent, and fragrance component in cosmetics (CTFA 2002a). External analgesic and skin conditioning agent, miscellaneous are functions of Capsicum Frutescens Fruit Extract (CTFA 2003b) and Capsicum Frutescens Resin (CTFA 2002c) in cosmetics.

Tincture of Capsicum has been used as a rubefacient (agent that reddens the skin by producing active or passive hyperemia) in hair tonics at concentrations ranging from 1% to 5% (Balsam et al. 1972).

Scope and Extent of Use in Cosmetics

Frequency of use data reported by industry to the Food and Drug Administration (FDA) indicate that Capsicum, Capsicum Extract, Capsicum Frutescens Extract, and Capsicum Oleoresin are used in a total of 19 cosmetic products, as described in Table 3 (FDA 2002).

TABLE 3
Product formulation data on Capsicum ingredients (FDA 2002; CTFA 2001)

Product category (number of formulations reported to FDA) (FDA 2002)	Number of formulations containing ingredient (FDA 2002)	Maximum use concentration range (CTFA 2001)
Capsicum		
Tonics, dressings, and other hair-grooming aids (598)	3	—
Other hair preparations (277)	1	—
2002 total uses/ranges for Capsicum	4	—
Capsicum Extract		
Tonics, dressings, and other hair-grooming aids (598)	5	—
Face and neck (excluding shaving) (310)	1	—
Body and hand (excluding shaving) (840)	2	—
2002 total uses/ranges for Capsicum Extract	8	—
Capsicum Annuum Fruit Extract		
Other bath preparations (196)	—	0.2%
Other hair preparations (277)	—	0.5%
Body and hand (excluding shaving) (840)	—	1%
2002 total uses/ranges for Capsicum Annuum Fruit Extract	—	0.2–1%
Capsicum Annuum Fruit Powder		
Bath oils, tablets, and salts (143)	—	5%
2002 total uses/ranges for Capsicum Annuum Fruit Powder	—	5%
Capsicum Frutescens Fruit Extract		
Bubble baths (215)	—	0.015%
Shampoos (noncoloring) (884)	—	0.003%
Other hair preparations (277)	—	1%
2002 total uses/ranges for Capsicum Frutescens Fruit Extract	—	0.003–1%
Capsicum Frutescens Extract		
Other bath preparations (196)	2	—
Shampoos (noncoloring) (884)	1	—
Cleansing (775)	1	—
Face and neck (excluding shaving) (310)	1	—
2002 Total uses/ranges for Capsicum Frutescens Extract	5	—
Capsicum Oleoresin		
Shampoos (noncoloring) (884)	2	—
2002 total uses/ranges for Capsicum Oleoresin	2	—

Although not reported to FDA, industry has provided current use concentration data on Capsicum Annuum Fruit Extract, Capsicum Annum Fruit Powder, and Capsicum Frutescens Fruit extract as a function of ingredient and product type, as shown in Table 3 (CTFA 2001).

Industry (CTFA 2002a, 2003b) has provided additional use concentration data on Capsicum Annuum Fruit Extract (from two suppliers) and Capsicum Frutescens Fruit Extract (from five suppliers), but the product categories were not stated. Capsicum Annuum Fruit Extract is used in cosmetics at concentrations up to 0.05% (as Capsaicin). A trade name mixture (name not stated) under which this ingredient is marketed is used at concentrations up to 0.2%. The following use concentrations for Capsicum Frutescens Fruit Extract in cosmetics were provided by five different suppliers: 0.1% to 0.3% (100% Capsicum Frutescens Fruit Extract); 0.05% maximum (Capsaicin) (Capsicum Frutescens Fruit Extract standardized to 0.5% Capsaicin); 1% to 10% (unnamed trade name mixture containing 10% to 25% Capsicum Frutescens Fruit Extract); 1% to 3% (unnamed trade name mixture containing 5% to 15% Capsicum Frutescens Fruit Extract); and 2% to 5% (unnamed trade name mixture containing unspecified concentration of Capsicum Frutescens Fruit Extract).

Capsicum Tincture (a.k.a. Capsicum Frutescens Fruit Extract, according to Pepe et al. 2002) is included on the list of ingredients restricted for content in all cosmetics that are marketed in Japan. For Capsicum Tincture, the maximum content per 100 g of product allowed is 1.0 g (as total) (Ministry of Health, Labor and Welfare [MHLW] 2001a). Capsicum Tincture is also included on the list of ingredients of quasi-drugs that are marketed in Japan. Some cosmetic products are considered quasi-drugs. For example, in Japan, products used to improve such symptoms as chapped skin, prickly heat, sores, corns, calluses, and dry skin are among those classified as quasi-drugs (MHLW 2001b).

The ingredients that are included in this safety assessment are not included among the substances listed as prohibited from use in cosmetic products marketed in the European Union (European Economic Community 1999, 2002).

Based on the information in Table 3, cosmetic products containing Capsicum and Capsicum Oleoresin are applied to the hair only. Products containing Capsicum Extracts are applied to the hair as well as most areas of the body, and cosmetic products containing Capsicum Annuum Fruit Powder are applied to most areas of the body, but not to the hair. These products may incidentally come in contact with the ocular and nasal mucosae. Additionally, cosmetic products containing Capsicum, Capsicum Extracts, Capsicum Annuum Fruit Powder, or Capsicum Oleoresin could be used on a daily basis and applied frequently over a period of several years. Capsicum Annuum Fruit Extract, Capsicum Annuum Fruit Powder, Capsicum Frutescens Fruit Extract, and Capsicum Frutescens Extract are used in some bath products. These products are added to bath water and thus are diluted prior to coming in contact with most areas of the body.

Noncosmetic Use

Information on the over-the-counter (OTC) drug review status of Capsicum and related ingredients (FDA 2003) is included in Table 4.

Capsaicin (supplied as Zostrix; two strengths: 0.025% and 0.075% Capsaicin) is used to treat the neuropathic pain of postherpetic neuralgia (Facial Neuralgia Resources 2003).

In 1993, the Food and Drug Administration published a final rule indicating that Capsicum and other active ingredients are not generally recognized as safe and effective and are misbranded when present in certain OTC drug products. This ruling was based on inadequate data for establishing general recognition of the safety and effectiveness of these ingredients for the specified uses. The ingredients and types of drug products for which this ruling is applicable are digestive aid drug products (Capsicum and Capsicum, fluid extract of), external analgesic drug products for fever blister and cold sore treatment (Capsicum, and Capsicum Oleoresin), and orally administered menstrual drug products (Capsicum Oleoresin). It was noted that this final rule affects only the marketing of ingredients that are listed as active ingredients in specific types of OTC drug products, for which unproven medical claims are being made, and that this rule does not affect the continued use and marketing of these ingredients in vitamin, mineral, and food supplement products (FDA 1993).

The spices Capsicum (plant source: *Capsicum frutescens* L. or *Capsicum annuum* L.) and paprika (plant source: *Capsicum annuum* L.) are among the spices and other natural seasonings and flavorings that are generally recognized as safe (GRAS) for their intended use in food (21CFR182.10; 582.10). Capsicum and paprika are also listed among the essential oils, oleoresins (solvent-free), and natural extractives (including distillates) that are GRAS for their intended use in food (21CFR182.20; 582.20). According to the *International Cosmetic Ingredient Dictionary and Handbook*, Capsicum is among the technical names for both Capsicum Annuum Resin and Capsicum Frutescens Fruit (Pepe et al. 2002).

The European Commission Scientific Committee on Food concluded that the available data in the published literature did not allow it to establish a safe exposure level for capsaicinoids in food (European Commission 2002).

Paprika (ground dried pod of mild capsicum [*Capsicum annuum* L.]) may be used safely for the coloring of foods, generally, in amounts that are consistent with good manufacturing practice (21 CFR:73.340).

According to the *International Cosmetic Ingredient Dictionary and Handbook,* Paprika Oleoresin is a trade name for Capsicum Annuum Resin (Pepe et al. 2002). For the purpose of identity as a color additive, Paprika Oleoresin is defined as the combination of flavor and color principles obtained from paprika (*Capsicum annuum* L.) by extraction, using any one or a

TABLE 4
OTC drug review ingredient status report for Capsicum and Capsicum derivatives (FDA 2003)

Ingredient	OTC panel	OTC use	Pharmaceutical use	Proposed OTC category[a]	*Final OTC category*[a]
Capsicum	Dental	Relief of Oral Discomfort	Counterirritant (External)	IIIE	Pending
Capsicum	Dental	Relief of Oral Discomfort	Toothache Relief	IISE	Pending
Capsicum	Miscellaneous External	External Analgesic	Fever Blister (Topical)	IISE	IIE 21CFR:310.545 (a)(10)(v)
Capsicum	Miscellaneous Internal	Digestive Aid	Digestive Aid (Immediate Postprandial Upper Abdominal Distress—IPPUAD)	—	IISE 21CFR:310.545 (a)(8)(ii)
Capsicum	Topical Analgesic	External Analgesic	Counterirritant	I	Pending
Capsicum Oleoresin	Miscellaneous External	External Analgesic	Fever Blister (Topical)	IISE	IIE 21CFR:310.545 (a)(10)(v)
Capsicum Oleoresin	Miscellaneous Internal	Menstrual/Diuretic	Diuretic	IISE	IISE 21CFR:310.545 (a)(8)(ii)
Capsicum Oleoresin	Topical Analgesic	External Analgesic	Counterirritant	I	Pending
Capsicum, Fluid Extract Of	Miscellaneous Internal	Digestive Aid	Digestive Aid (IPPUAD)	—	IISE 21CFR:310.545 (a)(18)(ii)

[a] Category I—conditions under which OTC drug products are generally recognized as safe (S) and effective (E) and are not misbranded; category II—conditions under which OTC drug products are not generally recognized as safe and effective or are misbranded; category III—conditions for which the available data are insufficient to permit final classification.

combination of the following solvents: acetone, ethyl alcohol, ethylene dichloride, hexane, isopropyl alcohol, methyl alcohol, methylene chloride, and trichloroethylene. The color additive, Paprika Oleoresin, is exempt from certification requirements because certification of this color additive is not necessary for the protection of public health (21CFR73.345).

According to the Joint Food and Agriculture Organization (FAO) of the United Nations/World Health Organization (WHO), the use of oleoresins of paprika as a spice will be self-limiting and governed by good manufacturing practice (Joint FAO/WHO Expert Committee on Food Additives 1970).

The active ingredients, pungent components, of pepper spray aerosols are usually described as being derived from Capsicum Oleoresin, and include Capsaicin, dihydrocapsaicin, and nordihydrocapsaicin (Haas et al. 1997).

Pesticide products containing Capsaicin (oleoresin of capsicum, from dried ripe fruits of *Capsicum frutescens* L.) as an active ingredient have been registered since 1962. In 1991, EPA classified Capsaicin as a biochemical pesticide because it is a naturally occurring biological substance and has a nontoxic mode of action. It is used as an animal and insect repellant, and Capsaicin repellants are also used outdoors to protect fruit and vegetable crops, flowers, ornamental plants, shrubbery, trees, and lawns (EPA 1992).

TOXICOLOGY

Acute Oral Toxicity

Capsicum Frutescens Fruit Extract

Fresh *Capsicum frutescens* fruits were homogenized in ethyl alcohol, and the extract was filtered and then concentrated under reduced pressure. The ethanolic fraction was partitioned between water and hexane, and the aqueous layer was further extracted with chloroform, and then with ethyl acetate. Hexane, chloroform, and ethyl acetate portions were then concentrated under reduced pressure. The following results for groups of five Swiss Webster albino mice (weights = 20 to 25 g) were reported following single oral doses of *Capsicum frutescens* L. extract fractions: hexane fraction (200 mg/kg, death of all mice), chloroform fraction (200 mg/kg, death of all mice), and ethyl acetate fraction (200 mg/kg, death of all mice). Mortalities also occurred (number not stated) after dosing with 100 mg/kg hexane fraction and 25 mg/kg chloroform fraction (Villaseñor and de Ocampo 1994).

Short-Term Inhalation Toxicity

Capsicum Oleoresin

Debarre et al. (1999) evaluated the short-term inhalation toxicity of aerosolized Capsicum Oleoresin (7% solution, solvent

not stated) using male Wistar rats (6 to 8 weeks old; weights = 180 to 200 g). On the first day of the study (pre-exposure training session), the animals were exposed to ambient air and to sodium chloride aerosol. At the end of the pre-exposure session, the animals were exposed to the solvent (nose only) once for 20 min and then exposed (nose only) four times to Capsicum Oleoresin aerosol at durations of 5, 10, 15, and 20 min, respectively. The interval between exposures was 24 h. Ninety percent of the particles had an aerodynamic equivalent diameter of between 1.5 and 2 μm. Breathing flow patterns were monitored during exposure. Each animal served as its own control. After the last exposure, the animals were anesthetized and the lungs and trachea were removed and subjected to microscopic examination.

A significant reduction ($p < .05$) in minute ventilation was observed after exposure to Capsicum Oleoresin (50 $\pm$ 6% of pre-exposure minute ventilation) or its solvent (56 $\pm$ 7% of pre-exposure minute ventilation). A comparison of the reduction in minute ventilation between Capsicum Oleoresin and solvent-exposed animals did not reveal any significant differences. Reductions in minute ventilation were caused by a reduction in tidal volume and respiratory frequency. At microscopic examination, an increase in mucus secretion in the trachea and interstitial edema in the lungs were reported for animals exposed to Capsicum Oleoresin.

Short-Term Oral Toxicity

Capsicum Annuum

Jang et al. (1992) evaluated the short-term oral toxicity of ground red chili (*Capsicum annuum*) using groups of male B6C3F1 mice (seven groups of five; 6 weeks old). Six groups were fed ground chili (in meal diet) at 0.5%, 1.0%, 2.5%, 5.0%, 7.5%, or 10.0%. Control mice were fed meal only. The feeding period for test and control mice was 4 weeks. At 10 weeks of age, all mice were killed by CO_2 inhalation. A complete necropsy was performed. The liver, kidneys, and thymus were weighed, and organ-to-body weight ratios were calculated. The following tissues were examined microscopically: liver, kidney, stomach, small intestine, colon, heart, lungs, salivary glands, spleen, and thymus.

None of the animals died, and no treatment-related physical signs or significant, dose-related growth retardation were noted. Except for the 7.5% dose group, average daily consumption of test diets decreased. No dose-response relationship for the decrease in diet consumption was noted. Data on changes in body weight or relative organ weight indicated no significant difference between test and control groups. Except for mild glycogen depletion and anisocytosis of hepatocytes (in 10% dose group), no treatment-related microscopic changes were observed in any of the tissues evaluated. The authors concluded that red chili (*Capsicum annuum*) was relatively nontoxic at the doses tested (Jang et al. 1992).

Capsicum Frutescens Fruit

Qarawi and Adam (1999) evaluated the short-term oral toxicity of Capsicum Frutescens Fruit using three groups of eight male Wistar rats (weights = 165 to 175 g). The control group was fed a commercial diet with the following composition: corn oil (5.0%), starch (59.2%), granulated sugar (5.0%), protein (22.5%), cellulose powder (3.0%), DL-methionine (0.3%), mineral mix (4.0%), and vitamin mix (1.0%). The remaining two groups were each fed a diet containing 2% (*w*/*w*) or 10% (*w*/*w*) ground Capsicum Frutescens Fruit. Diets were fed for 8 weeks. At 4 weeks, blood samples were obtained from four rats in each group, after which the animals were killed. The remaining animals in each group were killed at 8 weeks. At necropsy (all animals), the intestine, liver, kidneys, heart and spleen were examined for gross lesions.

Compared to the control group, rats fed 10% Capsicum Frutescens Fruit had a lower average food intake, body weight, and feed efficiency ($p < .05$–.001) within 8 weeks of feeding. However, the average food intake and growth were not adversely affected in the group fed 2% Capsicum Frutescens Fruit in the diet.

No significant differences in erythrocytic series were observed in either treatment group at 4 weeks. However, it is important to note that the increase ($p < .05$) in white blood cell values in rats fed 10% Capsicum Frutescens Fruit was due to an increase ($p < .05$) in numbers of lymphocytes. At 8 weeks, differences in the following hematological parameters between the two Capsicum Frutescens Fruit feeding groups were noted: lower hemoglobin concentration, packed cell volume, red blood cell count, and lymphocytes ($p < .05$–.001), and higher mean corpuscular volume and neutrophils ($p < .05$–.001) in rats fed 10% Capsicum Frutescens Fruit. No changes in eosinophil, basophil, or monocyte numbers were noted.

When the two Capsicum Frutescens Fruit feeding groups were compared, changes in the following enzyme activities were evident: increased aspartate transaminase, alanine transaminase, and lactic dehydrogenase ($p < .05$–.001), and decreased total protein, albumin, cholesterol, and copper ($p < .05$–.001) in rats fed 10% Capsicum Frutescens Fruit in the diet. These findings were noted at 4 and 8 weeks. No significant differences in alkaline phosphatase, urea, iron, or globulin were reported for rats fed 2% or 10% Capsicum Frutescens Fruit.

In animals necropsied at 4 weeks, packing of the intestinal lamina propria with lymphocytes and slight cytoplasmic fatty vacuolation of the centrilobular hepatocytes were observed in the 10% Capsicum Frutescens Fruit feeding group. No lesions (at 4-week necropsy) were detected in rats fed 2% Capsicum Frutescens Fruit in the diet.

After 8 weeks of feeding, the following changes were observed in animals fed 10% Capsicum Frutescens Fruit in the diet: exfoliation of the intestinal epithelium into the lumen, with lymphocytic accumulation, cytoplasmic fatty vacuolation and centrilobular necrosis of hepatocytes, and aggregates of

lymphocytes in the portal areas. No significant changes were observed in the organs of rats fed 2% Capsicum Frutescens Fruit for eight weeks (Qarawi and Adam 1999).

Capsicum Extract

Monsereenusorn (1983) studied the short-term oral toxicity of Capsicum Extract (crude fruit extract) using two groups of rats (strain not stated; weights = slightly over 100 g). One group served as the control. Both groups were subdivided into seven subgroups (10 to 14 rats per group). Test animals received Capsicum Extract (0.5 g/kg day^{-1}) by stomach tube for 60 days. Control rats received an equivalent volume of saline solution. No deaths were observed in test or control groups.

At day 40 of feeding, a significant reduction in body weight gain was noted in groups dosed with Capsicum Extract. A significant decrease in the following blood plasma parameters was also noted in these groups after dosing for more than a month: blood urea nitrogen, glucose, phospholipids, triglycerides, total cholesterol, free fatty acids, glutamic pyruvic acid transaminase, and alkaline phosphatase. Hematological analyses (i.e., red blood cells, hemoglobin, hematocrit, white blood cells, and prothrombin time) were normal. Additionally, tests for urinary glucose, blood, bile salts and lactones were negative, and quantities of protein and microscopic constituents were similar between test and control groups.

At necropsy, no significant gross pathologic changes were noted in most organs. However, at 60 days, slight hyperemia (without hemorrhage) was observed in the livers of animals dosed with Capsicum Extract. Additionally, the gastric mucosa of animals dosed with each test substance was reddened and mucus materials were increased. Compared to controls, there were no statistically significant changes in the following relative organ weights after 60 days of dosing: liver, stomach, pancreas, spleen, intestine, caecum, lung, heart, kidney, thyroid, adrenal, and gonad. The author concluded that a longer period of dosing with Capsicum Extract may have had a mild effect on the animals tested (Monsereenusorn 1983).

Capsicum Frutescens

In a study by Srinivasan et al. (1980), groups of 8 to 10 weanling Wistar rats (mean weights = 38.6 to 38.7 g) were fed basal diets supplemented with whole red pepper (*Capsicum frutescens*, 0.3% Capsaicin content) at concentrations of 0.5%, 1.0%, 2.0%, and 5.0% for 4 or 8 weeks. Control rats (mean weight = 38.5 g) were fed basal diet only. Compared to controls, rats in the highest dose group had a significant increase in feed intake, weight gain, and in the feed efficiency ratio (FER) ($p < .05$) in 4- and 8-week experiments. In the 4-week experiment, there was no difference in food intake or FER between the four dose groups. However, in the 8-week experiment, the FERs for the 2% and 5% dose groups were significantly greater ($p < .05$) than control values. At hematological evaluation, no differences (compared to controls) in the following blood parameters were observed: red blood cells, white blood cells, differential counts, hemoglobin, or serum proteins. At microscopic examination, the large intestines, livers, and kidneys of animals in each test group were normal. However, pathological changes, such as destruction of taste buds and keratinization and erosion of the mucosal layer of the gastrointestinal tract, were observed in all treatment groups (incidence per group not stated).

Chronic Oral Toxicity

Capsicum Annuum Powder (Paprika Powder)

In a study by Lee (1963), two groups of nine and seven healthy white rabbits (strain not stated; average body weight ≈2 kg) received a standard diet with Capsicum Annuum Powder (Paprika Powder, 5 mg/kg body weight day^{-1}) and without Paprika Powder, respectively, daily for 12 months. The animals were killed after 12 months of feeding, and livers and spleens were examined microscopically.

Spleen and liver weights were increased in the test group. At necropsy, the liver was brown to pale in color, firm, and had increased vascular markings, with blunt edges. At microscopic examination, the liver contained multiloculated pseudolobules surrounded by portal fibrous tissue with cellular proliferation, mainly lymphocytes and plasma cells. Massive central eosinophilic necrosis with infiltration of inflammatory cells was also observed. Hepatic cells were described as having a moderate to marked degree of extracellular and intracellular fatty infiltration, and were associated with parenchymatous hyaline degeneration and necrotic process. Microscopically, the splenic capsule and trabeculae were diffusely thickened due to increased hyalinized fibrous connective tissue, markedly dilated and congested red pulp, and markedly atrophied, scattered white pulp.

The author concluded that prolonged (12 months) administration of a large dose of Paprika Powder (5 mg/kg body weight day^{-1}) induced liver and spleen damage (Lee 1963).

Capsicum Frutescens

In separate 9- and 12-month feeding experiments conducted by Srinivasan et al. (1980), groups of 20 weanling Wistar rats (mean weights = 38.6 to 38.7 g) were dosed with basal diets supplemented with whole red pepper (*Capsicum frutescens*, 0.3% Capsaicin content) at concentrations of 0.5%, 1.0%, 2.0%, and 5.0%, respectively. Control rats (mean weight = 38.5 g) were fed basal diet only. In the first experiment, the rats were dissected and blood constituents analyzed at 3- and 9-month intervals. These procedures were performed at 6- and 12-month intervals in the second experiment. After up to 1 year of feeding, no changes in food intake or body weight were reported. Growth rates for all four groups were close to the control value. At microscopic examination, the large intestines, livers, and kidneys of animals in each dose group were normal. In rats fed red pepper in the diet, there was no evidence of variation from the control values regarding any of the blood parameters (red blood cell, white blood cell, differential counts, hemoglobin, serum proteins, and

disc electrophoretic pattern of the latter) that were evaluated. The results of nitrogen balance studies, conducted before animals were killed, indicated that red pepper in the diet favors the absorption and retention of nitrogen, mainly due to increased food intake.

In another experiment in this study, groups of weanling rats (eight per sex) were fed red pepper (*Capsicum frutescens*) at a concentration of 0.05% in a basal diet (concentration comparable to human intake, based on body weight). The following parameters were monitored at 3, 6, and 12 months: growth, red blood cells, white blood cells, differential counts, and hemoglobin. Control rats received basal diet only. No abnormalities relating to growth, blood parameters (red blood cells, white blood cells, differential counts, hemoglobin, or serum proteins) or organ weights were noted. At microscopic examination, the large intestines, livers, and kidneys of test animals were normal (Srinivasan et al. 1980).

Skin Irritation

Capsicum Annuum Fruit Extract

The skin irritation potential of two trade-name chemicals (Capsicum Tincture and Capsicum Tincture SH) under which Capsicum Annuum Fruit Extract is marketed was evaluated using one white rabbit. Each of the two trade-name samples was described as a solution of Capsicum Annuum Fruit Extract dissolved in 70% ethanol, and test concentrations of Capsicum Tincture and Capsicum Tincture SH ranged from 0.1% to 1%. Additional details concerning the test procedure were not included. Reactions were scored at 24, 48, and 72 h post application according to the following scales: 0 (no erythema) to 4 (severe erythema [beet redness] to slight eschar formation) and 0 (no edema) to 4 (severe edema). Over the range of concentrations tested, Capsicum Tincture and Capsicum Tincture SH) were classified as nonirritants (Maruzen Pharmaceuticals Co., Ltd. 2002).

Cytotoxicity

Capsicum Frutescens Extract

The effect of Capsicum Frutescens Extract on the proliferation of a human buccal mucosa fibroblast cell line was evaluated. The cell line was incubated with test substance concentrations ranging from 25 to 300 μg/ml for 6 days and 400 and 500 μg/ml for 18 days. At concentrations of 300 to 500 μg/ml, a decrease in cell proliferation was evident as early as day 3. Total cell death was noted at 16 days (test concentration = 300 μg/ml) and at 6 days (test concentrations of 400 and 500 μg/ml). At lower test concentrations (25 to 150 μg/ml), daily cell counts were lower when compared to controls (fibroblasts not exposed to test substance), but the difference was not statistically significant. It was concluded that the cytopathic effect of Capsicum Frutescens Extract on fibroblasts was concentration dependent (van Wyk et al. 1995).

GENOTOXICITY

Studies using bacterial and mammalian test systems to evaluate the genotoxicity/mutagenicity of red chillies (*Capsicum annuum* or *Capsicum frutescens*) and ingredients derived from the Capsicum plant are included in Table 5. In one bacterial test system, positive results were found, but in another the results were negative. Mixed results also were reported in different mammalian test systems.

Antimutagenicity

Using *Salmonella typhimurium* strain YG1024, González de Mejía et al. (1998) demonstrated the antimutagenicity of carotenoids extracted from five different varieties of *Capsicum annuum* against the following mutagens: 1-nitropyrene, 1,6-dinitropyrene, and 1,8-dinitropyrene.

CARCINOGENICITY

Studies on the carcinogenicity/tumorigenicity of chilies (*Capsicum annuum* and *Capsicum frutescens*) are summarized in Table 6.

In a study by Hoch-Ligeti (1951), 30 rats were fed chilies (*Capsicum frutescens* and *Capsicum annuum*) at a concentration of 10% in a semisynthetic diet. The authors noted that chilies contain 0.14% Capsaicin. In addition to chilies, the diet consisted of casein (17 g), fat (14 g), sugar (21 g), and starch (43 g) per 100 g of food, mineral salts, and cod liver oil. After 2 years of feeding, changes in the liver that resembled incipient hepatomas and cholangiomas developed in seven animals. Of the seven, malignant tumors were observed in three rats (one mammary tumor at month 14, one hepatic tumor at month 16, and one pancreatic tumor at month 18). Due to the late appearance of the tumors, the authors considered the findings inconclusive.

A second experiment was conducted to determine whether the combination of a diet (with chilies) devoid of all first-class protein would accelerate the development of liver changes. Therefore, casein (in the original diet) was replaced with ardein, a purified protein of the ground nut. Initially, this was the only modification of the original diet. Rats fed the modified diet lost weight and developed muscular weakness. However, weight gain was increased after the diet was supplemented with a vitamin B compound (0.2 mg riboflavin, 3.0 mg nicotinamide per rat/day) or white bread. At 4 months into the study, the rats were subdivided into the following three groups: group 1 (diet supplemented with bread), group 2 (diet supplemented with vitamin B compound), and group 3 (no dietary supplement).

At necropsy, gross fatty changes in the liver constituted the most prominent pathological finding, whether the diet contained chilies or not. From month 6 to study termination, neoplastic changes in the liver were found in rats fed chilies in the diet. Seemingly, male rats were more prone to tumor development than females. The highest and lowest number of tumors occurred in rats fed vitamin B–supplemented and bread-supplemented diets, respectively. The following four types of tumors were

TABLE 5
Genotoxicity studies

Strain/cell type	Test substance	Procedure	Results	References
Salmonella typhimurium TA98, TA100, and TA1538	Capsicum Oleoresin in DMSO; contained 1.7% *w*/*w* Capsaicin	*Salmonella*/mammalian microsome mutagenicity assay with and without metabolic activation at 2.5 to 25,000 μg/plate	Negative results (no increased reversion rate) with or without metabolic activation	Buchanan et al. 1981
S. typhimurium TA98 (strains 510, 4, and 1018) and TA100 (strains 1, 2, 3, and 7823); *E. coli* trp$^-$ her$^-$ (strains 3, 13, and 14)	Red chilies ethanolic extract. Genus and species not stated	Extract (40 μl) incubated with each strain (on sterile paper disc) for 2 to 3 days	Extract strongly mutagenic to strains 4 and 510; positive (with moderate background) for strain 1018; slightly mutagenic (with very high background) for strain 7823; negative for remaining strains	Shashikanth and Hosono 1987
Bone marrow cells from Swiss Webster Albino mice	Capsicum Frutescens Extract. Four different extracts prepared using hexane, chloroform, ethyl acetate, or water	Groups of 5 mice received single i.p. doses at 50 mg/kg for the hexane extract, 12.5 mg/kg for the chloroform extract, 160 mg/kg for the ethyl acetate extract, and 200 mg/kg for the water extract; followed by micronucleus test	At the maximum tolerated doses, the chloroform extract of *Capsicum frutescens* induced the highest incidence of micronucleated polychromatic erythrocytes (comparable to tetracycline), indicating that it was the most clastogenic fraction. For the other extracts, the mean incidence of micronucleated polychromatic erythrocytes was half or less of that reported for the chloroform extract	Villaseñor and de Ocampo 1994
Bone marrow cells from Swiss Webster Albino mice	Acetamido-2-methyltetradecane was the major component of an extract of fruit of *Capsicum frutescens*	Micronucleus test on acetamido-2-methyltetradecane	Positive results	Villaseñor et al. 1995
Bone marrow cells from male Swiss mice	*Capsicum frutescens* extract (Chili extract)	Micronucleus test at 146.4 mg/kg	No increase in micronuclei after dosing with chili extract	Nagabushan and Bhide 1985
Bone marrow cells from female Swiss albino mice	Red Pepper (Capsicum Frutescens Fruit)	Three groups of 6 mice received oral doses of 200, 300, and 400 mg/kg daily for 30 consecutive days. Dosing followed by chromosomal aberrations assay	Dose-related increase in % of cells with chromosomal abnormalities. Statistically significant findings included chromosome breakage, polyploidy, and aneuploidy. Inhibition of spindle formation also noted	John and Abraham 1994
V79 Chinese hamster cells	Chili extract (in DMSO)	Assay for induction of 8-azaguanine-resistant mutants with and without metabolic activation at 1.83 mg/ml	No induction or resistant colonies	Nagabhushan and Bhide 1985

TABLE 6
Carcinogenicity/tumorigenicity studies

Species/strain	Test substance	Procedure	Results	References
30 rats (strain, ages, or weights not stated)	Chilies (*Capsicum frutescens* and *Capsicum annuum*), containing 0.14% Capsaicin	Chilies fed at a concentration of 10% in a semisynthetic diet containing casein for 2 years	Changes in the liver that resembled incipient hepatomas and cholangiomas developed in 7 animals; malignant tumors were observed in 3 of these 7 (1 mammary tumor at month 14, 1 hepatic tumor at month 16, and 1 pancreatic tumor at month 18). Due to late appearance of the tumors, the findings were considered inconclusive, but it was concluded that chilies in the feed was the determining tumorigenic factor	Hoch-Ligeti 1951
30 rats (strain, ages, or weights not stated)	Chilies (*Capsicum frutescens* and *Capsicum annuum*) containing 0.14% Capsaicin	Chilies fed at a concentration of 10% in a semisynthetic diet (casein replaced with ardein, purified protein of the ground nut, in diet) for 2 years. Beginning at 6 months, the rats were killed at intervals until the end of the study	From month 6 to study termination, neoplastic changes in the liver were observed, with males more prone to tumor development than females—cystic cholangiomas, solid cholangiomas, adenocarcinomas, and hepatomas were observed	Hoch-Ligeti 1951
Group of 20 male Swiss mice; mean body weight of 24.9 ± 2.0 g	Chilies (*Capsicum annuum*)	Feeding of standard diet containing chilies (100 mg chilies/mouse/day) continuously for 12 months. Animals observed for up to 2 years. Control group fed standard diet only	Adenocarcinoma of abdomen observed (total tumor incidence in 2 years = 7/20 (35% tumor incidence)). No tumors in control mice	Balachandran and Sivaramkrishnan 1995
Groups of 30 male Wistar rats; 60 to 150 g	Red chili powder (*Capsicum annuum*) in a commercial diet containing 20% peanut oil	Daily dietary dose of red chili powder was 8 mg/day/100 g body weight for 30 days. 30 positive-control rats fed 1,2-dimethylhydrazine (DMH). 30 negative-control rats fed commercial diet only	Group incidences of intestinal tumors/polyps: 0/30 (negative control), 27/30 (positive control), and 25/30 (red chili powder)	Nalini et al. 1997
Groups of 30 male Sprague-Dawley albino rats; 120 to 150 g	Red chili (Capsaicin) powder	Control rats fed commercial diet; positive control fed DMH and commercial diet; test group fed red chili powder (8 mg/day/100 g body weight in water) and commercial diet; and positive control/test group fed DMH with chili and commercial diet—animals killed at end of 30 weeks	Tumor incidence (intestine and colon) in positive control 28/30, test group 27/30, and positive control/test group 29/30—authors concluded that chili not only promotes carcinogenesis, but, in the presence of a carcinogen, accelerates the process	Chitra et al. 1997

observed: cystic cholangiomas, solid cholangiomas, adenocarcinomas, and hepatomas. Based on results from the preceding two experiments, it was concluded that the feeding of chilies was the determining tumorigenic factor (Hoch-Ligeti 1951).

In a study by Nalini et al. (1997), 30 male Wistar rats (weights = 60 to 150 g) were fed red chili powder (*Capsicum annuum* Linn.) with a commercial diet containing 20% peanut oil daily for 30 weeks. Water was given ad libitum. The daily dietary dose of red chili powder (described as 8 mg/day/100 g body weight in water) corresponded to the average human dietary intake, in the form of curry powder, in India. Positive-control animals (30 rats, of which 10 were from another study) were fed 1,2-dimethylhydrazine (DMH; a procarcinogen). DMH (20 mg/kg body weight) was dissolved in 1 mM EDTA, and the pH was adjusted to 6.5 with $NaHCO_3$. Negative control animals (30 rats) were fed commercial diet only.

Results relating to the incidence of intestinal tumors/polyps in rats fed red chili powder, compared to negative- and positive-control groups, are as follows: negative-control group (0/30), positive-control group (27/30), and test group (25/30). Additional results indicated that red chili induced profound alterations in lipid metabolism, as a prerequisite for colon carcinogenesis. Compared to the control group, the cholesterol content of the intestines and liver for animals fed red chili was significantly increased. Increased HMG-CoA (β-hydroxy-β-methylglutaryl–coenzyme A) reductase, the rate-limiting enzyme in cholesterol biosynthesis, activity was also reported for this group. Effects on lipid metabolism were overcome considerably when coconut kernal was included in the diet (Nalini et al. 1997).

Chitra et al. (1997) evaluated the tumorigenicity of red chili (Capsaicin) powder using groups of 30 male Sprague-Dawley albino rats (weights = 120 to 150 g). Four groups were defined as follows: group 1 (control rats fed commercial diet); group 2 (DMH and commercial diet); group 3 (red chili powder and commercial diet) (8 mg/day/100 g body weight in water); and group 4 (DMH with chili and commercial diet). DMH was administered subcutanesously (s.c.) at a dose of 20 mg/kg body weight once per week for 15 weeks, and feed and water were made available ad libitum. At the end of 15 weeks, dosing with DMH was discontinued and rats received commercial diet only. The animals were killed at the end of 30 weeks, and tissues were examined microscopically.

The tumor incidence per group was as follows: group 2 (28 of 30 rats); group 3 (27 of 30); and group 4 (29 of 30). Tumors in both the intestine and colon were noted in all three groups. Polyploidal, sessile, or fungating growth on the mucosa (1 to 2 cm in diameter) was observed in animals injected with DMH. At microscopic examination, areas of dysplasia, but no areas of overt malignancy, were observed in groups treated with chili powder. However, an invasive adenocarcinoma (marked pleomorphism with papillary growth pattern) was observed in groups treated with DMH. Animals of chili + DMH–treated groups had colonic tissue changes that were associated with both substances. A transitional zone with areas of marked dysplasia, followed by an infiltrating adenocarcinoma, were observed. The authors concluded that chili not only promotes carcinogenesis, but also, in the presence of a chemical carcinogen (e.g., DMH), accelerates the process (Chitra et al. 1997).

In a study by Balachandran and Sivaramkrishnan (1995), 20 male Swiss mice (mean body weight = 24.9 ± 2.0 g) were fed a standard diet containing chilies (100 mg chilies/mouse/day). The diet was fed continuously for 12 months, and the animals were observed for up to two years. The control group was fed the standard diet only. Moribund animals (due to tumor formation) were killed and necropsied. The remaining animals were killed at the end of 2 years. Compared to controls, statistically significant growth retardation was noted after 18 months of feeding. Mean weights were 39.1 ± 3.2 g (controls) and 28.2 ± 3.9 g (group fed chilies) after 18 months. Growth was not affected during the first 6 months of feeding. Whether or not the inclusion of chilies in the diet had any effect on food intake was not stated. Adenocarcinoma in the abdomen was noted in mice fed chili (total tumor incidence in 2 years = 7/20, 35% tumor incidence). Tumors were not observed in control mice.

Cocarcinogenicity

Capsicum Annuum

Nalini et al. (1998) evaluated the effect of red chili (*Capsicum annuum*) on β-glucuronidase and mucinase, with or without DMH, known colon carcinogen, using groups of 10 male Wistar rats (weights = 120 to 150 g). β-Glucuronidase and mucinase are present in the colon microflora. Mucinase is the enzyme system that is responsible for degrading the protective mucins in the colon, and β-glucuronidase is the enzyme that hydrolyzes biliary glucuronides after they reach the colon. Furthermore, when a conjugated carcinogen reaches the colon mucosa, it may be hydrolyzed, releasing free carcinogen. The authors noted that if glucuronide hydrolysis is a rate-limiting step in this process, then the activity of microbial β-glucuronidase in the colon may influence the risk of colon carcinogenesis.

One group of rats was fed red chili in water at a dose of 8 mg/day/100 g body weight (corresponding to the average daily intake [humans] of spices in India). A second group received red chili + DMH. DMH (20 mg/kg body weight) was dissolved in 1 mmol/L EDTA (pH adjusted to 6.5) and injected s.c. once per week for 15 weeks, after which the rats received commercial diet only. Other groups of rats were fed black pepper or cumin with or without DMH in the diet. Control rats were fed a low-fat commercial diet and the positive-control group received DMH. The animals were killed at the end of 30 weeks, and neoplasms of the intestine and colon were counted.

At the end of the experiment, weight gains of 210 g (DMH), 218 g (chili), and 238 g (chili + DMH) were reported. The number of rats with tumors per total number of rats for the three groups was stated as follows: chili (25/30, 83.3% tumor incidence), DMH (27/30, 90% tumor incidence), and chili + DMH

(28/30, 93.3% tumor incidence). Compared to these results, decreased numbers of tumors were observed in rats fed cumin + DMH in the diet and in those fed black pepper + DMH in the diet.

Compared to control rats, rats fed red chili and those fed chili + DMH had a significant increase in β-glucuronidase activity in the colon and the colon/liver, respectively. Mucinase activity was significantly increased in rats fed DMH in the diet, and a similar increase was noted in rats fed chili in the diet. However, when chili was supplemented with DMH in the diet of rats, the increase in mucinase activity was greater than that noted in the group fed DMH in the diet. These results indicate that chili induced a profound alteration in the activity of β-glucuronidase and mucinase in the presence or absence of DMH in rats fed high-fat diets. In this study, chili intake resulted in the promotion of DMH-induced colon carcinogenesis.

The authors concluded that cumin or black pepper in the diet protected the colon in the presence of the procarcinogen DMH, as a possible result of the decreased activity of β-glucuronidase and mucinase, whereas red chili supplementation had the opposite effect in the proximal intestine and proximal colon (Nalini et al. 1988).

Capsicum

Kim et al. (1985) evaluated the cocarcinogenicity of red pepper using groups of 8 to 10 ACI or Fisher inbred rats. A 1% red pepper diet was fed to 10 ACI rats, and 10 control ACI rats were fed diet only. Ten and eight Fisher rats were fed 1% and 3% red pepper in the diet, respectively, and a control group of 10 Fisher rats was fed the diet only. Red pepper diets for ACI and Fisher rats also contained *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine [MNNG] (100 μg/ml), and the diets were fed for 37 weeks. All survivors were killed, and gross and microscopic examination performed at 40 weeks.

ACI inbred rats fed 1% red pepper + MNNG solution in the diet had a tumor incidence of 57% (compared to 44% in the control group). A tumor incidence of 63% (compared to 43% in control group) was reported for Fisher inbred rats fed 1% or 3% red pepper + MNNG solution in the diet. It was concluded that red pepper alone may not be carcinogenic to the stomach, but may enhance MNNG-induced carcinogenesis over a prolonged period of time (Kim et al. 1985).

Park and Kim (1993) evaluated the effect of red pepper (plant species not stated) on gastroduodenal carcinogenesis induced by MNNG in an oral feeding study using groups of Wistar rats. Animals were 6 weeks old, with weights from 130 to 150 g. Groups were standard diet and distilled water (group A, 18 rats), standard diet with 3.0% red pepper and distilled water (group B, 28 rats), standard diet and MNNG (group D, 27 rats), and standard diet with 3.0% red pepper and MNNG (group E, 27 rats). After continuous feeding for 40 weeks, the animals were killed at week 41.

Mortality was as follows: 3 of 18 rats (group A), 3 of 28 rats (group B), 4 of 27 rats (group D), and 3 of 27 rats (group E). No statistically significant differences in survival between the groups were noted. Many tumors were observed in groups fed MNNG in the diet; however, no tumors were observed in groups in which MNNG was absent from the diet.

All tumors were located in the glandular stomach and duodenum. Incidences of adenocarcinoma in the glandular stomach per group were 18 of 23, or 78.3% (group D, MNNG only) and 17 of 24, or 70.8% (group E, MNNG + red pepper). Incidences of adenocarcinoma in the duodenum were 1 of 23, or 4.3% (group D) and 10 of 24, or 41.7% (group E), and the increased incidence in group E was statistically significant ($p < .05$). The authors reported that the results suggest that red pepper had a promoting effect on duodenal carcinogenesis, but had no cancer-promoting effect in the glandular stomach. The authors suggested that the Capsaicin in red pepper increases cancer occurrence as a promoter (Park and Kim 1993).

Tumor Promotion Activity

Capsicum Frutescens Fruit Extract

Agrawal et al. (1986) evaluated the tumor promoting activity of Capsicum Frutescens Fruit Extract (chili extract) using inbred male and female Balb/c mice (6 to 8 weeks old). Animals were exposed (tongue application) to chili, with and without atropine, and treatment was also combined with methyl(acetoxymethyl)nitrosamine [DMN-OAc]. For treatment with chili extract without atropine (21 mice), 10 μl were applied to the tongue 2 days/week (50 μg/week, up to 16 months of age). For treatment with chili extract (with atropine), 10 μl were applied to the tongue (according to the same procedure) after the mice consumed 1% atropine solution in drinking water 4 h earlier. In both experiments, the animals were observed until death.

Combined treatment with DMN-OAc + chili extract without atropine involved 22 mice receiving a single treatment of DMN-OAc (2 mg/kg) on the tongue. At 12 h after carcinogen treatment (single treatment of DMN-OAc [2 mg/kg]) on the tongue, 10 μl of chili extract were applied 2 days per week (50 μg/week) up to 16 months of age. Combined treatment with DMN-OAc + chili extract with atropine involved 23 mice receiving 1% atropine solution in drinking water 4 h prior to the application of chili extract twice a week (50 μg/week) up to 16 months of age.

In two other experiments, animals were exposed to the carcinogen, benzene hexachloride (BHC), with and without chili extract, in drinking water. In the first experiment, 25 mice received a diet containing 500 ppm BHC for 4 months, after which chili extract (in drinking water) was administered for 4 months. In the second experiment, 31 mice received a diet containing 500 ppm BHC for 4 months, after which chili extract in drinking water (25 μg Capsaicin/mouse/week) was administered up to 16 months of age.

The untreated control group consisted of 50 animals, and the three positive-control groups were described as follows: 24 mice were dosed with 2 mg/kg DMN-OAc fortnightly, up to 12 months of age (DMN-OAc alone); 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (1 μg in 10 μl of acetone) was applied to the

tongues of 21 mice twice weekly, up to 16 months of age (TPA alone); 19 mice received a single application of DMN-OAc (2 mg/kg), on the underside of tongue, followed by application of TPA (1 μg in 10 μl of acetone; started 24 h after carcinogen treatment) 2 days per week, up to 16 months of age.

In each experiment, animals that died or that were killed during the study were examined for gross abnormalities, and the liver, stomach, tongue, and kidney were examined microscopically.

Fourteen of the 34 mice receiving applications of DMN-OAc (positive control, 2 mg/kg) to the tongue every 2 weeks, up to 12 months of age, had tongue and stomach tumors. The remaining mice had tumors on the tongue or in the stomach. The total tumor incidence in this group of 34 mice was 89%. Tumors of the tongue were classified as well-differentiated, squamous cell carcinomas. Tumors in the stomach were identified as papillomas and carcinomas.

In the positive-control group of 19 mice that received a single application of DMN-OAc (2 mg/kg) on the tongue, followed by applications of TPA (1 μg in 10 μl of acetone) 2 days per week (up to 16 months of age), the overall tumor incidence was 63%. This tumor incidence was higher than that reported for the 59 untreated control mice (two lung tumors and one liver tumor), or the 34 mice treated with a single dose of DMN-OAc alone (11% tumor incidence), or the 28 mice treated 2 days per week with TPA (10% tumor incidence).

Either no lesions or various different types of lesions were observed on the tongues of untreated animals or those that received DMN-OAc alone or chili extract alone (with and without atropine). The lesions (not counted) included hyperplasia, keratin dysplasia, and cellular infiltrations. With the exception of one tumor in the liver and one in the stomach (chili with and without atropine, respectively), no treatment-related tumors were observed in groups that received chili with or without atropine. It was also noted that lung tumors were common in the mouse strain used.

In groups that received DMN-OAc + chili with atropine (23 mice) and without atropine (22 mice), significantly higher tumor incidences of 48% (11 of 23 mice) and 36% (8 of 22 mice), respectively, were reported. These results were compared with results for the 14 control mice that received chili extract with atropine (14% tumor incidence, 2 of 14 mice) and the 21 control mice that received chili extract without atropine (5% tumor incidence, 1 of 21 mice). Most of the tumors in the two groups with the higher tumor incidence were observed in the forestomach, and were characterized microscopically as papillomas and well-differentiated squamous cell carcinomas. The increase in tumor incidence in these groups was also significantly higher when compared to the 34 control mice that received DMN-OAc alone and the untreated control group of 59 mice.

Microscopic lesions in the liver, such as necrosis, inflammation, fatty change, and hemangiomas, were observed in groups that received chili extract alone (22 mice: no hemangiomas; one stomach papilloma observed) or with atropine (14 mice: one hemangioma), and in groups that received DMN-OAc + chili extract (22 mice: no hemangiomas; three stomach papillomas observed) or DMN-OAc + chili extract and atropine (23 mice: one hemangioma, one stomach papilloma, and one stomach carcinoma). Hemangiomas were the only lesions of the liver that were counted. No hepatic lesions were observed in the 59 untreated mice or in the 34 mice that received a single dose of DMN-OAc.

Compared to the untreated control group (5% total tumor incidence, 3 of 59 mice), the total tumor incidences for mice exposed to BHC were as follows: BHC (8% tumor incidence: one hepatocarcinoma and one liver hemangioma; 2 of 25 mice) and BHC + chili (29% tumor incidence: five hepatocarcinomas and three hepatomas; 9 of 31 mice).

The authors concluded that the results indicated a promoter effect of chili extract in the following two systems: (1) induction of stomach tumors in male mice initiated with DMN-OAc (a potent carcinogen) and (2) induction of liver tumors in male mice treated with BHC, a known hepatocarcinogen in mice (Agrawal et al. 1986).

Agrawal and Bhide (1988) studied the histopathological changes after chronic administration of Capsicum Frutescens Extract, alone or in combination with a tumor promoter. The substances administered are defined in the preceding study. Chili (*Capsicum frutescens*) Fruit Extract, containing 20 μg Capsaicin, was placed in the right cheek pouch of each of 36 hamsters (weights = 95 to 100 g) 5 days per week for 14 months. Very few animals survived beyond 16 months of age. Furthermore, the animals did not gain weight and some developed diarrhea. Compared to the 30 untreated control hamsters, test animal weights were decreased. Note: the findings for the solvent control group (dosing with alcohol), the untreated control group, and the positive-control group (dosing with DMN-OAc) are included near the end of the discussion of this study.

Gross findings for the cheek pouch of test animals included thickened mucosa and bleeding in focal areas, leading to shortening of the cheek pouch wall. One or more of the following lesions was observed at microscopic examination: hyperplasia, keratosis, papillary formation (early and advancing stage), inflammation, and polyp formation. Specifically, of the 17 cheek pouches studied, mild hyperplasia with early papillary formation was noted in 7 and advancing papillary formation with hyperplasia and keratosis was noted in 6. Polyp formation was observed in one hamster.

Other gross/microscopic findings in hamsters included cirrhosis (in 14 of the 29 livers examined); an increase in liver weight by 1 to 2 g; kidneys with a white, nodular surface (in most of the treated animals) and degeneration of the glomeruli (65% incidence); and a reddish and thickened forestomach wall. On microscopic examination of the stomach, one or more of the following lesions were noted: hyperplasia, papillary formation (early and advancing stage), and keratinization and infiltration of epithelial cells into the connective tissue. One animal developed a gastric ulcer after 11 months of treatment, and one had

moderate hyperplasia (with papillary formation) in the forestomach. No significant changes were observed in the forestomachs of animals dosed with alcohol only, animals dosed with DMN-OAc, or untreated control animals. Changes in the kidneys (degeneration of glomeruli) of hamsters dosed with Capsicum Frutescens Fruit Extract were significantly increased ($p < .05$) when compared to each of these three groups.

In another experiment described in this same study, a single dose of 20 μl of DMN-OAc solution (16 mg/ml distilled water) was placed in the right cheek pouch of each of 30 hamsters (weights = 95 to 100 g). Atropine was injected prior to dosing, to prevent salivation for a few hours. At 24 h post application of DMN-OAc, 20 μl of chili (*Capsicum frutescens*) extract was applied to the mucosa of the right cheek pouch five times per week for 14 months. Animals were observed until death. The purpose of this experiment was to determine whether chilies have a promoter action on cheek pouch mucosa initiated with a single treatment of DMN-OAc.

The animals survived for a maximum of 16 months. No weight gain was reported, and some of the animals developed diarrhea. Gross findings for the cheek pouch of test animals included thickened mucosa and bleeding in focal areas, leading to shortening of the cheek pouch wall. One or more of the following lesions was observed at microscopic examination: hyperplasia, keratosis, papillary formation (early and advancing stage), inflammation, and polyp formation. Specifically, of the 20 cheek pouches studied, mild hyperplasia with early papillary formation was noted in 10 and hyperplasia with advancing papillary formation and keratosis was noted in 5. Polyp formation was observed in three hamsters, and squamous cell carcinoma was observed in one hamster.

Other gross/microscopic findings in hamsters included cirrhosis (in 9 of the 25 livers examined); an increase in liver weight by 1 to 2 g; kidneys with a white, nodular surface (in most of the treated animals) and degeneration of the glomeruli (50% incidence, compared to 65% for animals treated with Capsicum Frutescens Extract only); and a reddish and thickened forestomach wall. On microscopic examination of the stomach, one or more of the following lesions were noted: hyperplasia, papillary formation, and keratinization and infiltration of epithelial cells into the connective tissue. Specifically, extensive hyperplasia with papillary formation (classified as advanced changes) was noted at microscopic examination. These advanced changes were not observed in animals treated with Capsicum Frutescens Extract only. Significant changes were not observed in the forestomachs of animals dosed with alcohol only, animals dosed with DMN-OAc, or untreated control animals. It is important to note that changes in the kidney were significantly different ($p < .05$) when compared to each of these three groups.

A single dose of 20 μl of DMN-OAc solution (16 μg/ml distilled water) was placed in the cheek pouch of each of 21 positive control hamsters (weights = 95 to 100 g). Atropine was injected 4 h prior to treatment. All animals were observed until death. Mild hyperplasia (with early papillary formation) was observed in 6 of the 25 cheek pouches examined. No advanced changes were noted.

In the solvent-control group (19 hamsters; weights = 95 to 100 g), 20 μl of absolute alcohol were placed in the right cheek pouch five times per week for 14 months. The animals were observed until death. Of the 19 hamsters, early papillary formation was observed in the cheek pouch epithelium of 11 animals.

The untreated control group consisted of 25 hamsters that were observed until death. Of the 25, 6 had early papillary formation in the cheek pouch epithelium.

In summarizing their findings, the authors noted that only one gross tumor was present in the cheek pouch of a hamster that was treated with Capsicum Frutescens Extract + DMN-OAc, and no tumors were noted in the group treated with Capsicum Frutescens Extract only. Though Capsicum Frutescens Extract induced toxic changes in several tissues, the authors concluded that no carcinogenic effect was apparent at the dose that was tested. They did note, however, that the extensive hyperplasia (with papillary formation) observed in the cheek pouch or stomach epithelium are indicative of early precancerous lesions, which may lead to malignant changes with longer survival of the animals (Agrawal and Bhide 1988).

The above studies are summarized in Table 7.

CLINICAL ASSESSMENT OF SAFETY

Inhalation Toxicity

Capsicum

Lankatilake and Uragoda (1993) evaluated the respiratory function of 25 male employees (chili grinders, exposed to chili dust) of five chili-grinding factories in India. The source of the chili (Capsicum species) was not stated. An average period of service of 6.6 years for the workers (age range = 17 to 53; mean age = 28.5 years) was reported. The total airborne dust concentration, estimated as an 8-h time-weighted average, ranged from 0.24 to 4.58 mg/m^3 (personal sampling) and 0.23 to 2.73 mg/m^3 (static sampling). Respirable dust values for personal and static sampling were 0.11 to 0.52 mg/m^3 and 0.03 to 0.09 mg/m^3, respectively. The control group consisted of workers at a printing company, matched for age, sex, height, and smoking habits.

Symptoms of cough, sneezing, and runny nose were reported for 15 (or 60.0%) of the chili factory workers shortly after initiation of employment, and persisted anywhere from 3 weeks to 6 months. The following chronic respiratory symptoms were reported for 11 (or 44.0%) of the workers: phlegm (11 cases), cough (8 cases), and breathlessness on exertion (3 cases). All chest radiographs were normal. Chronic respiratory symptoms were not reported for the control group. Additionally, no statistically significant differences in ventilatory measurements were noted between test and control groups (Lankatilake and Uragoda 1993).

TABLE 7
Tumor promotion activity

Animals tested	Test substance	Procedure	Results	References
Groups of 8 to 10 ACI or Fisher inbred rats	1% and 3% red pepper diet (whether *Capsicum annuum* or *Capsicum frutescens* not stated)	Red pepper diets containing *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG, 100 μg/ml). Gross and microscopic examination performed at 40 weeks	ACI inbred rats fed 1% red pepper + MNNG in diet had tumor incidence of 57%, compared to 44% in group fed red pepper. Tumor incidence of 63% in the 1% or 3% red pepper + MNNG group, compared to 43% in red pepper alone group	Kim et al. 1985
Groups of 21 to 23 inbred male and female BALB/c mice (6 to 8 weeks old)	Capsicum Frutescens Fruit Extract (chili extract)	Animals of some groups exposed to chili extract with and without atropine, and treatment was also combined with methyl(acetoxymethyl)nitrosamine (DMN-OAc) in other groups. Applications made to tongue. Daily dosing up to 16 months of age	Promoter effect of chili extract in the following two systems: (1) induction of stomach tumors in male mice initiated with DMN-OAc (potent carcinogen); and (2) induction of liver tumors in male mice treated with benzene hexachloride, a known carcinogen	Agrawal et al. 1986
Groups of 30 to 36 hamsters, 95 to 100 g	Chili (*Capsicum frutescens*) extract containing 20 μg Capsaicin	Test substance placed in right cheek pouch of each animal 5 days per week for 14 months (36 hamsters). Treatment also combined with DMN-OAc (30 hamsters)	Only one gross tumor in cheek pouch of a hamster treated with Capsicum Frutescens Extract + DMN-OAc. Mild hyperplasia (with early papillary formation) in 6 of 25 hamsters dosed with DMN-OAc only. No tumors in group treated with Capsicum Frutescens; but lesions (e.g., hyperplasia) observed at microscopic examination	Agrawal and Bhide 1988

Capsicum Oleoresin

Respiratory responses to Capsicum Oleoresin spray in humans include: burning of the throat, wheezing, dry cough, shortness of breath, gagging, gasping, inability to breathe or speak, and, rarely, cyanosis, apnea, and respiratory arrest (Steffee et al. 1995).

In a study by Chan et al. (2002), 34 subjects (24 men, 10 women; mean weight = 79.1 kg) participated in experimental trials over two separate days in a university medical center pulmonary function laboratory. For each trial, subjects were exposed to Capsicum Oleoresin, via inhalation, using a 5 × 3 × 3-foot exposure box. A hood for the subject was attached to one end of the exposure box, and a small opening for delivery of the spray was created at the opposite end. This method allowed for uniform and reproducible delivery from a standard distance of 5 feet. Capsicum Oleoresin was delivered via a commercially available, standard duty aerosol spray canister that is widely used by law enforcement agencies nationwide. The aerosol contained 5.5% Capsicum Oleoresin (0.92% capsaicinoids), 64% isopropyl alcohol carrier agent, and 30.5% isobutane/propane propellant. Placebo spray was delivered by a similar canister containing carrier only and propellant gases (68% isopropyl alcohol and 31.5% isobutane/propane). Capsicum Oleoresin or placebo spray was delivered for 1 s, and the subject's head remained in the hood of the exposure box for an additional 5 s. At the conclusion of exposure, the subjects were immediately seated.

Exposure to Capsicum Oleoresin spray did not cause any significant differences in oxygenation or any hypoxemia when compared to the placebo. A decrease in arterial carbon dioxide levels was noted following exposure. No evidence that inhalation of Capsicum Oleoresin spray caused respiratory compromise was found in this study (Chan et al. 2002).

Skin Irritation

Capsicum

According to Rietschel and Fowler (1995), the five spices that most commonly cause dermatitis in the United States are Capsicum, cinnamon, cloves, nutmeg, and vanilla. When ingested or inhaled, all five can induce flares at the healed sites of allergic contact dermatitis. These spices may also produce urticaria when ingested with foods or drink, or when inhaled.

Capsicum Frutescens Fruit Extract

In a study by Institut D'Expertise Clinique (1995), the epicutaneous local tolerance to Vegetol Capsicum LC 481 Huileux (trade name mixture containing Capsicum Frutescens Fruit Extract) was evaluated using 10 healthy volunteers (7 women, 3 men; 20 to 52 years old). The composition of Vegetol Capsicum LC481 Huileux is described as follows: Capsicum Frutescens Fruit Extract (1% to 5%), prunus armeniaca (apricot) kernel oil (10% to 25%), and mineral oil (70% to 89%) (CTFA 2002b). The trade name mixture was diluted with 20% *v*/*v* kernel stone oil (effective concentration range for Capsicum Frutescens Extract in diluted trade name mixture = 0.2% to 1%). The diluted mixture (0.02 ml) was placed on a disc of filter paper and applied to the back (50 mm^2 area) of each volunteer. The disc, covered with an occlusive patch (Finn chambers on scanpor), remained in place for 48 h. A patch without the test article served as the negative control. At 30 min after patch removal, cutaneous macroscopic examinations were performed. Reactions were scored: 0 (no erythema) to 4 (purpuric erythema); 0 (no edema) to 4 (severe edema at least 1 mm thick, on a surface greater than the area of application); 0 (no papules, vesicles, bullae, pustules) to 4 (bullae with clear liquid); and 0 (no dryness or desquamation) to 4 (severe desquamation, i.e., presence of thick squamae or at least 3/4 of the application area, with possibility of tegument fissuration). Scales for detergent effect and reflectivity were also used.

Very slight erythema was observed in 1 of the 10 volunteers tested. No pathological irritation reaction or significant cutaneous intolerance was observed. It was concluded that a single application of the diluted trade name mixture was well-tolerated (Institut D'Expertise Clinique 1995).

Skin Irritation and Sensitization

Capsicum Frutescens Fruit Extract

In a study provided by the cosmetics industry (Reliance Clinical Testing Services, Inc. 2000), the skin irritation/sensitization potential of a product ("Pulse Point Gel") containing 0.05% of a raw material consisting of approximately 50% Carthamus Tinctorius (Safflower) Seed Oil and 50% Capsicum Frutescens Fruit Extract (effective concentration = 0.025%) (CTFA 2003c) was evaluated using 122 subjects (110 females, 12 males; ages 18 to 69 years). Of the122, 103 subjects completed the study. Sixteen withdrew for reasons that were unrelated to conduct of the study. Furthermore, two subjects withdrew due to a protocol violation and one withdrew because of skin reactivity (heat rash, not test substance related) at patch test sites. An occlusive patch containing the product was applied to the back for 24 h on Mondays, Wednesdays, and Fridays (total of nine applications). The application site was between the scapulae and waist, adjacent to the spinal midline. Tuesday and Thursday removals were followed by 24-h nontreatment periods, and Saturday removals were followed by 48-h nontreatment periods. After a 2-week nontreatment period, following induction, the challenge phase was initiated. Challenge patches were applied to new test sites, and reactions were scored at 24 and 72 h post application. The grading scale for reactions observed and study results are included below.

All reactions were graded according to the following scale: 0 (no evidence of any effect) to 4 (severe—deep-red erythema with/without vesiculation or weeping). During the induction phase, transient, barely perceptible erythema was observed in seven subjects. An eighth subject had a 2 reaction (moderate—pink-red erythema uniform in the entire contact site) on day 2 of

induction and a + reaction (barely perceptible—minimal, faint, uniform, or spotty erythema) during the challenge phase. The early onset of skin reactivity during induction was considered evidence of preexisting sensitivity to an ingredient(s) in the product and not sensitivity induced by the product. It was concluded that neither the incidence nor level of peak severity (barely perceptible) of the low-grade responses observed was considered evidence of clinically meaningful irritation, nor were they considered evidence of induced allergic contact dermatitis (Reliance Clinical Testing Services, Inc. 2000).

Epidemiology

Capsicum

Notani and Jayant (1987) conducted a case-control study to assess the role of dietary factors in the incidence of cancers of the oral cavity, pharynx, esophagus, and larynx. The case group, from India, consisted of males with the following cancers: oral cavity (278 patients), pharynx (225 patients), esophagus (236 patients), and larynx (80 patients). The two control groups consisted of 215 patients without cancer and a comparable group of 177 subjects from the general population. The use of red chili powder emerged as a risk factor for all of the cancers (two- to threefold risk, with dose-response relationship), compared to population controls.

A case-control study by Buiatti et al. (1989), conducted in Italy, involved 1016 patients ($\leq$75 years old) with histologically confirmed gastric cancer. The patients were from seven hospitals. The controls were randomly selected from 5-year age and sex strata of the general population of each center. The measure of association between gastric cancer risk and the dietary and other exposure variables was the odds ratio (OR). Adjusted OR estimates and corresponding 95% confidence intervals were obtained, and significant trends (over the tertiles) evaluated. Intakes of individual food items and of food groups were categorized into tertiles defined by weekly frequency of consumption among all controls. Multivariate logistic regression analyses were also conducted.

In addition to other foods, prominent trends in gastric cancer risk were associated with the consumption of chili (OR = 1.0, 0.8, and 0.6 for tertiles 1 [low], 2, and 3, respectively; $p <$ .001). The authors concluded that dietary factors contribute to the regional variation of stomach cancer occurrence in Italy, and offer clues for further etiologic and prevention research (Buiatti et al. 1989).

A case-control study by López-Carrillo et al. (1994) was conducted in the Mexico City metropolitan area, where hot chili peppers (genus = *Capsicum*) containing Capsaicin are heavily consumed. The 267 cases constituted subjects (>20 years old) with newly diagnosed stomach cancer. Of the cases, slides from 220 were reviewed and classified as follows: intestinal-type (98 cases), diffuse-type (95 cases), and indeterminate (27 cases) adenocarcinomas of the stomach. Controls consisted of an age-stratified random sample of residents (752) of the Mexico City metropolitan area.

Study results indicated a significant correlation between hot pepper consumption and the incidence of gastric cancer in the Mexican population. Specifically, a highly significant trend of increasing risk ($p = 2 \times 10^{-7}$) with increasing self-rated level of consumption (low, medium, and high) was noted. However, when consumption was expressed in terms of frequency per day, a significant trend among consumers was not observed. The authors concluded that chili pepper consumption may be a strong risk factor for gastric cancer, and that further studies are needed in order to test this hypothesis (López-Carrillo et al. 1994).

Another epidemiological study was conducted by Archer and Jones (2002) using four cookeries in the United States that are noted for their high pepper content: Mexican-American, Cajun, white Creole, and black Creole. It was noted that each cookery is largely confined to a single ethnic-cultural group that is concentrated in some counties. Age-adjusted mortality data by site and county for the 1970–1979 period and by sex and race (white or black) were used. In these data, Mexican-Americans and Cajuns were classified as white. Total cancer mortality was included to determine the overall influence of pepper intake on cancer rates in general. Mortality rate ratio patterns for 'all cancer' and cancer at nine sites (liver, stomach, esophagus, pancreas, rectum, colon, lung, breast [females], and urinary bladder), arranged in the order of estimated Capsaicin exposure, were presented.

Mortality rate ratios exhibited four different patterns. The first pattern (referred to as the Capsaicin curve), exhibited by cancers of the stomach and liver among all white groups, was defined as decreasing mortality rate ratios associated with decreasing Capsaicin exposure. All mortality rate ratios were significantly higher than 1.0, with the highest indicating excess mortality of 60% to 80%. For black Creoles, who did not fit the curves for white subjects, the stomach cancer rate was 15.2 (14.4 to 16.0) per 100,000 versus 10.5 (10.2 to 10.9) for their controls (black subjects). The mortality rate ratio for this group was consistent with that of white subjects, despite the fact that their rates were nearly twice as large. Additionally, the black Creole mortality rate ratios and rates for liver cancer were both inconsistent with data on white subjects.

Cancers of the lung, breast, urinary bladder, and 'all cancer' comprised the second mortality rate ratios pattern. For 'all cancer' and these three sites, there appeared to have been no association between pepper exposure and mortality rate ratios.

The third mortality rate ratio pattern was exhibited by cancers of the pancreas and esophagus. The two highest Capsaicin exposure groups (counties with >50% Mexican ancestry and counties with 35% or more Cajun ancestry, respectively) had mortality rate ratios that were significantly greater than 1. The authors expressed the view that the high Cajun mortality rate ratios for cancers of the pancreas and esophagus may have been due to the excessive heating and browning of food (increasing the heterocyclic amine mutagen content).

The fourth mortality rate ratio pattern was exhibited by colon cancer. There was an overall upward trend with decreasing

Capsaicin exposure. The three highest Capsaicin exposure groups had mortality rate ratios that were significantly below 1 (i.e., a negative association with Capsaicin). The mortality rate ratios for rectal cancer yield also indicated an upward trend with decreasing Capsaicin exposure.

The authors concluded that, using county population and mortality data, significantly higher rates for stomach and liver cancer were found in counties inhabited by the four ethnic-cultural groups (Mexican-American, Cajun, white Creole, and black Creole) than in matched control counties. The cancer increase was dependent on the concentration of these groups in the county. The authors noted that the results in this study strengthen and extend an earlier case-control study (preceding study by López-Carrillo et al. 1994) that found odds ratios above 5 for the stomach cancer association with Capsaicin pepper. Furthermore, the authors considered data in the current study as further evidence that Capsaicin is a human carcinogen (Archer and Jones 2002).

In a study by Dasgupta et al. (1998), no premalignant or malignant changes were observed in bladder biopsies of 20 patients who received repeated intravesical instillations of Capsaicin (1 to 2 mmol/L) for the treatment of intractable detrusor hyperreflexia. The patients (mean age = 52.5 years) were treated from 1991 to 1996. It was noted that because the morphological effects of chemical carcinogens may not be apparent for 10 years, surveillance of the patients is being continued.

In a prospective case-control study by Mathew et al. (2000), dietary risk factors for stomach cancer were evaluated. One hundred ninety-four stomach cancer patients and 305 controls, from India, participated in the study. The patients ranged in age from 20 to 75+ years. Data were analyzed using a multiple logistic regression model, and odds ratios (OR = measure of association between stomach cancer risk and all dietary variables) were estimated. Increased risks for stomach cancer that remained significant on multivariate analysis were as follows: high consumption of rice (OR = 3.5; 95% confidence interval [CI] = 1.1 to 10.8 for 2 to 4 times/week and OR = 6.7; 95% CI = 2.3 to 19.8 for daily users); food with very high temperature (OR = 4.1; 95% CI = 2.0 to 8.3); and high chili consumption in food (OR = 5.1; 95% CI = 2.5 to 10.4).

In a case-control study by Pandey and Shukla (2002), 64 newly diagnosed cases of carcinoma of the gall bladder (mean patient age = 51 ± 1.2 years) were compared with 101 control patients with cholelithiasis (mean age = 40.9 ± 1.1 years) in India. Dietary data were collected according to a standard 30-day recall method using a preset food frequency questionnaire, which contained items regarding intake of the following: cereals, pulses (lentils), beverages, milk and milk products, vegetables (seasonal and others), fruits, meat, and cooking oil. Statistical analysis was carried out by calculating OR and 95% CI for the odds ratio. No effort was made to ascertain changes in diet over the years.

A considerable lowering of gallbladder cancer risk was associated with the consumption of vegetables of the cruciferous family. A slight increase in the odds ratio was associated with the consumption of cauliflower (OR = 1.67; 95% CI = 0.5 to 5.2), Capsicum (OR = 2.2; 95% CI = 1.1 to 4.5), green peas (OR = 1.5; 95% CI = 0.5 to 4.3), and red chili (OR = 1.29; 95% CI = 0.6 to 2.7). Except for Capsicum, these increases were not statistically significant (Pandey and Shukla 2002).

Case Reports

Capsicum Annuum

A 23-year-old surgical nurse with hand eczema, a cough, asthma, and a family history of atopy had a strong positive prick test reaction (wheal 2× histamine control) to a commercial latex extract and a freshly prepared latex glove extract. Over the following weeks, her cough and asthma worsened and she also had severe episodes of Quinke's and glottal edema after eating pickled pepper. Prick test results for fresh yellow pepper, *Capsicum annuum*, were positive (wheal > histamine control) (Gallo et al. 1997).

Capsicum Annuum Resin

Watson et al. (1996) conducted a study involving 81 subjects (mean age = 27.6 ± 7.9 years) who visited a hospital emergency room after exposure to Capsicum Annuum Resin (Capsicum Oleoresin) during law enforcement action. Ocular burning (45 subjects) and conjunctival injection (36 subjects) were the most common symptoms. Dermal reactions (burning, 20 subjects; erythema, 12 subjects) were also reported. A heart rate of greater than 100 beats/min was reported for 32 subjects, and a respiratory rate of greater than 20 breaths/min was reported for 16. It was suggested that a relatively small percentage of subjects exposed to Capsicum Oleoresin spray during an encounter with law-enforcement personnel will have clinically significant toxicity. It was noted that pulmonary and ocular toxicity should be considered in cases of prolonged exposure.

Of the 100 cases of pepper spray (containing Oleoresin Capsicum) exposure identified by Brown et al. (2000), 7 patients had sustained corneal abrasions.

In a study by Vesaluoma et al. (2000), the safety of Oleoresin Capsicum spray was evaluated using 10 police officers (ages 24 to 50). A mixture with the following components was sprayed (at a distance of 1.5 to 2.5 m) into the face of each subject for a duration of 0.5 to 1.5 s: 5.5% Oleoresin Capsicum, 30% isobutane (as a propellant), and 64% isopropyl alcohol (as a carrier). Mild to moderate facial hyperemia and conjunctival hyperemia (mean duration of 9.8 h; Draize score = 1) were observed in all subjects at 20 min post exposure. The focal epithelial cell damage observed in six corneas healed within 24 h. Mild chemosis (Draize score = 1) was observed in two subjects after exposure, but was not observed on the following day. Additionally, the mean heart rate increased significantly from a basal value of 79.7 ± 13.3 beats/min to 116.0 ± 19.1 beats/min (at 1 min), and then decreased to 73.0 ± 17.9 beats/min in 10 min.

Paprika (Spice from Capsicum Annuum)

Sastre et al. (1996) reported symptoms of rhinitis and asthma in a 27-year-old subject 1 year after preparing a type of sausage that contained paprika. The results of a skin prick test for paprika (dry powder of Capsicum Annuum; 10% *w*/*v*) were positive. Additionally, results of a bronchial inhalation challenge with paprika extract indicated an immediate asthmatic reaction.

Kanerva et al. (1996) evaluated 1000 patients with occupational skin disease (hand or finger dermatitis), 5 of whom had spice-induced allergic contact dermatitis. Of the five, paprika induced a weak allergic patch test reaction (<2+ reaction) in two patients.

Foti et al. (1997) reported wheals on the hands and forearms of a 25-year-old female after 2 years of employment at a biscuit factory. The wheals developed whenever she came in contact with paprika powder. Prick test results indicated a positive wheal-and-flare reaction to paprika. In a control group of nine subjects (five atopic and four nonatopic), prick test results for paprika were negative. However, an irritant reaction to paprika was observed in one non-atopic subject.

Vega de la Osada et al. (1998) reported an episode of anaphylaxis after the intake of paprika in a patient employed as a spices-and-condiment seller. Occupation-related rhinoconjunctivitis symptoms were also reported. All skin test results (fresh solanaceous and extracts, paprika included) were positive. The results of a conjunctival challenge test for paprika were also positive. In a laboratory study, specific immunoglobulin E (IgE) to all solanaceous (paprika included) was detected and histamine-release test results were positive. Cross-reactivity between the paprika antigenic determinant recognized by the patient and the one that was presented by the rest of solanaceous could not be detected using RAST (radio allergosorbent test)-inhibition studies.

PART 2: CAPSAICIN

CHEMISTRY

Definition and Structure

Capsaicin (CAS no. 404-86-4) is defined as the organic compound that conforms to the following formula (Pepe et al. 2002) shown in Figure 1. Other names for this ingredient (Budavari 1989; Committee on Revision of the United States Pharmacopeal Convention 2000; Pepe et al. 2002) are:

- Capsaicin;
- Capsaicine;
- N–[4-Hydroxy-3-Methoxyphenyl)Methyl]-8-Methyl-6-Nonenamide;
- 6-Nonenamide, N–[(4-Hydroxy-3-Methoxyphenyl) Methyl]-8-Methyl-;
- 6-Nonenamide, 8-methyl-*N*-canillyl-, (E)-;
- 6-Nonenamide, (E)-N-[(4-Hydroxy-3-methoxy-phenyl) methyl]-8-methyl;
- (E)-8-Methyl-*N*-vanillyl-6-noneamide
- (E)-N-[(4-Hydroxy-3-methoxyphenyl)-methyl]-8-methyl-6-nonenamide;
- *trans*-8-methyl-*N*-vanillyl-6-nonenamide; and
- *N*-(4-hydroxy-3-methoxybenzyl)-8-methylnon-*trans*-6-enamide.

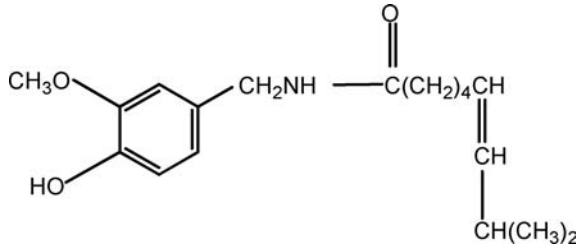

FIGURE 1
Chemical formula for Capsaicin (CAS no. 404-86-4) from Pepe et al. (2002).

The Capsaicin content of red chilies varies depending on the variety. The red chilies *Capsicum annuum* and *Capsicum frutescens* are sources of cosmetic ingredients that are included in this review. The content of Capsaicin in *Capsicum annuum* is 1.67 mg/g dry weight and in *Capsicum frutescens*, 0.45 mg/g dry weight (Glinsukon et al. 1980).

Chemical and Physical Properties

Capsaicin is a crystalline pungent alkaloid with a molecular weight of 305.42. Other properties of this ingredient are included in Table 8.

Composition/Impurities

According to the *United States Pharmacopeia* (USP), Capsaicin contains not less than 90.0% and not more than 110.0% of the labeled percentage of total capsaicinoids. The content of capsaicin ($C_{18}H_{27}NO_3$) is not less than 55%, and that the sum of the contents of capsaicin and dihydrocapsaicin ($C_{18}H_{29}NO_3$) is not less than 75%. Additionally, the content of other capsaicinoids is not more than 15%, all calculated on the dried basis. The following caution statement is also included with the preceding composition statement on Capsaicin: "Caution—Handle Capsaicin with care. Prevent inhalation of particles of it and prevent its contact with any part of the body." (Committee of Revision of the United States Pharmacopeial Convention 2000).

Budavari (1989) indicates that, based on its empirical formula, Capsaicin consists of carbon (70.78%), hydrogen (8.91%), nitrogen (4.59%), and oxygen (15.72%).

Products resulting from the hydrolysis of Capsaicin have been identified as vanillylamine and an unsaturated fatty acid. Furthermore, the reaction of vanillylamine and the acid component from natural Capsaicin results in the formation of a product with the same physical, chemical, and pungent properties as natural Capsaicin (Cordell and Araujo 1993).

TABLE 8
Chemical and physical properties of Capsaicin

Property	Value	References
Form	Crystalline pungent alkaloid; rectangular plates, monoclinic prisms, or scales from petroleum ether	D'Amelio 1999; Budavari 1989; Lide 1993
Molecular weight	305.42	Grasselli 1975
Loss on drying	Not more than 1.0% loss of weight when dried in a vacuum over phosphorus pentoxide at 40°C for 5 h	Committee of Revision of the United States Pharmacopeial Convention 2000
Solubility	Insoluble in water; very soluble in ethyl alcohol; soluble in acetone, benzene, ether, petroleum ether, and chloroform; slightly soluble in carbon disulfide	Grasselli 1975; Budavari 1989; Lide 1993
Melting range	Between 57°C and 66°C	Committee of Revision of the United States Pharmacopeial Convention 2000
Melting point	65°C	Grasselli 1975
Boiling Point	210–220°C	Grasselli 1975
Residue on ignition	Not more than 1.0%	Committee of Revision of the United States Pharmacopeial Convention 2000
Distillation range	Distillation in vacuum of 0.01 mm at 210–220°C	Molnar, no date
Sublimation temperature	115°C	Molnar, no date

Methods of Production

Commercially available Capsaicin is extracted from natural sources (Carter 1991).

Analytical Methods

Capsaicin has been analyzed/identified by the following methods:

- thin-layer chromatography (Makara et al. 1967; Spanyár and Blazovich 1969; Srinivasan et al. 1981; Todd et al. 1975; Azizan and Blevins 1995);
- thin-layer chromatography–colorimetric method (Karawa et al. 1968);
- multiband thin-layer chromatography (Pankar and Magar, 1977);
- gas chromatography (Sagara et al. 1980; Krajewska and Powers 1987; Azizan and Blevins 1995);
- reverse-phase high-performance thin-layer chromatography (Suzuki 1980);
- high-performance liquid chromatography (Sticher et al. 1978; Saria et al. 1981);
- high-performance liquid chromatography with electrochemical detection (Kawada et al. 1985);
- liquid chromatography (Parrish 1996);
- liquid chromatography–thermospray mass spectrometry (Wilkins 1993);
- gas-liquid chromatography (Bennett and Kirby 1968; DiCecco 1976);
- high-pressure liquid chromatography (Law 1983; Gannett et al. 1988);
- paper chromatography (Govindarajan and Ananthakrishna 1974);
- mass fragmentography (Lee et al. 1976);
- micellar electrokinetic capillary chromatography (Khaled et al. 1993);
- mass spectroscopy and nuclear magnetic resonance (NMR) spectroscopy (Bennett and Kirby 1968); and
- infrared spectroscopy (Grasselli 1975).

Capsicum oleoresins have been analyzed/identified by liquid chromatography (Parrish 1996), high-performance liquid chromatography (Cooper et al. 1991), and high-performance liquid chromatography–mass spectrometry and field desorption mass spectrometry (Games et al. 1984).

UV Absorption

The results of a spectral analysis (170 to 600 nm) of Capsaicin in ethanol indicated absorption peaks at 281 ± 1 and 227 ± 1 nm (Grasselli 1975). Similar results were presented in another UV spectral analysis (Lee and Kumar 1980).

Stability/Reactivity

The reaction of Capsaicin with potassium ferricyanide yielded a dimer (Lawson and Gannett 1989).

Capsaicin is oxidized by pepper (*Capsicum annuum*) peroxidase isoenzyme B_6 in a reaction that is strictly dependent on

H_2O_2. This reaction is totally inhibited by antibodies that are raised against horseradish peroxidase (Bernal et al. 1994).

Capsaicin (250 mg) plus dihydrocapsaicin were nitrosated (at pH 2.0) with 5.5 g sodium nitrate in 100 ml of water. The following products (nitrophenols) were determined using high performance liquid chromatography: 4-nitroguaiacol, 4,6-dinitroguaiacol, nitrocapsaicin, and nitrodihydrocapsaicin. Formation of nitrosamides was not observed (Mende et al. 1994).

USE

Purpose in Cosmetics

In cosmetic products, Capsaicin functions as an external analgesic, a fragrance ingredient, and as a skin-conditioning agent—miscellaneous (Pepe et al. 2002).

Scope and Extent of Use in Cosmetics

Although listed as a cosmetic ingredient in the *International Cosmetic Ingredient Dictionary and Handbook* (Pepe et al. 2002), Capsaicin is not currently used in cosmetics. Capsaicin is not included in FDA's frequency of use data base on cosmetic ingredients (FDA 2002), and use concentration data on this ingredient were not reported in a 2001 industry-wide survey on cosmetic ingredient use concentrations (CTFA 2001). Capsaicin is present in cosmetic ingredients that are derived from *Capsicum annuum* and *Capsicum frutescens*, as indicated in Part 1 of this safety assessment.

Capsaicin is not included on the list of ingredients restricted for content in all cosmetics that are marketed in Japan (Ministry of Health, Labor and Welfare [MHLW] 2001a). It also is not included among the substances listed as prohibited from use in cosmetic products marketed in the European Union (European Economic Community 1999, 2002).

Noncosmetic Use

Information on the OTC drug review status of Capsaicin (FDA 2003) is included in Table 9.

In 1993, the FDA published a final rule (FDA 1993) indicating that Capsaicin and other active ingredients are not generally recognized as safe and effective and are misbranded when present in certain OTC (over-the-counter) drug products. This ruling was based on inadequate data for establishing general recognition of the safety and effectiveness of these ingredients for the specified uses, and, regarding Capsaicin, relates to external analgesic drug products for fever blister and cold sore treatment. It was noted this final rule affects only the marketing of ingredients that are listed as active ingredients in specific types of OTC drug products, for which unproven medical claims are being made, and that this rule does not affect the continued use and marketing of these ingredients in vitamin, mineral, and food supplement products.

OTC Capsaicin-containing creams (Zostrix, Zostrix-HP, and Axsain) have been shown to be effective in managing the following painful conditions: rheumatoid arthritis (Deal et al. 1991), osteoarthritis (McCarthy and McCarty 1992), diabetic neuropathy (Donofrio et al. 1991; Tandan et al. 1992), postherpetic neuralgia (Watson et al. 1988; Bernstein et al. 1989), postmastectomy pain syndrome (Watson et al. 1989), cluster headache (Sicuteri et al. 1989), and reflex sympathetic dystrophy (Chesire and Snyder 1990). Two strengths of Zostrix (0.025% and 0.075% Capsaicin) are used to treat the neuropathic pain of postherpetic neuralgia (Facial Neuralgia Resources 2003).

BIOLOGICAL PROPERTIES

Absorption, Distribution, Metabolism, and Excretion

In Vivo Studies

Kim and Park (1981) reported that after intravenous (i.v.) administration of a single dose of Capsaicin (dose = 4 mg/kg) to rabbits, the elimination phase was short (average half-life of 17.35 min). Urinary excretion of Capsaicin during a 2-h period after dosing was 0.004% to 0.04% of the administered amount. In another experiment, the maximum plasma concentration after oral administration of 300 mg/kg Capsaicin was 4×10^{-7} g/ml at 40 min.

Saria et al. (1982) injected Capsaicin intravenously (i.v. dose = 2 mg/kg) into the femoral vein of Sprague-Dawley rats (males and females; weights = 200 to 250 g) that had been anesthetized with pentobarbitone (dose = 35 mg/kg). Stock solutions contained 20 mg/ml Capsaicin in 0.9% NaCl with 10% ethanol and 10% Tween 80 (Polysorbate 80). Appropriate

TABLE 9
OTC drug review ingredient status report for Capsaicin (FDA 2003)

Ingredient	OTC panel	OTC use	Pharmaceutical use	Proposed OTC category[a]	Final OTC category[a]
Capsaicin	Miscellaneous External	External Analgesic	Fever Blister (Topical)	IISE	IIE 21CFR:310.545(a)(10)(v)
Capsaicin	Topical Analgesic	External Analgesic	Counterirritant	I	Pending

[a]Category I—conditions under which OTC drug products are generally recognized as safe (S) and effective (E) and are not misbranded; category II—conditions under which OTC drug products are not generally recognized as safe and effective or are misbranded; category III—conditions for which the available data are insufficient to permit final classification.

dilutions for i.v. injection were made in 0.9% saline. Subsequently, the animals were placed under light pentobarbitone anesthesia (dose = 25 mg/kg) and Capsaicin was injected subcutaneously (dose = 50 mg/kg) into the dorsal skin. The animals were killed, and the following tissues homogenized and centrifuged: whole brain, spinal cord, liver, kidney, and omentum. Supernatants were evaporated and redissolved in methanol, and Capsaicin was determined using reverse-phase high-performance liquid chromatography.

When blood concentrations of Capsaicin at three minutes after i.v. dosing (581 ± 230 ng/g) were compared to concentrations in other tissues, an approximately fivefold higher concentration of Capsaicin was found in the brain (2763 ± 412 ng/g) and spinal cord (2670 ± 280 ng/g). The concentration of Capsaicin in the liver was approximately threefold higher (1736 ± 355 ng/g). At 10 min post dosing, Capsaicin concentrations in the blood (48 ± 18 ng/g) and liver (46 ± 14 ng/g) were greatly decreased. However, concentrations in the spinal cord (916 ± 192 ng/g) and brain continued to be higher than those found in the blood and liver.

Following a single subcutaneous injection of Capsaicin, the concentration in the blood appeared to follow a Bateman function and reached a maximum at 5 h. Up to 100 min post dosing, Capsaicin concentrations in the brain and spinal cord were almost as high as those in the blood. Thereafter, blood concentrations were higher than those in the brain and spinal cord. The highest concentrations of Capsaicin were found in the kidney, whereas concentrations in the liver remained very low. It is important to note that as early as 10 min post dosing, Capsaicin was detectable in all tissues that were investigated, except for adipose tissue (omentum). In adipose tissue, measurable concentrations of Capsaicin were not found earlier than 30 min post dosing. After 17 h, Capsaicin concentrations in all tissues (except for the blood) decreased to levels that were undetectable (Saria et al. 1982).

Leelahuta et al. (1983) evaluated the absorption and excretion of Capsaicin using fasted albino rats (number not stated; weights = 150 to 220 g). The rats were dosed orally with Capsaicin (dose = 47.5 mg/kg), after which each segment of the gastrointestinal tract (GI) was ligated. The luminal content was collected, and Capsaicin was determined using a colorimetric method after elution from silica gel. In excretion experiments, urinary Capsaicin (in free form and liberated from glucuronides) was determined using a colorimetric method after separation on a thin layer plate.

At 1 h post dosing, up to 75.5% of the administered oral dose of Capsaicin was readily absorbed, and 22.0% and 2.5% remained in the small intestine and stomach, respectively. At 3 h post dosing, less than 2.0% remained in the small intestine. In the excretion experiments, a parent Capsaicin was excreted as such in free form (~7.6% in urine and ~10.2% in feces) and in glucuronide form (4.9% in urine and 5.1% in feces) within 24 h post dosing. During the second day, only a small amount of the parent compound was excreted in free form. Other Capsaicin metabolites were not estimated in this study.

The absorption of Capsaicin in situ was also evaluated. Capsaicin (dose = 2.0 mg/kg, dissolved in ethanol–Tween 80–saline, 1:1:25) was injected into a ligated segment of the GI tract. After 1 h, the luminal content was collected and Capsaicin was determined using a colorimetric method after elution from silica gel. Capsaicin was absorbed in both the stomach and small intestine. The greatest absorption was observed in the jejunum (91.4%), followed by the ileum, duodenum, and stomach. Following absorption from the jejunum, Capsaicin was detected in the blood at a concentration as high as 1.55 mg % (Leelahuta et al. 1983).

In a study by Kawada et al. (1984), fasted male Wistar rats were fed a basal diet containing 3 mg of a commercial Capsaicin mixture (85% Capsaicin and 15% dihydrocapsaicin) by stomach tube. The content of Capsaicin and dihydrocapsaicin in the GI tract was determined using high-performance liquid chromatography (HPLC). Capsaicin was rapidly absorbed from the stomach and small intestine. At 3 h, only 15% of the dose administered remained in the entire GI tract (i.e., 85% absorption at 3 hours). No metabolite of Capsaicin or dihydrocapsaicin was found in the gut contents. After 48 h, the amount of Capsaicin and dihydrocapsaicin in the feces was less than 10% of the administered dose.

In situ experiments (in situ loop method of Kimura et al. 1973) in the preceding study indicated that Capsaicin and dihydrocapsaicin were absorbed more readily from the jejunum and ileum than from the stomach. The rate of disappearance of Capsaicin and dihydrocapsaicin from the jejunal loop was 2.0% of the dose per minute per length (in cm) of loop. This rate was approximately equal to the rate of appearance of radioactivity in the bloodstream.

No significant reduction in the uptake of [^{3}H]dihydrocapsaicin was noted in systems in which either of the metabolic inhibitors, 2,4-dinitrophenol (uncoupler of oxidative phosphorylation) or sodium cyanide (respiratory chain inhibitor), was added to the emulsion that was injected into ligated jejunal loops. If an active transport mechanism were necessary for the absorption of [^{3}H]dihydrocapsaicin, then its uptake should have been depressed in the presence of these inhibitors. These results indicate that an active transport mechanism is not involved in the absorption of Capsaicin and its analogs, and, thus, absorption is a nonactive process.

[^{3}H]Dihydrocapsaicin was administered into the ligated intestinal loops in situ, and radioactive substances were subsequently extracted from venous blood. Approximately 85% of the portal blood radioactivity was detected as [^{3}H]dihydrocapsaicin and approximately 15% was detected as 8-methyl nonoanoic acid (dihydrocapsaicin metabolite), bound to albumin fraction. Additionally, dihydrocapsaicin-hydrolyzing enzyme was found in jejunal tissue. These results support the possibility that Capsaicin and its analogs undergo a first-pass effect (metabolism of the compound following initial absorption in the GI tract) (Kawada et al. 1984).

Donnerer et al. (1990) studied the absorption and metabolism of capsaicinoids using groups of male Sprague-Dawley rats (4 to 6/group). In the first experiment, Capsaicin (50 or 500 μg dissolved in 1 ml of the solvent, 10% Tween 80 and 10% ethanol in saline) was labeled with 12 μCi of [^{3}H]dihydrocapsaicin and injected into the stomach via an esophageal cannula. In another group, 2 mg/kg Capsaicin (0.2 ml of a 1 mg/ml solution per 100 g body weight) was labeled with 20 μCi [^{3}H]dihydrocapsaicin and injected i.v. Animals in another group were injected s.c. with 50 mg/kg Capsaicin (0.5 ml of a 10 mg/ml solution per 100 g body weight labeled with 20 μCi [^{3}H]dihydrocapsaicin).

Following intragastric administration, approximately 30% to 50% of Capsaicin and dihydrocapsaicin were absorbed from the stomach, with practically no degradation (based on HPLC analysis) taking place. Furthermore, after dosing with 50 μg Capsaicin (plus tracer amounts of [^{3}H]dihydrocapsaicin), absorption from the whole intestine amounted to approximately 90%. Approximately 75% of the 500-μg dose was absorbed, with considerable degradation taking place in the intestine. Additional data from this experiment imply that the percentage of unchanged labeled dihydrocapsaicin decreased as follows: GI lumen > portal vein blood > trunk blood and brain.

After either parenteral route of administration (i.v. or s.c.), approximately 50% unchanged Capsaicin (labeled with [^{3}H]dihydrocapsaicin) was identified in the trunk blood and brain. The authors concluded that, in the rat, Capsaicin and dihydrocapsaicin are readily absorbed from the GI tract (Donnerer et al. 1990).

In Vitro Studies

Lee and Kumar (1980) evaluated the metabolism of Capsaicin in vitro using liver homogenates and microsomes from three groups of female rats (mixed strain; weights = 250 to 300 g) that had been treated with sodium pentobarbital for non-specific mixed function oxidase induction, with Capsaicin for substrate induction, and with saline (control), respectively. Capsaicin and other capsaicinoids were shaken with either the supernatant fraction from liver homogenate enriched with NADP and glucose-6-phosphate or liver microsomes supplemented with NADPH. Acidification and extraction with ethyl acetate were used to terminate enzyme reactions. The capsaicinoids, Capsaicin, and nanoyl vanillylamide were incubated with the liver homogenates and thin-layer chromatography (TLC) was performed in order to characterize the metabolites of capsaicinoids. Metabolite portions from TLC plates were eluted with ethyl acetate and subjected to gas chromatography–mass spectroscopy after silylation. Based on the mass spectrum, the proposed structure of the metabolite detected corresponded to *N*-(4,5-dihydroxyl-3-methoxybenzyl)-acylamides. The authors concluded that Capsaicin is metabolized in the liver by a mixed function oxidase system.

Leelahuta et al. (1983) studied the metabolism of Capsaicin in vitro using tissues from Swiss albino mice (number not stated; weights = 30 to 40 g), albino rats (number not stated; weights = 150 to 220 g), and Syrian golden hamsters (number not stated; weights = 100 to 150 g). The incubation mixture consisted of the following: Capsaicin (1.0 μmole), NADP (2.6 μmoles), glucose-6-phosphate (7.09 μmoles), and tissue subcellular fraction equivalent to 100 mg wet weight of tissues. Capsaicin and its metabolites were determined either using a colorimetric method or qualitative analysis by gas chromatography. Capsaicin was metabolized using rat, mouse, or hamster liver fractions. The use of other subcellular fractions from the rat indicated that Capsaicin was also metabolized in the kidney, lung, and intestinal mucosa. At least three metabolites of Capsaicin were identified. One of the metabolites was believed to be vanillylacetamide, and, the other, one of two possible metabolites (5-hydroxy-capsaicin or 3,4-dihydroxybenzyl-8-methyl-6-nonenamide). The third metabolite was not identified.

In a metabolism experiment by Lawson and Gannett (1989), the incubation of Capsaicin with microsomes (from Syrian golden hamsters) resulted in Capsaicin dimer formation. The incubation mixture had the following composition: glucose-6-phosphate (5 mM), glucose-6-phosphate dehydrogenase (20 units), microsomal protein (1 mg), nicotinamide adenine dinucleotide phosphate (2.5 mM) and substrate (1.0 mM) in 1.0 ml of 100 μM phosphate buffer (pH 7.4) containing 125 mM sucrose and 5.5 mM magnesium chloride.

In an investigation of the oxidation of Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) using peroxidase enzyme assays, Boersch et al. (1991) reported that Capsaicin appears to form a fluorescent dimer that is comparable to those that are known to be formed from other compounds containing the vanillyl (4-hydroxy-3-methoxybenzyl-) group.

In a metabolism experiment by Surh et al. (1995a), the incubation of Capsaicin with phenobarbital-induced rat liver postmitochondrial supernatant enriched with an NADPH-generating system resulted in production of the metabolite, *N*-(4,5-dihydroxy-3-methoxybenzyl)-(E)-6-noneylamide.

Percutaneous Absorption

The in vitro penetration of Capsaicin through rat skin was evaluated using a diffusion cell that is similar to the Franz horizontal diffusion assembly. Fluxes ranged between 7 and 11 μg/cm^2/h. The low pH value of the aqueous donor phase (pH 4.2) resulted in a slightly higher flux relative to pH's in the range of 5 to 8 (Tsai et al. 1994).

Fang et al. (1995) performed percutaneous absorption experiments on hydrophilic and commercial ointment bases containing 0.075% Capsaicin using various skin (full-thickness) types. The following *in vitro* permeability coefficients (cm/h) were reported for Capsaicin (from hydrophilic ointment base): 0.49 $\pm$ 0.09 (human skin), 2.91 $\pm$ 0.47 (rat skin), 3.92 $\pm$ 0.49 (mouse skin), 2.55 $\pm$ 0.55 (rabbit skin), and 0.71 $\pm$ 0.08 (pig skin). Permeability coefficients (cm/h) for Capsaicin (from commercial ointment base) were as follows: 0.88 $\pm$ 0.13 (human skin), 3.17 $\pm$ 0.13 (rat skin), 8.43 $\pm$ 0.51 (mouse skin), 2.96 $\pm$ 1.01 (rabbit skin), and 1.27 $\pm$ 0.20 (pig skin).

Fang et al. (1996) evaluated the penetration of Capsaicin (from gel bases) through pig skin in vitro. The composition of each gel base used was as follows: Gel Base 1 (20% *w*/*w* propylene glycol; 1.20% Carbopol 940; 2, 4, or 8% triethanolamine [TEA]; and purified water accounted for remaining composition), Gel Base 2 (Gel Base 1 with 8% triethanolamine and 10%, 20%, or 30% isopropanol [solubilizing agent] added), and Gel Base 3 (Gel Base 1 with 8% triethanolamine and 10%, 20%, or 30% ethanol [solubilizing agent] added). Shaved skin from male pigs (1 to 2 weeks old; weights = 5 to 6 kg) was mounted in a vertical Keshary-Chien glass diffusion cell. Skin was mounted on the receptor compartment, with the stratum corneum side facing downward into the receptor compartment. The donor compartment was filled with 2 g gel containing 0.060% Capsaicin. The receptor fluid was withdrawn (500-μl aliquots) and replaced with an equal volume of fresh receptor solution at appropriate intervals.

Values for Capsaicin flux (μg/cm^2/h) in the presence of different concentrations of TEA in Gel Base 1 were as follows: 2.17 $\pm$ 0.43 (with 2% TEA), 2.28 $\pm$ 0.47 (with 4% TEA), and 1.97 $\pm$ 0.43 (with 8% TEA). The results of an analysis of variance (ANOVA) indicated no significant difference in Capsaicin flux ($p > .05$) in the presence of different concentrations of TEA.

The addition of isopropanol or ethanol to Gel Base 1, resulting in Gel Bases 2 and 3, respectively, reduced the penetration fluxes of Capsaicin (ANOVA not included). Values for Capsaicin flux (μg/cm^2/h) in the presence of isopropanol were as follows: 0.93 $\pm$ 0.21 (with 10% isopropanol), 0.57 $\pm$ 0.21 (with 20% isopropanol), and 0.56 $\pm$ 0.20 (with 30% isopropanol). The following values for Capsaicin flux in the presence of ethanol were also reported: 1.22 $\pm$ 0.37 (with 10% ethanol), 1.07 $\pm$ 0.33 (with 20% ethanol), and 0.49 $\pm$ 0.13 (with 30% ethanol) (Fang et al. 1996).

In a similar study, Fang et al. (1997) evaluated the in vitro percutaneous absorption of Capsaicin using excised dorsal skin from male pigs (1 to 2 weeks old; weights = 4 to 5 kg). Capsaicin (0.06%) was incorporated into a gel formulation, the composition of which was as follows: propylene glycol (20.0% *w*/*w*), triethanolamine (2.0%), Carbopol 940® (0.6%), benzalkonium chloride (0.0 to 0.2%), and purified water (added to 100.0%). The penetration flux for gel base was determined using a vertical glass diffusion cell. The diffusion cell was filled with gel (2 g) and 20 ml of 1:1 (*v*/*v*) ethanol (pH 7.4), and the top of the cell was covered with paraffin paper. McIlvain buffer was used as the receptor medium. Pig dorsal skin was mounted on the receptor compartment, such that the stratum corneum side was facing upward into the donor compartment. Capsaicin was analyzed using HPLC. The difference between applied and recovered amounts corresponded to the cumulative absorbed amount. The total amount of Capsaicin that penetrated through the unit diffusion surface and into the receptor was calculated and plotted as a function of time.

A reduction in Capsaicin flux was observed as the concentration of benzalkonium chloride was increased from 0.1% to 0.2%. The formation of benzalkonium chloride micelles for Capsaicin at the higher concentrations to impede transfer across the skin barrier was offered as a possible reason for this phenomenon. The maximum flux of Capsaicin over the range of gel concentrations of benzalkonium chloride was approximately 3 μg/cm^2/h (benzalkonium chloride concentration $\approx$0.05%). The lowest flux of Capsaicin was approximately 1.5 μg/cm^2/h (presence of 0.1% benzalkonium chloride). The results of a human in vivo study evaluating the percutaneous absorption of Capsaicin (in same gel base) and skin irritation potential are included in the section "Clinical Assessment of Safety" later in the report (Fang et al. 1997).

Magnusson and Koskinen (2000) conducted in vitro diffusion studies using human epidermal membranes in an automated system of miniature diffusion cells with flow-through receptor compartments. The skin penetration of Capsaicin was determined using a donor solution consisting of 1% Capsaicin in 50% ethanol/water (volume = 50 μl). The receptor fluid consisted of 4% bovine serum albumin in phosphate-buffered saline or 50% ethanol in water. Percutaneous steady-state penetrations of Capsaicin were 28.2 $\pm$ 2.7 and 29.6 $\pm$ 2.9 μg/cm^2 using the former and latter receptor fluids, respectively.

Skin Penetration Enhancement

Degim et al. (1999) evaluated the in vitro skin penetration of naproxen (nonsteroidal anti-inflammatory agent) in the presence of Capsaicin (in ethanol) using full-thickness, human skin (from female; underlying fatty tissue removed) that was obtained after abdominal surgery. The skin was mounted in a Franz-type diffusion cell. Ex vivo experiments using isolated perfused rabbit ear skin were also performed. Naproxen fluxes were averaged over a period of 60 (human skin) and 6 (rabbit skin) h. Capsaicin enhanced the skin permeation of naproxen through human skin by approximately twofold. For rabbit skin, an enhancement ratio of 3.8 was reported for naproxen in the presence of Capsaicin. No gross changes in structural features of the stratum corneum were observed. Evidence of skin thickening was observed, but not to a large extent.

Effect on Protein Kinase Activity

Barber and Vasko (1996) evaluated the augmentation of Capsaicin-induced peptide release by protein kinase C (PKC) activation using rat sensory neuronal cultures (from dorsal root ganglion). Initially, untreated neuronal cultures were exposed to 50 nM Capsaicin for 10 min, which resulted in an approximately 8- to 10-fold increase in the release of substance P (SP) and calcitonin gene–related peptide (CGRP). Neuron cultures were also pretreated for 10 min with 1 nM phorbol 12,13-dibutyrate (PDBu, a phorbol ester that activates PKC) prior to and throughout Capsaicin stimulation. PDBu had no effect on resting release of SP and CGRP, but facilitated Capsaicin (50 nM)-induced release of these peptides. SP release in the presence of Capsaicin increased from 273.36 $\pm$ 11.3 (control neurons) to 511.4 $\pm$

32.1 fmol/10 min per well. Capsaicin-induced CGRP release increased from 1279.1 ± 99.0 to 2500 ± 199.9 fmol/10 min per well.

PDBu augmentation of Capsaicin-induced SP and CGRP release was significantly attenuated by down-regulation of PKC. Neuron cultures were pretreated with 1 mM PDBu for 48 h. In the presence of 1 nM PDBu, Capsaicin-stimulated release of SP and CGRP was 258 ± 23.5 and 1170.1 ± 198.8 fmol/10 min per well, respectively. PKC down-regulation had no effect on resting release of SP or CGRP, and also did not alter Capsaicin-induced release of CGRP. However, it significantly decreased the Capsaicin-induced release of SP from 273.4 ± 11.3 to 187.7 ± 12.7 fmol/10 min per well. The fact that PKC down-regulation significantly decreased the Capsaicin-induced release of SP suggests that Capsaicin may activate PKC in sensory neurons (Barber and Vasko 1996).

This finding is supported by results from another study (Harvey et al. 1995) indicating that resiniferotoxin (Capsaicin analogue) activates PKC in sensory neurons from adult rats.

Effect on Collagenase Release

The following effects of Capsaicin on rheumatoid arthritis synoviocytes were evaluated in vitro: DNA synthesis, RNA synthesis, collagenase release, and prostaglandin (PGE_2) synthesis. The effect of Capsaicin on collagenase release and prostaglandin synthesis will be summarized in this section and the following section, respectively. Effects on DNA and RNA synthesis are summarized in the section "Effects on DNA/RNA Synthesis" later in the report. Synovial tissue was obtained from rheumatoid arthritis patients who were undergoing reconstructive joint surgery. The release of collagenase from synoviocytes was significantly increased at Capsaicin concentrations of 10^{-8} to 10^{-3} mol/L. The greatest stimulation of collagenase release occurred at a concentration of 10^{-8} mol/L ($p < .05$). The stimulation of collagenase synthesis was less, but still significant ($p < .05$), at higher doses of Capsaicin (Matucci-Cerinic et al. 1990).

Effect on Prostaglandin Synthesis

Collier et al. (1975) evaluated Capsaicin-induced stimulation of prostaglandin biosynthesis in bull seminal vesicles. The test substance was incubated with arachidonic acid in bull seminal vesicle homogenate. Prostaglandin-like material was extracted in ethyl acetate after acidification with citric acid. In the presence of Capsaicin (2.8 μg/ml), prostaglandin production was increased by 50%.

Matucci-Cerinic et al. (1990) evaluated the effect of Capsaicin on PGE_2 synthesis in rheumatoid arthritis synoviocytes. PGE_2 synthesis in synoviocytes increased with increasing concentrations of Capsaicin. Compared to controls, maximal stimulation of PGE_2 production was noted at a Capsaicin concentration of 10^{-8} mol/L. Inhibition of PGE_2 synthesis was observed at a concentration of 10^{-3} mol/L.

Mohr et al. (1975) have reported that synovial tissue proliferates rapidly, and, according to Dayer et al. (1976), synovial tissue produces high concentrations of collagenase (produced by fibroblasts) and prostaglandins. Harris (1985) reported that collagenase can initiate the breakdown of type I, II, and III interstitial collagens. Furthermore, according to Matucci-Cerinic et al. (1990), the production of collagenase and PGE_2 by rheumatoid arthritis synoviocytes is of potential importance in the degradation of articular cartilage and adjacent joint structures.

Effect on Protein Synthesis

Cochereau et al. (1996) demonstrated that Capsaicin inhibits the aminoacylation of tyrosine. Cochereau et al. (1997) reported that Capsaicin-induced inhibition of protein synthesis was inhibited in the presence of tyrosine in Vero cells.

Richeux et al. (1999) evaluated the effect of Capsaicin on protein synthesis in human neuroblastoma SHSY-5Y cells at test concentrations ranging from 12.5 to 125 μM. Protein synthesis was not inhibited at a test concentration of 12.5 μM. However, from that point on, the percentage of inhibition increased with increasing concentrations of Capsaicin. At the highest concentration (125 μM), 88% inhibition of protein synthesis was noted. An IC_{50} of 60 μM was reported for Capsaicin.

Creppy et al. (2000) evaluated the effect of Capsaicin on protein synthesis in vitro using Vero kidney cells, tissue culture cells derived from the kidney of an African green monkey (*Cercopithecus aethiops*). Aliquots of cell suspensions were incubated for 24 h with 97 μM Capsaicin and different concentrations of tyrosine (97, 194, 388, and 776 μM, respectively). Protein synthesis was assessed by monitoring the incorporation of [^{3}H]L-leucine into the kidney cells (counts/min/mg total protein). Vero cell cultures without Capsaicin served as controls. Capsaicin inhibited protein synthesis in Vero cells, and the inhibition was reversed when a molar ratio of 4/1 (tyrosine/Capsaicin) was reached. Compared to the control, the incorporation of [^{3}H]L-leucine was significantly less in cultures incubated with 97 μM Capsaicin + 97 μM tyrosine or 97 μM Capsaicin + 194 μM tyrosine ($p < .001$) (Creppy et al. 2000).

Antimicrobial Activity

Molina-Torres et al. (1999) evaluated growth the following liquid bacterial/yeast cultures in the presence of Capsaicin: *Escherichia coli* (DH5α Difco ATCC 25922; test concentrations of 200 and 300 μg/ml Capsaicin), *Pseudomonas solanacearum* (test concentrations of 75, 150, and 300 μg/ml), *Bacillus subtilis* (test concentrations of 50, 75, and 150 μg/ml), and*Saccharomyces cerevisiae* (test concentrations of 25, 150, and 300 μg/ml Capsaicin). All inocula were obtained from cultures growing exponentially in the same medium, and growth was followed by determining turbidity at 650 nm in a spectrophotometer.

The following effects were noted: retardation of *E. coli* growth (Capsaicin concentrations up to 200 or 300 μg/ml), 20%

reduction in growth of *P. solanacearum* (300 μg/ml Capsaicin), and inhibition of *B. subtilis* growth (beginning at 25 μg/ml Capsaicin). The effect of Capsaicin on *S. cerevisiae* was not clear. Short-term cellular growth was stimulated at concentrations as high as 150 and 300 μg/ml. However, Capsaicin had no effect on the long-term (24 h) growth of *S. cerevisiae* at concentrations as high as 300 μg/ml (Molina-Torres et al. 1999).

DNA Binding

Teel (1991) reported that Capsaicin concentrations of 25, 50, and 100 μM decreased the binding of aflatoxin (AFB_1) to calf thymus DNA by 19%, 44%, and 71%, respectively, in the presence of metabolic activation. At concentrations of 50 and 100 μM, Capsaicin reduced the formation of AFB_1-DNA adducts by 53% and 75%. These effects correlated with a decrease in S9-mediated metabolism of AFB_1 in the presence of Capsaicin that was concentration dependent. Capsaicin also altered the formation of water-soluble conjugates of AFB_1. The results of these experiments suggest that Capsaicin inhibited the biotransformation of AFB_1 by modifying phase I hepatic enzyme activity.

Capsaicin Receptor

The ability of Capsaicin, the hot component of chili peppers, to induce pain has been known for many years (Szallasi and Blumberg 1999). Pharmacological and physiological studies have demonstrated that Capsaicin, which contains a vanillyl moiety, produces its sensory effects by activating a Ca^{2+}-permeable ion channel on sensory neurons (Szallasi and Blumberg 1999; Wood et al. 1988). Studies on structure-activity relationships of agonists and the development of the antagonist capsazepine have shown that the activation of this ion channel is mediated by a classical receptor-based mechanism (Szallasi and Blumberg 1999; Bevan et al. 1992).

Capsaicin is a known activator of vanilloid receptor 1 (VR1, a heat-gated ion channel) (Szallasi and Blumberg 1999; Sterner and Szallasi 1999) and has numerous acute biologic effects, which include the induction of pain, the modulation of thermoregulation, and the production of neurogenic inflammation. These responses to Capsaicin may lead to desensitization, depending on the dose of Capsaicin and the duration of exposure (Holzer 1991). Capsaicin responses are believed to involve its activation of a subset of sensory neurons with thin unmyelinated (C-fiber) and thin myelinated (Aδ-fiber) processes (Biro et al. 1997). The VR1 receptor was initially isolated from a cDNA library from rat sensory neurons (Caterina et al. 1997), and studies have indicated the following distribution in humans: sensory neurons (dorsal root ganglia and trigeminal ganglia), pancreas, brain, spinal cord, bladder, kidney, liver, spleen, testis, lung, and bowel (Caterina et al. 1997; Hayes et al. 2000; Mezey et al. 2000; Birder et al. 2001; Yiangou et al. 2001). Vanilloids, such as Capsaicin, are the only known activators of the VR1 receptor (Gunthorpe et al. 2002).

Other Biological Effects

A wide range of other Capsaicin-induced biological effects (in vivo or in vitro) have been reported, ranging from cough elicitation in guinea pigs to the suppression of inflammation in arthritic rats. These data are grouped by species or test system in Table 10.

TOXICOLOGY

Acute Intratracheal Toxicity

For anesthetized male Swiss albino mice (25 to 35 g) dosed intratracheally (into tracheal lumen; dose volume = 0.5 ml/kg) with Capsaicin in DMSO, a mean LD_{50} of 1.60 mg/kg was reported (Glinsukon et al. 1980).

Acute Oral Toxicity

Glinsukon et al. (1980) administered Capsaicin (in ethanol, Tween 80, and saline), by stomach tube, to 30 Swiss male albino mice (25 to 35 g) at a dose volume of 16.7 ml/kg. An LD_{50} range of 60 to 75 mg/kg was reported. In another experiment, 63 male mice of the same strain and weight range were dosed with Capsaicin in DMSO according to the same procedure. An LD_{50} of 190 mg/kg was reported. Desquamatic necrosis, with increased mucous material, of the gastric mucosa was noted microscopically (number of animals with these findings not stated). Additionally, some of the chief and parietal cells were vacuolated and had a pale, basophilic cytoplasm. No significant histopathological changes were observed in other organs.

Saito and Yamamoto (1996) evaluated the acute oral toxicity of Capsaicin (in propylene glycol) using 120 rats (CD strain; 149 to 174 g [males] and 117 to 138 g [females]) and 120 mice (Crj:ICR strain; 24 to 30 g [males] and 19 to 26 g [females]). Mice and rats were 6 weeks old. For rats or mice, five groups (10 males, 10 females/group) received the following single oral doses of Capsaicin, respectively: 96, 116, 139, 167, and 200 mg/kg. The control groups (10 males, 10 females/group; five group per strain) were dosed with vehicle (propylene glycol) only. All animals were necropsied after death or on day 8 post dosing, and gross changes recorded.

For mice, LD_{50} values of 118.8 mg/kg (males, 95% confidence limit 96.9 to 145.6 mg/kg) and 97.4 mg/kg (females, 95% confidence limit 68.8 to 137.4 mg/kg) were reported. More than 60% of the mice (6/10 to 9/10) died in all groups, with the exception of the 96 and 116 mg/kg dose groups (4/10). Body weight gains were comparable to the controls. The following signs were reported after dosing: salivation, convulsions (tonic and clonic convulsions included), tremor, dyspnea, cyanosis, staggering gait, bradypnea, lateral position, prone position, and erythema of the skin.

For rats, LD_{50} values of 161.2 mg/kg (males, 95% confidence limit 126.1 to 206.3 mg/kg) and 148.1 mg/kg (females, 95% confidence limit 120.5 to 182.1 mg/kg) were reported. Body weight gains were comparable to the controls. When all dose

TABLE 10
Other biological effects of Capsaicin

Reported effects	References
Rats	
Profound hypothermia (at doses down to 1.7 mg/kg), associated with skin vasodilation, in rats injected subcutaneously (s.c.) or intraperitoneally (i.p.)	Jancsó-Gabór et al. 1970
i.p. administration (dose = 5 mg/kg) to rats resulted in increased microsomal cytochrome P450 content and NADPH–cytochrome P450 activity	Kim et al. 1979
Long-lasting fall in body temperature in rats injected s.c. (dose = 5 mg/kg), and moderate decrement in hypothermic effect after a second injection 4.5 h after the first	Rabe et al. 1980
Hypocholesterolemic effect in rats after oral dosing with diet containing 15 mg %	Sambaiah and Satyanarayana 1980
Significant increase in concentrations of histamine and 5-hydroxytryptamine in dorsal skin of hindpaw and dorsal spinal cord of newborn rats injected s.c. (dose = 50 mg/kg)	Holzer et al. 1981
Significant reduction in mean systemic arterial blood flow in adult male rats dosed s.c. (dose = 50 mg/kg)	Virus et al. 1981
Significant reduction in baroreceptor and chemoreceptor reflex activity in anesthetized adult rats that had been treated neonatally (s.c. dose = 50 mg/kg)	Bond et al. 1982
Decreased blood pressure, decreased heart rate, and apnea in Sprague-Dawley rats pretreated with i.v. dose (50 mg/kg) and subsequently dosed i.v. with 1 μg	Donnerer and Lembeck 1982
Decreased blood pressure, but no effect on heart rate or respiration in Wistar rats pretreated with i.v. dose (50 mg/kg) and subsequently dosed i.v. with 1 μg	Donnerer and Lembeck 1982
Dose-dependent (1 to 10 mg/kg) hypothermia in male rats dosed s.c.	Szikszay et al. 1982
Antiinflammatory effect in rats innoculated with *Mycobacterium butyrium* and dosed s.c. (effect at doses down to 20 mg/kg)	Colpaert et al. 1983
Significant prolongation of pentobarbital-induced sleeping time in adult female rats, following s.c. injection (dose = 10 mg/kg)	Miller et al. 1983
Stimulation of ventilation following intrathecal injection (into subarachnoid space) into rats (50 μg injected)	Bervoets and Colpaert 1984
Prolonged bleeding time significantly in male Sprague-Dawley rats dosed orally (doses down to 5 mg/kg)	Hamid and Flurry-Rowersi 1984
Reduction of spinal content of substance P and increased tail-flick latency in male rats receiving intrathecal injection (75 μg injection)	Jhamandas et al. 1984
Apnea, bradycardia, and hypotension following intravenous injection into male rats (effects at doses down to 30 μg/kg)	Mitchell et al. 1984
Contraction of rat urinary bladder in vivo after topical application to bladder (2 μg applied)	Maggi et al. 1984, 1986
Increased gastrointestinal absorption of acetaminophen in adult male rats dosed orally (doses down to 1 mg/kg)	Metwally and Kandil 1985
Enhanced energy metabolism in male rats injected i.p. (dose = 6 mg/kg)	Kawada et al. 1986
Decreased blood pressure and heart rate, followed by pressor response, in rats after i.v. dosing (1 μg injected)	Chahl and Lynch, 1987
Dose-dependent hypotension and bradycardia in male rats that received microinjections of capsaicin into the nucleus tractus solitarii (doses of 9.82 and 98.2 nmol)	Lukovič et al. 1987
Stimulation of liver triglyceride secretion (from liver to serum) in rats receiving a diet containing 0.2 mg %	Sambaiah and Satyanarayana 1987
Lipid lowering action in rats fed high fat diets containing 0.2 mg %	Srinivasan and Satyanarayana 1987
Significantly increased total lipid and decreased phospholipid and cholesterol content of rat lung (adult male rats) after i.p. injection (1.6 mg/kg doses)	De and Ghosh 1988a
Reduction of capillary skin blood flow in plantar area of rat legs after topical treatment of sciatic nerve with 1% Capsaicin in vivo	Sann et al. 1988
Enhancement of adrenal medullary catecholamine secretion in male Wistar rats injected i.v. with doses down to 20 μg/kg	Watanabe et al. 1988

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TABLE 10
Other biological effects of Capsaicin *(Continued)*

Reported effects	References
Significant increase in rat pulmonary superoxide dismutase, catalase, and peroxidase activities with short-term treatment (i.p. dosing of 1.68 mg/kg), and significant decrease in pulmonary catalase and peroxidase activities with long-term treatment (i.p. dosing of 0.42 mg/kg)	De and Ghosh 1989
Slight (but not statistically significant) reduction in P-450 content and enzyme activities of aniline hydroxylase, aminopyrine demethylase, UDP-glucuronyl transferase, sulfotransferase, and glutathione *S*-transferase in male rats dosed orally (50 mg/kg doses)	Iwama et al. 1990
Capsaicin aerosol (stock solution of Capsaicin [100 μg/ml] diluted to 10 and 1 μg/ml prior to aerosolization; median particle size = 6 μm) administration induced an increase in arterial blood pressure in rats at concentrations down to 1 μg/ml	Palecek et al. 1990
Concentration-dependent increase (test concentrations down to 80 μM) in blood flow after topical application to gastric mucosa of male Sprague-Dawley rats	Wallace et al. 1992
Significant increase in gastric mucosal blood flow in Wistar rats receiving an intragastric dose of 0.5 mg/kg	Brzozowski et al. 1993
Protection against trinitrobenzene sulfonic acid-induced colitis (in the presence of 640 μM Capsaicin) in rats following topical application to lumen of colon	Goso et al. 1993
Inhibition of gastric motility (>0.6 mg/ml), increased mucosal blood flow (>0.03 mg/ml), and stimulation of HCO_3^- secretion (>0.3 mg/ml) after intragastric administration to male rats	Matsumoto et al. 1993
Decreased blood pressure and bradycardia in male Wistar rats after intraarterial and i.v. injection (dose = 25 μg/kg)	Yang et al. 1993
Capsaicin (5 mg/kg doses) in the diet lowered production of reactive oxygen species in peritoneal macrophages from rats	Joe and Lokesh 1994
Apnea, bradycardia, and hypotension following i.v. dosing (1 μg/kg) in young rats of either sex	Lee and Lundberg 1994
Increased gastric mucosal blood flow after topical treatment (with 160 μM) of rat gastric mucosa	Podolsky et al. 1994
Increased cutaneous blood flow following intraplantar injection (0.3 μg/10 μl)	Herbert and Holzer 1994
Increased blood flow (vasodilation) in plantar skin of rat hindpaw after intraplantar injection (0.03 μg/10 μl Capsaicin). Capsaicin injection preceded by intraplantar injection with tumor necrosis factor α (TNFα), which had no consistent effect on local cutaneous blood flow	Herbert and Hering 1995
Suppression of inflammation in arthritic male rats after s.c. injection (dose = 50 mg/kg)	Cruwys et al. 1995
Antiarrhythmic and antiischemic activity in isolated, perfused rat heart preparations in situ (concentrations down to 10 μM Capsaicin)	D'Alonzo et al. 1995
Depression of the micturition reflex in male rat bladders infused with Capsaicin in vivo (concentration = 1000 μM)	Kuo 1997
Mice	
Significant reduction in croton oil and 12-*O*-tetradecanoyl-phorbol-13-acetate-induced inflammation after topical application (1.75 μmoles/application) to CD-1 mice	LaHann 1986
Inhibitory effect on intestinal thiamine absorption in mouse intestinal loops in situ (concentrations down to 7 mg %)	Toskulkao et al. 1992
Inhibitory effect on intestinal thiamine absorption in mice dosed orally (doses down to 1 mg/kg body weight)	Toskulkao and Hatthachote 1992
Guinea pigs	
Elicitation of cough in guinea pigs after aerosol exposure (300 μM Capsaicin aerosol; particle size = 1.04 to 1.1 μm mass median aerodynamic diameter)	Bolser et al. 1955
Bronchial spasms in guinea pigs injected s.c. (10 mg of solution containing 0.1 g Capsaicin)	Hadžćovic et al. 1964

(Continued)

TABLE 10
Other biological effects of Capsaicin *(Continued)*

Reported effects	References
Elicited contractions of the isolated guinea pig ileum and positive inotropic and chronotropic effects on the isolated guinea pig auricle (concentrations down to 5×10^{-8} g/ml)	Molnár et al. 1969
Bronchoconstrictor action following i.v. administration to guinea pigs (threshold dose for this effect varied between 0.5 and 2 μg/kg)	Molnár et al. 1969
Profound hypothermia and skin vasodilation in guinea pigs injected subcutaneously (dose of 1.7 mg/kg)	Jancsó-Gabór et al. 1970
Marked inhibition of retrograde axoplasmic transport of nerve growth factor following s.c. injection of 50 mg/kg	Miller et al. 1982
Plasma extravasation and protein leakage in selected tissues (i.e., trachea, esophagus, nasal mucosa, paw skin, and ureter) from guinea pigs dosed i.v. with 0.5 μmol/kg	Saria et al. 1983
Dose-dependent increase in arterial blood pressure after application (0.3 to 30 μM) to guinea pig nasal mucosa in vivo	Lundblad et al. 1984
Slight reduction in blood pressure and tachypnea in guinea pigs after i.v. dosing (effects at doses down to 5 μg/kg)	Pórszász and Szolcsányi 1992
Significant and reproducible increase in microvascular leakage in tracheas and bronchi of male barrier-bred HD guinea pigs after intratracheal infusion of Capsaicin (0.2 nM)	Gallico et al. 1993
i.v. dosing induced dose-dependent bronchoconstriction (0.1 to 3.0 μg/kg) in male Hartley guinea pigs	Hsiue et al. 1993
Dose-dependent (1, 10, and 100 μg/kg) increase in airway resistance in anesthetized tracheostomized male guinea pigs	Martins et al. 1993
Dose-dependent (20 to 50 nmol) increase in plasma extravasation in the guinea pig conjunctiva after instillation into conjunctival sac	Figini et al. 1995
Antiarrhythmic and antiischemic activity in isolated, perfused guinea pig heart preparations in situ (doses down to 10 μM)	D'Alonzo et al. 1995
Dogs	
Bradycardia, hypotension and apnea in dogs following i.v. dosing (50 μg/kg)	Pórszász et al. 1955
Bradycardia and decreased systemic arterial pressure after injection into dog femoral vein (doses down to 5 μg/kg)	Coleridge et al. 1963
Bradycardia and decreased aortic pressure after injection (0.2 or 0.3 mg) into right cardiac chambers, or main pulmonary artery of dogs	Brender and Webb-Peploe 1969
Transient increase in mean arterial blood pressure, followed by sustained decrease, in dogs after i.v. dosing (doses down to 10 μg/kg)	Toda et al. 1972
Injection into right ventricle of dog significantly decreased airway diameter, heart rate, aortic pressure, and pulmonary artery pressure; injection into left ventricle did not alter airway diameter, but decreased heart rate and pulmonary artery pressure (dose injected = 20 μg/kg)	Russel and Lai-Fook 1979
Injection into left circumflex coronary artery of mongrel dogs (doses down to 0.3 μg/kg) caused either systemic hypotension and bradycardia, a pressor response associated with tachycardia, or a biphasic effect, with an initial increase and then a decrease in blood pressure and heart rate; intravenous injections (doses down to 3 μg/kg) resulted in a cardioinhibitory and depressor response	Staszewska-Woolley et al. 1986
Capsaicin aerosol (stock solution of Capsaicin [100 μg/ml] diluted to 10 and 1 μg/ml prior to aerosolization, median particle size = 6 μm) administration induced no consistent changes in either breathing pattern or cardiovascular parameters in dogs	Palecek et al.1990
Application to pericardium (open-chest) of dogs caused dose-related (0.1 to 100 μg), reflex increases in blood pressure and heart rate	Staszewska-Woolley et al. 1991
Bronchial vasodilation after injection (10 μg/kg) into dog right atrium	Pisarri et al. 1993

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TABLE 10
Other biological effects of Capsaicin *(Continued)*

Reported effects	References
Cats	
Bradycardia, hypotension and apnea in cats following i.v. dosing (200 μg/kg)	Pórszász et al. 1955
Hypotension, hypertension, negative inotropic effect, and increased pulmonary arterial pressure in cats after i.v. injection (doses down to 5 μg/kg). After left atrial injection of 5 μg/kg, a pressor response was observed	Molnár and György 1967
Reduction in systemic vascular resistance (i.e., a decrease in systemic perfusion pressure) in cats following administration (dose of 1 μg) into coronary arteries (open-chest experiments)	Osadchii et al. 1967
Rabbits	
Hypotension in rabbits after i.v. dosing (10 to 100 μg/kg). Decrease in blood pressure varied with dose	Toda et al. 1972
Pupillary constriction after retrobulbar injection into rabbit eye (concentrations down to 1.6 μmol). Topical application (300 nmol in 50 μl) pre-treatment greatly reduced the miotic response to retrobulbar injection of 1.6 μmol Capsaicin, but not 16 μmol Capsaicin	Wahlestedt et al. 1984
Dose-dependent (3.10×10^{-8} to 3.10×10^{-7} mol/site) increase in blood flow in rabbit skin following intradermal injection	Buckley et al. 1992
In vitro studies—guinea pig	
Inotropic effect on guinea pig atrium in vitro (concentration = 1.6×10^{-9} M)	Fukuda and Fujiwara 1969
Positive inotropic and chronotropic effects on guinea pig atrium in vitro (threshold dose of 10^{-9} M)	Lundberg et al. 1984
Stimulation of cyclic AMP accumulation in tissue slices from guinea pig spinal cord in vitro (1 μM Capsaicin)	Northam and Jones 1984
Smooth muscle relaxation of guinea pig carotid artery and thoracic aorta in vitro (induced by 3×10^{-7}M Capsaicin) only after vessels were precontracted with norepinephrine	Duckles 1986
Produced slowly developing, tonic contraction of isolated guinea pig gallbladder in vitro (concentration = 1 μM)	Maggi et al. 1989
Dose-dependent (5 nM to 1μM) stimulation of cAMP formation in guinea pig dorsal spinal cord slices, but not ventral spinal cord slices in vitro	Uhlén and Wikberg 1989
In vitro studies—hamster	
Inhibition of glucose transport from the mucosal to the serosal side of everted hamster small intestine in vitro (14 mg % Capsaicin); intestinal glucose transport was diminished by reducing the pH of the incubation medium from 7.4 to 5.0	Monsereenusorn 1979
Significant inhibition of lysosomal enzyme leakage from peritoneal macrophages obtained from mature hamsters (concentrations down to 1 nM)	De et al. 1992
In vitro studies—rat	
Labilized rat liver lysosomes, but not rat epidermal lysosomes in vitro (concentration = 10^{-3} M)	Smith et al. 1970
Inhibition of mitochondrial oxidative phosphorylation in rat liver mitochondria in vitro (50 μg Capsaicin)	Chudapongse and Janthasoot 1976
Increased adenylate cyclase activity in the rat brain (preoptic area of the hypothalamus, cerebral cortex, and cerebellum) in vitro (concentrations down to 10^{-7} M)	Jancsó and Wollemann 1977
Inhibitory action on energy metabolism by rat liver mitochondria in vitro by retarding electron flow from NADH to coenzyme Q (300 μg Capsaicin)	Chudapongse and Janthasoot 1981
Inhibition of ethylmorphine demethylation in rat liver microsomes in vitro (concentrations down to 25 μM), suggesting that Capsaicin is a potent inhibitor of microsomal cytochrome P450–mediated biotransformation reactions	Miller et al. 1983
Dose-related (300 nM to 10 μM) depolarization of rat sciatic nerve in vitro	Hayes et al. 1984
Time, concentration (0.001 to 10 μM), and Ca^{2+}-dependent increase in cyclic AMP accumulation in tissue slices from rat spinal cord in vitro	Northam and Jones 1984

(Continued)

TABLE 10
Other biological effects of Capsaicin *(Continued)*

Reported effects	References
Inhibiton of collagen-induced platelet (from rats) aggregation in vitro (minimal effect at 35 μg/ml; maximal effect at 175 μg/ml)	Wang et al. 1984
Dose-dependent (1 to 300 μM) inhibition of $^{45}Ca^{2+}$ uptake in cultured rat aortic smooth muscle and mouse neuroblastoma cell lines in vitro	Monsereenusorn and Kongsamut 1985
Concentration-related transient inhibition of the field-stimulation–induced motility of rat vas deferens (male albino rats) in vitro (0.01 to 3 μM Capsaicin)	Maggi et al. 1987
Prolonged duration of action potential in dorsal root ganglion neurons from neonatal rats in vitro (concentration = 3×10^{-4}M)	Godfraind et al. 1980
Concentration-dependent (10 to 320 μM) inhibition of platelet aggregation in rat arterial blood in vitro	Hogaboam and Wallace 1991
Fluidization of rat peritoneal mast cell membranes in vitro (40 to 320 μM Capsaicin)	Meddings et al. 1991
Prolongation of rat ventricular action potential in vitro, an effect associated with inhibition of potassium currents (concentration = 10 μM)	Castle 1992
Dose-dependent (effective concentration (EC_{50}) $\approx$ 160 nM) stimulation of cyclic GMP production in rat dorsal root ganglion neurons in vitro	Bauer et al. 1993
Arteriolar dilation in right cremaster muscle (male rats) suspended in tissue bath (containing 0.1 μg/ml Capsaicin)	White et al. 1993
Dose-dependent (50 to 1000 nM) stimulation of cyclic GMP production in rat dorsal root ganglion neurons in vitro	Bauer et al. 1995
Inhibition of Ca^{2+} -triggered phospholipase A_2 (PLA_2) activity in rat macrophages in vitro. Inhibitory concentration (IC_{50}) = 20 μM	Savitha and Salimath 1995
Concentration-dependent (10^{-9} to 10^{-5} M) inhibition of contractile tension in isolated rat ventricular papillary muscle in vitro	Yamato et al. 1996
Inhibition of the incorporation of arachidonic acid into rat peritoneal macrophage lipids in vitro (at Capsaicin concentrations down to 1 μM), leading to decreased formation of prostaglandin E_2, leukotriene B_4, and leukotriene C_4	Joe and Lokesh 1997
Reduced secretion of lysosomal enzymes (at 10 μM Capsaicin), such as collagenase, elastase, and hyaluronidase, in activated rat peritoneal macrophages in vitro	Joe and Lokesh 1997
Inhibition of phospholipase C–mediated Ca^{2+} increase (at 50 μM Capsaicin) in rat pheochromocytoma PC12 cells *in vitro*	Choi and Kim 1999
In vitro studies—rabbit	
In isolated atrial preparations from the rabbit, no effect on rate or force of contraction (test concentrations of 2×10^{-8} to 2×10^{-6} g/ml). Mostly no increased tension in strips of rabbit aorta, main pulmonary artery, or superior mesenteric artery (at concentration of 10^{-5} g/ml)	Toda et al. 1972
Concentration-dependent (3×10^{-7} to 3×10^{-5} M) contractile responses in albino rabbit iris sphincter muscle in vitro	Ueda et al. 1984
Fluidization of plasma membranes of platelets from rabbits (40 to 320 μM Capsaicin)	Meddings et al. 1991
Pulmonary edema in isolated, perfused rabbit lungs in vitro (test concentration of 10^{-4} M)	Delaunois et al. 1993
Release of serotonin from cerebrovascular mast cells (from adult male rabbits) in vitro (at test concentrations down to 10^{-7} M)	Reynier-Rebuffel et al. 1994
In vitro studies—cat	
Contraction of vascular smooth muscle of cat middle cerebral artery in vitro (test concentration = 3×10^{-7} M)	Duckles 1986
Vasoconstriction of cat middle cerebral and basilar arteries in vitro (at concentrations down to 3×10^{-8} M)	Saito et al. 1988

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TABLE 10
Other biological effects of Capsaicin *(Continued)*

Reported effects	References
In vitro studies—dog	
In isolated atrial preparations from the dog, no effect on rate or force of contraction (at test concentrations of 2×10^{-8} to 2×10^{-6} g/ml). Increased tension of spiral strips of mesenteric and renal arteries. No response in strips of aorta and pulmonary artery (test concentration of 10^{-5} g/ml)	Toda et al. 1972
In vitro studies—*bovine*	
Inhibition of electron-transfer activity of NADH–coenzyme Q oxidoreductase, isolated from beef heart mitochondria, in vitro (test concentration of 10 mM)	Shimomura et al. 1989
Inhibition of aldosterone production (stimulated by angiotensin II in vitro) by bovine glomerulosa cells in the presence of Capsaicin ($IC_{50} = 23 \pm 6$ μmol/L, concentration that inhibited 50% of the stimulated increase in aldosterone)	Hadjokas and Goodfriend 1991
In vitro studies—human	
Contraction of human segmental bronchi in vitro (test concentration of 10^{-5} M)	Lundberg et al. 1983
Inhibition of 5-hydroxyeicosatetraenoic acid biosynthesis in human neutrophils ($IC_{50} = 100$ μM). Capsaicin inhibited 5-lipoxygenase activity in vitro	Flynn et al. 1986
Vasodilation of human coronary arteries in vitro (test concentration of 10^{-6} M)	Franco-Cereceda and Rudehill 1989
Inhibition of platelet aggregation in human whole blood in vitro (test concentration of 20 mg/ml)	Sylvester and LaHann 1989
Contraction of human bronchial smooth muscle in vitro (test concentration of 10^{-5} or 10^{-4} M)	Honda et al. 1991
Fluidization (at test concentration of 40 μM) or reduction in fluidity (at test concentration of 320 μM) of human red blood cell membranes in vitro	Meddings et al. 1991
Stimulation of migration of human polymorphonuclear cells in vitro (test concentrations down to 10^{-8} M)	Partsch and Matucci-Cerinic 1993
Pretreatment of human myelomonoblastic leukemia cells (ML-1a cells) with Capsaicin (up to 300 μM) blocked TNF (an inflammatory cytokine)-mediated activation of NF-κB in vitro. [Note: viral replication, immune regulation, and induction of various inflammatory and growth-regulatory genes require the activation of a nuclear transcription factor, (NF)-κB.]	Singh et al. 1996
Inhibition of plasma membrane NADH oxidase (partially purified from surface of HeLA cancer cells) activity by 1 μM Capsaicin in vitro	Kishi et al. 1999
Increased [Ca^{2+}] and subsequent synthesis and release of inflammatory cytokines in SV-40/adenovirus-transformed human bronchial epithelial cells (BEAS-2B) in the presence of 10 μM Capsaicin in vitro	Veronesi et al. 1999
In vitro studies—Liposomes	
Modification of UV-induced lipid peroxidation in liposomal membranes in vitro (at doses down to 0.01 μg/ml)	De et al. 1993

groups were considered, the occurrence of mortalities was dose dependent. The signs reported for mice in this study were also reported for rats. However, compared to mice, clonic convulsion, cyanosis, lateral position, and dyspnea occurred more frequently in rats.

Hemorrhage of the gastric fundus was reported for some of the animals that died. Pathological changes were not observed in animals that survived. At microscopic examination, focal, slight erosion or ulceration with increased mucus was observed in the stomach. Other organs from both dead and surviving animals appeared normal (Saito and Yamamoto 1996).

Acute Dermal Toxicity

Glinsukon et al. (1980) evaluated the acute dermal toxicity of Capsaicin (in DMSO) using 16 Swiss male albino mice (25 to 35 g). The test substance was applied to shaved skin of the neck and back. An LD_{50} of >512 mg/kg was reported, and there were no signs of toxicity.

Acute Intravenous Toxicity

Glinsukon et al. (1980) reported a mean i.v. LD_{50} of 0.56 mg/kg (55 male Swiss albino mice; 25 to 35 g) after dosing with Capsaicin, in DMSO, at a dose volume of 1.67 ml/kg. In

another study, Kim and Park (1981) reported an acute i.v. LD_{50} of 0.4 mg/kg (confidence limits = 0.37 to 0.43 mg/kg) for mice.

Biggs and Goel (1985) reported that single intravenous doses of Capsaicin (0.25 to 16 μg/kg) caused dose-dependent increases in pulmonary flow resistance and dynamic thoracic elastance, indicative of potent bronchospastic activity, and a decrease in mean arterial blood pressure in guinea pigs (males or females, weights = 400 to 850 g). (Concerning the finding of increased pulmonary flow resistance, results from an earlier publication indicated that i.v. administered Capsaicin causes a marked increase in airway resistance that may be due to neurokinin release [Lundgberg and Saria 1982].) These responses to Capsaicin were similar after decentralization, bilateral vagotomy, or glossopharyngealotomy, and after the administration of autonomic blockers. It was concluded that Capsaicin induced bronchospasm without stimulating any afferent receptors in a centrally mediated bronchospastic reflex arc.

In a study by Bertrand et al. (1993) involving male Hartley guinea pigs (pretreated i.v. with atropine), it was determined that the activation of NK_1 and NK_2 tachykinin receptors contributed to the noncholinergic increase in total pulmonary resistance that was induced by Capsaicin.

In a study by Kopczyńska and Szereda-Przestaszewska (1998), Capsaicin, in isotonic saline with ethanol and Tween 80, was injected i.v. as a bolus (single injection; dose = 10 μg/kg [0.032 μmol/kg]) into the right femoral vein of each of ten adult cats (mean weight 3.2 kg). Prior to injection, the cats were anesthetized i.p. with sodium pentobarbitone, which was later supplemented with an i.v. dose of alpha-chloralose. Expiratory apnea was induced in all dosed animals, and the mean duration was 8.7 $\pm$ 0.98 s for intact animals and 8.7 $\pm$ 1.3 s after division of the superior laryngeal nerves. Frequently, the apnea was associated with bradycardia and mild hypotension prior to midcervical vagotomy, which abolished the apnea. A typical recorded response to i.v. administration of Capsaicin (for one cat) indicated that the expiratory arrest of breathing occurred prior to a decrease in blood pressure.

Acute Intraarterial Toxicity

After Capsaicin (in isotonic saline with ethanol and Tween 80, 10 μg/kg bolus dose) was injected into the aortic arch of cats, expiratory apnea was induced in all animals. A typical recorded response to aortic arch administration of Capsaicin (for one cat) indicated that the expiratory apnea coincided with an increase in blood pressure. Mean values for the duration of apnea were expressed as follows: four intact cats (5.75 $\pm$ 1 s); three cats with divided superior laryngeal nerves (8.8 $\pm$ 0.8 s); and five cats after midcervical vagotomy (8.6 $\pm$ 0.8 s) (Kopczyńska and Szereda-Przestaszewska 1998).

Acute Intraperitoneal Toxicity

In a study by Glinsukon et al. (1980), LD_{50} values using rats, hamsters, guinea pigs, rabbits, and mice were reported.

Female rats of the Fischer-derived strain (adults, 130 to 150 g; weanling, 45 to 55 g) were dosed intraperitoneally (i.p.) with Capsaicin. The mean acute LD_{50} values were reported as 10.40 mg/kg (Capsaicin in dimethylsulfoxide [DMSO], 56 adult rats); 13.20 mg/kg (Capsaicin in propylene glycol, 62 weanling rats); and 9.50 mg/kg (Capsaicin in DMSO, 76 adult rats). The test substance was administered at a dose volume of 1.67 ml/kg, after which the animals became excited and convulsed within approximately 1 to 2 min. Death by respiratory failure was noted within 2 to 5 min. Hyperemia (without hemorrhage) in the visceral organs and muscular wall of the peritoneal cavity, and a slight increase in peritoneal fluid were noted at necropsy (number of animals not stated).

Electrocardiogram, mean arterial pressure, and respiratory rates for anesthetized female rats (number not stated) were recorded. Respiratory rates increased slightly during the first minute after dosing. Tidal volume decreased to 10% to 20% of the control value within 3 to 4 min and respiration ceased. Changes in respiration were accompanied by a gradual decrease in heart rate, and electrocardiograph signals were not observed at approximately 6 to 14 min. Mean arterial pressure was variable; hypotension observed initially was followed by hypertension. Convulsion was not observed in rats that received a lethal dose of Capsaicin.

These authors also reported an i.p. LD_{50} of >120 mg/kg for male Syrian golden hamsters (20 animals; 55 to 75 g) dosed with Capsaicin in DMSO. The test substance was administered at a dose volume of 1.67 ml/kg.

For male albino guinea pigs (30 animals; 350 to 440 g) dosed i.p. with Capsaicin in DMSO, a mean LD_{50} of 1.10 mg/kg was reported. The test substance was administered at a dose volume of 0.2 ml/kg.

An i.p. LD_{50} of >50 mg/kg was reported for male and female albino rabbits (12 animals; 500 to 600 g) dosed with Capsaicin in DMSO. The test substance was administered at a dose volume of 0.2 ml/kg.

For male mice (70 animals; 25 to 35 g) dosed i.p. with Capsaicin in DMSO, these authors reported a mean LD_{50} of 7.65 mg/kg. The test substance was administered at a dose volume of 1.67 ml/kg. Hyperemia (without hemorrhage) in the visceral organs and muscular wall of the peritoneal cavity and a slight increase in the amount of peritoneal fluid were noted at necropsy (number of animals not stated) (Glinsukon et al. 1980).

Kim and Park (1981) reported an acute i.p. LD_{50} of 18.63 mg/kg for mice.

Muaralidhara and Narasimhamurthy (1988) injected i.p. groups of six adult male mice of the CFT strain (35 to 30 g) with Capsaicin in DMSO. An LD_{50} (computed statistically using probit analysis) of 8.0 mg/kg body weight was reported.

Acute Subcutaneous Toxicity

Glinsukon et al. (1980) reported a mean LD_{50} of 9.0 mg/kg (50 Swiss male albino mice; 25 to 35 g) after s.c. injection (dorsal

region of skin) with Capsaicin in DMSO. The test substance was administered at a dose volume of 1.67 ml/kg.

In a study by Atkinson and Chaggar (1983), groups of 10 anesthetized, pregnant female mice (of 15-day duration dated pregnancy; strain and weights not stated) were injected s.c. with Capsaicin (in 10% absolute alcohol, 10% Tween 80, and 80% isotonic saline) at doses up to 50 mg/kg. The following mortalities were reported: 8 of 10 mice (50 mg/kg dose); 5 of 10 mice (30 mg/kg dose); and 1 of 10 mice (25 mg/kg dose). In another experiment in which pregnant females were dosed with less than 6 mg/kg Capsaicin, two mice developed respiratory distress and died. It was stated that the results of this experiment may have been due to a cumulative effect of general anesthesia and Capsaicin. Whether or not respiratory distress was noted at higher doses was not stated.

In a study by Shimizu et al. (1984), groups of ddY mice (weights not stated) were injected s.c. with 12.5 to 100 mg/kg Capsaicin (in a solution consisting of 10% ethanol, 10% Tween 80:80% physiological saline (0.9%) saline [*v*:*v*:*v*]) at various times after birth. Signs in mice dosed with 50 mg/kg on day 2 of life included excited behavior (i.e., struggling, screaming, or rotation), apnea, hyperventilation, and moderate, generalized cyanosis. At 15 min post injection, respiration returned to normal and cyanosis was not noted. A mild degree of transient cyanosis was observed in neonatal mice injected with low doses of Capsaicin (12.5 and 25 mg/kg). Signs in mice receiving 100 mg/kg Capsaicin on day 2 of life were not reported. Mortality data for 12.5 and 25 mg/kg dose groups were not reported. However, none of the mice injected with 50 or 100 mg/kg Capsaicin at day 2 of life died. Additional experiments indicated that as the time of injection after birth was delayed, increased mortality was noted.

Eight of 15 mice injected with 50 mg/kg Capsaicin on day 16 of life died within 24 h. For two groups of adult mice injected with Capsaicin (50 mg/kg) during the eighth week of life, mortality incidences of 12 of 19 mice and 18 of 20 mice were reported. Deaths were reported within 15 min after the following signs were observed: depressed respiration with severe cyanosis and panting, occasionally accompanied by spasmodic running and jumping.

Signs reported for neonatal control mice injected with vehicle (solution consisting of 10% ethanol, 10% Tween 80:80% physiological saline (0.9%) saline [*v*:*v*:*v*]) on day 2 of life included "wandering" type of behavior and moderately hyperemic skin over the entire body. Within 10 min, the animals became calm and drowsy. Cyanosis was not observed.

None of the Balb/c mice (7 males, 11 females; weights not stated) injected s.c. with 50 mg/kg Capsaicin on the second day of life died. Similar results were reported for male Wistar rats dosed with 12.5 mg/kg (10 rats), 25 mg/kg (11 rats), or 50 mg/kg (7 rats) Capsaicin according to the same procedure (Shimizu et al. 1984).

Santicioli et al. (1985) injected Capsaicin s.c. at 50 mg/kg (in 10% ethanol, 10% Tween, and 80% NaCl [0.9%]) into anesthetized albino rats (Wistar-Morini strain, 1 to 3 days old). Experiments were performed at 2 months post dosing. Cystometrograms (tracings of pressure exerted as urinary bladder is filled) for test and control animals were compared. Mean body weights for rats dosed with Capsaicin and their age-matched controls were 272 ± 11 g(18 rats) and 244 ± 14 g (12 rats). The difference in mean body weight between the two groups was not statistically significant. However, mean weight of the urinary bladder in Capsaicin-treated rats (110 ± 4; units not stated) was significantly higher when compared to the vehicle control group (93 ± 6; units not stated). An explanation of this finding was not provided. Capsaicin markedly altered the cystometrogram recordings for urethane-anesthetized, 2-month-old rats. Specifically, phasic contractions no higher than 8 to 12 mm were reported for seven of eight rats tested.

These authors also injected adult, Wistar-Morini rats (360 to 400 g) with Capsaicin at 50 mg/kg (in solvent described above) at 4, 14, or 30 days before cystometrograms were obtained. Significant differences in body weight or bladder weight between test and vehicle control groups were not observed. In cystometrograms (for urethane-anesthetized rats), there was no apparent effect of Capsaicin on the amplitude of rhythmic contractions (Santicioli et al. 1985).

Wallengren et al. (1991) evaluated the effect of Capsaicin dosing on dinitrochlorobenzene-induced sensitization using female guinea pigs (Sahlin, Malmö, Sweden) weighing 200 g. Results concerning this effect are included in the section "Effect on Contact Hypersensitivity" later in the report. The first and second experiments involved 20 and 21 guinea pigs, respectively. In both experiments, Capsaicin (dissolved in ethanol and Tween 80 and then mixed with physiological saline) was injected s.c. into the neck at a concentration of 6.6 mg/ml (dose of 50 mg/kg or 10 mg/animal). Another s.c. injection (16.6 mg/ml; dose = 250 mg/kg or 50 mg/animal) was made 24 h later. Six of 20 animals in group A and 5 of 21 animals in group B died after the first Capsaicin injection. Exerted respiration, decreased breathing frequency, and itching were noted prior to death. No deaths occurred after the second injection.

Santoni et al. (2000) injected Capsaicin s.c. at 50 mg/kg (in 10% ethanol and 10% Tween 80) into specific pathogen–free, Wistar rats of both sexes (2 days old; number not stated). The animals were killed on days 7, 14, and 28 post injection. No toxic effects or changes in body weight were observed. However, a significant reduction in thymus weight and thymus cell numbers ($p < .01$) was noted. These changes were evident at day 7 and persisted to day 28 post injection.

Acute Intramuscular Toxicity

The acute intramuscular toxicity of Capsaicin in DMSO was evaluated using 84 Swiss male albino mice (25 to 35 g). The test substance was administered at a dose volume of 1.67 ml/kg. A mean LD_{50} of 7.80 mg/kg was reported (Glinsukon et al. 1980).

Acute Intracisternal Toxicity

Capsaicin was injected intracisternally (30 μg in 50 μl of solvent, artificial cerebrospinal fluid) into 12 adult, Sprague-Dawley rats of the CFY strain. Immediately after injection, apnea and marked vasodilation of skin blood vessels (excessive redness of ears) was observed. The duration of the apnea was 25 to 40 s, and violent scratching (5-min duration) of the cheek, nose, and hind legs was noted 3 to 4 min later. Additionally, marked skin hyperaesthesia developed during the next 20 to 25 min. Subsequently, the animals behaved normally; however, a practically complete abolition of chemical pain sensitivity induced by Capsaicin persisted for several months (Jancsó 1981).

Short-Term Oral Toxicity

Nopanitaya (1973) evaluated the effects of Capsaicin on fat absorption and growth in a study involving 70 male Sprague-Dawley rats (100 to 110 g). The 70 rats were divided into six dietary groups (four groups of 10 and two groups of 15). The diets (all isocaloric, 4.0 kcal/kg) contained varying proportions of protein/carbohydrate, and three of the diets were supplemented with 0.014% Capsaicin. Control animals were fed a diet consisting mainly of casein (25%) and corn starch (57%). At the conclusion of the 28-day and 56-day feeding periods per group, two rats from each group were selected for a fat absorption study. The rats were fasted for 48 h, slightly anesthetized, and sections of the duodenum were excised. Frozen tissue sections were stained for fat, and fat absorption was evaluated based on the quantity of visible fat droplets stained with Oil Red O within lacteals of the intestinal villi and epithelial absorptive cells. Twenty stained sections per animal were evaluated.

Differences in food intake reported for the groups tested were not statistically significant. An analysis of growth performance indicated that rats fed the low-protein high-carbohydrate diet supplemented with Capsaicin had the slowest rate of growth (statistically significant [$p < .05$], compared to control). The lowest food efficiency ratio (FER) was also reported for this group. Additionally, differences in body weight between this group and the control group were significantly different ($p < .05$).

Results from the fat absorption study indicated that less fat was found in the mucosa of rats maintained on diets supplemented with Capsaicin for 28 and 56 days. Similar results were reported for the low-protein diet without Capsaicin. In this study, the low-protein diet caused the reduction in the absorptive ability of the intestinal epithelial cells, which led to the slow growth of young rats, and these effects were intensified when the diet was supplemented with Capsaicin (Nopanitaya 1973).

In a study by Nopanitaya and Nye (1974), 10 male Sprague-Dawley rats (weights = 250 to 300 g) were anesthetized and dosed orally (intragastric feeding) with 0.014% Capsaicin in 0.85% saline (volume = 2.0 ml; dose = 1 mg Capsaicin/kg). Dosing periods (continuous dosing) for the 10 animals were 2, 6, 15, 30, 45, and 60 min, respectively. Ten control rats were dosed with 0.85% saline (2.0 ml) according to the same procedure. The animals were killed at the end of the dosing period and a portion of the duodenum was removed for light and electron microscopy.

Using light and electron microscopy, the duodenal villi of control animals were unremarkable and appeared normal. Capsaicin had direct toxic effects on the rat duodenal absorptive cell. Cellular damage was observed using light and electron microscopy. The damage observed increased with the duration of dosing. The following ultrastructural changes were observed: swollen mitochondria with rarefied matrix and disorganized cristae, increased numbers of free ribosomes and lysosomes, dilatation of endoplasmic reticulum and Golgi complexes, and shrunken nuclei with chromatin clumped and marginated at the nuclear envelope. In another experiment, injection of the same Capsaicin solution into a ligated duodenal segment (2-min exposure) resulted in changes that were similar to those induced via intragastric administration (6- and 15-min exposures) (Nopanitaya and Nye 1974).

Mann (1977) administered 100 mg/kg Capsaicin (suspended in 1% methyl cellulose) to fasted male albino rats (10 rats; weights = 150 to 200 g) by esophageal intubation. Four equally divided doses (1 h apart) were given. The vehicle-control group was dosed with 1% methylcellulose. The animals were killed 1 h after administration of the last dose. Stomachs were removed and sectioned along the lesser curvature. The number of erosions was then counted. Capsaicin induced acute gastric erosions (total of 31 erosions; mean 3.1 ± 0.140 per animal). At microscopic examination, superficial mucosal necrosis and submucosal congestion were observed. Gastric erosions were not observed in control rats dosed with 1% methyl cellulose.

Srinivasan et al. (1980) performed short-term feeding experiments using basal diets supplemented with natural Capsaicin (concentrations of 1.5, 3.0, and 15.0 mg %) and basal diets supplemented with synthetic Capsaicin analogue (concentrations of 1.5, 3.0, and 15.0 mg %). Groups of 8 to 10 weanling Wistar rats (mean weights = 38.6 to 38.7 g) were fed for four or eight weeks.

Compared to the control group, feeding with natural Capsaicin (3.0 and 15.0 mg %) or the synthetic Capsaicin analogue (15 mg %) for 4 weeks caused a significant decrease ($p < .05$) in food intake. This effect was overcome at 8 weeks in animals that received 3 mg % natural Capsaicin alone. In the highest dose group (natural Capsaicin or synthetic analogue), weight gain was significantly reduced ($p < .05$) at 4 and 8 weeks. Additionally, the FER associated with natural Capsaicin feeding reverted to the control value, whereas the FER continued to be significantly low ($p < .05$) at 4 and 8 weeks of feeding with the synthetic Capsaicin analogue. Significantly lower FER values were associated with natural Capsaicin (3 and 15 mg % dose groups) and the synthetic analogue (15 mg % dose group) at 4 weeks, and with the synthetic analogue only (3 and 15 mg % dose groups) at 8 weeks.

At eight weeks, growth rates in groups dosed with natural Capsaicin or the synthetic analogue were lower than the control

value, and the lowest growth rate was associated with the highest dose group (15 mg % natural Capsaicin or 15 mg % synthetic analogue).

The results of hematological analyses indicated that at 8 weeks, total cholesterol concentrations in the serum were significantly lower in rats fed natural Capsaicin (3 and 15 mg % dose groups) or the synthetic Capsaicin analogue (1.5, 3, and 15 mg % dose groups). Liver cholesterol concentrations remained normal in these groups. Additionally, compared to controls, there were no variations in the following blood parameters at 4 or 8 weeks: white blood cells, red blood cells, differential counts, hemoglobin, and serum proteins. This was true for all groups tested. At microscopic examination, the large intestine, liver, and kidneys of animals in each test group were normal. However, pathological changes such as destruction of taste buds and keratinization and erosion of the mucosal layer of the GI tract were observed in 3 and 15 mg % dose groups (incidence per group not stated).

According to the authors, the results of nitrogen balance studies, conducted before animals were killed, indicated that the highest dose of Capsaicin (15 mg %) seemed to have retarded nitrogen retention, although it did not affect absorption (Srinivasan et al. 1980).

Monsereenusorn (1983) studied the short-term oral toxicity of Capsaicin using two groups of rats (slightly over 100 g; strain not stated). One group served as the control. Both groups were subdivided into seven subgroups (10 to 14 rats per group). Test animals received Capsaicin (50 mg/kg day^{-1}) by stomach tube for 60 days. Control rats received an equivalent volume of saline solution. No deaths were observed in test or control groups.

At day 40 of feeding, a significant reduction in body weight gain was noted in groups dosed with Capsaicin. A significant decrease in the following blood plasma parameters was also noted in these groups after dosing for more than a month: blood urea nitrogen, glucose, phospholipids, triglycerides, total cholesterol, free fatty acids, glutamic pyruvic acid transaminase, and alkaline phosphatase. Hematological analyses (i.e., red blood cells, hemoglobin, hematocrit, white blood cells, and prothrombin time) were normal. Additionally, tests for urinary glucose, blood, bile salts and lactones were negative, and quantities of protein and microscopic constituents were similar between test and control groups.

At necropsy, no significant gross pathologic changes were noted in most organs. However, at 60 days, slight hyperemia (without hemorrhage) was observed in the livers of animals dosed with Capsaicin. Additionally, the gastric mucosa was reddened and mucus materials were increased. Compared to controls, there were no statistically significant changes in the following relative organ weights after 60 days of dosing: liver, stomach, pancreas, spleen, intestine, caecum, lung, heart, kidney, thyroid, adrenal, and gonad. The author concluded that a longer period of dosing with Capsicum Extract or Capsaicin may have had a mild effect on the animals tested (Monsereenusorn 1983).

Jang et al. (1989) evaluated the effect of Capsaicin on tumor development induced by benzo(a)pyrene (BP) or 7,12-dimethylbenz[2]anthracene (DMBA) (two carcinogens) using newborn Swiss Webster mice (non-inbred strain, less than 24 h old). Mean weights at 1 week after birth ranged from 10.1 to 14.4 g (males) and 10.3 to 13.8 g (females). The four experimental groups used in this study were defined as follows: BP (69 mice), BP combined with 0.01% Capsaicin in food pellets (64 mice), DMBA (27 mice), and DMBA combined with 0.01% Capsaicin in food pellets (46 mice). Two additional groups were defined as gelatin (60 mice) and gelatin + Capsaicin (58 mice). Details concerning the test procedure and results regarding the anticarcinogenicity of Capsaicin are included in the section "Antimutagenicity/Anticarcinogenicity."

No mortality was attributable to treatment with Capsaicin or carcinogen. Overall weight gain (over 6-week period) was comparable between control and test animals, and the same was true with respect to lung and liver weights. However, compared to male mice treated with DMBA alone, the pulmonary weight of mice dosed with Capsaicin + DMBA was significantly decreased (Jang et al. 1989).

Brzozowski et al. (1993) reported that in Wistar rats of both sexes (180 to 220 g), intragastric application of Capsaicin at 0.5 mg/kg did not induce mucosal lesions. Additionally, Capsaicin induced a significant increase in gastric blood flow in these animals consistent with its potent vasorelaxant effect.

Short-Term Intraperitoneal Toxicity

In a study by Muralidhara and Narasimhamurthy (1988), groups of male mice (8 weeks old; number per group not stated) were injected with Capsaicin (2 mg/ml in DMSO) at doses of 0.4, 0.8, or 1.6 mg/kg body weight day^{-1}, respectively, for 5 consecutive days. Control mice were dosed with 0.05 ml/mouse day^{-1} DMSO only. The animals were killed 35 days after the last day of dosing, and tissues examined microscopically. Results for microscopic evaluations are included in the section "Reproductive and Developmental Toxicity" later in the report. No signs of toxicity were observed during the study.

Mandal et al. (1994) injected groups of eight adult male Charles Foster albino rats (120 g) i.p. with Capsaicin in 10% Tween 80, 10% ethanol, and saline (1:1:8). The following groups were used: group A (control), group B (1.68 mg/kg for 1 day), group C (1.68 mg/kg for 3 days), group D (0.42 mg/kg for 10 days), and group E (0.42 mg/kg for 15 days). For each group, the animals were killed 2 h after the last injection. None of the animals had any clinical signs, and there were no mortalities during the study. Compared to the control group, weight loss was reported for groups B and C. Dosing with Capsaicin for 1 or 3 days (groups B and C, respectively) induced lesions of the alveolar cell lining and widening of the alveolar lumen. In these two groups (B and C), Capsaicin dosing significantly increased the concentration of total lipids and decreased the cholesterol and phospholipid content of the lungs, compared to results for

groups A, D, and E. Dosing with Capsaicin for 10 or 15 days normalized the histological alterations observed following short-term dosing and stabilized the lung membrane lipid matrix (i.e., protective effect of long-term treatment).

Short-Term Intravenous Toxicity

Makara et al. (1967) evaluated circulatory and respiratory responses to Capsaicin (98% to 99% pure) using three groups of female Wistar rats (230 ± 7 g). The two test groups consisted of seven rats (group 2) and six rats (group 3), respectively. The control group (group 1) consisted of eight animals. Each animal in group 2 was injected s.c. (pretreatment) with 4, 8, and 16 mg of Capsaicin. The three doses were administered at 12-h intervals. Group 2 animals received the same three doses of Capsaicin plus a final dose of 200 mg/kg (same pretreatment procedure). Control animals were injected s.c. with 0.2, 0.4, and 0.8 ml of a control solution (composition not stated) according to the same procedure. At 13 days after pretreatment, the animals were anesthetized with chloralose (intravenous dose at 50 mg/kg), and the trachea, femoral artery, and right jugular vein were cannulated. A solution of 2% Capsaicin, 5% alcohol, and 10% Tween 80 in 0.9% saline was prepared and diluted for intravenous injection. Respiration, blood pressure, and heart rate were recorded. The hypotensive effect of Capsaicin was evaluated by interpolating (using logarithmic scale) the dose that caused a 20-mm Hg decrease in blood pressure ($ED_{20\ mm\ Hg}$).

In group 1 (control group), the administration of Capsaicin at intravenous doses of 0.5 to 2 μg/kg, or greater, caused a rapid decrease in blood pressure and heart rate, accompanied by apnea. The duration of hypotension was 30 to 120 s. The $ED_{20\ mm\ Hg}$ was 1.1 μg/kg. This triad effect was abolished by bilateral surgical vagotomy; however, occasionally, the administration of high doses continued to result in hypotension. In group 2, systemic blood pressure, from 90 to 130 mm Hg, was the same as that noted in control and Capsaicin-pretreated groups; bradycardia was slightly inhibited. At a dose of 2 μg/kg, apnea was observed only in one of seven animals. The $ED_{20\ mm\ Hg}$ of 2.4 μg/kg was slightly greater than the value reported for group 1, but the two values were not significantly different.

In group 3, the effects of intravenous dosing with Capsaicin were significantly inhibited. Compared to group 1, an approximately sixteenfold increase in the $ED_{20\ mm\ Hg}$ to18.0 μg/kg ($p < .001$) was reported, and an approximately eightfold increase in the dose of Capsaicin administered was required to achieve the same decrease in heart rate. Furthermore, a 4 μg/kg dose of Capsaicin was required for the induction of apnea in group 3 animals. At doses of 8 to 16 μg/kg, a transient increase in blood pressure occurred in 4 of 6 animals. A biphasic response (transient decrease, followed by increase in pressure) was noted at higher doses (Makara et al. 1967).

Short-Term Subcutaneous Toxicity

Malmgren et al. (1990) injected seven female Sprague-Dawley rats (205 to 330 g; average 247 g) s.c. with Capsaicin solutions, up to a total dose of 125 mg/kg. Diluted solutions containing 1 and 0.1 mg/ml Capsaicin were made from the following stock solution: Capsaicin (10 mg/ml), 10% alcohol, 10% Tween 80, and saline. Over a period of 2 days, five doses per day were administered at 2-h intervals in the following order: 20 μg/kg, 200 μg/kg, 2 mg/kg, 20 mg/kg, and 20 mg/kg (day 1) and 2 mg/kg, followed by four doses of 20 mg/kg (day 2). Twenty control rats (weight range = 192 to 280 g; average weight = 233 g) were injected with vehicle only.

There was no mortality in either test or control groups. Dosing with Capsaicin resulted in a significant increase in urinary bladder weight (73.3 ± 2.4 mg) in the test groups, compared to the control group (63.5 ± 1.9 mg). These results were presented in a study evaluating the morphological effects of Capsaicin treatment on innervation of the urinary bladder. Capsaicin-induced depletion of substance P and calcitonin gene-related peptide in the urinary bladder did not result in supersensitivity to these peptides (Malmgren et al. 1990).

In a reproductive toxicity study by Perfumi and Sparapassi (1999), Capsaicin at 50, 100, or 200 mg/kg (in 10% ethanol, 10% Tween 80, and 80% isotonic saline) was injected s.c. into the thoracic region of anesthetized specific pathogen-free, pregnant rats (six groups of five). The fourth group was dosed with vehicle only. Injections were made every other day on gestation days 7 through 15. Animals were evaluated daily for signs of toxicity. Two additional groups were injected with Capsaicin (50 mg/kg) or vehicle on gestation day 15. Two females in the 200 mg/kg dose group died; no other mortality was reported. Clinical signs of morbidity were not observed in dams from either of the treatment groups. At gestation day 20, there was no significant difference in maternal body weight between the control group and either of the groups dosed with Capsaicin. Additionally, maternal body weight gain during gestation was not significantly different from the control group. Results relating to reproductive effects are included in the section "Reproductive and Developmental Toxicity."

In another experiment in the preceding study, 2-day-old rats (2nd postnatal day) were injected s.c. with Capsaicin (50 mg/kg) or the vehicle. Data were recorded after the animals reached 1 month of age. Compared to controls (102 ± 3.1 g), a notable decrease in body weight was reported for rats dosed with Capsaicin (77.3 ± 3.5 g). The rats also had cutaneous lesions, especially in the ventral region of the neck, in the periorbital region, in the retroauricular area, and around the nostrils (Perfumi and Sparapassi 1999).

Subchronic Oral Toxicity

Wanichanon et al. (1996) evaluated Capsaicin-induced ultrastructural changes in intestinal absorptive cells using groups (12 to 15/group) of male Swiss albino mice (20 to 25 g). The vehicle for Capsaicin was absolute ethanol, Tween 80, and 0.9% NaCl, at a 10:10:80 ratio. In addition to the regular chow diet, two groups received oral doses of 1 and 2 mg Capsaicin/kg body weight,

respectively, daily for 12 weeks. Two control groups received water and vehicle, respectively. After 12 weeks, the animals were anesthetized and the abdomen of each was opened. The upper jejunum was isolated, and an intestinal loop prepared. The rate of thiamine disappearance from the intestinal lumen and the percent inhibition were calculated. The intestinal loop was subsequently excised, and thick sections of the jejunal wall examined using light microscopy. Thin sections were examined using electron microscopy.

Differences in the growth rate between control groups and groups dosed with Capsaicin were not statistically significant. Compared to the vehicle control group, thiamine absorption was inhibited by 2.7% in mice dosed with 1 mg/kg Capsaicin, and significantly inhibited by 12.25% ($p < .001$) in mice dosed with 2 mg/kg Capsaicin.

The most striking ultrastructural changes in intestinal absorptive cells were observed in mice dosed with 2 mg/kg Capsaicin, with the mitochondria being affected the most. Compared to the control, the number of mitochondria in most cell profiles was generally increased and mitochondria with dilated cristae were more numerous. Degenerating mitochondria frequently showed myelin figures in the vicinity of the dilated cristae. The authors stated that the ultrastructural changes of intestinal absorptive cells may have been associated with the inhibition of thiamine absorption (Wanichanon et al. 1996).

Akagi et al. (1998) evaluated the subchronic oral toxicity of a mixture of capsaicinoids (64.5% Capsaicin and 32.6% dihydrocapsaicin) using six groups of 10 B6C3F1 mice (6 months old). Five groups were fed powdered diets containing 0.0625%, 0.125%, 0.25%, 0.5%, and 1.0% capsaicinoids, respectively, for 13 weeks. The sixth group (control) was fed powdered diet only. Animals were killed at the end of the study and necropsied. Major organs were weighed and examined microscopically.

Reduced body weight gain, especially females, was noted in all dose groups. With the exception of male mice fed 0.0625% capsaicinoids, mean weights for male rats in the other dose groups were significantly lower when compared to the control group. Furthermore, with the exception of female mice fed 0.125% capsaicinoids, mean weights for females in the other dose groups were significantly lower, compared to the control group. For both sexes, dosing with capsaicinoids (all dose groups) induced an increase in the liver/body weight ratio, compared to the control group. Significant increases in liver/body weight ratio were reported for the following dose groups: 0.0625% (females), 0.125% (males), 0.25% (males and females), 0.5% (males and females), and 1% (males and females).

Kidney/body weight ratios (males and females) in the dose groups were slightly decreased, but not statistically significant when compared to the control group. At microscopic examination, focal tubular dilatation of the kidney was reported for males dosed with 1% capsaicinoids, but not for males in other dose groups.

Based on the results of this study, it was determined that 0.25% capsaicinoids is the highest concentration that should be evaluated for carcinogenicity. The results of this carcinogenicity study are included in the section "Carcinogenicity/Tumorigenicity" later in this report (Akagi et al. 1998).

Ocular Irritation/Toxicity

Ocular irritation/toxicity studies on Capsaicin are summarized in Table 11.

In a study by Makara et al. (1967), blepharospasm was observed in all eight female Wistar rats (pretreated with control solution) after 2% Capsaicin was instilled into the eye. The composition of the control solution, injected subcutaneously, was not stated. However, blepharospasm was not observed in either of the two groups (six to seven rats/group) pretreated with Capsaicin (same procedure) prior to the instillation of 2% Capsaicin.

Fujita et al. (1984) evaluated Capsaicin-induced changes in the cornea using 2-day-old mice of the ddY strain. In the first set of experiments (four groups of 10 newborn mice), the animals were injected s.c. with Capsaicin (in 0.9% saline containing 10% ethanol and 10% Tween 80) at doses of 12.5, 25.0, 50.0, or 100.0 mg/kg, respectively. A fifth group was injected with vehicle only. Ocular anterior segments were observed using a slit-lamp microscope. Corneal lesions were observed approximately 20 days after Capsaicin injection. Changes in the cornea were observed, regardless of the dose administered. At the lower doses (12.5 or 25.0 mg/kg), the corneal lesion was generally mild and had healed within several weeks. However, with the larger dose (50 or 100 mg/kg), the lesion was moderate or severe. Though the lesion eventually healed in these two groups, frequently, opaque scars with or without vascularization remained.

Shimizu et al. (1984) evaluated the toxicity of Capsaicin (in a solution consisting of 10% ethanol, 10% Tween 80:80% physiological saline (0.9%) saline [v:v:v]) to the cornea using neonatal mice of the ddY and Balb/c strains and Wistar rats.

Male ddY mice (groups of 10) were injected s.c. with Capsaicin (doses of 12.5, 25, 50, and 100 mg/kg) on the second day of life. Nine female ddY mice were injected with 50 mg/kg Capsaicin on day 2 of life. Control animals were injected with vehicle only. Using a slit-lamp biomicroscope, anterior ocular segments were initially examined on the 14th day of life (i.e., when the animals opened their eyes) and then daily for the next 10 days. At the conclusion of the 10-day examination period, observations were made regularly until day 85 (mice) or day 63 (rats). Corneal lesions were graded on a scale of 0 (normal cornea) to 4 (deep stromal opacity with ulceration and vascularization).

Capsaicin injection induced corneal lesions (confined to central area primarily) in male ddY mice of all four dose groups. The intensity of lesions in the two higher dose (50 and 100 mg/kg) groups was greater than that of the two lower dose groups (12.5 and 25 mg/kg). Lesions were observed in two to five mice per group after a relatively long latency of approximately 20 days. By day 30, the incidence of corneal lesions was 100% in all dose groups. In the 50 and 100 mg/kg dose groups, this high incidence persisted to day 85. In 12.5 and 25 mg/kg dose groups,

TABLE 11
Ocular irritation/toxicity studies on Capsaicin

Test substance	Animals tested	Procedure	Results	References
2% Capsaicin	8 female Wistar rats	Instillation into eye	Blepharospasm in all animals	Makara et al. 1967
100 mg/kg Capsaicin	Neonatal Wistar rats	Hypodermic injection on day 2 after birth	Poor attachment of corneal epithelium. Decreased corneal nerve ending density	Katakami and Yamamoto 1996
50 mg/kg Capsaicin in 0.9% saline containing 10% ethanol and 10% Tween 80	Newborn offspring of Sprague-Dawley rats (83 pups from 13 different litters)	i.p. injection on day of birth, day 1, and day 2, or on days 1, 2, and 3	Keratitis in all but one animal. Additional corneal changes (most common): cloudiness (edema) of the epithelium and stroma, granulation or stippling of corneal surface, and neovascularization	Marfurt et al. 1993
50 mg/kg Capsaicin in 0.9% physiological saline	Neonatal Wistar rats	s.c. injection	Disintegrated epithelial cells. Corneal sensitivity reduced to extent of losing corneal reflex	Fujita et al. 1987
12.5, 25, or 50 mg/kg Capsaicin in 10% ethanol, 10% Tween 80:80% physiological saline (0.9%) saline [*v*:*v*:*v*])	3 groups of 7 to 11 Male Wistar rats	s.c. injection on day 2 of life	Corneal lesions in all animals. Dose-related increase in intensity of lesions	Shimizu et al. 1984
12.5, 25, 50, or 100 mg/kg Capsaicin in 0.9% saline containing 10% ethanol and 10% Tween 80	2-day old ddy mice (4 groups of 10)	s.c. injection	Moderate to severe corneal lesions (dose-related) at day 20 post injection	Fujita et al. 1984
12.5, 25, 50, and 100 mg/kg Capsaicin in 10% ethanol, 10% Tween 80:80% physiological saline (0.9%) saline (*v*:*v*:*v*)	4 groups of 10 male ddY mice	s.c. injection on day 2 of life	Corneal lesions (confined to central area primarily) in all dose groups. Dose-related increase in intensity of lesions	Shimizu et al. 1984
50 mg/kg Capsaicin in of 10% ethanol, 10% Tween 80:80% physiological saline (0.9%) saline (*v*:*v*:*v*)	Balb/c neonatal mice (7 males, 11 females)	s.c. injection on day 2 of life	Corneal lesions in all animals at day 20 post injection	Shimizu et al. 1984
12.5, 25, and 50 mg/kg, Capsaicin in 10% ethanol, 10% Tween 80, 80% physiological saline (0.9%) saline (*v*:*v*:*v*)	3 groups of 8 male ddy neonatal mice	s.c. injection with 0.9% saline vehicle with 0.1% ascorbic acid or saline vehicle with Capsaicin	Corneal lesions observed at approximately day 20 post injection. Intensity of lesions varied in dose-dependent manner	Shimizu et al. 1987
0.5 ml of a 1% Capsaicin in solution containing 99.5% ethanol, 850 μl Tween, and 9 ml saline	10 adult pigmented rabbits (mixed strains)	Retrobulbar injection into left eye	Prompt inflammatory reaction, with conjunctival hyperemia, chemosis, and dense aqueous flare	Bynke 1983
0.33% Capsaicin in 75 μl of 99.5% ethanol, 425 μl of Tween 80 and 14.5 ml of saline	8 adult pigmented rabbits	Retrobulbar injection	Miosis and breakdown of blood-aqueous barrier, manifested as an aqueous flare response	Bynke et al. 1983

the incidence of corneal lesions decreased gradually after day 30. At 6 months, corneal lesions were not observed in any of the mice dosed with 12.5 or 25 mg/kg. Compared to male ddY mice dosed with 50 mg/kg Capsaicin (average intensity score = 0.9), similar corneal lesions (average intensity score = 1.3) were observed in female ddY mice at 21 days after dosing with 50 mg/kg Capsaicin. No corneal lesions were observed in control animals.

Because of the possibility that loss of lacrimation may cause corneal lesions, the left eyelids of three anesthetized male ddY mice were sutured on the twentieth day of life. Capsaicin (50 mg/kg) was injected s.c. within 30 min after the procedure. The suture was removed on the day in which corneal lesions were observed in contralateral eyes (no sutures) or 7 days later. Results indicated that the corneal lesions observed after injection with Capsaicin in the preceding experiments were not due to traumatic effects caused by loss of lacrimation or dryness.

Other male ddY mice were injected with Capsaicin (50 mg/kg) on day 10 (7 mice), day 20 (10 mice), or during the eighth week of life (9 mice), to determine the effects of age at the time of injection. Results indicated that corneal lesions were more evident as the age of the animal increased.

Balb/c mice (7 males, 11 females) were injected s.c. with 50 mg/kg Capsaicin on day 2 of life to determine any strain-related differences in susceptibility to Capsaicin-induced corneal damage. At day 20 post injection, corneal lesions were observed in all animals. The corneas of these animals were more severely involved, compared to the results for ddY mice. For example, on day 21, average intensity scores of 3.8 (7 Balb/c male mice) and 0.9 (10 ddY male mice) were reported. Thus, Balb/c mice were more sensitive to the development of corneal lesions induced by Capsaicin than ddY mice.

The potential for Capsaicin-induced corneal lesions in rats was also evaluated. On day 2 of life, male Wistar rats were injected s.c. with the following doses of Capsaicin: 12.5 mg/kg (10 rats), 25 mg/kg (11 rats), or 50 mg/kg (7 rats). Corneal lesions were observed in all animals, and the intensity of the lesions was dose related. On the day in which the eyes opened, corneal opacity was observed in all rats that had been injected with 50 mg/kg Capsaicin. Four months later, corneal opacity persisted in four of seven animals. Corneal abnormalities were not observed in the ten control rats that were injected with vehicle only (Shimizu et al. 1984).

Shimizu et al. (1987) injected three groups of eight male ddY neonatal mice (control groups) s.c. with 0.9% saline containing 0.1% ascorbic acid or 0.9% saline, followed by dosing with 12.5, 25, and 50 mg/kg Capsaicin, respectively. Corneal lesions were noted around postnatal day 20. By day 30, the incidence of corneal changes reached a maximum of 100% at all doses administered, with the intensity of lesions varying in a dose-dependent manner. For the highest dose of Capsaicin administered (50 mg/kg), the high incidence and intensity of corneal changes remained until the final day of observation (day 75 of life). For lower doses (12.5 and 25 mg/kg), the intensity and incidence of corneal lesions decreased gradually, with recovery from moderate to mild lesions. Corneal changes primarily involving the corneal epithelium were noted at lower doses and, stromal opacities, at the highest dose.

In a study by Fujita et al. (1987), edematous lesions were observed initially, while degenerative opacities of the corneal epithelium and stroma were observed in neonatal Wistar rats injected s.c. with Capsaicin in 0.9% physiological saline (dose = 50 mg/kg, single injection). Disintegrated epithelial cells were observed at microscopic examination. Additionally, the animals lost their corneal reflex to touch.

Marfurt et al. (1993) injected newborn offspring of Sprague-Dawley rats (83 pups from 13 different litters) i.p. with Capsaicin. The pups were anesthetized and then injected i.p. with Capsaicin (in 0.9% saline, containing 10% ethanol and 10% Tween 80; dose = 50 mg/kg) either on the day of birth, day 1, and day 2, or on days 1, 2, and 3. The pups were anesthetized prior to injection. Twenty control rats were injected with vehicle only, and an additional 30 served as untreated controls. Sixty-five pups from 10 different litters were killed, and corneas (from pups whose eyes had already opened) were examined to assess the severity of keratitis. For pups less than 13 to 14 days old (eyelids still fused), corneas were examined for keratitis after enucleation.

Of the rat pups dosed neonatally with Capsaicin and allowed to survive for at least 3 weeks, keratitis was observed in all but one animal. Generally, keratitis was first observed between days 10 and 25 of life. This means that some of the animals developed corneal anomalies prior to eyelid opening. The most common corneal changes were as follows: cloudiness (edema) of the epithelium and stroma; granulation or stippling of the corneal surface; and neovascularization (i.e., ingrowth of blood vessels into the normally avascular cornea). Keratitis and neovascularization gradually increased in severity during the first six weeks of life, and typically peaked in intensity by 6 to 8 weeks (Marfurt et al. 1993).

Katakami and Yamamoto (1996) injected neonatal Wistar rats with 100 mg/kg Capsaicin on day 2 after birth. After 7 to ~28 days, corneas were excised and subjected to gold chloride staining, [^{3}H]thymidine autoradiography, or immuno-histochemistry using anti-substance P antibody. Results (compared to control animals) were as follows: poor attachment of corneal epithelium, decreased corneal nerve ending density, decreased [^{3}H]thymidine uptake by epithelial cells, and decreased expression of substance P in the epithelial layer.

In a study by Bynke (1983), a 1% Capsaicin solution (0.5 ml) was administered to 10 adult pigmented rabbits (mixed strains) by retrobulbar injection into the left eye. The solution was prepared by dissolving 100 mg of Capsaicin in 150 μl of 99.5% ethanol, 850 μl of Tween 80, and 9 ml of saline. Control rabbits were injected with vehicle only. Capsaicin induced a prompt inflammatory reaction with conjunctival hyperemia, chemosis, and a dense aqueous flare, which subsided gradually over a period of 1 to 2 days. A slight aqueous flare was observed in control

eyes. The response in test eyes was significantly different ($p < .001$) from that observed in control eyes.

Both eyes of adult pigmented rabbits (mixed strain; 8 rabbits) were injected with Capsaicin (150 μl of a 0.33% solution). Capsaicin was dissolved in 75 μl of 99.5% ethanol, 425 μl of Tween 80, and 14.5 ml of saline, yielding a concentration of 0.33% Capsaicin. During retrobulbar injection, the test substance was introduced through the center of the lower eyelid and around the interior aspect of the globe to a depth of 10 mm into the vitreous chamber. Capsaicin induced miosis and breakdown of the blood-aqueous barrier, manifested as an aqueous flare response (Bynke et al. 1983).

Skin Irritation

De and Ghosh (1988b) conducted a study in which Capsaicin (in 10% ethanol + 10% Tween 80 + normal saline; proportion = 1:1:8 *v*/*v*/*v*) was injected into the plantar region of the right hind paw of each of 8 Wistar albino rats (weights = 120 to 130 g) at doses ranging from 0.84 to 84 μg/kg (constant dose volume = 0.05 ml). Paw volume was measured both before and after 30, 60, and 90 min of Capsaicin injection according to a mercury displacement method. Results were expressed in terms of edema volume. A dose-related edema response was noted.

De and Ghosh (1990) reported that Capsaicin induced rat paw edema within 10 min of injection into the hind paw. The edema peaked after 30 min, remained steady for 180 min, and then began to decline. Pretreatment with the antihistamine compound, diphenhydramine (DPH) or the substance P antagonist (D-Pro^{2,} D-Trp^{7,9})-SP significantly inhibited Capsaicin-induced paw edema. The authors concluded that the results of this study offered a proposed mechanism for Capsaicin-induced skin irritation and inflammation, considering that study results indicated that Capsaicin induced a biphasic inflammatory reaction. They suggested that the early phase (10 to 60 min) involves histamine only and the later phase (60 to 180 min) may involve both histamine and substance P.

Kinnman and Levine (1995) reported skin reactions in a study on Capsaicin-induced hyperalgesia involving 35 male Sprague-Dawley rats. Results relating to skin irritation were reported for six of the rats. Capsaicin (30 μg in a volume of 6 μl 5:5:90 [*v*:*v*:*v*] Polysorbate 80–ethanol-saline) was injected intradermally into an area that was proximal to the anterior two foot pads on the left hind paw. Vehicle (6 μl 5:5:90 [*v*:*v*:*v*] Polysorbate 80–ethanol-saline) was injected into the corresponding area on the right hind paw. A flare response on the plantar surface of the paw was observed in all rats that were injected with Capsaicin. The flare response did not extend beyond the two most proximal foot pads.

In a study by Gábor and Rázga (1992), Capsaicin in 90% alcohol (10 μl) was applied to the right ear (inner surface) of CFLP mice (three groups of six) at doses of 30, 40, and 50 μg, respectively. The untreated control group consisted of 8 to 10 mice. At intervals over a period of 1 to 24 h post application, the mice were killed and ears removed. Disks of ear tissue were excised, and tissue weights determined. The degree of edema that resulted was expressed in terms of the difference in weight between inflamed and control ears. Edema formation reached a maximum by 1 h post application, after which a continuous decrease was noted. Similar degrees of edema were observed after administration of 30, 40, or 50 μg of Capsaicin.

In a study by Inoue et al. (1995), Capsaicin was dissolved in acetone at a concentration of 12.5 mg/ml, and 20 μl (250 μg per ear, 41 mM) of this solution was applied topically to both surfaces of one ear of 6-week-old male ddY mice (weights = 30 to 35 g). The magnitude of the edema response was indicated by measuring ear thickness (in 0.001-mm units) with dial calipers. Ear thickness prior to Capsaicin treatment was 0.251 $\pm$ 0.020 mm (26 groups, $n = 6$ to 7). Erythema appeared immediately after application. Edema reached a maximum at 30 min post application, and values for ear thickness approached baseline values at 24 h post application. The magnitude of the edema observed when Capsaicin was reapplied to the ears at 4, 24, and 48 h after the initial application was not the same as that observed initially. Following reapplication at 4 h and at 24 h, edema reached a maximum at 15 min post application. For reapplication at 48 h after the initial application, edema reached a maximum at 30 min. The second edema response that was induced after reapplication of Capsaicin was significantly suppressed ($p < .01$ or $p < .001$) for up to 30 days after the initial application. The reapplication of Capsaicin at 40 or 50 days after the initial application resulted in a second edema, the magnitude of which was up to 64% and 78% of control edema, respectively.

Edema was induced after Capsaicin application in a study by Blazsó and Gábor (1995). Capsaicin (10 μl/40 μg) was dissolved in 90% ethanol and the solution was applied to the right ear of male outbred mice of the CFLP strain.

Mucous Membrane Irritation

Makara et al. (1965) evaluated the effect of Capsaicin on gastric ulcer formation using female albino rats (100 to 140 g) in a series of three experiments. Capsaicin had been dissolved in isotonic saline with one drop of ethanol and one drop of Tween 80. In the first experiment, 71 rats were deprived of food for 36 h and allowed free access to water. The pylorus of each anesthetized animal was ligated, and the treatment groups were described as follows: Capsaicin (1 mg in 0.5 ml physiological saline) administered intragastrically and physiological saline (2 ml) injected intraduodenally (25 rats); Capsaicin (1 mg in 2 ml saline) administered intraduodenally and saline (0.5 ml) administered intragastrically (23 rats); saline (0.5 ml) administered intragastrically; and saline (2 ml) injected intraduodenally (23 control rats). At 12 h after surgery (ligation of pylorus), the animals were placed under anesthesia and stomachs removed. The volume and pH of gastric juice were then determined and total acidity was estimated.

Compared to the control value, intragastric administration of Capsaicin resulted in an 89% increase in the ulcer index.

No significant change in the total acidity or quantity of gastric juice was indicated. Intraduodenal administration of Capsaicin did not affect the ulcer index, and also did not have any effect on the quantity or pH of gastric juice. However, intraduodenal administration of Capsaicin induced a significant increase ($p <$ .02) in total acidity.

The treatment groups for the second experiment were described as follows: Capsaicin (1 mg in 1 ml of physiological saline) administered intragastrically (nine rats); saline (1 mg) administered intragastrically (nine rats); and Capsaicin (1 mg in 1 ml of saline) administered by stomach tube (four control rats). The test procedure for the two groups of nine rats also included, simultaneously, four consecutive daily doses of reserpine (1 mg/kg body weight) by s.c. injection. On the fifth day, the animals from all three treatment groups were killed, and stomachs were examined for gross lesions.

Subcutaneous injection of reserpine alone yielded an ulcer index of 9.2. An increase in the ulcer index to 15.0 resulted when reserpine was administered in combination with the intragastric administration of Capsaicin. Ulceration in the glandular part of the stomach was observed in both groups. In the group that received reserpine and Capsaicin, microscopic examination revealed round-cell infiltration around the vessels of the muscle layer of the mucosa. Neither gross nor microscopic changes were observed in the four control rats that were dosed intragastrically with Capsaicin only.

In the third experiment, 95 rats were fasted for 24 h and allowed free access to water, after which each was injected i.p. with reserpine (dose = 5 mg/kg body weight). At 48 h, 15 rats were decapitated, and stomachs examined for ulcerations. The two remaining treatment groups (pretreated with reserpine) consisted of 39 rats each. One group was subjected to intragastric administration of daily doses of sunflower oil (0.15 ml), and, the other, paprika oil containing 1 mg of Capsaicin (0.05 ml) emulsified in physiological saline (0.3 ml). Ten control rats were dosed intragastrically with an emulsion of saline (0.3 ml) and sunflower oil (0.2 ml). At 24 h after the 3rd, 6th, 9th, and 12th dose, respectively, 10 rats from each of the two groups (test and control) were killed, and stomachs were examined for gross lesions.

Compared to the incidence of reserpine-induced gastric ulcer (13 per 15 control rats), the ulcer incidence was two per nine rats after 12 days of dosing with paprika oil (containing 1 mg of Capsaicin) and three per nine rats after dosing with sunflower oil. Thus, Capsaicin and sunflower oil reduced the incidence of reserpine-induced gastric ulcer (Makara et al. 1965).

Effect on Contact Hypersensitivity

Wallengren et al. (1991) evaluated the effect of Capsaicin on dinitrochlorobenzene (DNCB)-induced sensitization in two experiments using female guinea pigs (Sahlin, Malmö, Sweden) weighing 200 g. In the first experiment, the effect of Capsaicin when administered prior to sensitization was evaluated using 20 guinea pigs (group A). Capsaicin was administered, between induction and challenge, to 21 guinea pigs (group B) in the second experiment. In both experiments, Capsaicin (dissolved in ethanol and Tween 80 and then mixed with physiological saline) was injected subcutaneously into the neck at a concentration of 6.6 mg/ml (dose of 50 mg/kg or 10 mg/animal). Another s.c. injection (16.6 mg/ml; dose = 250 mg/kg or 50 mg/animal) was made 24 h later. The sensitization procedure involved the intracutaneous injection (sacral area) of guinea pigs with 0.1 ml of 0.005% DNCB in propylene glycol. During the challenge phase, a pipette was used to distribute 20 μl of DNCB in 95% ethanol to each of three shaved sites on the flanks of each animal (two sites on one flank, and one on the other). Group A animals were tested with 0.1%, 0.25%, and 0.5% DNCB. Group B animals were tested with 0.25%, 0.5%, and 1.0% DNCB. Ethanol was applied to the fourth flank site. At 24 h post challenge, the intensity of allergic contact dermatitis was evaluated by visual inspection and palpation (grading scale: 0 to 3). After the animals were killed, sites on the flank were excised and inflammatory edema was assayed.

In group A, 0.1% DNCB did not induce dermatitis. At a concentration of 0.25% DNCB, the reaction was the same with and without Capsaicin. Additionally, application of the highest DNCB concentration (0.5%) did not produce scores that were significantly different when compared to the control group. When allergic contact dermatitis was assessed using tissue wet weight, the values obtained were higher (compared to controls) at all DNCB concentrations. At a concentration of 0.5% DNCB, the difference was highly significant ($p <$ .01).

In group B, no animal had a dermatitis reaction to DNCB sham exposure. Reactions to 0.25%, 0.5%, and 1.0% DNCB in the presence of Capsaicin were observed. However, compared to the control group, statistically significant differences were not noted. Tissue wet weight determinations indicated higher values (compared to controls) for 0.25% DNCB ($p <$ .05) and 0.5% DNCB ($p <$ .01) in the presence of Capsaicin, but not for 1.0% DNCB in the presence of Capsaicin. The authors concluded that compared to controls, DNCB-induced contact dermatitis was enhanced in the presence of Capsaicin (Wallengren et al. 1991).

Veronesi et al. (1995) evaluated the effect of Capsaicin on dinitrofluorobenzene (DNFB)-induced ear swelling using a group of 8 to 12 Balb/c female mice (8 weeks old). Capsaicin was administered to the upper cervical dorsal region (i.p. or s.c. injection) twice daily over a period of 3 days (cumulative dose = 30 mg/kg). At 1 to 2 days after the last injection, DNFB or acetone vehicle was applied to the ears, and measurements were taken at 4 and 24 h post exposure. Changes in ear thickness reported relate to results for the control group of five mice in which DNFB was applied to the front and back of the ear (20 μl per side). In mice injected s.c. with Capsaicin, ear thickness was significantly decreased by 44% and 43% at 4 and 24 h post exposure, respectively. In mice injected i.p., ear swelling was significantly decreased by 42% at 24 h post exposure.

Veronesi et al. (1998) reported that the i.p. administration of 50 mg/kg Capsaicin (in 1:18 solution of Tween 80:95%

ethanol:phosphate-buffered saline) to female Balb/c mice on postnatal days 2 to 3 resulted in the denervation of neuropeptide (i.e., tachykinin)-containing sensory C fibers (see Table 13). The effect of chemical denervation on the elicitation and induction phases of contact hypersensitivity (CHS) was studied. The elicitation phase of CHS was analyzed in mice using ear swelling as an end point, and the induction phase was analyzed by measuring lymph node cell (LNC) proliferation. Test and control groups used in the mouse ear swelling assay and the LNC proliferation assay were defined as follows: untreated, sensitized with 2,4-dinitrofluorobenzene (O/DNFB); denervation, sensitized (Capsaicin/DNFB); denervated controls (Capsaicin/O); and untreated controls (O/O). Cytokine release from LNC was also evaluated.

In the ear swelling assay (groups of nine mice), ears from sensitized mice (Capsaicin/DNFB and O/DNFB) were significantly thicker ($p < .05$) at 24 and 48 h post exposure, compared to O/O and Capsaicin/O-treated controls. It is important to note that ears of mice in the Capsaicin/DNFB treatment group were approximately 2.4-fold thicker at 24 and 48 h, compared to O/DNFB mice. There was no significant difference in ear swelling between the two control groups (O/O and Capsaicin/O).

In the LNC proliferation assay (groups of six mice), significant increases in LNC proliferation ($p < .05$) were observed in both groups sensitized with DNFB (Capsaicin/DNFB and O/DNFB groups), compared to nonsensitized controls (Capsaicin/O and O/O groups). Additionally, LNC proliferation in the denervated, sensitized groups (Capsaicin/DNFB) was significantly increased ($p < 0.05$), compared to normal, sensitized mice (O/DNFB) and both control groups (Capsaicin/O and O/O groups). There was no significant difference in LNC proliferation between these two control groups.

Cytokine release (groups of three mice) was measured in the nutrient fluid of LNC cultured from auricular lymph nodes. A slight, nonsignificant increase in interleukin (IL)-1β release was reported for normal, DNFB-sensitized (O/DNFB) mice, compared to nonsensitized controls (O/O). However, a 30-fold increase ($p < .05$) in IL-1β release was reported for the Capsaicin/DNFB group, compared to normal controls (O/O). Tumor necrosis factor alpha (TNFα) levels were also significantly increased ($p < .05$) in normal, sensitized (O/DNFB) and denervated, sensitized (Capsaicin/DNFB) mice, compared to normal and denervated controls (Capsaicin/O and O/O groups). However, compared to normal, sensitized mice (O/DNFB), TNFα levels were significantly higher in denervated, sensitized mice.

The results of flow cytometry (groups of five mice) indicated no differences in the percentages of T cells or B cells in DNFB-sensitized mice (O/DNFB and Capsaicin/DNFB groups). However, both sensitized groups had increased numbers of B cells.

Study results indicated that neuropeptide denervation by neonatal administration of Capsaicin altered the induction and elicitation phases of contact hypersensitivity, and thus may modify sensitivity to chemically induced contact hypersensitivity (Veronesi et al. 1998).

Wille et al. (1999) presented results for Capsaicin in a study evaluating the immunosuppressive effect of *cis*-Urocanic Acid on the induction phase of the contact hypersensitivity reaction, using groups of eight Balb/c mice. The ability of Capsaicin (1% in hydroxypropyl methylcellulose [HPMC] gel) to inhibit DNCB sensitization was studied. Finn chambers were placed on shaved abdominal skin, to which 1% Capsaicin in HPMC gel had been applied. The chambers were applied 24 h prior to sensitization with a single application of 1% DNCB (20 μl) to one ear. The positive-control group was sensitized by a single application of 0.1 ml of 1% DNCB in acetone on shaved abdominal skin, followed by a single application of 1% DNCB (20 μl) to one ear 5 days later in order to elicit a sensitization response. The negative-control group was sensitized according to a similar procedure, with the exception that the animals were challenged with acetone. In the vehicle-control group, Finn chambers were placed on shaved abdominal skin that contained HPMC gel (0.2 ml). In all groups, ear thickness measurements were conducted at 0, 24, 48, and 72 h after challenge. When 1% Capsaicin in vehicle was applied 24 h prior to DNCB, >30% inhibition ($p \leq .05$) of DNCB sensitization was reported.

Immune System Effects

Effects of Capsaicin on the immune system are summarized in Table 12.

Sandberg and Ljungdahl (1986) evaluated the mitogenic responses of B and T lymphocytes from the spleens of newborn, albino NMRI mice dosed with Capsaicin (in stock solution consisting of 10% ethanol and 10% Tween 80, diluted in saline to a 10% solution). Control mice were injected with vehicle only. Test mice were injected s.c. with a single dose of Capsaicin (dose = 50 mg/kg) and examined after 4 to 6 weeks. Control mice were injected with vehicle only. The following five mitogens (in 10 μl of distilled water; one mitogen per culture) were added to spleen cell cultures: lipopolysaccharide (LPS), pokeweed mitogen (PWM), dextran sulfate, concanavalin A (ConA), and phytohemagglutinin (PHA). Prior to harvest at 48 h, cultures were treated with a 2-h pulse of tritiated thymidine. Incorporated radioactivity was determined by liquid scintillation counting. Spleen cell cultures from each animal were tested with all of the mitogens. Statistical analyses of mitogen responses were performed using the Wilcoxan test or student's *t* test. Each statistical comparison involved 18 mice dosed with Capsaicin versus 17 control mice.

Compared to control mice, the growth of mice injected with Capsaicin was significantly lower (83% of normal, $p < .01$) at day 28 post injection. Subsequently, test animals regained weight, and no difference in growth between the two groups was observed 35 to 42 days after injection. A significant reduction in spleen weight after Capsaicin injection (81% reduction, $p < .05$) was also noted, up to day 35 post injection. Differences in relative spleen weight between the groups tested were not observed. Dosing with Capsaicin had no effect on the size of the thymus.

TABLE 12
Effects of Capsaicin on the immune system

Animals	Test substance	Procedure	Results	References
Neonatal and adult rats, males and females, of the F_1 hybrid strain (BN × Wi/Fu)	50 mg/kg Capsaicin in 10% ethanol and 10% Polysorbate 80	Single s.c. injection after immunization with ovalbumin. Spleens removed, cells suspended, and suboptimal, optimal, and supraoptimal levels of ConA added	Significant decrease ($p < .01$) in lymphocyte proliferative response to suboptimal, but not supraoptimal, concentrations of ConA in cells from rats dosed with Capsaicin	Nilsson and Ahlstedt 1988
Neonatal and adult male specific pathogen-free rats of the F_1 hybrid strain (BN × Wi/Fu)	50 mg/kg Capsaicin in saline with 10% ethanol and 10% Polysorbate 80 for adults; 50 mg/kg in olive oil for neonates	Single s.c. injection before or after immunization with ovalbumin (s.c. injection or aerosol inhalation). Spleens removed and lymph nodes and spleen cells obtained	Increased and decreased serum levels of IgE in neonatal rats injected with Capsaicin after s.c.and aerosol immunization, respectively. No effect on levels of serum antibodies after dosing of adult rats with Capsaicin prior to immunization. Slight, significant decrease in IgG levels ($p < .05$), but not IgA or IgE, in adult rats dosed with Capsaicin after aerosol immunization	Nilsson et al. 1991
Neonatal specific pathogen–free Wistar rats	50 mg/kg Capsaicin in 10% ethanol and 10% Tween 80	Single s.c. injection on day 2 of life. Blood smears made and spleen cell suspensions prepared	Marked inhibition of natural killer cell– and antibody-dependent cytotoxic functions	Santoni et al. 1995
Neonatal specific pathogen–free Wistar rats	50 mg/kg Capsaicin in 10% ethanol and 10% Tween 80	Single s.c. injection on day 2 of life	Strong correlation between inhibition of cell proliferation and decreased numbers of $CD5^+$ and $CD4^+$	Santoni et al. 1996
Neonatal specific pathogen–free Wistar rats	50 mg/kg Capsaicin in 10% ethanol and 10% Tween 80	Single s.c. injection (dose = 50 mg/kg) on day 2 of life. Thymus cell suspensions prepared	Marked depletion of $CD5^+$ T lymphocytes at each time point post injection. Significant decrease in % of double negative and single positive $CD4^+$ and $CD8^+$ cells, but no alteration of % of double positive thymocytes	Santoni et al. 2000
Neonatal specific pathogen–free Wistar rats	50 mg/kg Capsaicin in 10% ethanol and 10% Tween 80	Single s.c. injection (dose = 50 mg/kg) on day 2 of life. Thymus cell suspensions prepared	Strong decrease in mitogen-induced thymocyte proliferative response	Santoni et al. 2000

(Continued)

TABLE 12
Effects of Capsaicin on the immune system *(Continued)*

Animals	Test substance	Procedure	Results	References
Specific pathogen–free Wistar rats	Capsaicin in 10% ethanol and 10% Tween 80	Single s.c. injection. Thymus cell suspensions prepared	Immunohistochemical analysis indicated TdT-mediated dUTP nick end labelling (TUNEL)-positive apoptotic cells in thymus sections. Study results suggest that Capsaicin administered at birth profoundly affects T-cell differentiation, likely through ability to activate apoptotic cell death	Santoni et al. 2000
Mature Wistar rats	1% Capsaicin in mixture of 10% ethyl alcohol, 10% Tween-80, and 80% physiologic saline	Doses of 25, 25, 50, and 50 mg/kg for two days. Total neurotoxic dose = 150 mg/kg	Neurotoxic dose produced phasic changes in functional activity of sensory neuropeptides and venous blood neutrophils. Early after Capsaicin injection, the sensitivity of neutrophils to bacterial stimuli decreased against the background of neuropeptide release from the sensory terminals	Zhukova and Makarova 2002
Neonatal Sprague-Dawley rats	50 mg/kg Capsaicin in 10% alcohol and 10% Tween 80 in saline	s.c. injections on days 2 to 4 of life. Regional lymph node response to antigen stimulus evaluated using plaque-forming cell assay	>80% reduction in number of cells secreting antigen-specific antibody (i.e., reduced response to antigenic stimulus)	Helme et al. 1987
Neonatal albino NMRI mice	50 mg/kg Capsaicin in solution containing 10% ethanol and 10% Tween 80, diluted in saline to 10% solution	Single s.c. injection. Spleen cultures prepared	Neonatal Capsaicin treatment did not affect overall immunocompetence of animals tested. Essentially normal responses to B- and T-lymphocyte mitogens	Sandberg and Ljungdahl 1986
Male albino Swiss mice	50 mg/kg Capsaicin in 20% ethanol and 10% Tween 80 in saline	single s.c. injection on day 4. PMN migration, in air pouches formed, evaluated	Selective inhibition of interleukin-1β–induced PMN migration	Perretti et al. 1993
C57/black mice (2 to 3 days old)	50 mg/kg Capsaicin	s.c. injection followed by immunization with cholera toxin at 6 weeks of age	Significant depression of intestinal antibodies to cholera toxin ($p < .001$)	Jarrah et al. 1995

(Continued on next page)

TABLE 12
Effects of Capsaicin on the immune system *(Continued)*

Animals	Test substance	Procedure	Results	References
Pathogen-free female Balb/c mice	5, 20, 50, or 100 ppm Capsaicin in the diet	Animals fed for 3 weeks	Number of splenocytes and antibody-producing cells increased and enhanced T-cell mitogen-induced lymphocyte proliferation in mice fed 20 ppm. Maximum stimulation of antibody and TNFα production at 20 ppm	Yu et al. 1998
Pathogen-free female Balb/c mice	5, 20, 50, or 100 ppm Capsaicin in the diet	Animals fed for 3 weeks	Increased serum immunoglobulin concentrations (IgG and IgM) in groups fed 20 and 50 ppm Capsaicin	Yu et al. 1998
Male Duncan Hartley guinea pigs	0.001% Capsaicin aerosol	Inhalation exposure until dyspnea occurred. Bronchoalveolar lavage fluid evaluated	186% increase in eosinophils and 100% increase in neurotrophils (both statistically significant, $p <$ .05) in lavage fluid at 24 hours post-exposure	Kudlacz and Knippenberg 1994
Jurkat T cells (human leukemic T-cell line)	Capsaicin in 50% ethanol/50% water	In vitro assay monitoring production of interleukin-2	Both T-cell receptor (TCR)-dependent and TCR-independent production of interleukin-2 suppressed by Capsaicin in a dose-dependent manner	Fischer et al. 2001

The six youngest animals examined (three per group) were excluded from comparisons relating to mitogen responsiveness because these animals either did not respond or responded very weakly to the mitogens that were tested, and because spleen cells from these animals were too few for complete testing.

No statistically significant differences in mitogen responses were noted for LPS, PWM, dextran sulfate, or ConA. The maximum response to PHA was induced at a dose of 40 μg/ml in control mice, and at a dose of 20 μg/ml in mice dosed with Capsaicin. To evaluate the statistical significance of the shift in dose-response relationship, a weighted mean of the optimal dose was calculated for each animal using the four highest doses of mitogen used. Results indicated a statistically significant difference between test and control groups ($p < .02$, Wilcoxan test). The maximal response to PHA was obtained with a significantly lower dose in animals dosed with Capsaicin. The authors concluded that neonatal Capsaicin treatment did not affect the overall immunocompetence of animals tested, because essentially normal responses to B- and T-lymphocyte mitogens were obtained (Sandberg and Ljungdahl 1986).

In a study by Helme et al. (1987), neonatal Sprague-Dawley rats were injected s.c. with 50 mg/kg Capsaicin in 10% alcohol and 10% Tween 80 in saline on days 2 to 4 of life. At 6 to 12 weeks of age, the regional lymph node response to an antigen stimulus was evaluated using the plaque-forming cell (PFC) assay. Antibody-secreting cells capable of forming hemolytic plaques in a liquid monolayer of complement-sensitive red blood cells are analyzed in the PFC assay. Antigen stimulation occurred by injection of fresh sheep red blood cells into both hind footpads of anesthetized rats. Capsaicin caused a >80% reduction in the number of cells secreting antigen-specific antibody. This effect was reversed by s.c. infusion of substance P after antigen stimulation. The authors suggested that the reduced response of Capsaicin-treated rats to an antigenic stimulus was due to an effect of Capsaicin on the substance P–containing primary afferent nerves, instead of a toxic effect of Capsaicin on the immune system.

Nilsson and Ahlstedt (1988) evaluated the effect of Capsaicin on lymphocyte proliferation using groups of five immunized (with ovalbumin), specific pathogen–free rats. Suboptimal, optimal, or supraoptimal concentrations of ConA were used. For neonatal and adult rats, the lymphocyte proliferation rate in the presence of suboptimal concentrations of ConA was significantly lower in spleen mononuclear cells (SMCs) from rats dosed with Capsaicin than in cells from untreated rats ($p < .05$ for nonimmunized rats; $p < .01$ for rats immunized s.c. or by aerosol inhalation). Proliferative responses of SMC to supraoptimal concentrations of ConA (10 μg/ml) were

not decreased significantly in animals dosed with Capsaicin. The authors concluded that, compared to normal rats, rats with Capsaicin-induced neurologic dysfunction have an altered immunologic response.

Perretti et al. (1993) studied the effect of Capsaicin on IL-1β–induced polymorphonuclear leucocyte (PMN) migration into a murine air pouch. Air pouches were formed by s.c. injection of air (2.5 ml on days 0 and 3) into anesthetized, male albino Swiss mice (weights = 20 to 22 g). Capsaicin (in 20% ethanol and 10% Tween 80 in saline) was injected (dose = 50 mg/kg) s.c. into each animal on day 4. Control mice were injected with vehicle only (dose = 3 ml/kg). On day 8, IL-1β was injected into air pouches of control and test animals, and PMN migration evaluated. Injection of IL-1β into the air pouch induced substantial PMN recruitment at the 4-h time point (net migration = 8.86×10^6 PMNs/mouse). Selective inhibition of IL-1β-induced migration ($42 \pm 6\%$ inhibition) was observed in mice pretreated with Capsaicin.

In a study by Jarrah et al. (1995), Capsaicin (dose = 50 mg/kg) was injected s.c. into nine, 2- to 3-day-old C57/black mice. At 6 weeks of age, the mice were immunized with cholera toxin. Three weeks later, the animals were purged and the stool cholera toxin–specific IgA was estimated using an enzyme-linked immunosorbent assay (ELISA) (results expressed in optical density units). The control group consisted of five mice immunized with cholera toxin. The injection of Capsaicin resulted in a significant decrease in the intestinal immune response ($p < .001$), i.e., significant decrease in intestinal antibodies to cholera toxin.

Yu et al. (1998) evaluated the modulation of immune responses by dietary Capsaicin. Groups of pathogen-free female Balb/c mice were fed diets containing 5, 20, 50, or 100 ppm Capsaicin for 3 weeks. Control mice received diet only. No significant differences in growth rate or weight gain between test and control groups were reported. Additionally, relative weights of the liver and spleen were not found to be significantly different when all test groups were compared. In mice fed 20 ppm Capsaicin, the number of splenocytes and antibody-producing cells was increased. Mice fed 20 ppm Capsaicin also had enhanced T-cell mitogen–induced lymphocyte proliferation. Maximum stimulation of antibody and TNFα production was also associated with this dietary concentration of Capsaicin (20 ppm). Increased serum immunoglobulin (IgG and IgM) concentrations were reported for groups fed 20 and 50 ppm Capsacin in the diet. The authors suggested that dietary Capsaicin may differentially enhance immune status as well as select immune functions.

Nilsson et al. (1991) studied the effect of Capsaicin on the immune response using male, specific pathogen–free rats of the F_1 hybrid strain (BN $\times$ Wi/Fu, neonatal and adult rats). Antibody function in vivo and in vitro was used to evaluate the immune response. Neonatal rats (1 to 2 days old) were injected s.c. with 50 mg/kg Capsaicin in olive oil during ether anesthesia. After treatment with bronchodilators, adult rats were injected s.c. with 50 mg/kg Capsaicin in saline with 10% ethanol and 10% polysorbate 80 during anesthesia, either 5 days before or 3 days after immunization with ovalbumin (s.c. injection or aerosol inhalation). All animals were immunized at 6 weeks of age. The animals were killed and lymph node and spleen cells were prepared by mechanical dispersion. Cells from neonatal rats were pooled for each group, cultured for 7 days, and supernatants collected. In the immunoglobulin analysis, IgE antibodies to ovalbumin were measured using a radio allergosorbent test (RAST). Immunoglobulin concentrations were determined using an enzyme-linked immunosorbent assay (ELISA).

Serum concentrations of IgE in neonatal rats dosed with Capsaicin and immunized s.c. were increased, whereas, the opposite effect was observed following aerosol immunization ($p < .05$). In adult rats, dosing with Capsaicin prior to immunization had no effect on the concentration of serum antibodies. However, dosing with Capsaicin after aerosol immunization induced a slight, significant decrease in the concentration of IgG ($p < .05$), but not IgA or IgE.

Capsaicin induced a decrease in serum IgA, regardless of the method of immunization, in neonatal rats. Concentrations of IgE and IgG were said to have been affected less. Neither the concentration of IgA nor IgG was affected by dosing of adult rats with Capsaicin before immunization (s.c. or aerosol administration). However, the concentration of IgE was increased in adult rats dosed with Capsaicin prior to aerosol immunization. Other results indicated that adult rats dosed with Capsaicin after aerosol immunization had an increased concentration of IgA, whereas, IgE was decreased in rats immunized s.c.

Different results for immunoglobulin synthesis were obtained, depending on whether Capsaicin was administered to neonatal rats or adult rats, or before or after immunization. The effects of dosing with Capsaicin on immunoglobulin synthesis in mesenteric lymph nodes, peripheral blood lymphocytes, and the spleen were very similar. However, it appeared that a pool of axillary, brachial, and mediastinal lymph nodes was less sensitive to Capsaicin.

The authors concluded that serum concentrations of immunoglobulins and the capacity of lymphocytes to synthesize immunoglobulins in rats are affected by treatment with Capsaicin (Nilsson et al. 1991).

In a study by Kudlacz and Knippenberg (1994), eosinophil and neutrophil influx into bronchoalveolar lavage (BAL) fluid was noted for male Duncan Hartley guinea pigs (five to six per group; weights = 300 to 350 g) 24 h after exposure to Capsaicin aerosol (0.001% Capsaicin). The animals were exposed until dyspnea occurred. Control guinea pigs were exposed to vehicle aerosols. A 186% increase in eosinophils and a 100% increase in neutrophils were reported after Capsaicin inhalation. Both increases were statistically significant ($p < .05$).

In a study by Santoni et al. (1995), newborn specific pathogen-free Wistar rats were injected s.c. with 50 mg/kg Capsaicin (in 10% ethanol and 10% Tween 80) on day 2 of life. The animals were killed at various times during a 90-day period post injection. Blood smears for hematological evaluations of cell morphology and differential counts were made. Peripheral

blood lymphocytes were isolated by centrifugation. Spleen cell suspensions were also prepared. YAC-1 (tissue culture cell line of YAC [Moloney virus-induced lymphoma of A/Sn origin]) was used as the target cell for natural killer cell activity. P815 (chemically induced mastocytoma of DBA/2 origin) coated with a rabbit anti-P815 antiserum was used as a target cell for antibody-dependent cytotoxic functions.

Compared to control rats, the total number of peripheral blood and spleen lymphocytes was not significantly different during the first 90 days after dosing. Capsaicin treatment resulted in marked inhibition of natural killer cell activity and antibody-dependent cytotoxicity. Inhibition was evident as early as 15 days post dosing and persisted to day 90 in the spleen. By day 90, peripheral blood lymphocyte-mediated cytotoxicity had returned to control levels (Santoni et al. 1995).

Santoni et al. (2000) evaluated the effect of neonatal Capsaicin treatment on the distribution and function of rat thymocyte subsets and apoptosis of thymus cells using specific pathogen–free Wistar rats of both sexes (age = 2 days). Test animals were injected s.c. with 50 mg/kg Capsaicin in 10% ethanol and 10% Tween 80 on day 2 of life. Controls were injected with vehicle only. The animals were killed on day 7, 14, or 28 post injection, and thymus cell suspensions prepared.

The purpose of the first experiment was to determine whether neonatal Capsaicin treatment caused changes in the total T-cell population in the thymus. Thymocyte subpopulations (from untreated, vehicle-, and Capsaicin-treated rats) were evaluated by two-color immunofluorescence and flow cytometric analysis using the following mouse monoclonal antibodies (mAbs): phycoerythrin (PE)-conjugated anti-rat CD5 (clone OX19), PE-conjugated anti-rat CD8a (clone OX8), fluorescein isothiocyanate (FITC)-conjugated anti-rat CD4 (cloneW3/25), FITC-conjugated anti-rat T-cell Receptor (TCR) γ/δ, and FITC-conjugated anti-rat TCR α/β. PE-mouse IgG1 and FITC-mouse IgG2a served as negative controls. The thymocytes (incubated with appropriate mAbs) were analyzed for relative fluorescence intensity. Marked depletion of $CD5^+$ T lymphocytes was noted at each time point after neonatal Capsaicin treatment. Additionally, Capsaicin induced a significant decrease in the percentage of double-negative (DN) and single-positive (SP) $CD4^+$ and $CD8^+$ cells (persisting to day 28), but did not alter the percentage of double-positive (DP) thymocytes.

Neonatal Capsaicin treatment affected the expression of TCR α/β or TCR γ/δ on $CD5^+$ T cells. A marked reduction in absolute numbers of $CD5^+$ TCRα/β^+ and $CD5^+$ TCRγ/δ^+ cells was observed in the thymus. The reduction (81.5%) of $CD5^+$ TCRγ/δ^+ was more pronounced, implying that this T-cell subset is particularly sensitive to Capsaicin treatment.

In the proliferation assay, thymocytes from untreated, vehicle-, and Capsaicin-treated rats were cultured with concanavalin A or phytohemagglutinin, and then with tritiated thymidine ([^{3}H]TdR) during the last 6 h of the 72-h incubation period. Capsaicin treatment refers to neonatal rats that received a single s.c. injection (dose = 50 mg/kg, in 10% ethanol and 10% Tween 80) of Capsaicin on day 2 of life. Capsaicin strongly decreased the mitogen-induced thymocyte proliferative response. It did not significantly affect spontaneous [^{3}H]TdR uptake by unstimulated thymus cells.

TdT-mediated dUTP nick end labeling (TUNEL) reactions on thymus sections were also evaluated. Frozen thymi from untreated, vehicle-, and Capsaicin-treated rats (five per group) were evaluated. After incubation with TUNEL reaction mixture, slides were subsequently covered with sheep anti-fluorescein Fab–horseradish peroxidase (POD)-conjugated antibody and incubated. The number of TUNEL-positive thymocytes (determined over 10 random cortical and medullary thymus fields/section/per rat using an image analysis program) was expressed as proportional area (PA). PA represented the number of grains/scan area, and was averaged to yield the mean proportional area (MPA). An immunohistochemical analysis revealed TUNEL-positive apoptotic cells only in thymus sections from Capsaicin-treated rats. Significant differences in the distribution of apoptotic cells within the thymus were noted. Compared to the thymic medulla, more apoptotic cells were present in the outer subcapsular region of the thymic cortex. The authors concluded that Capsaicin, when administered at birth, profoundly affects T-cell differentiation, likely through its ability to activate apoptosis (Santoni et al. 2000).

The results of a study by Fischer et al. (2001) indicated that Capsaicin (in 50% ethanol/50% water) blocked receptor-stimulated Ca^{2+} entry in Jurkat T cells (human leukemic T-cell line), and this effect was dose-dependent. The IC_{50} for Capsaicin-induced inhibition of Ca^{2+} entry was $\cong$23 μM. Capacitative calcium entry (CCE) through Ca^{2+} channels is an absolute requirement for normal activation of T lymphocytes. In whole cell voltage clamp experiments (Jurkat cells), Capsaicin rapidly blocked voltage-gated K^+ current in a nonvoltage-dependent manner ($IC_{50} \cong 18$ μM).

The relationship between blockage of inward Ca^{2+} current by Capsaicin and functional immunosuppressive activity–TCR activation was evaluated using Jurkat cells. Both TCR-dependent and TCR-independent production of IL-2 (an immune modulator) was suppressed by Capsaicin in a dose-dependent manner. IC_{50}s for TCR-dependent and TCR-independent Capsaicin-induced suppression of IL-2 production were $\cong$18 μM and 75 μM, respectively. The dose-dependence of Capsaicin-induced reduction of IL-2 was comparable to its blockage of inward Ca^{2+} current, indicating the functional relevance of Capsaicin-induced blockage of lymphocyte capacitative calcium entry. Based on the results of this study, the authors stated that Capsaicin and its analogs may have potential use as immunomodulatory drugs (Fischer et al. 2001).

Zhukova and Makarova (2002) evaluated the effect of Capsaicin on the dynamics of neutrophil functional activity in venous blood from Wistar rats. It was noted that the release of neuropeptides induced by a toxic dose of Capsaicin can provoke essential changes in the blood. With this in mind, a study was conducted to determine how the functional state of

Capsaicin-sensitive neurons affects biocidal activity of venous neutrophils and the total antioxidant potential of the plasma. Capsaicin (1% in mixture of 10% ethyl alcohol, 10% Tween 80, and 80% physiologic saline) was injected subcutaneously into mature Wistar rats. Doses of 25, 25, 50, and 50 mg/kg were injected for 2 days at 12-h intervals. The total neurotoxic dose of Capsaicin was 150 mg/kg. Blood samples were obtained on days 7, 14, and 21 after Capsaicin injection.

The production of biooxidizers by neutrophils was evaluated using spontaneous and induced nitro blue tetrazolium (NBT) reduction. For the spontaneous production evaluation, 25 μl of Hanks' solution and 25 μl of 0.2% NBT solution were added to 50 μl of heparinized (10 U/ml) blood. For the induced production evaluation, the blood was incubated with 25 μl of inductor solution (instead of Hanks' solution). The inductors were prodigiosan (lipopolysaccharide from *Serratia marcescens*) and killed *Staphylococcus aureus* vaccine. Total and differential leukocyte counts were determined routinely. Neutrophils that reduced NBT into dark-blue NBD (nitro blue diformazan) were counted using a light microscope. Plasma antioxidant activity was determined as a reciprocal value to chemiluminescence intensity. Chemiluminescence was recorded using an SKIF-0306 chemiluminometer.

By day 14 after Capsaicin injections (when neuropeptide stores in sensory terminals were depleted), the activity of peripheral blood neutrophils (assessed by the spontaneous NBT test) had increased by twofold (compared to control, $p < .01$). In tests involving stimulation with prodigiosan or killed *Staphylococcus aureus* vaccine, the activity of peripheral blood neutrophils increased 1.4- and 2-fold, respectively.

The authors stated that the increased leukocyte count in venous blood, noted 2 weeks after injection of the neurotoxic dose of Capsaicin, was determined by lymphocytes because the neutrophil count was significantly decreased ($2.70 \pm 0.47 \times 10^9$/L versus $5.20 \pm 0.60 \times 10^9$/L in the control, $p < .01$). Additionally, the percentage of lymphocytes in experimental rats exceeded the corresponding parameter in controls ($72.0 \pm 6.27\%$ and $59.0\% \pm 3.26\%$, respectively, $p < .05$). Compared to controls, the percentage of neutrophils was lower ($28.0 \pm 5.9\%$ and $39.0\% \pm 3.42\%$ in experimental and control rats, respectively).

On day 14 after Capsaicin dosing, the total antioxidant activity of venous plasma decreased 1.6-fold (compared to control, $p < .05$). This decrease was accompanied by enhanced production of biooxidizers by polymorphonuclear leukocytes, and probably resulted from the inactivation of sensory neuropeptides following the injection of a neurotoxic dose of Capsaicin. On day 21 post dosing, the percentage of NBT-positive neutrophils returned to the control level. However, in the test with inductors, the percentage of NBT-positive neutrophils decreased to values that were 2.3 times lower than the control value ($p < 0.05$; test with prodigiosan) and 1.6 times lower than the control value ($p < .05$; test with killed *Staphylococcus aureus* vaccine). Under these conditions, no significant changes in plasma antioxidant activity were observed.

The authors concluded that a neurotoxic dose of Capsaicin produced phasic changes in functional activity of sensory neurons and venous blood neutrophils of Wistar rats. Early after Capsaicin injection, the sensitivity of neutrophils to bacterial stimuli decreased against the background of neuropeptide release from the sensory terminals (Zhukova and Makarova 2002).

Cytotoxicity

Reinhardt et al. (1985) evaluated the cytotoxicity of Capsaicin using detachment and growth inhibition assays. The cell detachment assay used was a modification of the procedure by Reinhardt et al. (1982). In this assay, the lowest concentration of Capsaicin that induced a significant toxic effect (CD_{low}) was determined by measuring the number of detached baby hamster kidney fibroblasts (BHK-21/C13). The test substance was serially diluted in medium prior to cell exposure. A CD_{low} of 0.38 mM (strongly toxic) was reported for Capsaicin.

In the growth inhibition assay (BHK-21/C13 cells), cells were exposed to Capsaicin, serially diluted with full medium, for 48 h. The data were expressed as percentages of the initial cell numbers that were determined at the beginning of the incubation period. The lowest concentration of Capsaicin that induced a significant toxic effect in the growth inhibition assay was defined as the GI_{low}. A GI_{low} of 0.2 mM (mild to moderate growth inhibition) was reported for Capsaicin (Reinhardt et al. 1985).

Hamid and Boelsterli (1989) evaluated the effect of Capsaicin, in vivo and in vitro, on red blood cell fragility using female Sprague-Dawley rats (240 to 280 g). In the in vitro experiment, red blood cells were incubated with Capsaicin at concentrations ranging from 10^{-4} to 10^{-2} M for 60 min. The mean corpuscular fragility (MCF) was determined graphically and expressed as the sodium chloride concentration at which 50% of the red blood cells hemolyze. Capsaicin had no effect on the osmotic fragility of red blood cells at any of the concentrations tested.

Female Sprague-Dawley rats were dosed orally (5 or 50 mg/kg) or s.c. (1 or 3 mg/kg) with Capsaicin daily for 10 days. MCF was determined according to the procedure in the preceding experiment. Capsaicin had no effect on the osmotic fragility of red blood cells (Hamid and Boelsterli 1989).

Hamid and Reinhardt (1989) evaluated the cytotoxicity of Capsaicin in vitro in cell detachment and cell growth assays, using the following three cell lines: BHK-21/C13 (baby hamster kidney cells), Keller cells (human diploid fibroblasts derived from an arm biopsy of an adult female), and MRC-5 cells (human embryonic lung fibroblasts). In the cell detachment assay, detached cells were removed from each well at the end of a 4-h incubation period with 0.0002 to 2.0 mM (2×10^{-7} to 2×10^{-4} M) Capsaicin. The number of detached cells was subtracted from the total number of cells counted in the control wells. The mean and 95% confidence limits of four parallel experiments were calculated. Concentration-dependent cell detachment was observed. The percent detached cells reached a maximum within a relatively narrow concentration range (0.2 to 2 mM for MRC

and BHK cells; 0.2 to 0.6 mM for Keller cells). In the growth assay, cells (same three cell types) were incubated with Capsaicin for 48 h. Cells were then counted, and the percentage of the total number of cells observed at the beginning of incubation was calculated. Significant growth inhibition was noted only at concentrations >0.02 mM (steep decline in growth). It was concluded that Capsaicin was a potent cell-detaching agent, with no effect on cell growth.

De et al. (1993) reported that Capsaicin induced a dose-related increase in hemolysis of human red blood cells in vitro over the range of concentrations tested (1 to 100 nM). The incubation period ranged from 10 to 60 min. This effect was due to Capsaicin-induced changes in the erythrocyte membrane, namely, a significant increase in total lipids and a decrease in phospholipid and cholesterol content. Significant, dose-related inhibition of erythrocyte membrane acetylcholinesterase activity was also noted, reaching a maximum at a concentration of 50 nM Capsaicin.

Wolvetang et al. (1996) reported that approximately one-half of the human Daudi and BL-29 lymphoblastoid cells incubated with 200 μM Capsaicin underwent apoptosis within 8 h. Using plasma membranes isolated from fresh rat liver, Capsaicin ($\sim$25 to 300 μM concentration range) was also shown to be an effective inhibitor of the NADH-oxidase function of the plasma membrane NADH-oxidoreductase (PMOR) system. The authors suggested that the PMOR system is a redox sensor that can, depending on the ambient redox environment and the availability of growth factors, regulate plasma membrane calcium fluxes and signal for apoptosis through calcineurin.

In a study by Ko et al. (1998), fibroblast and keratinocyte cell cultures were exposed separately to the following concentrations of Capsaicin: 0.025%, 0.05%, 0.1%, 0.15%, and 0.2% weight/volume solutions. Capsaicin in 10% ethanol was diluted with Dulbecco's modified Eagle's medium.

Capsaicin-induced cytotoxicity was both time- and concentration-dependent in fibroblast and keratinocyte cell cultures. After 4 h of incubation, fibroblast cultures treated with 0.15% and 0.20% Capsaicin had cell counts that were significantly lower ($p < .05$) when compared to controls. After 24 and 48 h of incubation, this finding was true for the entire range of concentrations tested. Results for keratinocyte cultures indicated concentration-dependent cytotoxicity during the first 6 h of incubation. After 2 h of incubation, a 30% reduction in the number of viable keratinocytes was observed at a concentration of 0.20% Capsaicin. At 6 h after incubation, a significant reduction in the number of live keratinocytes ($p < .05$) was observed over the entire range of concentrations tested when results were compared to control cultures.

In another experiment in the preceding study, fibroblasts were seeded on rat-tail collagen matrix and grown to confluence on the surface and inner layer of the matrix for 3 days. Cell samples were incubated with the Capsaicin concentrations indicated above, and matrixes were examined microscopically after 24 h. At concentrations $\geq$0.1% weight/volume, Capsaicin penetrated the collagen matrix, resulting in fibroblast degeneration on both the surface and inner layer of the matrix (Ko et al. 1998).

Macho et al. (1999) evaluated the role of mitochondria and reactive oxygen species (ROS) in the apoptotic pathway induced by Capsaicin (250 μM), using a cytofluorimetric method and the following three tumor cell lines: Jurkat (human leukemic T-cell line), an erythroleukemic cell line, and 293T, a human embryonic, kidney-derived cell line expressing the SV40 large T antigen. After treatment of the cells for 6 h, dissipation of the transmembrane mitochondrial potential ($\Delta\psi_m$) and ROS generation were detected by double staining experiments. In these experiments, hydroethidine (HE; nonfluorescent) that becomes ethidium (Eth; red fluorescent) after oxidation via ROS and 3,3′-dihexyloxacarbocyanine iodide [$DiOC_6(3)$], green fluorescent, cationic probe that accumulates in mitochondria as a function of its potential, were used. Hypodiploidy (loss of fragmented DNA), a marker for apoptosis, was analyzed by propidium iodide (PI) staining after 18 h of treatment.

Untreated cells had a high $\Delta\psi_m$ ($DiOC_6(3)^{high}$) and low levels of intracellular ROS $(HE \rightarrow Eth)^{low}$. Capsaicin induced an increase in the percentages of $DiOC_6(3)^{low}$ /$(HE \rightarrow Eth)^{high}$ and $DiOC_6(3)^{high}$/$(HE \rightarrow Eth)^{high}$ in all three cell lines. Compared to untreated cells, there was no change in the percentage of $DiOC_6(3)^{low}$/$(HE \rightarrow Eth)^{low}$ in these cell lines. These results indicate that ROS are first generated, and that generation is followed by $\Delta\psi_m$ breakdown. The appearance of $DiOC_6(3)^{low}$ cells correlates with a significant increase in the number of hypodiploic apoptotic cells. In this study, Capsaicin was described as a vanilloid quinone analog that inhibits the plasma membrane electron transport system and induces apoptosis in transformed cells. It was determined that Capsaicin induces a rapid increase in ROS, followed by disruption of the $\Delta\psi_m$, and DNA nuclear loss in transformed cell lines and in mitogen activated human T cells (Macho et al. 1999).

In a study by Rizvi and Srivastava (1999), a protective effect of Capsaicin on the osmotic fragility of human erythrocytes was reported. Compared to the control (normal blood), a significant ($p < .001$) decrease in mean erythrocyte fragility (MEF) was observed when erythrocytes were incubated with 10^{-4} and 10^{-5} M Capsaicin. However, no significant alteration in MEF was observed at lower Capsaicin concentrations (10^{-6} and 10^{-7} M). MEF values were not affected when the incubation period was increased from 30 to 60 min.

Vascular Toxicity

Capsaicin-induced gastric vascular damage was evaluated using groups of six anesthetized Wistar rats of either sex (weights = 150 g and 180 g). Capsaicin was administered orally at doses of 0.8, 8.0, and 80 mg/kg. The animals were killed at 10 min post dosing, and stomachs were removed. Gastric contents and the glandular portion of the stomach were maintained in HCl. After 18 h, Evan's Blue was extracted in chloroform and estimated spectrophotometrically at 620 nm. Evan's Blue is a dye that is

strongly bound to plasma albumin, and will leak into tissue only in the presence of vascular damage. Consistently greater leakage of Evan's Blue dye was observed with increasing doses of Capsaicin, indicating a dose-dependent increase in gastric vascular damage (Thatte et al. 1988).

Renal Effects

Perfumi and Sparapassi (1999) evaluated the effect of prenatal or neonatal dosing with Capsaicin (in 10% ethanol, 10% Tween 80, and 80% isotonic saline) on urine excretion by dams and neonates, following water load. Groups of 15 specific pathogen–free, Wistar rats (dams) were injected s.c. with Capsaicin (50, 100, or 200 mg) on gestation days 7 to 15. For all dose groups, the urine excretion in response to water load was similar to that of controls at the different times of observation. In another analysis of the data, the results for 23 dams injected with 50 mg/kg Capsaicin on gestation day 15 (prenatal treatment) and 8 neonates injected with the same dose on postnatal day 2 were compared with related controls. No difference in urine excretion was noted in rats treated prenatally. However, for neonatal rats, diuresis was markedly decreased for the entire observation period in rats dosed postnatally. Cumulative 24-h values were 5.10 ± 0.42 ml/100 g body weight for controls and 0.391 ± 0.07 ml/100 g body weight for rats treated neonatally ($p < .001$). Thus, the authors concluded that treatment with Capsaicin during gestation did not modify diuresis, whereas, diuresis was impaired following treatment after birth.

Neurotoxicity/Nervous System Effects

Neither oral nor dermal neurotoxicity studies were identified, but the results of numerous studies using injection routes of exposure indicate that Capsaicin is a neurotoxin. Brief summaries of studies on the neurotoxicity of Capsaicin are included in Table 13.

In addition, Buck and Burks (1986) and Sternini et al. (1987) reported that chronic administration of Capsaicin to neonatal or adult animals destroys and depletes the neurochemical markers of primary afferent neurons. Chard et al. (1995) proposed a mechanism of action in which Capsaicin kills a subpopulation of sensory neurons (cultured dorsal root ganglion neurons from neonatal rats) by activating a receptor-operated channel. The resulting Ca^{2+} influx causes large increases in $[Ca^{2+}]$ and the activation of Ca^{2+}-sensitive proteases. They concluded that this model provides support for the role of $[Ca^{2+}]$ as the orchestrator of delayed neuronal degeneration. Bringing the possible mechanism full circle, Kopanitsa et al. 1995) reported the blocking action of Capsaicin on Ca^{2+} channels in isolated rat trigeminal and hippocampal neurons.

GENOTOXICITY

Studies (bacterial and mammalian test systems) on the genotoxicity/mutagenicity of Capsaicin are summarized in Table 14. In both test systems, both positive and negative findings are reported.

Antimutagenicity

Capsaicin significantly inhibited the mutagenicity of the tobacco-specific nitrosamine (product of the nitrosation of nicotine and its derivatives during tobacco processing), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in *Salmonella typhimurium* strain TA1535 at doses of 0.1, 0.2, and 0.4 μmol/plate (with metabolic activation) (Miller et al. 1994). The mechanism for this antimutagenic effect is suggested in the following study.

Zhang et al. (1993) evaluated the effect of Capsaicin on the in vitro metabolism of NNK by hamster and rat liver microsomes. The metabolism assay for NNK, a tumorigen in rodents, is a modification of the procedure by Smith et al. (1990). Metabolic activation is required for the mutagenicity and carcinogenicity of NNK (Hecht and Hoffmann 1988; Crespi et al. 1991), and study results have indicated that the metabolism of NNK occurs via the following three major pathways (Castonguay et al. 1984): (1) carbonyl reduction, (2) *N*-oxidation of the pyridine ring, and (3) α-methylene hydroxylation.

At each of the concentrations of Capsaicin tested (0.25, 0.5, and 1.0 mM, in DMSO), significant inhibition ($p < .05$) of all three major pathways for NNK metabolism was noted using hamster liver microsomes. Dose-dependent inhibition of NNK reduction was noted using rat microsomes. Capsaicin also significantly inhibited α- hydroxylation of NNK by rat microsomes at concentrations of 0.5 and 1.0 mM.

In another experiment in the preceding study, the effect of Capsaicin (0.5 M) on the formation of metabolites of [^{14}C]testosterone by hamster microsomes and rat microsomes was evaluated. It was noted that testosterone is subject to site-specific hydroxylation reactions by several isozymes of cytochrome P450. Results indicated that Capsaicin inhibited the formation of [^{14}C]testosterone, thereby suggesting that the activity of cytochrome P450 isozymes was inhibited.

The authors suggested that the antimutagenic and anticarcinogenic properties of Capsaicin are due to its inhibition of xenobiotic-metabolizing enzymes (Zhang et al. 1993).

Surh et al. (1995b) reported that Capsaicin (0.42 mM) attenuated the mutagenicity of vinyl carbamate and *N*-nitrosodimethylamine (NDMA) in strain TA100 of *Salmonella typhimurium*. The authors also suggested that Capsaicin suppressed vinyl carbamate and NDMA-induced mutagenesis, in part, through inhibition of the cytochrome P450 IIE1 isoform that is responsible for the activation of these two carcinogens.

Effect on Gene Expression

In a study by Pelto-Huikko (1991), a single dose of Capsaicin (25 mg/kg), dissolved in 10% Tween 80 and 10% ethanol in physiological saline, was injected s.c. into adult male Sprague-Dawley rats (175 to 200 g; number not stated). Vehicle-injected

TABLE 13
Effects of Capsaicin on the nervous system

Animals	Test substance	Procedure	Results	References
Groups of 3 adult male CFY rats (200 to 250 g)	50, 100, and 200 mg/kg Capsaicin in 10% ethanol and 10% Tween 80 in physiological saline	s.c. injection	Dose-dependent, substantial decrease in numbers of unmyelinated axons and moderate loss of myelinated axons in peripheral nerves	Király et al. 1986
Sprague-Dawley rats (10 days old; number not stated)	50 to 150 mg/kg Capsaicin in 10% alcohol/10% Tween 80	s.c. injection	Microscopic examination of tissues (cupric silver staining technique) revealed degeneration (mainly nerve terminals and axon fragments) in loci throughout brain, spinal cord, and retina	Ritter and Dinh 1990
Sprague-Dawley rat pups (10 to 30 days old; number not stated)	75 or 100 mg/kg Capsaicin in 10% alcohol/10% Tween 80			
Adult Sprague-Dawley rats (75 days to 11 months old; number not stated)	100 mg/kg Capsaicin in 10% alcohol/10% Tween 80			
24 Neonatal male Sprague-Dawley rats	50 mg/kg Capsaicin	Dosing on day 2 of life (method not stated)	Marked decrease in density of corticotropin releasing factor, substance P, vasoactive intestinal peptide, and cholecystokinin-containing neurons in the dorsal spinal cord, the substantia gelatinosa, and lateral border of the spinal trigeminal nucleus toward and in the spinal trigeminal tract	Skofitsch et al. 1985
6 Neonatal Sprague-Dawley rats; 6 male adult rats (250 to 300 g)	50 mg/kg Capsaicin in 10% ethanol, 10% Tween 80, and 80% saline	s.c. injection on day 2 of life	Immunocytochemistry revealed that, compared to neonatal (8 rats) and adult (6 rats) control groups, practically no corticotropin-releasing factor–like immunoreactivity in regions of CNS rich in primary sensory neurons. No effect on immunoreactivity of higher areas of brain that do not contain sensory neurons	Skofitsch et al. 1984

(Continued)

TABLE 13
Effects of Capsaicin on the nervous system *(Continued)*

Animals	Test substance	Procedure	Results	References
Sprague-Dawley rats (males and females 2, 10, or 20 days old; number not stated)	50 mg/kg Capsaicin	s.c. injection.	Substance P immunoreactivity studied by applying a mouse x rat monoclonal antibody; results were a long-lasting loss of substance P immunoreactive material in fibers of primary sensory neurons in the spinal cord and medulla oblongata	Cuello et al. 1981
20 Neonatal Sprague-Dawley rats (males and females)	50 mg/kg Capsaicin in 10% ethanol and 10% Tween 80 in isotonic saline	s.c. injection	Radioimmunoassay procedure revealed that, compared to control group of 14 rats, Capsaicin treated rats had a statistically significant reduction in concentration of immunoreactive substance P (55% decrease; $p < .02$) and immunoreactive somatostatin (84% decrease; $p < .01$) in the sciatic nerve	Chad et al. 1983
18 Sprague-Dawley rat pups; 5 adult rats (weights not stated)	50 mg/kg Capsaicin (1.5% in 10% alcohol, 10% Tween 80, and 80% saline)	s.c. injection on postnatal day 1	In rat pups, neuronal apoptosis and mitochondrial swelling in trigeminal ganglion (also DNA fragmentation) identified by light and electron microscopy of tissues. Mitochondrial swelling, but no neuronal apoptosis in trigeminal ganglia from adult rats. Authors suggested an apoptotic mechanism involved in Capsaicin-induced primary neuronal death	Sugimoto et al. 1998
18 Neonatal male Sprague-Dawley rats	50 mg/kg Capsaicin in10% Tween 80 and 10% absolute ethanol in Gey's balanced solution	s.c. injection	Compared to vehicle controls, 92.4% decrease in calcitonin gene–related peptide imnunoreactive, unmyelinated sensory afferent fibers in airway epithelium, vascular smooth muscle, and perivascular adventitial layer of lung tissue	Davies et al. 1994

(Continued on next page)

TABLE 13
Effects of Capsaicin on the nervous system *(Continued)*

Animals	Test substance	Procedure	Results	References
Neonatal Sabra rats (number not stated)	50 mg/kg Capsaicin in 10% ethanol and 10% Tween 80 in isotonic saline	s.c. injection	Electron microscopy of tissues revealed that, compared to control rats, the largest saphenous nerve axons (diameter = 1.6 to 1.7 μ) were absent in treated rats	Scadding, 1980
8 rat pups (strain and weights not stated)	50 mg/kg Capsaicin in vehicle consisting of 10% ethanol, 10% Tween 80, and 80% saline	s.c. injection at back of neck	Compared to 8 control pups, significant decrease in brain and olfactory bulb weights ($p < .005$)	Pérez et al. 1991
Neonatal rats, mice, and dogs (number, strains/breed, and weights not stated)	Capsaicin in 10% ethanol and 10% Tween 80 in isotonic saline	s.c. injection of 50 mg/kg on day 2 of life.	Axon degeneration in medulla oblongata and spinal cord demonstrated in serial frozen sections, using silver impregnation techniques; selective degeneration of small to medium-sized primary sensory neurons in treatment animals	Jancsó et al. 1985
5 Adult male Hartley guinea pigs (250 to 400 g)	1, 5, 50, 200, 200, 500, and 500 mg/kg Capsaicin (solvent not indicated)	consecutive daily doses (s.c. injection) on days 1 to 7	Significant depletion of substance P from dorsal root ganglia, and nonsignificant, slight decrease in substance P in dorsal cord	Buck et al. 1981
6 male, inbred Wistar-Furth rats (average body weight = 250 g)	65% Capsaicin (in saline/ethanol vehicle)	i.p. injection of 12.5 and 2.5 mg/kg on days 1 and 2, followed by dose of 50 mg/kg for 2 days	Eye-wipe response taken as index of destruction of small trigeminal afferents; animals failed to wipe ammonium chloride from their eyes, indicating damage to small trigeminal afferent	Bernstein 1996
Neonatal Wistar rats	50 (55 rats) or 100 mg/kg (40 rats) Capsaicin mixture (1.5% *w*/*v* Capsaicin, 10 % *v*/*v* ethanol, and 10% *v*/*v* Tween 80 in 0.9% *w*/*v* saline)	i.p. injection on days 2 and 3 of life	Compared to controls (25 rats), doses of 50 and 100 mg caused a reduction in the number of calcitonin gene–related peptide–immunoreactive cells (in sensory neurons projecting to viscera or skin) to 47% and 39%, respectively	Kashiba et al. 1990

(Continued)

TABLE 13
Effects of Capsaicin on the nervous system *(Continued)*

Animals	Test substance	Procedure	Results	References
Female Porton rats (6 to 8 animals; weights = 200 ± 10 g)	30 μg/μl Capsaicin per side, in DMSO diluted with water	injected bilaterally into the substantia nigra reticulata	Compared to DMSO-treated control rats, substance P concentrations in the substantia nigra remained unchanged, but 5-hydroxytryptamine concentrations were significantly lower on days 1 and 3 after surgery	Dawbarn et al. 1981
Neonatal CBA mice (number not stated)	50 mg/kg Capsaicin mixture in DMSO diluted with water	i.p. injection on days 2 and 5 of life	Electron microscopy revealed active degeneration of large diameter axons (1.1 to 1.6 μm) of sural nerve	Scadding 1980
Neonatal female Balb/c mice	50 mg/kg Capsaicin in 1:18 solution of Tween 80:95% ethanol:phosphate-buffered saline	i.p. injection on postnatal days 2 to 3	Denervation of neuropeptide (tachykinin) containing sensory C fibers.	Veronesi et al. 1998

and untreated rats served as controls. The animals were killed at 10, 20, 30, and 60 min post injection, after which adrenal glands were rapidly dissected out and frozen sections prepared. The expression of c-*fos* and c-*jun* mRNA in the adrenal gland was studied using in situ hybridization histochemistry. The c-*fos* and c-*jun* genes encode DNA-binding transcription factors whose protein products interact with specific nucleotide sequence elements in gene promoter regions, thereby regulating mRNA synthesis. Concentrations of c-*fos* and c-*jun* mRNAs in the adrenal gland increased after dosing with Capsaicin.

Kashiba et al. (1997) studied the effects of systemically administered Capsaicin (mixture) on expression of the following receptor family genes in dorsal root ganglion neurons of male Wistar rats: β-preprotachykinin, γ-preprotachykinin, somatostatin, calcitonin gene–related peptide, vasoactive intestinal polypeptide, galanin, neuropeptide Y, and neurotrophin receptor family (trkA, trkB, trkC) genes. The study was conducted using 17 adult male Wistar rats (8 to 9 weeks old), and the Capsaicin mixture consisted of the following: Capsaicin (1.5% *w*/*v*) with ethanol (10% *v*/*v*) and Tween 80 (10% *v*/*v*) in saline (0.85% *w*/*v*). Capsaicin (950 mg) was injected s.c. into the dorsum (at cervical and upper thoracic levels) over a period of 5 days. Twelve control rats were dosed with vehicle only according to the same procedure. The animals were killed 6 days after the last administered dose.

The number of neurons in which β/γ-preprotachykinin and calcitonin gene–related peptide messenger RNAs were expressed decreased to approximately 50% and 70% of control values, respectively, at 6 days after the end of Capsaicin dosing. At the same time, the number of neurons expressing trkA messenger RNA decreased to approximately 70% of the control level. However, the number of neurons expressing trkB and trkC messenger RNAs was not affected. The expression of vasoactive intestinal polypeptide and galanin messenger RNAs, but not neuropeptide Y messenger RNA, was noted in approximately 10% of dorsal root ganglion neurons after Capsaicin dosing. Systemic Capsaicin administration had no effect on the expression of somatostatin messenger RNA. The authors suggested that the expression of β/γ-preprotachykinin, calcitonin gene–related peptide, and trkA messenger RNAs is depressed after systemic administration of Capsaicin to adult rats. Additionally, they suggested that dosing with Capsaicin induces expression of vasoactive intestinal peptide and galanin messenger RNAs in sensory neurons (Kashiba et al. 1997).

Effects on DNA/RNA Synthesis

Nagabhushan and Bhide (1985) evaluated the effect of Capsaicin on testicular DNA biosynthesis using four groups of six Swiss mice (ages and weights not stated). The two test groups were injected i.p. (single dose) with Capsaicin at doses of 1.8 and 7.5 mg/kg, respectively. The two control groups were injected with DMSO (0.1 ml) and distilled water (0.1 ml), respectively. Compared to DMSO controls, Capsaicin significantly inhibited ($p < .05$) DNA synthesis at both doses.

TABLE 14
Capsaicin genotoxicity studies

Strain/cell type	Test substance	Procedure	Results	References
S. typhimurium TA97, TA98, TA100, and TA102	Capsaicin in DMSO at 10 mg/ml	Ames plate incorporation assay	Low level mutagenicity in TA98; detection limited by Capsaicin toxicity	Toth et al. 1984
S. typhimurium TA98, TA100, TA1535, TA1537, and TA1538	Capsaicin (no solvent indicated) at 0.04 mg/plate	Ames assay with and without metabolic activation	Mutagenic with metabolic activation in TA98, TA100, and TA1535; not mutagenic without metabolic activation for all strains	Nagabhushan and Bhide 1985
S. typhimurium TA98, TA100, TA1535, and TA1538	Capsaicin in DMSO at 0.5 to 5000 μg/plate	Ames assay with and without metabolic activation	No increased reversion rate with or without metabolic activation over entire concentration range	Buchanan et al. 1981
S. typhimurium TA98, TA100, TA1535, TA1537, and TA1538	Capsaicin (no solvent indicated) at 19.5 to 2500 μg/plate	Ames assay with and without metabolic activation	Not mutagenic (with or without metabolic activation) in any of the strains tested	Hamid et al. 1989
S. typhimurium TA100	Capsaicin in DMSO at 20, 30, and 40 μg/plate or in acetone at 10 to 40 μg/plate	Modified Ames assay with and without metabolic activation	Weak mutagenic response, with metabolic activation using 0.5 ml S9, at doses of 20, 30, and 40 μg/plate, but with a dose response; not mutagenic with 0.25 ml S9; not mutagenic with the acetone vehicle; not mutagenic in the absence of metabolic activation	Azizan and Blevins 1995
Bone marrow cells from male Swiss mice	Capsaicin in DMSO at i.p. doses of 1.8 and 7.5 mg/kg	Micronucleus test	Statistically significant increase ($p < .05$) in the incidence of MN-PCE at 7.5 mg/kg, but not 1.8 mg/kg	Nagabushan and Bhide 1985
Bone marrow cells from male NIH mice	Capsaicin in DMSO at i.p. doses of 1.46 and 1.94 mg/kg (latter dose was the maximum sublethal dose in protocol, corresponding to 1/15 of LD_{50})	Micronucleus test	Animals dosed with 1.46 mg/kg had a statistically significant increase in the number of peripheral blood cells with micronuclei, only at the last day of sampling. Significant, constant increase in the frequency of micronucleated cells in animals dosed with 1.94 mg/kg	Arceo et al. 1995
Bone marrow cells from Swiss Webster albino mice	Capsaicin in DMSO at i.p. doses of 1.22 mg/kg	Micronucleus test, with metabolic activation	Not mutagenic	Villaseñor et al. 1993

(Continued)

TABLE 14
Capsaicin genotoxicity studies *(Continued)*

Strain/cell type	Test substance	Procedure	Results	References
Lymphocytes from human blood	Capsaicin in DMSO at 10 to 200 μM	Cytokinesis-block human lymphocyte micronucleus assay with and without metabolic activation	Micronuclei formed in a dose-dependent manner in the cytokinesis-blocked lymphocytes, with and without metabolic activation, but was more evident in the absence of metabolic activation	Marques et al. 2002
Bone marrow cells from male NIH mice	Capsaicin in DMSO at 10 to 200 μM	Sister chromatid exchange assay	In 1.94 mg/kg dose group, moderate, but significant, increase in frequency of sister-chromatid exchanges, but within control range in group dosed with 1.46 mg/kg	Arceo et al. 1995
Lymphocytes from human blood	Capsaicin in DMSO at 10 to 200 μM	Sister-chromatid exchange assay with and without metabolic activation	Sister-chromatid changes induced in a dose-dependent manner with and without metabolic activation, but more evident without metabolic activation	Marques et al. 2002
V79 Chinese hamster cells	Capsaicin in DMSO at 0.04 mg/ml	Assay for induction of 8-azaguanine–resistant mutants with and without metabolic activation	No induction of resistant colonies	Nagabhushan and Bhide 1985
V79 Chinese hamster cells (hepatocytes)	Capsaicin at 1 to 10 μg/ml	Mutagenicity (ouabain and 6-thioguanine resistance)	Mutagenic	Lawson and Gannett 1989
Bone marrow cells from Swiss albino inbred mice	Capsaicin in vehicle containing Tween 80 (10%), ethanol (10%), and physiological saline, at a 1:1:8 ratio at i.p. doses of 0.42, 1.68, or 3.36 mg/kg followed by cyclophosphamide	Chromosome aberrations assay	Weak clastogenic effects in animals dosed with Capsaicin alone and in the vehicle control group; Capsaicin inhibited cyclophosphamide-induced chromosomal aberrations	De et al. 1995
		DNA damage assay	Capsaicin alone had no significant effect on DNA breakage. DNA breakage increased with increasing doses of cyclophosphamide	De et al. 1995

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TABLE 14
Capsaicin genotoxicity studies

Strain/cell type	Test substance	Procedure	Results	References
Human SHSY-5Y neuro blastoma cells	Capsaicin in 50:50 aqueous solution of ethanol and 0.9% sterile sodium chloride at 25, 50, 75, and 100 mM	Comet assay—extent of individual cell nucleic DNA damage quantified by measuring length of stained DNA and the tail moment.	Treated cells had elongated comet in most of the cells. Frequencies of comet and tail moments of cells exposed to Capsaicin significantly greater, compared to controls, at all test concentrations	Richeux et al. 1999
Human SHSY-5Y neuroblastoma cells	Capsaicin in 50:50 aqueous solution of ethanol and 0.9% sterile sodium chloride at 25, 50, 60, 75, and 100 μM	DNA fragmentation assay	For cells attached to the cell surface, DNA fragmentation noted at all test concentrations	Richeux et al. 1999
Calf thymus and plasma DNA	Capsaicin (1 mM)	DNA damage assay	Capsaicin induced strand breaks in calf thymus and plasma DNA in the presence of Cu(II). After 6 hours of incubation with Capsaicin, DNA degradation was <2%. DNA strand breakage was mediated by reactive oxygen species, especially the hydroxyl radical	Singh et al. 2001
Groups of adult male and female Swiss albino mice	Capsaicin in DMSO given i.p. at doses up to 1.6 mg/kg day^{-1}	Males dosed for 5 consecutive days then mated with adult virgin females for 8 weeks	The pregnancy index, mean number of implants or live embryos per male, mean number of post-implantation deaths per male, and dominant lethal mutations in male germ cells unaffected by Capsaicin	Muralidhara and Narasimhamurthy 1988

In a study by Matucci-Cerinic et al. (1990), the effects of Capsaicin on the following processes in rheumatoid arthritis synoviocytes were evaluated in vitro: DNA synthesis, RNA synthesis, collagenase release, and prostaglandin (PGE_2) synthesis. DNA and RNA effects will be summarized in this section. Effects on collagenase release and PGE_2 synthesis are summarized in the section "Biological Properties" earlier in the report. Synovial tissue was obtained from rheumatoid arthritis patients who were undergoing reconstructive joint surgery. The mitogenic effect of Capsaicin was measured using [^{3}H]thymidine as a precursor for DNA. Regarding the study of DNA synthesis, cell proliferation was analyzed by measuring thymidine incorporation into synoviocytes in the presence of increasing concentrations of Capsaicin (10^{-8} to 10^{-4} mol/L). RNA synthesis was evaluated by monitoring increases in [^{3}H]uridine incorporation into confluent synovial cells.

Compared to baseline controls, thymidine incorporation into synoviocytes was stimulated by Capsaicin at a concentration of 10^{-6} mol/L. The maximal significant increase ($p < .05$) in thymidine incorporation was noted at this concentration.

Compared to the control, Capsaicin concentrations of 10^{-8}, 10^{-4}, and 10^{-3}mol/L had no significant influence on DNA synthesis (i.e., no significant increase in thymidine incorporation). These data suggest that Capsaicin can induce cell proliferation at fairly low concentrations (10^{-6} mol/L), while higher concentrations restore cellular DNA synthesis to control levels. In the evaluation of RNA synthesis, Capsaicin did not induce a statistically significant change in uridine incorporation at any of the concentrations tested, although the data were not shown (Matucci-Cerinic et al. 1990).

Effect on Mitosis

Godlewski and Kozakiewicz (1999) evaluated the effects of Freund's complete adjuvant, Capsaicin (in ethanol, Tween 80, and saline; concentration not stated), and solvent on the frequency of mitotic cells, the frequency of cells in early G1 (PCNA [proliferating cell nuclear antigen] positive) in the spinous layer, and the frequency of PCNA-positive cells in the basal layer of the gingival epithelium using male Lewis rats. Saline (0.9%) served as the control. The four groups of 10 rats (control group included) that were used ranged in weight from 220 to 300 g. Each group received injections (100-μl aliquots) into the gingival muscosa on the right side of the mandible. The animals were killed at 8 h post injection. At 2 h before the animals were killed, colchicine was injected i.p. at 100 mg/kg to induce mitotic arrest. Gingival samples were obtained from both sides of the mandible (injection and contralateral side). The following cell frequencies were determined: Mitotic cell and PCNA-positive cell frequencies were determined from 500 successive basal layer and basal + spinous layer keratinocytes, respectively.

Up to 95% of basal layer keratinocytes was PCNA positive, and the immunostaining was strong and uniformly distributed. Immunostaining in the spinous layer (less intense, compared to basal layer) was diffusely distributed in the nuclei and rarely appeared as scattered grains. The results for Capsaicin and the saline control were reported as follows: frequency of mitotic cells (8.75 ± 5.12% [saline control]; 6.32 ± 2.45% [Capsaicin]); frequency of cells in early G1 (PCNA positive) in spinous layer (66.4 ± 3.07% [saline control]; 52.9 ± 21.28% [Capsaicin]), and frequency of PCNA-positive cells in basal layer (96.55 ± 3.07% [saline]; 94.55 ± 4.71% [Capsaicin]). The following results were reported after the administration of Freund's complete adjuvant: frequency of mitotic cells (4.21 ± 3.04%), frequency of cells in early G1 (PCNA positive) in spinous layer (51.2 ± 14.06%), and frequency of PCNA-positive cells in basal layer (96.65 ± 2.42%). The analysis of data included distribution tests (χ^2 and Kolmogrov-Smirnoff), significance test of the difference between groups (normal distribution) with student's t test, and χ^2 test for comparison between PCNA-positive frequency and mitotic cell rate. Compared to the saline control, significant decrease in the frequency of mitotic cells and PCNA positive cells were noted after Capsaicin administration as well as after the administration of Freund's complete adjuvant (Godlewski and Kozakiewicz 1999).

CARCINOGENICITY

Studies on the carcinogenicity/tumorigenicity of Capsaicin are summarized in Table 15.

Toth et al. (1984) evaluated the tumorigenicity of Capsaicin using six groups of Swiss albino mice (6 weeks old; four males, four females/group). Five groups were fed Capsaicin (in a powdered diet) at one of the following concentrations for 35 days: 1% (average daily intake of Capsaicin = 26 mg [females] and 30 mg [males]), 0.5% (average daily intake = 16.5 mg [females] and 22 mg [males]), 0.25% (average daily intake = 9.25 mg [females] and 9.75 mg [males]), 0.125% (average daily intake = 4.5 mg [females] and 4.8 mg [males]), and 0.0625% (average daily intake = 2.37 mg [females] and 3.18 mg [males]). The untreated control group consisted of 100 male and 100 female mice that were observed throughout their life. At the end of the 35-day feeding period, the animals were either allowed to die or were killed (when moribund) with ether. Complete necropsy was performed on all animals. All organs were examined microscopically.

Compared to the untreated control group, Capsaicin in the diet had no effect on survival. Of the five groups fed Capsaicin in the diet, four mice developed adenocarcinomas of the duodenum (combined tumor incidence = 10%, $p < .004$) at 72, 91, 100, and 126 weeks of age, respectively. The distribution of these tumors in the five treatment groups was as follows: 0.5% Capsaicin (one tumor, male mouse), 0.25% Capsaicin (one tumor, male mouse), 0.125% Capsaicin (one tumor, male mouse), and 0.0625% (one tumor, female mouse). Duodenal tumors were not observed in animals fed 1.0% Capsaicin in the diet or in the untreated control group. Other tumors observed were as follows: females dosed with 1% Capsaicin (one adenocarcinoma of the breast, one lung adenoma, and two malignant lymphomas [lymphocytic]); males dosed with 1% Capsaicin (two lung adenomas); females dosed with 0.5% Capsaicin (one lung adenoma, one lung adenocarcinoma, one breast adenocarcinoma, and one uterine angioma); and males dosed with 0.5% Capsaicin (one lung adenoma). Tumors were also observed in 0.0625%, 0.125%, and 0.25% Capsaicin dose groups. Data for the untreated control group (100 female and 100 male Swiss albino mice) were reported in a study by Toth et al. (1980). The tumor incidence in control animals was greatest in the liver (26%—males and 25%—females). It was concluded that Capsaicin administered at concentrations of 0.5%, 0.25%, 0.125%, and 0.0625% in a powdered diet induced tumors of the duodenum (Toth et al. 1984).

Toth and Gannett (1992) evaluated the carcinogenicity of Capsaicin using 100 Swiss albino mice (50 males, 50 females; 6 weeks old). Capsaicin was defined as follows: 65% Capsaicin, 31% dihydrocapsaicin, 0.9% nordihydrocapsaicin, 1% homocapsaicin, 0.6% homodihydrocapsaicin, 0.5% norcapsaicin, and 0.3% nornorcapsaicin. The animals were fed Capsaicin (0.03125%) in the diet ad libitum for life. The untreated

TABLE 15
Carcinogenicity/tumorigenicity of Capsaicin

Animals tested	Test substance	Procedure	Results	References
Six groups of Swiss albino mice (6 weeks old; 4 males, 4 females/group)	Capsaicin in a powdered diet at 1% (average daily intake 26 mg [females] and 30 mg [males]); 0.5% (average daily intake 16.5 mg [females] and 22 mg [males]); 0.25% (average daily intake 9.25 mg [females] and 9.75 mg [males]); 0.125% (average daily intake 4.5 mg [females] and 4.8 mg [males]); and 0.0625% (average daily intake 2.37 mg [females] and 3.18 mg [males])	Capsaicin fed daily for 35 days; animals were either allowed to die or were killed with ether when moribund	4 mice developed adenocarcinomas of the duodenum at all but the highest test concentration; no duodenal adenocarcinomas in untreated control group	Toth et al. 1984
Four groups of B6C3F$_1$ mice (50 males, 50 females/group; 6 weeks old)	Mixture of capsaicinoids (64.5% Capsaicin and 32.6% dihydrocapsaicin) at 0.025, 0.083, and 0.25%; effective concentrations of 0.02, 0.05, and 0.16% Capsaicin, respectively	Three groups fed diet with capsaicinoids for 79 weeks, followed by feeding with basal diet for 4 weeks; control group fed basal diet only; animals were allowed to die or were killed with ether when moribund	Number of tumor-bearing mice significantly lower (p <.02) in 0.25% dose group (females only), but not in other dose groups; dose-dependent decrease in incidence of hepatocellular carcinomas in males (p < .05) and hepatocellular adenomas + carcinomas in females (p <.05). A relative dose-dependent increase in the incidence of hepatocellular adenomas (males) was noted. Renal cell adenomas in one mouse each from 0.025% and 0.25% capsaicinoids dose groups	Akagi et al. 1998
100 Swiss albino mice (50 males, 50 females; 6 weeks old)	Capsaicin at 0.03125% (contained 65% Capsaicin, 31% dihydrocapsaicin, 0.9% nordihydrocapsaicin, 1% homocapsaicin, 0.6% homodihydrocapsaicin, 0.5% norcapsaicin, and 0.3% nornorcapsaicin	Capsaicin fed in the diet ad libitum for life. Untreated control group (50 males, 50 females) fed semisynthetic diet only for life	Of the 50 females dosed with Capsaicin, 11 (22%; p <.05) had polyploid adenomas (17 tumors total) of the cecum. Seven male rats (14%) collectively had a total of 12 polyploid adenomas of the cecum. Incidences of this tumor in the control group were as follows: 4 females (5 polyploid adenomas) and 4 males (5 benign polyploid adenomas)	Toth and Gannett 1992

control group (50 males, 50 females; 6 weeks old) was fed a semisynthetic diet only for life. Complete necropsy was performed on all animals. Animals treated with Capsaicin lived longer than control animals. Animals that lived the longest (two mice) were 150 weeks old at the time of death. Of the 50 females dosed with Capsaicin, 11 (22%, $p < .05$) had polyploid adenomas (17 tumors total) of the cecum. Seven male rats (14%) collectively had a total of 12 polyploid adenomas of the cecum. Incidences of this tumor in the control group were as follows: four females (five polyploid adenomas) and four males (five benign polyploid adenomas). At microscopic examination, the polyploid adenomas of the cecum were classified as benign. Other tumor types were observed in the test group. However, because of the low incidence of these neoplasms, they were not considered treatment related. It was concluded that Capsaicin induced tumors of the cecum in Swiss albino mice.

Akagi et al. (1998) evaluated the carcinogenicity of a mixture of capsaicinoids (64.5% Capsaicin and 32.6% dihydrocapsaicin) using four groups of $B6C3F_1$ mice (50 males, 50 females/group; 6 weeks old). Three groups of animals were fed 0.025%, 0.083%, and 0.25% capsaicinoids (effective concentrations of 0.02%, 0.05%, and 0.16%, respectively) for 79 weeks, and then basal diet for 4 weeks. Surviving animals were killed (under anesthesia) during week 83. Necropsy was performed and tumor sizes recorded. For hepatocellular tumors, only those greater than 3 mm in diameter were recorded. Necropsy was also performed on mice that died before the end of the study or became moribund. At week 83, survival rates for males and females were 92% to 98% and 92% to 96%, respectively. One female mouse (0.025% capsaicinoids dose group) was killed accidentally at week 9 and two mice with advanced postmortem change were found dead at weeks 50 (control male) and 83 (female, 0.025% capsaicinoids dose group), respectively. These three animals were excluded from the effective number of mice in the study.

Compared to the control group, mean body weights for males were lower, but not significantly different. Mean body weights for females were significantly lower (compared to control) at doses of 0.025%, 0.083%, and 0.25% capsaicinoids. At the conclusion of the study, inhibition of body weight gain was more pronounced in females than in males. For males, the liver/body weight ratio was significantly reduced (compared to control) only in the 0.083% capsaicinoids dose group. However, for females, the liver/body weight ratio was significantly reduced in the 0.025%, 0.083%, and 0.25% dose groups. For male mice, the higher liver/body weight ratio in the control group was associated with the development of large liver tumors.

Compared to the control group, the number of tumor-bearing mice was significantly lower ($p < .02$) in the 0.25% dose group (females only), but not in the other dose groups. A dose-dependent decrease in the incidence of hepatocellular carcinomas in males ($p < .05$) and hepatocellular adenomas plus carcinomas in females ($p < .05$) was noted. The incidence of hepatocellular adenomas (males) showed a relative dose-dependent increase. Of the hepatocellular carcinomas noted in males (control and 0.025% dose groups), one in each group metastasized to the lungs.

In males, renal cell adenomas developed in one mouse each from the 0.025% (2-mm adenoma) and 0.25% (microscopic size adenoma) dose groups. Capsaicinoid-induced damage to the stomach mucosa was not observed in mice of either sex. Tumors of the intestinal tract (few in number) were observed in test and control groups. Additionally, a jejunal adenocarcinoma invading the subserosa was observed in a male mouse in the 0.25% dose group. No differences in other tumor incidences between test and control groups were noted.

The authors concluded that a mixture of capsaicinoids (64.5% Capsaicin and 32.6% dihydrocapsaicin) was not carcinogenic to $B6C3F_1$ mice. Given the renal toxicity (males) noted in the subchronic oral toxicity, dose range–finding study (see the section "Subchronic Oral Toxicity" earlier in the report) and the low incidence of renal cell adenomas (males) in this study, they suggested that further study of the renal carcinogenicity of capsaicinoids is required (Akagi et al. 1998).

Cocarcinogenicity

Jang et al. (1991) evaluated the effect of Capsaicin dosing on animals pretreated with three carcinogens (diethylnitrosamine [DEN], *N*,*N*-dibutylnitrosamine [DBN], and *N*-methylnitrosourea [MNU]) using four groups of male F344 rats (60 animals total; age = 6 weeks old). Two groups (groups 1 and 2) were pretreated with the carcinogens. The rats were initially injected i.p. with a single dose of DEN (100 mg/kg) dissolved in 0.9% sodium chloride solution. MNU (20 mg/kg) in citrate-buffered solution, adjusted to pH 6.0, was injected on days 2, 5, 8, and 11. DBN was administered at a concentration of 0.05% in drinking water during weeks 3 and 4. Group 3 received vehicles (without carcinogens), and group 4 served as the untreated control. Groups 2 and 3 were also fed a solid pellet diet containing 0.01% Capsaicin until week 20. At the conclusion of the study, animals that survived were killed, and major organs removed. Liver sections were subjected to quantitative analysis of glutathione *S*-transferase placental-form (GST-P^+) hepatic foci. Tissues were examined using immunohistochemistry.

Several rats died of pneumonia during the initiation stage and, thus, the survivors were grouped as follows: group 1 (DEN + MNU + DBN; 16 rats), group 2 (DEN + MNU + DBN and Capsaicin; 15 rats), group 3 (Capsaicin and vehicles; 10 rats), and group 4 (untreated control; 10 rats). No significant differences in mean body weight and relative organ weights were noted when test and control groups were compared. Dosing with 0.01% Capsaicin significantly inhibited the induction of GST-P^+ hepatic foci ($p < .05$ [number of foci/cm^2]; $p < .02$ [area of foci, expressed as mm^2/cm^2]) in rats dosed with carcinogens.

Rats treated with a combination of DEN, MNU, and DBN had neoplastic and preneoplastic lesions, induced primarily in the lung, thyroid, kidney, and urinary bladder. Capsaicin significantly decreased the incidence of lung adenoma (from 56% to

13%), but enhanced the incidence of papillary or nodular (PN) hyperplasia of the urinary bladder (from 13% to 53%; $p < .02$). The incidences of other carcinogen-induced lesions of the thyroid and kidney were not significantly different when compared to the group supplemented with Capsaicin. Neoplastic lesions were not induced in the absence of carcinogen pretreatment. The authors concluded that Capsaicin inhibited the development of GST-P$^+$foci in the liver and adenoma of the lung, but enhanced PN hyperplasia of the urinary bladder induced by DEN, MNU, and DBN. Thus, the modifying potential of Capsaicin on carcinogenesis was different, depending on the organ that was examined (Jang et al. 1991).

Two-Stage Carcinogenicity Models

Studies on the activity of Capsaicin in two-stage carcinogenicity models are summarized in Table 16.

Surh and Lee (1995) reported that Capsaicin acted as a tumor promoter for the development of diethylnitrosamine-initiated enzyme-altered foci in the livers of male Sprague-Dawley rats. A single intraperitoneal injection of diethylnitrosamine with subsequent administration of Capsaicin (0.002% in drinking water for 6 weeks) resulted in statistically significant enhancement of formation of glutathione *S*-transferase–positive foci in hepatocytes, as determined by increased numbers, areas, and maximal diameters.

Surh and Lee (1996) proposed a mechanistic basis for the carcinogenicity, cocarcinogenicity, and anticarcinogenicity effects of Capsaicin. They argued that although a minute amount of capsaicin displays few or no deleterious effects, heavy ingestion of the compound has been associated with necrosis, ulceration, and even carcinogenesis. They further noted that Capsaicin is considered to be metabolized by cytochrome P450-dependent mixed-function oxidases to reactive species (Gannett et al. 1990; Lee and Kumar 1980; Miller et al. 1983), which may subsequently interact with target cell DNA in an irreversible manner, thereby triggering mutagenicity and malignant transformation as shown by Surh and Lee (1995). In contrast, they suggested that the ability of Capsaicin to alter carcinogen metabolism would provide rationale for application of this compound to chemoprevention. They suggested that this latter idea is supported by findings of inhibitory effects of Capsaicin on metabolism, DNA binding, mutagenicity and tumorigenicity of certain chemical carcinogens.

Park and Surh (1997) evaluated the tumor initiating and promotional effects of Capsaicin using groups of 30 female ICR mice ($\approx$6 weeks old). Capsaicin (10 μmol) in 0.2 ml acetone was applied topically to shaved skin of the back. Dosing was followed by treatment with the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) for 22 weeks. Compared to acetone-treated controls, no appreciable increases in the incidence and multiplicity of skin papillomas in Capsaicin-treated mice were noted.

In the same study, the tumor promotion activity of Capsaicin was evaluated in a two-stage, mouse skin carcinogenesis assay. After initiation with 7,12-dimethylbenz[α]anthracene (DMBA; 0.2 μmol), Capsaicin (10 μmol) was applied topically twice weekly for 22 weeks. Compared to ICR mice treated with DMBA alone, this dosing procedure resulted in no significant enhancement of skin tumor formation. In another experiment, treatment with Capsaicin 30 min prior to each topical application of TPA lowered the incidence of DMBA-initiated mouse skin tumorigenesis by 22% (week 15). The multiplicity of skin tumors was affected slightly (7.1 ± 8.1 with Capsaicin versus 8.5 ± 7.3 without Capsaicin) (Park and Surh 1997).

In a study by Park et al. (1998), the tumor promotion and copromotion activity of Capsaicin was evaluated using groups of 25 female ICR mice. A single topical application of DMBA (0.2 μmol in 0.2 ml acetone), or the same volume of solvent alone, was made to shaved dorsal skin. At 1 week after initiation, 0.2 ml of acetone, or the same volume of acetone containing either TPA (15 nmol) or Capsaicin (10 μmol), was applied topically twice weekly over a period of 22 weeks. In order to evaluate the copromotion activity of Capsaicin, some of the animals received applications of TPA and Capsaicin simultaneously during the promotion stage. Animals were examined for the presence of papillomas (at least 1 mm in diameter) on a weekly basis, beginning at 1 week after promotion. Epithelial thickening, dermal inflammation, and the presence of atypical cells were evaluated using light microscopy. The ability of Capsaicin to induce epidermal ornithine decarboxylase (ODC) activity was also evaluated as a short-term biochemical measurement of tumor promotion.

At the end of the tumor promotion experiment, only 1 of the 25 mice (4%) that received multiple applications of Capsaicin during the postinitiation stage developed papillomas (average of 0.04 tumor/mouse). This response was not significantly different when compared to the DMBA-acetone group. In mice that received repeated applications of Capsaicin without DMBA initiation, no tumors were observed. In the group of mice initiated with DMBA, followed by TPA promotion, skin tumors were observed in all animals (multiplicity of 9.2 ± 5).

Compared to the DMBA-TPA group, simultaneous application of Capsaicin and TPA after DMBA initiation resulted in a slightly higher tumor incidence and multiplicity during the first 12 weeks of tumor promotion. The tumor response in the DMBA–Capsaicin + TPA group was slightly reduced after the first 12 weeks of tumor promotion. A possible explanation for this finding was that repeated applications of Capsaicin may lead to the regression of tumors that have already formed or the suppression of tumor promotion. Mice promoted with TPA alone continued to develop papillomas.

The results of microscopic examination indicated that repeated topical applications of Capsaicin did not alter the morphological features of untreated skin. However, treatment with TPA resulted in the following striking changes: epithelial hyperplasia, epithelial atypia at the basal and spinous cell layers, and chronic dermal inflammation.

TABLE 16
Capsaicin in two-stage carcinogenicity models

Animals tested	Test substance	Procedure	Results	References
Male Sprague-Dawley rats	Capsaicin (0.002% in drinking water)	Single i.p. injection of diethylnitrosamine, followed by Capsaicin dosing	Statistically significant enhancement of formation of glutathione *S*-transferase positive foci in hepatocytes	Surh and Lee 1995
Groups of 30 female ICR mice (ages $\approx$ 6 weeks old)	Capsaicin in acetone at 10 μmol	Capsaicin in 0.2 ml acetone applied topically to shaved skin of back, followed by treatment with TPA for 22 weeks	No appreciable increases in the incidence and multiplicity of skin papillomas in treated mice	Park and Surh 1997
		After initiation with 7,12-dimethyl-1,2-benzanthracene (DMBA; 0.2 μmol single topical application), Capsaicin applied topically twice weekly for 22 weeks	Compared to mice treated with DMBA alone, no significant enhancement of skin tumor formation	
		After initiation with DMBA, treatment with Capsaicin 30 minutes prior to each topical application of TPA for 22 weeks	Incidence of DMBA-initiated mouse skin tumorigenesis reduced by 22% at week 15 by Capsaicin treatment; multiplicity: 7.1 $\pm$ 8.1 with versus 8.5 $\pm$ 7.3 without Capsaicin	
Groups of 25 female ICR mice (ages or weights not stated)	Capsaicin in acetone at 10 μmol	Single topical application of DMBA (in acetone) or acetone alone applied to shaved dorsal skin. At 1 week post-initiation, acetone or acetone containing TPA (15 nmol) or Capsaicin applied topically twice weekly until end of 22-week study	Only 4% of mice that received multiple applications of Capsaicin during the postinitiation stage developed papillomas (average of 0.04 tumor/mouse); but this incidence was not significantly different compared to the DMBA-acetone group; tumors were not observed in mice that received repeated applications of Capsaicin without DMBA initiation	Park et al. 1998

Compared to the acetone-treated control, topical application of Capsaicin did not cause a significant increase in epidermal ODC activity. However, TPA caused a dramatic increase in enzyme activity. The authors suggested that Capsaicin lacks the ability to promote tumors in mouse skin (Park et al. 1998).

Anticarcinogenicity/Antitumorigenicity

LaHann (1986) evaluated the effect of Capsaicin on croton oil- and TPA-induced tumorigenesis using groups of 20 female CD-1 mice. DMBA (25 nmol) was applied to shaved skin of the back. At week 1 post initiation, the mice were treated twice weekly with TPA or vehicle. One group of 20 animals was also treated with Capsaicin (13.5 μmol/treatment) 1.5 and 24 hours prior to each TPA treatment (10 nmol/treatment). Another group of 20 was also treated with Capsaicin (13.5 μmol/treatment) 30 min prior to each TPA treatment (10 nmol/treatment). The rate and incidence of papillomas were recorded weekly for a total of 20 weeks. Capsaicin did not reduce the incidence or rate of croton oil- or TPA-induced tumor formation. However, Capsaicin reduced the time that was required for TPA-induced tumor development.

In a study by Modly et al. (1986), Capsaicin inhibited the metabolism of benzo(a)pyrene (BP) and the binding of [^{3}H]BP to DNA in Balb/c mouse and human keratinocytes in vitro. The authors suggested that Capsaicin may have anticarcinogenic effects.

Jang et al. (1989) evaluated the inhibitory effect of Capsaicin on lung tumor development in mice using groups of newborn, non-inbred Swiss Webster mice (<24 h old). The treatment groups (with mean weights at third week after birth) were as follows: BP + Capsaicin (34 males, 13.6 ± 1.5 g; 30 females, 13.8 ± 1.5 g); DMBA + Capsaicin (24 males, 12.8 ± 1.1 g; 22 females, 12.2 ± 1.2 g); 1% aqueous gelatin + Capsaicin (28 males, 14.4 ± 4.1 g; 30 females, 13.3 ± 2.9 g); BP alone (35 males, 13.6 ± 1.6 g; 34 females, 13.0 ± 1.2 g); DMBA alone (10 males, 10.1 ± 1.4 g; 17 females, 10.3 ± 2.0 g); and 1% aqueous gelatin alone (25 males, 14.0 ± 3.1 g; 35 females, 13.0 ± 2.0 g). Newborn mice were injected s.c. in scapular region with 0.2 ml of a suspension containing 1 mg of BP and 40 μg of DMBA in 1% aqueous gelatin. Capsaicin was dissolved in DMSO and used at a concentration of 0.01% in pelleted feed. Vehicle-control and Capsaicin test groups consisted of 60 and 58 mice, respectively. Capsaicin control mice were given diets containing 0.01% Capsaicin. The average daily intake of Capsaicin was approximately 13 to18 mg/kg body weight. Weaning rates of newborn mice treated with BP and DMBA were approximately 75%, and were greater than 90% in other experimental groups. At 9 weeks after birth, all of the mice were killed.

In male and female mice dosed with BP, incidences of lung adenoma were 20% and 23.5%, respectively. Compared to these results, dosing with BP + Capsaicin reduced the lung tumor incidence to 8.8% and 13.3% in male and female mice, respectively. In male and female mice that received DMBA alone, the mean numbers of lung adenoma were 25.9 and 21.9, respectively. Following combined treatment with DMBA and Capsaicin, the adenoma numbers in male and female mice were 8.3 and 17.6, respectively. The administration of Capsaicin in the diet after injection with BP and DMBA induced a significant decrease ($p < .05$ and $p < .01$, respectively) in the frequency of tumor-bearing mice (BP treatment group) and a dramatic decrease ($p < .001$) in the mean number of tumors (DMBA treatment group). Thus, in this study, dosing with Capsaicin had an inhibitory effect on the development of BP- and DMBA-induced lung tumors (Jang et al. 1989).

Surh et al. (1995b) studied the antitumorigenicity of Capsaicin using groups of 30 ICR mice (6 to 7 weeks old). Capsaicin (2.5 μmol) in 0.2 ml acetone was applied to shaved dorsal skin of each animal 10 min prior to topical application of vinyl carbamate (5.8 μmol) in 0.2 ml of acetone containing 15% DMSO. The control group received a topical application of vinyl carbamate only (5.8 μmol).

Diminution of vinyl carbamate-induced tumor formation in mouse skin was observed with Capsaicin pretreatment. The topical application of vinyl carbamate alone, followed by promotion with TPA, resulted in an average of 2.6 skin tumors/mouse at 22 weeks. The tumor response was expressed as the average number of papillomas per mouse (multiplicity) and the percentage of tumor-bearing animals (incidence). Pretreatment with Capsaicin lowered the tumor multiplicity by 62%. Furthermore, compared to control animals treated with vinyl carbamate alone, the tumor incidence in Capsaicin-pretreated mice was also reduced. The results of another assay suggested that Capsaicin suppressed vinyl carbamate-induced tumorigenicity, in part, through inhibition of the cytochrome P450 IIE1 isoform that is responsible for activation of the carcinogen, vinyl carbamate (Surh et al. 1995b).

Kim et al. (1997) reported the results of an in vitro study using a Korean human stomach cancer cell line (SNU-1) that indicated that Capsaicin induced apoptotic cell death. They concluded that this effect may have been mediated by the overexpression of p53 and/or c-*myc* genes.

Zhang et al. (1997) evaluated the effects of orally administered Capsaicin (in olive oil) on the in vitro metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in hamster lung and liver microsomes. Male Syrian golden hamsters (140 to 150 g) were used. Capsaicin was administered in doses of 2 and 10 mg/kg body weight, and the animals were killed at 6 and 24 h post administration. Lungs and livers were removed, and microsomes prepared. Assays of NNK metabolism were conducted according to the procedure in the preceding study. The results of this study indicated that a single oral dose of Capsaicin in olive oil altered the metabolism of NNK by hamster lung and liver microsomes in vitro. Inhibition of α-carbon hydroxylation pathways that methylate and pyridyloxobutylate DNA and protein was the most significant effect of Capsaicin administration. Both pathways were inhibited in lung

microsomes; however, only the methylating pathway was inhibited in liver microsomes. Based on the results of this study, it was suggested that any potentially chemo-preventive action of Capsaicin would be greater toward NNK-induced lung tumorigenesis than toward NNK-induced liver tumorigenesis.

Kim et al. (1999) evaluated the effects of Capsaicin dosing on *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG; alkylating agent)–induced c-*jun* proto-oncogene expression using groups of ten male Fisher-344 rats (ages and weights not stated). Specifically, the proto-oncogene c-*jun* encodes for a transcriptional activator, and the regulation of c-*jun* transcripts after dosing with MNNG and Capsaicin was evaluated. Two experiments were conducted. In the first experiment, group 1 rats were fed a Capsaicin-mixed diet (20 ppm) and water (1 ppm) for 13 weeks. The control group (group 2) was fed a normal diet for the same duration. After 6 weeks, five rats from group 1 were dosed orally with MNNG at 200 mg/kg. The remaining five rats in group 1 were not dosed with MNNG. The treatment of group 2 control rats was identical to that of group 1. Animals were killed on the last day of week 13.

The test procedure for the second experiment involved 10 male rats dosed orally with MNNG (single dose = 200 mg/kg). At 1 week post dosing, Capsaicin (20 ppm) was administered to five of the animals for 5 weeks. The remaining five were not dosed with Capsaicin. Animals were killed on the last day of week 6.

MNNG enhanced c-*jun* expression 1.5- to 3.0-fold in tissues of the spleen, heart, and intestine; however, c-*jun* transcripts were reduced in the liver, kidney, stomach, and lung. Capsaicin decreased c-*jun* mRNA levels in most organs, except for the spleen and intestine. The most significant Capsaicin-induced reduction of c-*jun* transcription was noted in the stomach and liver. A similar organ-specific c-*jun* expression pattern (induced by Capsaicin) between rats treated with Capsaicin before and after MNNG dosing (experiments 1 and 2, respectively) was noted. Because MNNG is a powerful carcinogen that is very effective in the induction of c-*jun* mRNA, the authors suggested that Capsaicin uptake in the diet could play a role in the inhibition of tumorigenesis induced by MNNG (Kim et al. 1999).

In a study by Teel and Huynh (1999), two groups of 20 female A/J mice were dosed intragastrically with Capsaicin (5 mg/kg, in corn oil) and corn oil (0.2 ml), respectively, for 3 consecutive days. In both groups, the last dose was followed by a single i.p. injection of NNK. The control group (20 mice) was dosed intragastrically with corn oil (no NNK dosing). The mice were killed 21 weeks after i.p. injection of NNK.

No statistically significant difference in the lung tumor incidence between the control group and either group dosed i.p. with NNK after intragastric dosing with Capsaicin or corn oil was demonstrated. Lung tumor incidences were 17.1 ± 1.8 lung tumors/mouse for the Capsaicin treatment group and 19.6 ± 2 lung tumors/mouse in the corn oil–control group.

The intragastric administration of Capsaicin, however, appeared to have had a small inhibitory effect on the number of NNK-induced lung tumors/mouse. It is important to note that 100% of the mice in both groups dosed i.p. with NNK after intragastric dosing had lung tumors. It was concluded that the intragastric administration of Capsaicin to female A/J mice did not affect the formation of NNK-induced lung tumors in a statistically significant manner (Teel and Huynh 1999).

Han et al. (2002) studied the effects of Capsaicin on TPA-induced activation of NF-κB (nuclear transcription factor kappa-B) and AP-1 (activator protein 1) in order to understand the molecular mechanisms that underlie the anti-tumor promoting property of Capsaicin (in DMSO). HL-60 cell cultures were treated with various concentrations of Capsaicin (up to 10 μM) for 30 min prior to treatment with TPA. The HL-60 cell line is a promyelocytic cell line that is derived from a patient with acute promyelocytic leukemia. These cells lack specific markers for lymphoid cells, but express surface receptors for Fc fragment and complement.

Study results indicate that Capsaicin suppressed TPA-stimulated activation of NF-κB DNA binding in a concentration-dependent manner. Capsaicin also inhibited TPA-induced activation of AP-1. The authors stated that it is likely that the suppression of AP-1 and NF-κB activation by Capsaicin is attributable, in part, to its antioxidant activity and may account for its anti–tumor-promoting effect (Han et al. 2002).

Effects on Tumor Cells

Morré et al. (1995) reported that Capsaicin, in cell cultures, inhibited the growth of HeLa, ovarian carcinoma, mammary adenocarcinoma and HL-60 leukemia cells (all of human origin). Specifically, without added epidermal growth factor (EGF), 50% growth inhibition of HeLa cells was noted in the presence of 1 μM Capsaicin. When the addition of EGF to HeLa cells was preceded by Capsaicin, complete growth inhibition was noted with 1 μM Capsaicin and 50% growth inhibition was noted at a Capsaicin concentration between 10 and 100 nM. Capsaicin was largely without effect in mammary epithelial cells, rat kidney cells, or HL-60 cells induced to differentiate with DMSO.

Additionally, in the presence of Capsaicin, NADH oxidase activity in the plasma membrane was inhibited in HeLa, ovarian carcinoma, mammary adenocarcinoma, and HL-60 cells. Specifically, an ED_{50} of 5 nM was reported for the inhibition of NADH oxidase activity in plasma membranes from HeLa cells. The activity of this enzyme was almost completely inhibited in the presence of 0.1 μM Capsaicin. NADH oxidase activity in plasma membranes of human mammary epithelial, rat liver, normal rat kidney cells or HL-60 cells induced to differentiate with DMSO was not inhibited by Capsaicin (Morré et al. 1995).

According to Morré et al. (1996), Capsaicin inhibited the growth of A-375 melanoma cells (human cell line) in culture.

After 48 h of growth, an EC_{50} of approximately 6 μM was reported. However, growth of primary melanocytes was not inhibited at a concentration of 10 μM Capsaicin, but was inhibited by approximately 20% in the presence of 1 mM Capsaicin. The human melanoma cell line SK-MEL-28 was resistant to Capsaicin.

Pyo et al. (1998) evaluated Capsaicin-induced apoptosis in a Korean human stomach cancer cell line (SNU-1 cells) by monitoring morphological changes that occurred after exposure. Exponentially growing cells were treated with 1 mM Capsaicin for 2, 4, 8, or 16 h. After treatment, cells were washed, fixed, and stained with hemotoxylin and eosin prior to microscopic evaluation. Morphological changes that were typical of apoptotic cells, condensation of the nucleus and membrane blebbing, were observed in cells treated for 16 h. To confirm that Capsaicin induced apoptosis in SNU-1 cells in the preceding experiment, nuclear DNA fragmentation after treatment with Capsaicin was studied. Using gel electrophoresis, DNA ladder formation was noted after 2 h of incubation with Capsaicin.

A third experiment was conducted to identify possible signal transduction pathways that may be involved in Capsaicin-induced apoptosis in SNU-1 cells. Intracellular calcium concentrations [Ca^{2+}] were investigated. Cells were labeled and fluorescence was measured using a spectrophotometer. Over the concentration range of 0 μM to 1 mM Capsaicin, a dose-dependent increase in [Ca^{2+}] was observed. In resting cells, [Ca^{2+}] was 185 nM. However, in cells treated with 1 mM Capsaicin, [Ca^{2+}] was 418 nM. The results of this experiment indicate that Capsaicin induced apoptosis by increasing [Ca^{2+}] in SNU-1 cells.

A fourth experiment was performed to determine whether Capsaicin induces changes in the level of *p*53 mRNA in SNU-1 cells. (The *p*53 tumor suppressor gene plays a pivotal role in the induction of apoptosis.) Compared to control data (not provided), Capsaicin concentrations of 10 and 100 μM increased the level of *p*53 mRNA by a factor of two.

The authors concluded that results from the preceding experiments in this study indicate that Capsaicin induces apoptosis in SNU-1 stomach cancer cells, and that the signal transduction pathways for apoptosis may involve an increase in [Ca^{2+}] and *p*53 transcription (Pyo et al. 1998).

Takahata et al. (1999) reported that Capsaicin inhibited cellular growth and increased the concentration of intracellular calcium in HeLa cells in vitro.

In a study by Jung et al. (2001), the treatment of SK-Hep-1 hepatocarcinoma cell cultures with various concentrations of Capsaicin for 72 h inhibited growth in a concentration-dependent manner ($IC_{50} \approx 90$ μM). This inhibitory effect was due mainly to the induction of apoptosis. Cells treated with the highest concentration of Capsaicin (200 μM) exhibited condensed and fragmented nuclei (i.e., DNA fragmentation), indicative of apoptosis. Other results indicated that apoptosis was induced via activation of the caspase-3 enzyme in SK-Hep-1 cells. Capsaicin reduced the ratio of antiapoptotic Bcl-2 to proapoptotic Bax, increasing caspase-3 activity. Bcl-2 and Bax are two key apoptosis-linked gene products.

Kang et al. (2003) evaluated the effect of Capsaicin in non-transformed (parental MCF10A) and *ras*-transformed (H-*ras* MCF10A) human breast epithelial cells. Cells in six-well plates were treated with Capsaicin (50 μM) for 1 h. Capsaicin selectively induced apoptosis in H-*ras*–transformed cells, but not in their normal cell counterparts.

Effect on UV-Induced Lipid Peroxidation

De and Ghosh (1993) evaluated the protective effect of Capsaicin against UV-induced lipid peroxidation using liposomal preparations. Results indicated that high doses of Capsaicin (0.5 to 1 μg) were more effective in inhibiting UV radiation–induced peroxidation of liposomal membrane lipids than lower doses (0.01 to 0.1 μg). The authors suggested that prooxidant activity was associated with lower doses, whereas, antioxidant activity was associated with higher doses of Capsaicin.

Effect on Cell Growth/Differentiation

Sasajima et al. (1987) evaluated the effect of Capsaicin, the tumor promotor TPA, and other chemicals on growth and differentiation of cultured human esophageal epithelial cells. The following parameters were studied: assessment of morphologic changes and measurement of clonal growth rate (population doublings per day), cross-linked envelope (CLE) formation, and the enzymatic activities of ornithine decarboxylase (ODC) and plasminogen activator (PA).

All chemicals tested reduced the clonal growth rate of human esophageal epithelial cells. The control clonal growth rate in LHC-8 medium was 0.9 ± 0.07 population doublings per day. None of the chemicals induced CLE formation. Treatment of cell cultures with TPA (1 to 100 nM) for 6 h did not cause terminal squamous differentiation of esophageal cells. TPA caused 50% growth inhibition at a concentration of approximately 10 nM and induced cell lysis at a concentration of 1 μM. No effect on spontaneous CLE formation by esophageal cells was noted at 1 to 100 nM TPA. A significant decrease (to approximately 40% of control value; $p < .05$) in ODC activity was induced by TPA. TPA (1 to 100 nM) also induced PA significantly ($p < .05$). Capsaicin (40 μM) resulted in 50% growth inhibition and significantly induced ODC at concentrations of 10 and 100 μM ($p < .05$). PA activity was induced by 100 μM Capsaicin (Sasajima et al. 1987).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Studies on the reproductive and developmental toxicity of Capsaicin are summarized in Table 17.

Kirby et al. (1982) evaluated the teratogenicity of Capsaicin using pregnant Wistar rats. Eight anesthetized dams were injected s.c. with 50 mg/kg Capsaicin (dissolved in a vehicle

TABLE 17
Reproductive and developmental toxicity of Capsaicin

Animals tested	Test substance	Procedure	Results	References
Specific pathogen–free pregnant rats (6 groups of 5)	Capsaicin (in 10% ethanol, 10% Tween 80, and 80% isotonic saline) injected s.c. into thoracic region	Three groups received doses of 50, 100, or 200 mg/kg, every other day, on gestation days 7 through 15; two additional groups injected with 50 mg/kg on gestation day 15	No significant difference in parturition times between test and control females; no external or skeletal malformations observed; compared to controls, no difference in number and weight of pups delivered	Perfumi and Sparapassi 1999
8 pregnant Wistar rats	Capsaicin (in 10% ethanol and 10% Tween in 0.9% sterile saline) injected s.c.	Capsaicin dose of 50 mg/kg in vehicle (3 ml injection volume) on gestation days 14, 16, 18, or 20, or on gestation days 15 and 16 or 16 and 17; dams killed on gestation day 21	No skeletal or soft tissue malformations in treatment groups; compared to control fetuses, fetal weight, the average number of fetuses per dam, and the incidence of resorptions were not significantly different; however, crown-rump length significantly reduced ($p < .05$) only in dams injected on day 18; decrease in fetal spontaneous activity and loss of fetal responsiveness to morphine with injection on days 16 and 17, but not on days 15 and 16	Kirby et al. 1982
Groups of 10 mice (strain not stated)	Capsaicin (in 10% absolute alcohol, 10% Tween 80, and 80% isotonic saline)	Group 1: Test substance (doses of 25, 30, or 50 mg/kg) injected s.c. into neck Group 2: Injection of 25 mg/kg dose through uterine wall, fetal membranes, and dorsal skin	Capsaicin crossed the placenta and exerted a neurotoxic effect (depletion of substance P) on primary afferent terminal field in spinal cord and peripheral nerves of fetuses and pregnant females at each administered dose	Atkinson and Chaggar 1983
Female Sprague-Dawley rats (number not stated)	Capsaicin (in 5% ethanol, 5% Tween, and 90% saline)	50 mg/kg injected s.c. on day 2 of life; control rats injected with solvent only; at 12 to 15 weeks of age, both groups mated with males	Compared to controls, growth rate in litters suckled by dams dosed with Capsaicin as neonates was significantly lower	Traurig et al. 1984
Groups of male mice (8 weeks old; number per group not stated)	Capsaicin (2 mg/ml in DMSO)	Doses (i.p. injections) of 0.4, 0.8, or 1.6 mg/kg body weight day^{-1}, respectively, for 5 consecutive days. Animals killed after last day of dosing and at 1, 3, 5, or 7 weeks after last day of dosing	Compared to control mice (DMSO injected), no significant changes in epididymal or testicular weights; for all dose groups, epididymal sperm counts similar to control values; no treatment-related changes in the % of abnormal sperms	Muralidhara and Narasimhamurthy 1988

(Continued on next page)

TABLE 17
Reproductive and developmental toxicity of Capsaicin *(Continued)*

Animals tested	Test substance	Procedure	Results	References
Male offspring of mated Sprague-Dawley rats (number not stated)	Capsaicin (in absolute alcohol, Tween 80, and saline [1:1:8])	Single dose of Capsaicin (not stated) injected s.c. into each newborn rat during 24-h period after birth. Rats killed at 28 to 30 days post injection to evaluate descent of testes. Normal testicular descent defined as descent of gonad into the scrotum	Abnormal testicular descent noted in 13 of 40 testes (32.5%) examined. No abnormal testicular descent in controls. In another experiment, Capsaicin increased the frequency of cryptorchidism in rats treated prenatally with flutamide, an antiandrogen	Shono and Hutson 1994
Three groups (3 to 7 pups per group) of female offspring of mated Sprague-Dawley rats	Capsaicin at 50 mg/kg in 5% ethanol, 5% Tween 80, and 90% saline	Female pups injected s.c. on day 2 of life; at 12 weeks of age, the estrous cyclicity of Capsaicin-treated and control rats monitored using daily lavage cytology	Compared to controls, plasma progesterone levels in Capsaicin-treated rats were not significantly different on the afternoon after diestrus, proestrus, or estrus	Traurig et al. 1988
12 Female Sprague- Dawley rats		Rats injected s.c.; electrical stimulation of uterine cervix at 1900 to 2000 h on proestrus served as the copulomimetic stimulus to induce pseudopregnancy	Following electrical stimulation, estrous cyclicity ceased in 11 of 12 control rats, and 8 of 9 treated rats developed a decidual response; 10 of 12 treated rats continued to cycle after cervical stimulation ($p < .01$); the mean plasma progesterone concentration in treated rats (9.3 ng/ml) 9 days later was significantly lower ($p < .001$) when compared to controls	
16 female Sprague-Dawley rats	Capsaicin at 50 mg/kg in 5% ethanol, 5% Tween 80, and 90% saline	16 female rats injected s.c. with Capsaicin and caged with males for up to 2 weeks; 23 control rats caged with males for up to 2 weeks	Of the 23 control rats caged with males, 22 became sperm-positive; 21 of the 22 delivered litters; compared to controls, 7 of 16 treated rats became sperm-positive during the 2-week period ($p < .05$), and 2 of the 7 delivered litters; in another group of sperm-positive rats that had been treated on the morning after mating, 4 of 5 delivered litters	Traurig et al. 1988

consisting of 10% ethanol and 10% Tween 80 in 0.9% sterile saline; 3 ml injection volume) on gestation days 14, 16, 18, or 20 according to the following dosing schedule: three dams (day 14), two dams (day 16), two dams (day 18), and one dam (day 20). Five control dams were dosed with vehicle on gestation days 14, 16, 18, and 20. The dams were killed on gestation day 21, and fetuses removed via laparotomy. No signs of skeletal or soft tissue malformations were observed in any of the four groups dosed with Capsaicin. Compared to control fetuses, fetal weight, the average number of fetuses per dam, and the incidence of resorptions were not significantly different. However, crown-rump length was significantly reduced ($p < .05$) only in dams injected with Capsaicin on day 18 of gestation.

In another experiment in the same report, another group of pregnant rats (number not stated) was injected with 50 mg/kg Capsaicin on gestation days 15 and 16 or 16 and 17. After injection on days 16 and 17, a decrease in fetal spontaneous activity and a loss of fetal responsiveness to morphine were noted. These effects were not observed after injection on days 15 and 16 (Kirby et al. 1982).

In a study by Traurig et al. (1984), 50 mg/kg Capsaicin was injected s.c. into female Sprague-Dawley rats on the second day of life. Control females were injected with solvent (5% ethanol, 5% Tween 80, and 90% saline) only. The number of Capsaicin-treated or control rats was not stated. At 12 to 15 weeks of age, both groups were mated with male Sprague-Dawley rats. Females treated with Capsaicin as neonates produced litters that were of normal size (9.4 ± 0.7 pups/litter). Control females produced 11.3 ± 0.7 pups per litter. Mean pup weights between Capsaicin-treated and control groups were also comparable after a normal gestational period.

The growth rate in litters suckled by dams dosed with Capsaicin as neonates was significantly lower when compared to control litters. Statistically significant differences in mean body weight were noted at day 5 of lactation ($p < .01$) and day 12 ($p < .001$). By day 12, mean pup body weight in litters of Capsaicin-treated dams was 81.4% of that reported for control litters. The basis for this finding relates to the observation that the quantity of milk obtained (determined over a 1-h suckling period on day 12 of lactation) from Capsaicin-treated lactating dams was significantly less ($p < .01$) when compared to control dams. The results of another experiment in this study indicated that the substance P immunoreactivity concentration in ventral abdominal skin and mammary nipple skin was markedly depleted (17% of control concentrations) in lactating rats that had been injected with Capsaicin as neonates. Study results suggest that Capsaicin-induced destruction of primary afferent C-fibers partially disrupted the neuroendocrine suckling reflex, resulting in a decrease in lactational performance due to reduced nipple sensitivity (Traurig et al. 1984).

Traurig et al. (1988) evaluated the effect of Capsaicin on reproductive function using young adult Sprague-Dawley rats. Female rats were mated to provide female pups for neonatal Capsaicin treatment. Female pups were injected s.c. with Capsaicin (dose = 50 mg/kg; vehicle = 5% ethanol, 5% Tween 80, 90% saline) on day 2 of life. Other female pups were injected with Capsaicin vehicle or served as untreated controls. Beginning at 12 weeks of age, the estrous cyclicity of Capsaicin-treated and control rats was monitored using daily lavage cytology. The cyclic secretion of progesterone during the estrous cycle in Capsaicin-treated rats was determined by comparing plasma progesterone levels in these animals to those of control rats.

On the afternoon after diestrus, proestrus, or estrus, plasma progesterone levels in rats injected with Capsaicin were not significantly different when compared to controls. Test and control groups were as follows: diestrus (7 controls, 4 Capsaicin-injected), proestrus (3 controls, 3 Capsaicin-injected), and estrus (14 controls, 7 Capsaicin-injected). Compared to diestrus, both control and Capsaicin-injected rats had significantly greater ($p < .01$) progesterone levels during proestrus.

Electrical stimulation of the uterine cervix at 1900 to 2000 h of proestrus was the copulomimetic stimulus used to induce pseudopregnancy in control (12 rats) and Capsaicin-injected rats (12 rats). Following electrical stimulation, estrous cyclicity ceased in 11 of 12 control rats, and 8 of 9 rats tested developed a decidual response. Rats designated for testing for the decidual response were subjected to uterine trauma on the morning of day 5, following cervical stimulation. The purpose of testing for the decidual response was to demonstrate further that pseudopregnancy had been initiated by cervical stimulation. In contrast, 10 of 12 proestrus, Capsaicin-injected rats continued to cycle following cervical stimulation ($p < .01$). The mean plasma progesterone concentration in these rats was 9.3 ng/ml 9 days later, and this value was significantly lower ($p < .001$) when compared to controls (mean plasma progesterone concentration $\approx$51.6 ng/ml). For the remaining two rats, cycling ceased following cervical stimulation and mean values for plasma progesterone were 65.7 and 12.5 ng/ml, respectively. A decidual response was also noted.

In a third experiment, cyclic control and Capsaicin-injected rats were housed with males for up to 2 weeks. Vaginal lavage cytology was examined for the presence of sperm each morning, and sperm-positive females were isolated and observed for parturition. Other sperm-positive Capsaicin-injected rats received progesterone implants on the first day of pregnancy. Trunk blood samples were obtained for progesterone radioimmunoassay, and the uteri were examined for fetal implantation sites on day 9 of pregnancy. Of the 23 control rats caged with males for up to 2 weeks, 22 became sperm positive and 21 delivered litters. Compared to controls, only 7 of 16 Capsaicin-injected rats became sperm positive during the 2-week period ($p < .05$) and 2 of the 7 delivered litters ($p < .05$). For another group of sperm-positive, Capsaicin-injected rats that had been treated with progesterone on the morning after mating, 4 of 5 delivered litters ($p < .05$; compared to 2 of 7 untreated [no progesterone] Capsaicin-injected rats). All control and untreated Capsaicin-injected rats that delivered litters had eight or more viable

pups. Additionally, all pregnant progesterone-treated rats (previously injected with Capsaicin) had nine or more implantation sites.

In another experiment, prolactin release through the hypothalamo-adenohypophyseal axis in Capsaicin-injected rats was tested by administering [α]-methyl-p-tyrosine (αMT). The authors stated that adequate prolactin secretion would stimulate luteal progesterone secretion, thereby inducing pseudopregnancy. αMT stimulates prolactin secretion by blocking catecholamine synthesis, thereby reversing the dopamine-dependent hypothalamic inhibition of adenohypophyseal prolactin release. It was concluded that hypothalamo-adenohypophyseal-ovarian interactions are normal in rats injected with Capsaicin. Other data indicated that adult female rats that had been injected with Capsaicin as neonates had decreased fertility after mating and decreased sensitivity to the induction of pseudopregnancy after electrical stimulation of the cervix. Additionally, corpus luteum progesterone secretion and uterine sensitivity to progesterone secretion were normal in rats injected with Capsaicin. The authors concluded that the results suggest that the reproductive dysfunction noted in rats injected with Capsaicin is due to destruction of the afferent limb of the neuroendocrine copulatory response, which facilitates the luteal progesterone secretion that is necessary for the support of pregnancy or pseudopregnancy (Traurig et al. 1988).

Shono and Hutson (1994) evaluated the effect of Capsaicin on the incidence of cryptorchidism using male offspring of mated Sprague-Dawley rats (number not stated). A single dose of Capsaicin in absolute alcohol, Tween 80, and saline (1:1:8 ratio) was injected s.c. into each newborn rat during the first 24 h after birth. The control group of newborn rats was dosed with vehicle only. At 28 to 30 days post injection, the rats were killed and dissected to evaluate descent of the testes. Normal testicular descent was defined as descent of the gonad into the scrotum. Abnormal testicular descent was not observed in control rats (40 testes analyzed). However, in Capsaicin-treated males, abnormal descent was noted in 13 of 40 testes (32.5%) examined. In another experiment, Capsaicin increased the frequency of cryptorchidism in rats treated prenatally with flutamide, an antiandrogen.

In a study by Perfumi and Sparapassi (1999), Capsaicin (in 10% ethanol, 10% Tween 80, and 80% isotonic saline) was injected s.c. into the thoracic region of anesthetized specific pathogen–free, pregnant rats (six groups of five). Three groups were dosed with 50, 100, or 200 mg/kg Capsaicin, respectively. The fourth group was dosed with the vehicle only. Injections were made every other day on gestation days 7 through 15. Animals were evaluated daily for signs of toxicity. Two additional groups were injected with Capsaicin (50 mg/kg) or the vehicle on gestation day 15. No significant difference in parturition times between control females and test groups was noted. None of the offspring died, neither external nor skeletal malformations were observed, and there were no skin ulcers or cutaneous lesions. Compared to controls, no difference in the number and weight of pups delivered was noted for either dose group. Up to postnatal day 31, test and control offspring followed a similar pattern of weight gain. Results relating to mortalities, clinical signs, and weight gain were included in the section "Short-Term Subcutaneous Toxicity" earlier in the report.

Atkinson and Chaggar (1983) evaluated the ability of Capsaicin (in 10% absolute alcohol, 10% Tween 80, and 80% isotonic saline) to cross the placenta and induce toxic effects using groups of 10 mice (strain not stated) in their 15th day of pregnancy. For one group of animals, the test substance (25, 30, or 50 mg/kg) was injected s.c. into the neck of anesthetized, pregnant females. Another group of anesthetized pregnant mice received an injection of 25 mg/kg Capsaicin through the uterine wall, fetal membranes, and dorsal skin. The abdomen was opened, and the uterus exteriorized prior to injection.

Capsaicin crossed the placenta and exerted a neurotoxic effect (depletion of substance P) on the primary afferent terminal field in the spinal cord and the peripheral nerves of fetuses and pregnant females at each administered dose. The authors stated that immunohistochemical analysis indicated that Capsaicin crossed the placenta in the direction of mother to fetus or fetus to mother (Atkinson and Chaggar 1983).

In a study by Muralidhara and Narasimhamurthy (1988), groups of male mice (8 weeks old; number per group not stated) were injected i.p. with Capsaicin (2 mg/ml in DMSO) at doses of 0.4, 0.8, or 1.6 mg/kg body weight day^{-1}, respectively, for 5 consecutive days. Control mice were dosed with DMSO only (maximum dose = 0.05 ml/mouse/day). The animals were killed 35 days after the last day of dosing. Epididymal and testicular weights were determined, and tissues examined microscopically. Compared to control mice, no significant changes in epididymal or testicular weights were noted in either of the dose groups. In a second experiment, additional mice dosed according to the same procedure were killed by cervical dislocation at 1, 3, 5, or 7 weeks after the last day of dosing, and sperm counts were determined. For all dose groups, epididymal sperm counts were similar to control values. No treatment-related changes in the percentage of abnormal sperms were observed. The authors concluded that Capsaicin did not affect normal sperm production or development.

CLINICAL ASSESSMENT OF SAFETY

Percutaneous Absorption

Fang et al. (1996) evaluated the percutaneous absorption of Capsaicin in vivo using six healthy male volunteers (average age = 24.7) with no existing or previous history of skin disease. Capsaicin (0.075% in Carbopol 940® gel, 2 g of gel) was applied to the volar forearm of each subject. The difference between applied and recovered amounts corresponded to the cumulated absorbed amount. Cutaneous blood flow was measured using laser Doppler flowmetry (LDF), and an evaporimeter was used to determine transepidermal water loss (TEWL). The mean value for Capsaicin flux was $2.71 \pm 0.80\ \mu g/cm^2/h$. Capsaicin induced a significant increase ($p < .05$) in TEWL.

Fang et al. (1997) evaluated the percutaneous absorption and skin irritation potential of Capsaicin using six healthy male volunteers (ages 25 to 27 years). Capsaicin (0.075%) was incorporated into a gel formulation, the composition of which was as follows: propylene glycol (20.0% *w/w*), triethanolamine (2.0%), Carbopol 940® (0.6%), benzalkonium chloride (0.0% to 0.2%), and purified water (added to 100.0%). None of the subjects had any previous or existing history of skin diseases. Using an occlusive dressing procedure, the gel (0.2 g) was spread uniformly over a sheet of cotton cloth. Pieces of cloth (four per subject) containing the gel were applied to both forearms and remained in place for 8 h.

At 8 h, the amount of Capsaicin absorbed was 22.65 ± 3.73 $\mu g/cm^2$ (flux $= 2.28 \pm 0.39$ $\mu g/cm^2/h$). No significant increase ($p > .05$) in Capsaicin flux was observed after the addition of benzalkonium chloride to the gel base, indicating that a concentration of 0.05% benzalkonium chloride may not be high enough to enhance the skin penetration of Capsaicin in human skin in vivo (Fang et al. 1997).

Cardiac and Vascular Effects

Franco-Cereceda et al. (1987) evaluated the effect of Capsaicin on the contractility of electrically driven human atrium using six patients (57 ± 5 years of age) undergoing cardiopulmonary bypass surgery for various reasons. The experimental procedure involved premedication, anesthesia, surgical removal, and postoperative delay. The right atrium was cannulated and connected to a heart-lung machine. Capsaicin (concentrations up to 10^{-5} M) did not induce stimulation of electrically driven atria from any of the patients.

May et al. (1998) noted increased regional cerebral blood flow in human subjects after Capsaicin (0.05 ml of a sterile 0.1% solution) was injected s.c. into the right forehead.

Effect on Histamine Release

Petersen et al. (1997) evaluated the effect of Capsaicin (in isotonic Kreb's Ringer bicarbonate containing 1.0% Tween 20) on histamine release using 14 healthy male volunteers (26 to 50 years old; mean age = 36 years). In the first experiment, the subjects were injected intradermally (into forearm) with 25-μl aliquots of increasing concentrations of Capsaicin (0.3 to 30 μM). The subjects were also injected with vehicle only. Two of eight subjects received doses up to 10 μM Capsaicin only because of the intensity of the pain induced. Six subjects received doses up to 30 μM. Histamine release in human skin was measured using a skin microdialysis technique. An increase in histamine concentrations (25 to 30 nM range) above those noted prior to Capsaicin injection was reported. However, compared to prechallenge histamine concentrations, the increase was not significantly different.

In the second experiment, 2.0% Capsaicin ointment was applied to a 1×1.5-cm area of intact forearm skin on each subject for 2.5 h. The site was covered with an occlusive dressing during the application period. Skin vasodilation was measured using laser Doppler flowmetry, and histamine release was measured using the same skin microdialysis technique. Capsaicin induced a significant increase in skin blood flow (maximum values detected at 60 to 90 min post application). Changes in dialysate histamine concentrations ($\approx$8 nM) were not significantly different from baseline values. The results of another series of experiments indicated that intradermal injection of Capsaicin did not result in substance P release in skin.

The authors concluded that neurogenic inflammation in the skin (in the form of vasodilation and plasma extravasation) does not involve the release of histamine from mast cells or detectable amounts of substance P release from sensory nerves in normal human skin (Petersen et al. 1997).

In a study by Krogstad et al. (1999), Capsaicin was used to investigate the involvement of neurogenic factors in the regulation of histamine release and blood flow in psoriatic plaque. Capsaicin is known to release neuropeptides from thin fibers and, after repeated treatment, to eliminate axon reflex vasodilation. It was hypothesized that if a local neural mechanism were active in the psoriatic plaque, then Capsaicin-induced increases in blood flow and histamine would be anticipated. Furthermore, it was anticipated that a reduction in blood flow would be associated with prolonged Capsaicin treatment. Twenty-two psoriatic patients (mean age = 44) participated in the study. Capsaicin (0.75% to 1% in cream) was applied epicutaneously to lesional and nonlesional skin for 1 or 24 h. Using a microdialysis procedure, two catheters were positioned in psoriatic skin and in adjacent lesion-free skin on each subject. Dialysates were collected for histamine analysis. Skin blood flow and perfusion were evaluated using the ^{133}xenon clearance technique and scanning laser Doppler, respectively.

Capsaicin (1-h application) caused an increase in perfusion, with a minor increase in histamine concentration and release in non-lesional and lesional psoriatic skin. A decrease in perfusion below the basal level was noted in lesional skin after 24 h of Capsaicin treatment. The authors stated that these results are compatible with the view that Capsaicin-sensitive nerves contribute to the increase in blood flow and may induce histamine release in psoriatic skin (Krogstad et al. 1999).

Inhalation Toxicity

Seventeen subjects (seven normal, five mildly asthmatic, and five smokers; mean age = 30) inhaled Capsaicin (2.4×10^{-10} mol and 2.4×10^{-9} mol) while seated in a sealed body plethysmograph. The mass median diameter of the particle size delivered by the nebulizer was 3.5 to 4.0 μm. Capsaicin caused a dose-dependent decrease in specific airway conductance, coughing, and retrosternal discomfort (Fuller et al. 1985).

Maxwell et al. (1987) reported that the inhalation of nebulized 10^{-7} M Capsaicin (particle size = 1 to 2 μm, mass median diameter), in ethanol and 0.9% *w/v* saline, by eight normal subjects (25 to 35 years old) caused an increase in mean inspiratory

flow (mean increase of 35 ± 6%), compared to inhalation of the diluent alone.

Neurotoxicity

Carpenter and Lynn (1981) reported that topical applications of Capsaicin (1% in 85% ethanol; applied at 2-h intervals) to skin of the flexor aspect of the forearm (nine subjects) prevented the development of flare (vasodilation) around a small injury in four subjects. Faint flare was observed in two subjects. Heat pain thresholds were also reduced. These results were attributed to Capsaicin blockage of the effector side of the axon reflex, via depletion of nerve terminals of substance P.

In a study by Simone et al. (1998), Capsaicin (in vehicle containing 7.5% Tween 80 in saline) was injected intradermally into the lateral aspect of the upper arm of each of eight subjects (six males, two females; 24 to 69 years old). Doses of 0.2, 2, or 20 μg in a volume of 20 μl or an equal amount of the vehicle were injected. A maximum of seven injections was made to each shoulder. Prior to each injection, the skin was anesthetized with an intradermal injection of 1% lidocaine (0.3 to 0.5 ml). Skin biopsies were obtained from vehicle- and Capsaicin-treated sites and prepared for immunocytochemistry. Images of protein gene product (PGP) 9.5–immunostained epidermal nerve fibers (ENFs) were analyzed by tracing nerve fibers in three dimensions. Individual ENFs were counted as they passed through the basement membrane. Capsaicin induced a rapid, dose-dependent degeneration of intracutaneous nerve fibers and a dramatic decrease in the sensation of pain produced by heat and mechanical stimuli. At all three doses injected, compared to vehicle-treated skin, Capsaicin caused a significant reduction ($p < .05$) in the mean number of ENFs. Nerve degeneration was evident at 24 h post injection. The magnitude and spatial extent of fiber loss were more pronounced at 72 h post injection.

Nolano et al. (1999) applied 0.075% Capsaicin cream to a 35-cm^2 area on the inner arm or volar forearm of 10 normal subjects (six males, four females; ages = 24 to 69 years). Applications were made four times daily for 3 weeks. Subjects were instructed to vigorously apply a liberal coat of the cream. Skin blisters were made by applying a suction capsule to cleansed, shaved skin. The entire blister roof was frozen and double immunostained to visualize ENFs, using antibodies to the pan-neuronal marker gene product 9.5 (PGP 9.5, rabbit polyclonal) and to Langerhans cells, using antibody to CD-1a (mouse monoclonal).

Topical application of Capsaicin cream resulted in transient sensations of cutaneous burning and itching. A striking loss of nerve fibers, most pronounced in the epidermis, was noted during examination of cutaneous innervation by immunolocalization of the PGP 9.5. Epidermal nerve loss was visualized clearly in blister preparations. The mean decrease in the number of ENFs was statistically significant ($p < .001$). It is important to note that, compared to normal skin, a 74% decrease in the number of PGP 9.5 ENFs ($p < .01$) was noted in blister specimens as early as 3 days after Capsaicin treatment. After 1 week of Capsaicin treatment, most of the ENFs had degenerated and cellular PGP 9.5 immunoreactivity colocalized with CD-1a immunoreactivity in Langerhans cells. Following 3 weeks of Capsaicin treatment, the average number of ENFs in blister preparations decreased (79% decrease; $p < .01$) from 392 ± 33 ENFs/mm^2 in control samples to 83 ± 28 fibers/mm^2 (Nolano et al. 1999).

Skin Irritation

Both provocative and predictive tests on the skin irritation potential of Capsaicin are summarized in Table 18.

Provocative Tests

Helme and McKernan (1984) evaluated flare responses in 77 subjects who received topical applications of Capsaicin. Most of the subjects were hospital inpatients. Twenty microliters of Capsaicin (up to 1 g/L in 70% ethanol) were pipetted onto a piece of blotting paper (1 cm^2), which was applied (affixed to the skin with paper tape) to the following skin sites: 7th cervical spinous process, mid part of the trapezoid ridge (applied bilaterally), antecubital fossae, volar aspect of wrist, medial aspect of knee, and extensor aspect of ankle. Each square was removed after a contact period of 30 min, and the area of each flare was determined. In six subjects, Capsaicin was applied at concentrations ranging from 0.001 to 40 g/L. Additionally, Capsaicin (1 g/L) was applied close to the midline of the forehead (10 subjects). It was stated that the evidence for plasma extravasation was not convincing, and, at most, a slight elevation of the skin to touch was observed. Furthermore, the red reaction at the site of Capsaicin application was frequently darker and more intensely red than the surrounding flare. Frequently, the flare was asymmetric and outlying areas of the flare were 1 to 2 mm dots of erythema.

The relative size of the flare was related to the site of application (e.g., 7th cervical spine and trapezoid ridge > cubital fossa > wrist or ankle). Many subjects had no response at the wrist (60% of subjects) or ankles (75% of subjects). Data on the relationship between flare size and test concentration (presented for three of the six subjects) indicated a threshold zone of concentrations for appearance of the flare. For the trapezoid ridge, the threshold was 10^{-3} to 10^{-2} g/L. The threshold was 10^{-2} to 10^{-1} g/L for the cubital fossa. Once a maximal flare size was reached, further increments in Capsaicin concentration over two orders of magnitude did not change the reaction. For the 10 subjects in which Capsaicin was applied to the midline of the forehead, it was determined that the flare was asymmetric. The authors stated that it was apparent that the territory of the opposite trigeminal nerve was not involved in the response (Helme and McKernan 1984).

Helme and McKernan (1985) applied Capsaicin (1 g/ml in 70% alcohol; volume = 20 μl) to blotting paper that had been placed on the skin of 220 subjects (50 student volunteers; remaining 170 were hospital inpatients and unpaid volunteers) for 30 min. An erythematous area underlying and surrounding the

TABLE 18
Capsaicin skin irritation studies

Subjects tested	Test substance	Procedure	Results	References
Provocative tests				
10 atopic subjects	Capsaicin (up to 0.5% in absolute ethanol)	On day 1, three applications of 0.05% Capsaicin solution (4 h apart) were applied to the whole flexor aspect of forearm. During next 3 days, Capsaicin concentration increased to 0.1%, 0.2%, and 0.5%, respectively. On day 5, intradermal injection of histamine, prostaglandin E_2, (PGE_2) and antigen (mixed grass pollen or dust-mite antigen) at separate sites on forearm	Acute burning sensation and flare reaction after Capsaicin application. No erythema on day of cutaneous challenge. Pretreatment of skin with topical Capsaicin attenuated the flare response to Capsaicin. Capsaicin did not inhibit the immediate flare response to intradermally injected PGE_2, and the effect of Capsaicin on the acute flare induced by antigens was inconsistent and failed to reach statistical significance	McCusker et al. 1989
138 male and female patients with diabetes mellitus	Capsaicin cream (0.075% Capsaicin)	Patients instructed to apply Capsaicin to areas corresponding to the neuropathy 4 times daily, and to return to medical center at weeks 2, 4, 6, and 8 for efficacy and safety evaluations	The 135 reactions in the test group were as follows: burning sensation (87 reactions), coughing-sneezing (16 reactions), rash-erythema (10 reactions), and exposure-irritation to other parts of the body (9 reactions). With the exception of coughing-sneezing, the frequency of other side effects was similar between test and placebo groups	Donofrio et al. 1991
99 cancer patients (18 to 74 years of age) with postsurgical neuropathic pain	Capsaicin cream (0.075% Capsaicin)	Patients instructed to rub cream into painful area four times daily for 8 weeks. During next 8 weeks, patients instructed to apply placebo cream according to same procedure	Compared to placebo cream, Capsaicin cream was associated with more burning and redness of the skin	Ellison et al. 1997
12 patients (mean age 27.5 years) with acute exacerbation of atopic eczema	Capsaicin (0.05%)	Applied for 5 days (3 times/day) to infrascapular region. On day 6 of pretreatment, Capsaicin applied 5 h prior to administration of histamine iontophoresis. Pretreatments were performed in reverse order one week later. Areas of wheal and flare reactions determined at 5, 10, and 20 min after histamine stimulus	Capsaicin pretreatment significantly reduced (compared to no pretreatment) the flare areas in control subjects, but not in atopic eczema patients. Capsaicin also suppressed histamine-induced itching in healthy skin, but had less of an effect in eczema patients	Weisshaar et al. 1998

(Continued on next page)

TABLE 18
Capsaicin skin irritation studies *(Continued)*

Subjects tested	Test substance	Procedure	Results	References
14 patients (59 to 91 years of age) with postherpetic neuralgia	Capsaicin cream (0.025% Capsaicin)	Applied to painful areas of skin 5 times daily for one week. After 1 week, application frequency changed to 3 times daily for 3 weeks	A mild to moderate burning sensation, noted intermittently by one patient at the time of application of the cream, was the only adverse effect that was reported	Bernstein et al. 1987
20 patients with history of notalgia paresthetica	Capsaicin cream (0.025% Capsaicin)	Patients instructed to apply Capsaicin cream to itchy or painful areas 5 times daily for 1 week. Applications made 3 times daily for remaining 3 weeks. After 2-week non-treatment period, a placebo cream was applied for 4 weeks according to same procedure	11 patients reported burning and stinging at application site. Burning and erythema of the back reported by another patient 24 hours after first application. These signs/symptoms were followed by burning and swelling of the face, which resembled an urticarial reaction	Wallengren and Klinker 1995
77 subjects (most of whom were hospital inpatients)	Capsaicin (in ethanol)	61 subjects: Twenty microliters of Capsaicin (up to 1 g/l in 70% ethanol) pipetted onto a piece of blotting paper, which was applied (affixed to skin with paper tape) to the 7th cervical spinous process, mid part of the trapezoid (applied bilaterally), antecubital fossae, volar aspect of wrist, medial aspect of knee, and extensor aspect of ankle for 30 min	The relative size of the flare observed was related to the site of application (7th cervical spine and trapezoid ridge > cubital fossa > wrist or ankle)	Helme and McKernan 1984
		6 subjects: Capsaicin applied at concentrations ranging from 0.001 g/L to 40 g/L to sites as above	Data presented for 3 of 6 subjects indicated a threshold zone of concentrations for appearance of the flare. For the trapezoid ridge, the threshold was 10^{-3} to 10^{-2} g/l, and for the cubital fossa, 10^{-2} to 10^{-1} g/l	
		10 subjects: Capsaicin applied close to midline of forehead	Asymmetric flare observed in the 10 subjects	

30 psoriatic patients and 16 patients with systemic scleroderma	Capsaicin (in 95% ethanol)	Application to forearm at concentrations increasing in geometric progression from 0.125 to 4.0 $\mu g/cm^2$. Time allowed for evaporation of solvent, after which test site covered with parafilm and adhesive tape. The mean Capsaicin response index was calculated as the sum of individual scores in the group divided by the number of subjects tested	$\geq 0.5 \mu g/cm^2$ needed in order to induce erythema in approximately two-thirds of psoriatic patients; erythema was induced at 0.125 or 0.25 $\mu g/cm^2$ in 16 healthy volunteers (controls); distribution of erythema in patients with systemic scleroderma did not differ from controls; reactions of patients with early onset psoriasis was similar to controls	Glinski et al. 1991
220 subjects (50 student volunteers; 170 hospital inpatients and unpaid volunteers)	Capsaicin (1 g/ml in 70% alcohol)	Capsaicin applied to blotting paper that was applied to the skin of the trapezoid ridges, forehead and cubital fossa for 30 min; effects assessed at 1 h	Erythematous area underlying and surrounding application site; threshold concentration for flare was between 0.001 and 0.05 g/L for the trapezoid ridges and forehead and between 0.01 and 0.1 g/L for the cubital fossa	Helme and McKernan 1985
Predictive tests				
8 subjects (ages not stated)	Capsaicin (in ethanol solution)	Intradermal injection of Capsaicin (250 and 500 pmol)	Flare reaction, but no wheal	Barnes et al. 1986
14 healthy male volunteers (mean age 36 years)	2.0% Capsaicin ointment application	For intradermal injection, Capsaicin (in 99% ethanol) diluted to final concentrations up to 30 μM in isotonic Krebs' Ringer bicarbonate containing 1.0% Tween 20 as solvent; 2.0% Capsaicin ointment applied to intact skin of forearm (under occlusive dressing) for 2.5 h; flare reactions evaluated at 5 and 20 min and wheal reactions at 20 min	After injection, dose-related flare reactions of 112 to 866 mm^2 (0.3 to 30 μM Capsaicin) due to histamine release, but no significant wheal reactions observed; topical administration did not result in flare or wheal	Petersen et al. 1997
10 healthy male volunteers (mean age 23 years)	1% Capsaicin in polysorbate 80 mixed with moisturizing cream	1.5 g Capsaicin applied under occlusion to volar forearm in five 15-min applications	8 subjects had visible flare response immediately after the second application; 2 subjects had a flare response after the second application	Mohammadian et al. 1998

(Continued on next page)

TABLE 18
Capsaicin skin irritation studies *(Continued)*

Subjects tested	Test substance	Procedure	Results	References
49 healthy volunteers, 10 of whom were controls (26 females, 23 males; 21 to 45 years old)	Hydrophilic cream containing 0.1% or 1% Capsaicin	39 subjects applied Capsaicin cream to the flexor area of the forearm; ointment base applied to other forearm as control; group 1 (6 subjects; 1 application over 1-day period), group 2 (12 subjects; 4 applications over 2-day period), group 3 (11 subjects; 10 applications over 4-day period), and group 4 (10 subjects; 16 applications over 4-day period); at 5 h after last application, histamine injected intradermally into same application site	Slight redness and a burning sensation at application sites treated with 0.1% and 1.0% Capsaicin. Symptoms persisted for 2 h. Flare responses to histamine injection increased with increasing applications of Capsaicin	Imai 1991
4 subjects (age range: 18 to 30 years)	Aqueous cream preparation containing 1% Capsaicin	Capsaicin cream applied to flexor area of one forearm; 8 applications made over 3 days; histamine acid phosphate (1 mg/ml), synthetic Substance P (SP), pure porcine vasoactive intestinal peptide (VIP), synthetic somatostatin, and synthetic bombesin injected intradermally (0.03 ml) at Capsaicin-treated sites	Erythema and burning sensations decreased with successive applications of Capsaicin. Capsaicin pretreatment significantly reduced or abolished all drug-induced flares and itch, but wheals were not affected	Anand et al. 1983
6 healthy normal volunteers (age range: 25 to 36 years)	Capsaicin (up to 0.5% in absolute ethanol)	On day 1, three applications of 0.05% Capsaicin solution (4 h apart) were applied to the whole flexor aspect of forearm; during next 3 days, Capsaicin concentration increased to 0.1%, 0.2%, and 0.5%, respectively; on day 5, intradermal injections (volume = 50 μl) of two coded solutions, histamine (1 μg) and platelet-activating factor (PAF; 200 ng), to two sites, respectively, were made	Pretreatment of human skin with topical Capsaicin attenuated the flare response to histamine and PAF	McCusker et al. 1989
10 healthy volunteers (5 males, 5 females)	Cream containing 0.075% Capsaicin	Application to middle of volar aspect of one forearm 4 times per day for 6 weeks	Each application caused mild burning pain in all subjects, which lasted 30 to 60 minutes. No visible flare in any of the subjects	Simone and Ochoa 1991

48 volunteers	Capsaicin (0.075% in an 80% ethanol/20% water vehicle)	Filter disk containing Capsaicin applied (with 125 μl of water, under occlusion) to volar forearm skin for 5 min	A burn and erythema response in all subjects. Degree of erythema not determined. After removal of the disk, the burning stopped immediately and the erythema began to fade	Reilly and Green 1999
10 volunteers (ages not stated)	0.025% Capsaicin	Application to dorsum of the hand (proximal to metacarpal phalangeal joint). Subjects instructed not to wash hands for 2 hours after application	Burning or itching (average score = 3; range = 0 to 7)	McCleane and McLaughlin 1998
10 subjects	Capsaicin (in ethanol)	Doses of 0.01, 0.1, 1.0, 10, and 100 μg injected into the forearms (3 injections of each dose into each forearm) of each subject. Dose volume for each injection = 10 μl	Flare response observed. Mean area of flare increased with increasing doses of Capsaicin. Capsaicin (1.0 μg) was lowest dose that produced a flare	Simone et al. 1989
27 healthy volunteers (12 males, 15 females; mean age 33)	Solution containing 1% Capsaicin in 7.5% Tween 80	Intradermal injection of solution (10 μl volume containing 100 μg Capsaicin)	Flare and hyperalgesia reported. Time between Capsaicin injection and detection of flare ranged between 1.9 and 10 s	Serra et al. 1998

application site was observed. The flare was always described as irregular. For some sites (such as the trapezoid ridge), the flare was ovoid, rather than circular. Concentration-dependent effects were assessed at 1 h. The threshold concentration for producing a flare was between 0.001 and 0.05 g/L (trapezoid ridges and forehead) and between 0.01 and 0.1 g/L (cubital fossa). The Capsaicin-induced flare size increased from 0 to nearly maximum over a concentration range of one order of magnitude. After the flare size had reached a maximum in an individual (usually at a concentration between 0.5 and 10 g/L), further increases in test concentration induced little or no further increases in flare size.

Bernstein et al. (1987) reported the results of a clinical trial for the use of topical Capsaicin in the treatment of postherpetic neuralgia (14 postherpetic neuralgia patients; age range = 59 to 91 years). Capsaicin (0.025% cream) was applied to painful areas of skin five times daily for 1 week, after which the frequency and duration of application were changed to three times daily for 3 weeks. All patients completed the study. Following 4 weeks of application, three of the patients classified their pain as 'much better,' three classified their pain as 'better,' and three of the patients indicated that the pain was 'completely gone.' The remaining three patients experienced no pain relief. A mild to moderate burning sensation, noted intermittently by one patient at the time of application of the cream, was the only adverse effect that was reported.

McCusker et al. (1989) evaluated the effect of topical Capsaicin (in absolute ethanol) on the cutaneous responses to inflammatory mediators and antigen using 6 healthy volunteers (25 to 36 years) and 10 atopic subjects (21 to 35 years). The results for normal subjects are included in the next section, "Predictive Tests." On day 1, three applications of 0.05% Capsaicin solution (4 h apart) were applied to the whole flexor aspect of the forearm. During the next 3 days, the Capsaicin concentration was increased to 0.1%, 0.2%, and 0.5%, respectively. On day 5, intradermal injections (50 μl) of the following coded solutions were made: histamine (1 μg), prostaglandin E_2 (PGE_2; 0.5 μg), and mixed grass pollen or dust-mite antigen, 1 or 10 BU (biologic unit; equivalent to 1/1000 histamine-equivalent prick test).

An acute burning sensation and flare reaction were reported after Capsaicin application. Erythema was not observed on the day of cutaneous challenge. Compared to normal subjects treated with vehicle (ethanol), the histamine-flare response in Capsaicin-treated atopic subjects was similar in magnitude. At all time points, Capsaicin significantly reduced the histamine-flare response in atopic subjects, although the inhibition was less marked than that observed in normal subjects. The flare response induced by PGE_2 in Capsaicin-treated atopic subjects was smaller ($p < .05$) than that induced by histamine. Capsaicin did not significantly inhibit the wheal-response to PGE_2 in atopic subjects. The immediate flare response to allergen was reduced in Capsaicin-treated skin; however, the results were not statistically significant. It was concluded that pretreatment of skin with topical Capsaicin attenuated the flare response to Capsaicin, that Capsaicin did not inhibit the immediate flare response to intradermally injected PGE_2, and that the effect of Capsaicin on the acute flare induced by antigens was inconsistent and failed to reach statistical significance (McCusker et al. 1989).

Donofrio et al. (1991) evaluated the treatment of painful diabetic neuropathy with topical Capsaicin in an 8-week study involving 138 male and female patients with stable diabetes mellitus. The patients were instructed to apply 0.075% Capsaicin to areas corresponding to the neuropathy four times daily and to return to the medical center at weeks 2, 4, 6, and 8 for efficacy and safety evaluations. The vehicle-treated group consisted of 139 patients who were instructed to use placebo cream (composition not stated) according to the same procedure. Of the 138 patients in the test group, 38 withdrew from the study. Twenty of the 139 patients in the placebo group withdrew. Eighteen of the 38 patients in the test group withdrew due to treatment side effects, whereas 5 of 20 in the placebo group withdrew for this reason. Overall, 149 of the patients who entered the study (108 test + 41 placebo) reported 184 side effects (135 reactions in test group; 49 in placebo group). The 135 reactions in the test group included burning sensation (87 reactions), coughing-sneezing (16 reactions), rash-erythema (10 reactions), and exposure-irritation to other parts of the body (9 reactions). With the exception of coughing-sneezing, the frequency of other side effects was similar between test and placebo groups. Treatment-related systemic side effects were not observed. The results suggested that topical Capsaicin cream is safe and effective in the treatment of painful diabetic neuropathy.

In a study by Glinski et al. (1991), Capsaicin was applied to the following groups of subjects: 30 patients with psoriasis, 16 patients with systemic scleroderma, and 16 healthy volunteers. The test substance (in 95% ethanol) was applied to the forearm at concentrations increasing in geometric progression from 0.125 to 4.0 $\mu g/cm^2$. Time was allowed for evaporation of the solvent, after which the test site was covered with parafilm and adhesive tape. Test sites were observed every 30 min for 3 h. The lowest concentration of Capsaicin that induced erythema was determined and expressed using the following 6-grade scoring system: 6 = 0.125 μg; 5 = 0.25 μg; 4 = 0.5 μg; 3 = 1 μg; 2 = 2 μg; and 1 = 4 μg. The mean Capsaicin response index was calculated as the sum of individual scores in the group divided by the number of subjects tested.

Capsaicin concentrations of $\geq$0.5 $\mu g/cm^2$ were needed in order to induce erythema in approximately two-thirds of the patients with psoriasis (mean index = 3.64 $\pm$ 1.40). Erythema was induced at lower concentrations of Capsaicin (0.125 or 0.25 $\mu g/cm^2$) in normal subjects. The distribution of erythema in patients with systemic scleroderma did not differ from that of normal controls (mean index = 5.0 $\pm$ 1.15). For patients in whom the age of onset of psoriasis was later than 21 years, larger doses of Capsaicin were required for the induction of erythema (mean index = 2.93 $\pm$ 1.39). The reaction to Capsaicin in patients with early onset psoriasis (mean index = 4.46 $\pm$ 0.88) was similar to that of normal controls (mean index = 5.44). Patients with

widespread psoriatic lesions (>40% of skin surface) appeared to have been unresponsive to low Capsaicin concentrations (mean index = 1.67 ± 0.95) (Glinski et al. 1991).

Wallengren and Klinker (1995) conducted a 10-week study on the effects of Capsaicin cream, involving 20 patients (16 females; mean age = 59 years) with a history of notalgia paresthetica. Symptoms of notalgia paresthetica included intermittent intense itch or pain in a well-defined patch of skin on the back. For the first week of the study, patients were instructed to apply Capsaicin cream (0.025% Capsaicin) to itchy or painful areas five times daily. Applications were to have been made three times daily for the remaining 3 weeks. After a 2-week nontreatment period, a vehicle (placebo) cream was applied over a period of 4 weeks according to the same procedure.

Eleven patients reported burning and stinging (main adverse reaction) at the application site. One patient (diagnosed with cardiac asthma) complained of coughing during the first week, which ceased after only a thin layer of cream was applied. Burning and erythema of the back were reported by another patient 24 h after the first application. These signs/symptoms were followed by burning and swelling of the face, which resembled an urticarial reaction (Wallengren and Klinker 1995).

Ellison et al. (1997) reported the results of a clinical trial for the use of topical Capsaicin cream (0.075% Capsaicin) involving 99 cancer patients (18 to 74 years) with postsurgical neuropathic pain. The patients were instructed to rub the cream into the painful area (on the arm) completely four times daily for a period of 8 weeks. For the next 8 weeks, patients were instructed to apply an identical-appearing placebo cream according to the same procedure. Compared to the placebo cream, the Capsaicin cream was statistically associated with more skin burning and redness and more coughing ($p < .0001$ for each sign/symptom). At the end of 8 weeks, the Capsaicin cream was responsible for substantially more pain relief, compared to the placebo cream.

Weisshaar et al. (1998) evaluated the effect of Capsaicin on histamine-induced itch using 12 patients (mean age 27.5 years) suffering from acute exacerbation of atopic eczema. The history of atopy for these patients ranged from 10 to 23 years (mean of 16.7 years). Twelve healthy volunteers with no personal or family history of atopy served as controls. Capsaicin (0.05%) was applied for 5 days (3 times/day) to the infrascapular region of each subject. On day 6 of pretreatment, Capsaicin was applied 5 h prior to the administration of histamine iontophoresis. The pretreatments were performed in the reverse order 1 week later. The areas of wheal and flare reactions were determined 5, 10, and 20 min after the histamine stimulus by tracing their borders onto acetate sheets. Capsaicin pretreatment significantly reduced (compared to no pretreatment) the flare areas in control subjects, but not in atopic eczema patients. Capsaicin also effectively suppressed histamine-induced itching in healthy skin, but had less of an effect in patients with atopic eczema.

Predictive Tests

Anand et al. (1983) conducted a study in which each of four subjects (18 to 30 years) applied an aqueous cream preparation containing 1% Capsaicin to the flexor area of one forearm. Eight applications were made over a period of 3 days. The control (aqueous cream) was applied to the other forearm according to the same procedure. Erythema and burning sensations decreased with successive applications of Capsaicin. The following drugs were injected intradermally (volume = 0.03 ml) at Capsaicin-treated sites: histamine acid phosphate (1 mg/ml), synthetic substance P (SP), pure porcine vasoactive intestinal peptide (VIP), synthetic somatostatin, and synthetic bombesin.

Wheal and flare responses in normal skin of all subjects were reported after injections of histamine, SP, somatostatin, and VIP. Capsaicin pretreatment significantly reduced or abolished all flares and itch, but wheals were not affected. The complete recovery of flares abolished by Capsaicin was evident anywhere from 3 to 4 weeks (Anand et al. 1983).

McCusker et al. (1989) studied the effect of topical Capsaicin (in absolute ethanol) on the cutaneous responses to inflammatory mediators and antigen using six healthy normal volunteers (25 to 36 years). On day 1, three applications of 0.05% Capsaicin solution (4 h apart) were applied to the whole flexor aspect of the forearm. During the next 3 days, the Capsaicin concentration was increased to 0.1%, 0.2%, and 0.5%, respectively. On day 5, intradermal injections (50 μl) of two coded solutions, histamine (1 μg) and platelet-activating factor (PAF; 200 ng), to two sites, respectively, were made.

An acute burning sensation and a flare reaction were noted after Capsaicin application. Erythema was not observed on the day of cutaneous challenge. Histamine induced a wheal-and-flare response at the vehicle-treated site. Following treatment with Capsaicin, the area of the flare was significantly reduced ($p < .01$), whereas wheal volume was unaffected. Similar reductions in area of the flare induced by PAF were observed in Capsaicin-treated skin; however, there was no effect on the wheal response. Pretreatment of human skin with topical Capsaicin attenuated the flare response to histamine and PAF (McCusker et al. 1989).

Simone et al. (1989) studied cutaneous responses to intradermally injected Capsaicin in 10 subjects. The subjects were injected intradermally with 0.01, 0.1,1.0, 10, and 100 μg of Capsaicin in ethanol vehicle or the vehicle alone. Six injections per subject were made to the forearm (three injections into each forearm), and the dose volume for each injection was 10 μl. Capsaicin dosing resulted in a flare response. The mean area of the flare increased with increasing doses of Capsaicin, with 1.0 μg as the lowest dose that produced a flare that was larger in area than the small area of local redness induced by the vehicle alone. Doses of 10 μg (flare area 20.55 cm^2) and 100 μg (flare area 31 cm^2) produced areas of flare that were significantly different from one another and from the area of the flare that was induced by 1 μg of Capsaicin.

Imai (1991) evaluated the effect of Capsaicin on the cutaneous reaction to histamine using 49 healthy volunteers (26 females, 23 males; 21 to 45 years old). Thirty-nine subjects applied a hydrophilic cream containing 0.1% or 1% Capsaicin to the flexor area of one forearm, and hydrophilic ointment base (control) was applied to the other. The 39 subjects were divided into group 1 (6 subjects; 1 application over 1-day period), group 2 (12 subjects; 4 applications over 2-day period), group 3 (11 subjects; 10 applications over 4-day period), and group 4 (10 subjects; 16 applications over 4-day period). All six subjects in group 1 were tested with 0.1% and 1.0% Capsaicin. In the remaining groups, half of the subjects was tested with 0.1% Capsaicin, and, the remaining half with 1.0% Capsaicin. The remaining 10 subjects served as untreated controls. At 5 h after the last application, histamine was injected intradermally into the same application site.

Slight redness and a burning sensation at Capsaicin application sites (0.1% and 1.0% Capsaicin) were reported by all subjects tested. Symptoms persisted for 2 h. Responses increased after test sites came in contact with warm water. No local reaction was observed after 8 to 10 applications. No remarkable reactions were observed at sites treated with hydrophilic ointment only. Following the application of 1% Capsaicin or after 10 or 16 applications of 0.1% Capsaicin, the area of histamine-induced flare was significantly reduced, compared to the area of the flare observed after treatment with hydrophilic base only. Flare responses to histamine injection increased with increasing applications of Capsaicin. Depending on the concentration of Capsaicin that was tested (0% [base], 0.1%, or 1%), significant differences ($p < .01$) between flare responses were found. However, in relation to the number of applications between the four groups (1, 4, 10, or 16 applications), differences in flare responses were not statistically significant ($p > .05$). The authors concluded that the histamine-induced flare response, as well as itching, were inhibited by Capsaicin, and that these responses (of short duration) were dependent upon the concentration of Capsaicin, rather than the number of applications (Imai 1991).

Simone and Ochoa (1991) conducted a study in which 10 healthy volunteers (5 males, 5 females) applied a cream containing 0.075% Capsaicin to a 4-cm^2 area on the middle of the volar aspect of one forearm. Vehicle cream was applied to the other forearm. Applications were made four times per day for 6 weeks. For the first 2 to 5 weeks, each Capsaicin application caused mild burning pain in all subjects, which lasted 30 to 60 min. With subsequent repeated applications, the burning pain decreased in both magnitude and duration. Application of the vehicle did not result in burning pain or any other sensation. Capsaicin did not induce a visible flare in any of the subjects tested. Additionally, skin temperature readings (using thermography) did not indicate any differences between vehicle-treated, Capsaicin-treated, and untreated adjacent skin throughout the study.

Fang et al. (1997) evaluated the skin irritation potential of Capsaicin using six healthy male volunteers (25 to 27 years of age). Capsaicin (0.075%) was incorporated into a gel formulation, the composition of which was as follows: propylene glycol (20.0% *w/w*), triethanolamine (2.0%), Carbopol 940® (0.6%), benzalkonium chloride (0.0% to 0.2%), and purified water (added to 100.0%). None of the subjects had any previous or existing history of skin diseases. Laser Doppler flowmetry (LDF), transepidermal water loss (TEWL; the rate at which water migrates from the viable dermal tissues and through the layers of the stratum corneum to the external environment), and colorimetry were used to quantify Capsaicin-induced skin erythema and irritation. Using an occlusive dressing procedure, the gel (0.2 g) was spread uniformly over a sheet of cotton cloth. Pieces of cloth (four per subject) containing the gel were applied to both forearms and remained in place for 8 h.

Capsaicin (0.075% in gel)-induced skin irritation was determined using LDF. During the first hour after application, an immediate increase in blood flow was observed. Peak blood flow was also noted during the same period. By 6 h post application, skin vasodilation and irritation were comparable to control (gel formulation only) values. The data presented represent mean values from a total of six experiments. Erythema color was measured using a colorimeter, which records color reflectance three-dimensionally. The degree of erythema was estimated and compared to normal, neighboring skin (color difference determination). As the erythema effect of Capsaicin developed, skin tone became darker and redness increased.

Skin damage was also assessed by evaluating TEWL. Compared to the control (gel formulation only), TEWL values for Capsaicin (in gel) were significantly higher ($p < .05$). At 1 h after removal of the Capsaicin gel, TEWL values leveled off immediately, and, thereafter, the level maintained was described as approximately constant (Fang et al. 1997).

In a study by Petersen et al. (1997), Capsaicin (2.0%) ointment was applied to a 1 × 1.5-cm area of intact forearm skin on each of 14 healthy male volunteers for 2.5 h. The site was covered with an occlusive dressing during the application period. Flare reactions were evaluated at 5 and 20 min, and wheal reactions at 20 min. After intradermal injection of Capsaicin, dose-related flare reactions of 112 to 866 mm^2 (0.3 to 30 μM Capsaicin) were observed.

Mohammadian et al. (1998) studied cutaneous reactions to 1.5 g of a moisturizing cream containing 1% Capsaicin. The cream was applied, under occlusion, to the volar forearm of each of 10 healthy male volunteers (mean age = 23 years) for 15 min and then removed with a swab. Capsaicin was dissolved in polysorbate 80 and mixed with moisturizing cream, yielding a final concentration of 1% Capsaicin. Each experiment involved five 15-min applications. Subjects rated the intensity of pain induced by Capsaicin and mapped the area of visible flare during each nontreatment period. The two subjects who perceived stroking with the swab as nonpainful after the first Capsaicin application did not have a visible flare reaction. However, a flare response was induced in these two subjects after the second application of Capsaicin. The remaining eight subjects all had a

visible flare response immediately after the first application of Capsaicin.

McCleane and McLaughlin (1998) applied a cream containing 0.025% Capsaicin to a 1-square-inch area on the dorsum of the hand, just proximal to the metacarpal phalyngeal joint, of each of 10 volunteers (ages not stated). The subjects were instructed not to wash their hands for 2 h after application, and asked to rate their burning discomfort on a visual analogue scale (0 to 10). Burning or itching (average score = 3; range = 0 to 7), but no other side effects, were reported.

In a study by Serra et al. (1998), 27 healthy human volunteers (12 males, 15 females; 19 to 46 years old [mean age 33 years]) were injected intradermally with a solution containing 1% Capsaicin in 7.5% Tween 80 (10 μl volume containing 100 μg of Capsaicin). The injection site encompassed skin of the anterior aspect of the forearm, midway between the elbow and wrist. A flare and hyperalgesia were reported after injection (number of subjects with reactions not stated), and the time between Capsaicin injection and detection of a flare response ranged between 1.9 and 10 seconds.

Reilly and Green (1999) applied a filter disk containing 0.075% Capsaicin in a vehicle of 80% ethanol/20% water (with 125 μl of water, under occlusion) for 5 min to the volar forearm skin of 48 volunteers, prior to blistering of the skin at the concentration of Capsaicin that is used in various medical preparations. The application of 0.075% Capsaicin to the skin caused a burn and erythema response in all subjects. Both responses persisted, with little or no change in intensity. The degree of severity of the erythema was not determined, because the bulky presence of the blister cup prevented the use of an erythema meter. After the vacuum was removed, the burning stopped immediately and erythema began to fade. A correlation between the levels of prostaglandin E_2 and interleukin-1α in blister fluids was made ($p < .01$; $n = 19$). The authors noted that these data are consistent with the proinflammatory mediators prostaglandin E_2 and interleukin-1α playing key roles in acute skin responses to mild irritants.

Mucous Membrane Exfoliation

According to Croft et al. (1966), estimation of the DNA content in stomach aspirates collected over a fixed period of time indicates the number of nuclei and, hence, the number of surface epithelial cells of gastric mucosa exfoliated during that period.

Desai et al. (1976) used this relationship to study the effects of Capsaicin on gastric surface epithelial cells. Capsaicin was added to 500 ml of normal saline and slowly infused (5, 7.5, or 10 mg Capsaicin/h) into the stomachs of subjects from each of the following three groups: group 1 (6 males, 2 females), group 2 (8 males), and group 3 (11 males, 1 female). Basal gastric aspiration was determined by slowly infusing 500 ml of normal saline (without Capsaicin). At the end of the 1-h infusion period, gastric aspirates were collected. The DNA content of each sample was measured using diphenylamine reagent. DNA values for gastric aspirates were obtained by comparison with DNA standard solutions that were prepared from highly polymerized calf-thymus DNA. The values for atoms of phosphorus in DNA were obtained using the following formula: 0.4 mg DNA = 0.836 μg DNA-P. Final values were stated as ng atoms DNA-P/min.

Compared to the basal level, no statistically significant difference in mean atoms of DNA-P after intragastric infusion of Capsaicin (5 or 7.5 mg/h) was noted. However, results were statistically significant for 10 mg Capsaicin/h ($p < .02$). In this group, an increase in DNA content of the gastric aspirate was noted in 10 of 12 subjects. For the two remaining groups, increased DNA content of the gastric aspirate was noted in one of eight subjects (5.0 mg Capsaicin dose group) and four of eight subjects (7.5 mg Capsaicin dose group). It was concluded that Capsaicin had a dose-dependent effect on the exfoliation of gastric surface epithelial cells. Exfoliation did not persist during the next hour after Capsaicin infusion was stopped (Desai et al. 1976).

Immune System Effects

Bunker et al. (1991) evaluated the effect of Capsaicin on mast cells in normal human skin using four normal subjects (two men, two women; mean age 31.5 years). Capsaicin (1%) was applied topically to the forearm (stratum corneum denuded). Mast cell degranulation was studied by microscopic examination of skin biopsies. Control skin sites were treated with saline. Both depletion and degranulation of mast cells were observed 6 hours after Capsaicin administration. Compared to control sites treated with saline, the reduction in numbers of mast cells was statistically significant ($p < .05$).

Case Reports

Wilkinson and Moore (1982) reported that Capsicum tinctures (containing a pungent principle, Capsaicin) have stimulatory effects on the growth of hair.

In a report by Szeimies et al. (1994), a topical ointment containing 0.05% Capsaicin was applied for 6 weeks to pruritic areas of skin on a 45-year-old male patient with severe, generalized pruritus. Excellent symptomatic relief was reported. Mild erythema and a transient burning sensation, the only treatment side effects, were reported during the first week of application.

Marciniak et al. (1995) reported that repeated applications of topical Capsaicin (0.025%) to a patient two to three times daily over a 14-day period resulted in the following symptoms among the nursing staff: coughing, shortness of breath, congestion, and rhinitis.

Williams et al. (1995) reported the case of a 31-year-old woman who complained of burning palms after contact with jalapeno peppers. The palms and volar surface of the fingers were erythematous. The authors concluded that Capsaicin is the ingredient in chili peppers that caused these symptoms.

Human Risk Evaluation

An EPA reregistration eligibility document on Capsaicin (oleoresin extract of *Capsicum* red pepper) as an active ingredient in pesticide products is available (EPA 1992). This assessment concluded that the long history of use as a food additive/component without any indication of deleterious health effects suggests that the potential risks to humans from both dietary and occupational exposure are negligible. In the absence of toxicological concerns from ingestion of Capsaicin, EPA waived the requirements for the submission of residue data. However, EPA did conclude that a tolerance exemption under Federal Food, Drug and Cosmetic Act Section 408 will be established for Capsaicin for all currently registered food uses.

SUMMARY—PARTS 1 AND 2

Capsicum-Derived Ingredients

Capsicum Annuum Extract, Capsicum Annuum Resin, Capsicum Annuum Fruit Powder, and Capsicum Frutescens Fruit Extract function as skin-conditioning agents—miscellaneous in cosmetics, although the resin does not appear to be in current use. Capsicum Annuum Fruit Extract functions as an external analgesic, flavoring agent, and fragrance component. Capsicum Frutescens Fruit Extract and Capsicum Frutescens Resin function as an external analgesic and skin-conditioning agent, miscellaneous. The function of Capsicum Frutescens Fruit is not known.

Production of Capsicum Annuum Fruit Extract, Capsicum Frutescens Fruit Extract, or their trade name mixtures involves extraction procedures that may utilize hexane, ethanol, or vegetable oil as solvents. It is likely that each contains the full range of phytocompounds that are found in the native plant (*Capsicum annuum* or *Capsicum frutescens*). Capsicum Annuum Resin or Capsicum Frutescens Resin (clear to dark red) is obtained by solvent extraction of the dried pods of *Capsicum annuum* or *Capsicum frutescens*. The resin may be decolorized following the extraction procedure. The ripe fruits of Capsicum frutescens are ground into a fine powder, and the oleoresin is obtained by distillation of the powder in an appropriate solvent. Information on the methods of production of Capsicum Annuum Extract, Capsicum Annuum Fruit Powder, or Capsicum Frutescens Fruit was not identified.

Capsaicin is the pungent principle in red hot chili peppers. The content of Capsaicin in red chilies (*Capsicum annuum* or*Capsicum frutescens*) varies depending on the variety. Reportedly, the content of Capsaicin in *Capsicum annuum* is 1.67 mg/g dry weight, and, 0.45 mg/g dry weight in *Capsicum frutescens*. According to another source, Capsaicin constitutes 0.03% of the total weight (using thin layer chromatography) of fresh *Capsicum annuum* or 1.27% of the total weight (using gas chromatography). Capsaicin content information provided by the cosmetics industry states that Capsicum Annuum Fruit Extract is standardized to contain 0.5% Capsaicin, and a very low concentration (unspecified) of Capsaicin is said to be present in a trade name mixture for Capsicum Annuum Fruit Extract that reportedly contains Polysorbate 80 (44%) and paprika oleoresin (20%). Capsicum Frutescens Fruit Extract (100%) contains Capsaicin at a concentration of 5%, and may be standardized to contain 0.5% Capsaicin. Furthermore, one unnamed Capsicum Frutescens Fruit Extract trade mixture contains 1% Capsaicin, and traces of Capsaicin are present in another. The latter trade mixture also contains the following preservatives: phenoxyethanol, methylparaben, ethylparaben, butylparaben, and propylparaben. The Capsaicin content of three grades of Capsicum Frutescens Resin is described as follows: 6.2% to 6.7% *w*/*w* Capsaicin, 9.5% to 10.5% *w*/*w* Capsaicin, and 19% to 21% *w*/*w* Capsaicin. Published information indicates that Capsicum Annuum Resin should contain not less than 6% *w*/*w* Capsaicin, and that Capsicum Frutescens Fruit contains Capsaicin at a concentration of 0.42%. Information on the composition of Capsicum Annuum Extract or Capsicum Annuum Fruit Powder was not identified.

Aflatoxin has been detected in samples of red pepper (*Capsicum annuum*) collected in Tokyo, Ethiopia, and Nigeria, and in red pepper (*Capsicum frutescens*) samples from Egypt. The insecticide, fluvalinate has been detected in red pepper samples from India, and *N*-nitroso compounds (*N*-nitrosodimethylamine and *N*-nitrosopyrrolidine) have been detected in chili samples from West Germany.

The UV absorption spectrum for Capsicum Annuum Fruit Extract indicates a small peak at approximately 275 nm, and a gradual increase in absorbance, beginning at approximately 400 nm.

Capsicum Annuum Fruit Extract is used at concentrations up to 1%, Capsicum Annuum Fruit Powder at concentration of 5%, and Capsicum Frutescens Fruit Extract at concentrations up to 1%. According to the cosmetic ingredient chemical description on Capsicum Annuum Fruit Extract, this ingredient is used in cosmetics at concentrations up to 0.05% (as Capsaicin) and a trade name mixture under which it is marketed (concentration of Capsicum Annuum Fruit Extract not stated) is used at concentrations up to 0.2%. The cosmetic ingredient chemical description of Capsicum Frutescens Fruit Extract, indicates that Capsicum Frutescens Fruit Extract is used at concentrations up to 0.3%.

In 1993, the U.S. Food and Drug Administration (FDA) published a final rule indicating that Capsicum and other active ingredients are not generally recognized as safe and effective and are misbranded when present in certain OTC (over-the-counter) drug products. This ruling was based on inadequate data for establishing general recognition of safety and effectiveness of these ingredients for the specified uses. In the United States, the spices Capsicum (plant source: *Capsicum frutescens* or *Capsicum annuum*) and paprika (plant source: *Capsicum annuum*) are generally recognized as safe by FDA for their intended use in food.

Groups of five Swiss Webster albino mice received up to 200 mg/kg single oral doses of hexane, chloroform, and ethyl acetate extracts of Capsicum Frutescens Fruit, respectively. Each extract resulted in death of all mice. Mortality also occurred after dosing with the hexane extract and chloroform extract.

In a short-term inhalation toxicity study, a reduction in minute ventilation was noted in male Wistar rats exposed to a 7% Capsicum Oleoresin solution and in control rats exposed to solvent only. The difference between the two groups was not found to be statistically significant. Microscopic findings for the test group included increased mucus secretion (trachea) and interstitial edema (lungs).

In a 4-week oral feeding study, red chilli (*Capsicum annuum*) in the diet at concentrations up to 10% was classified as relatively nontoxic in groups of male B6C3F1 mice. Exfoliation of the intestinal epithelium into the lumen, with lymphocytic accumulation, cytoplasmic fatty vacuolation and centrilobular necrosis of hepatocytes, and aggregates of lymphocytes in the portal areas were observed in male Wistar rats fed 10%, but not 2%, Capsicum Frutescens Fruit in the diet for 8 weeks. Groups of rats were fed 0.5 g/kg day^{-1} crude Capsicum Fruit Extract for 60 days and, for most organs, no significant gross pathology was noted at necropsy. However, slight hyperemia (without hemorrhage) of the liver and reddening of the gastric mucosa were observed. Compared to saline-treated controls, no statistically significant changes in relative organ weights (stomach, liver, or other organs) were observed in treated rats. In groups of weanling Wistar rats fed basal diets supplemented with whole red pepper (*Capsicum frutescens*, at concentrations up to 5.0%) for up to 8 weeks, the large intestines, livers, and kidneys were free of pathology at microscopic examination. However, destruction of the taste buds and keratinization and erosion of the GI tract were noted in groups fed 0.5% to 5.0% red pepper in the diet.

The results of 9- and 12-month feeding experiments involving groups of weanling Wistar rats and the same concentrations of red pepper (*Capsicum frutescens*, 0.5% to 5.0%) in the diet revealed no abnormalities (red blood cells, white blood cells, differential counts, hemoglobin, or serum proteins). At microscopic examination, the large intestines and kidneys of rats in each dose group were normal. Similar results were reported in another experiment (same study) in which weanling Wistar rats were fed red pepper (0.05% in the diet) for 12 months. In another chronic study, nine healthy white rabbits were fed Capsicum Annuum Powder (Paprika Powder, 5 mg/kg body weight day^{-1}) in the diet daily for 12 months. Damage to the liver and spleen were noted at gross and microscopic examination.

Two trade name chemicals under which Capsicum Annuum Fruit Extract is marketed (Capsicum Tincture and Capsicum Tincture SH, both described as Capsicum Annuum Fruit Extract dissolved in 70% ethanol) were evaluated in a skin irritation test at concentrations ranging from 0.1% to 1.0%. Both chemicals were classified as non-irritants.

Capsicum Frutescens Fruit Extract induced concentration-dependent (at 25 to 500 μg/ml) cytotoxicity in a human buccal mucosa fibroblast cell line. Total cell death was noted at 16 days (300 μg/ml) and at 6 days (400 and 500 μg/ml).

An ethanolic extract of red chili (Capsicum species not stated) was strongly mutagenic to strains 4 and 510 of *Salmonella typhimurium* TA98, and positive results were also reported for strain 1018. Negative results were reported for the following strains: TA100 strains 1, 2, and 7823, and *Escherichia coli* trp$^-$ her$^-$ strains 3, 13, and 14. When four different extracts of *Capsicum frutescens* (hexane, chloroform, ethyl acetate, and water) were administered to Swiss Webster albino mice, the chloroform extract (most clastogenic) induced the highest incidence of micronucleated polychromatic erythrocytes in bone marrow cells (at maximum tolerated dose). In an assay for the induction of 8-azaguanine–resistant mutants (with and without metabolic activation) in V79 Chinese hamster cells, Chili extract (1.83 mg/ml) did not induce resistant colonies. In groups of female Swiss albino mice, a dose-related (200, 300, and 400 mg/kg) increase in the percentage of cells with chromosomal abnormalities was noted following dosing with Capsicum Frutescens Fruit. Statistically significant findings included chromosome breakage, polyploidy and aneuploidy, and inhibition of spindle formation. Capsicum Oleoresin, containing 1.7% *w*/*w* Capsaicin, produced negative results in *S. typhimurium* strains TA98, TA100, and TA1538 both with and without metabolic activation.

Intestinal tumors/polyps were observed in 25 of 30 male Wistar rats fed red chili powder (*Capsicum annuum*, 8 mg/day/100 g body weight) in a commercial diet for 30 days. Tumors were not observed in the 30 control mice that were fed standard diet only. A tumor incidence (for intestine and colon) of 27/30 was reported for male Sprague-Dawley albino rats fed the same doses of red chili powder for 30 weeks. After 30 rats were fed chilies (*Capsicum frutescens* or *Capsicum annuum*) at a concentration of 10% in a semisynthetic diet for 2 years, changes in the liver that resembled incipient hepatomas and cholangiomas developed in 7 of 30 rats. Of the seven rats, malignant tumors were observed in three. The findings were considered inconclusive due to late appearance of the tumors. However, the feeding of chilies was considered the determining tumorigenic factor. In a group of 20 male Swiss mice fed standard diet containing chilies (*Capsicum annuum*, 100 mg chilies/mouse/day) for 12 months, adenocarcinoma of the abdomen was noted in seven mice. Tumors were not observed in control mice that were fed standard diet only.

Cumin or black pepper in the diet protected the colon in the presence of the procarcinogen 1,2-dimethylhydrazine (DMH), as a possible result of the decreased activity of β-glucuronidase and mucinase, whereas red chili supplementation (*Capsicum annuum*, 8 mg/day per 100 g body weight for 30 weeks) had the opposite effect in the proximal intestine and proximal colon. In two other studies, diets containing red pepper (up to 3.0%) and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) solution were fed to Fischer rats (37-week study) and Wistar rats (40-week study). In the 37-week study, it was concluded that red

pepper alone may not be carcinogenic to the stomach, but may enhance MNNG- induced carcinogenesis over a prolonged period of time. In the 40-week study, the results suggested that red pepper had a promoting effect on duodenal carcinogenesis, but had no cancer promoting effect in the glandular stomach. In another tumor promotion study, only one gross tumor was present in the cheek pouch of a hamster that was treated with Capsicum Frutescens Fruit Extract + DMN-OAc. No tumors were observed in the group that was treated with Capsicum Frutescens Extract only. The extensive hyperplasia that was observed in the cheek pouch or stomach epithelium was said to have been indicative of early precancerous lesions, which may lead to malignant changes with longer survival of the animals. A tumor promotion effect of Capsicum Frutescens Fruit Extract was observed in inbred male and female Balb/c mice dosed orally (tongue application) with methyl(acetoxymethyl)nitrosamine (carcinogen) or benzene hexachloride (hepatocarcinogen).

Symptoms of cough, sneezing, and runny nose were reported for 15 of 25 chili factory workers, beginning at the initiation of employment and persisting anywhere from 3 weeks to 6 months. No statistically significant differences in ventilatory measurements were noted between test and control groups. Burning of the throat, wheezing, dry cough, shortness of breath, gagging, gasping, inability to breathe or speak, and, rarely, cyanosis, apnea, and respiratory arrest in response to Capsicum Oleoresin spray have been reported.

A trade name mixture containing 1% to 5% Capsicum Frutescens Fruit Extract induced very slight erythema in one of ten volunteers patch tested (occlusive patches, i.e., Finn chambers) for 48 h. A total of 103 subjects completed a 24-h, repeated-insult patch test on a gel product containing Capsicum Frutescens Fruit Extract (effective ingredient test concentration = 0.025%). Neither the incidence nor level of peak severity (barely perceptible) of the low-grade responses observed was considered evidence of clinically meaningful irritation or allergic contact dermatitis.

One epidemiological study indicated that chili pepper consumption may be a strong risk factor for gastric cancer in populations with high intakes of chili pepper; however, other authors have not reported this strong association.

A study comprising 81 subjects who visited a hospital emergency room after exposure to Capsicum Oleoresin during law enforcement action was conducted. The most common symptoms included ocular burning (45 subjects) and conjunctival injection (36 subjects). Thirty-two subjects had a heart rate of >100 beats/min and 20 additional subjects had a respiratory rate of >16 breaths per minute. In another report, corneal abrasions were noted in 7 of 100 cases of pepper spray exposure.

Bronchial challenge with paprika (spice from *Capsicum annuum*) induced an immediate asthmatic reaction in a 27-year old subject who had prepared a sausage containing paprika a year earlier. Skin prick test results for paprika were positive. Paprika also induced a weak allergic patch test reaction (<2+ reaction) in two of the five patients with spice-induced allergic contact dermatitis. Two other case reports indicated a positive wheal-and-flare reaction and anaphylaxis after exposure to paprika.

Capsaicin

Capsaicin functions as an external analgesic, a fragrance ingredient, and as a skin-conditioning agent—miscellaneous in cosmetic products, but it is not in current use in cosmetic products.

Capsaicin is a crystalline pungent alkaloid that is extracted from natural sources, which include the red chilies *Capsicum annuum* and *Capsicum frutescens*. The content of Capsaicin in *Capsicum annuum* is 1.67 mg/g dry weight, and in *Capsicum frutescens*, 0.45 mg/g dry weight. United States Pharmacopoeia–grade Capsaicin contains not less than 90.0% and not more than 110.0% of the labeled percentage of total capsaicinoids. The content of Capsaicin ($C_{18}H_{27}NO_3$) is not less than 55%, and the sum of the contents of Capsaicin and dihydrocapsaicin ($C_{18}H_{29}NO_3$) is not less than 75%. Additionally, the content of other capsaicinoids is not more than 15%, all calculated on the dried basis.

In 1993, the U.S. Food and Drug Administration (FDA) published a final rule indicating that Capsicum and other active ingredients are not generally recognized as safe and effective and are misbranded when present in certain OTC (over-the-counter) drug products. This ruling was based on inadequate data for establishing general recognition of safety and effectiveness of these ingredients for the specified uses, and, regarding Capsaicin, relates to external drug products for fever blister and cold sore treatment. But, Capsaicin is considered to be in category I (safe and effective) for use as an external analgesic counterirritant in OTC drug products.

The results of an oral feeding study involving rats indicated that Capsaicin was rapidly absorbed from the stomach and small intestine. At 3 h, only 15% of the administered dose remained in the entire GI tract (i.e., 85% absorption at 3 h). No metabolite of Capsaicin was found in the gut contents, and, after 48 h, the amount of Capsaicin in the feces was less than 10% of the administered dose. In another oral feeding study (rats), 75.5% of the administered dose was readily absorbed at 1 h post dosing. Capsaicin was excreted in free form (~7.6% in urine and ~10.2% in feces) and in glucuronide form (4.9% in urine and 5.1% in feces) within 24 h post dosing. Following a single subcutaneous injection of Capsaicin into rats, the concentration in the blood reached a maximum at 5 h. As early as 10 min post dosing, Capsaicin was detectable in all tissues that were investigated, except for adipose tissue (omentum). The highest concentrations of Capsaicin were found in the kidney, whereas, concentrations in the liver remained very low. The results of in vitro experiments (rat, mouse, or hamster liver fractions) have suggested that Capsaicin is metabolized in the liver by a mixed function oxidase system and is also metabolized in the kidney, lung, and intestinal mucosa. The in vitro percutaneous absorption of Capsaicin has been demonstrated using the following skin types: human, rat, mouse, rabbit, and pig. Enhancement of the skin permeation of

naproxen (nonsteroidal anti-inflammatory agent) in the presence of Capsaicin has also been demonstrated.

The observation that protein kinase C (PKC) down-regulation significantly decreased the Capsaicin-induced release of substance P suggests that Capsaicin may activate PKC in rat sensory neurons. Pharmacological and physiological studies have demonstrated that Capsaicin, which contains a vanillyl moiety, produces its sensory effects by activating a Ca^{2+}-permeable ion channel on sensory neurons. Capsaicin is a known activator of vanilloid receptor 1 (VR1; a heat-gated ion channel), and has numerous biological effects, which include the induction of pain, the modulation of thermoregulation, and the production of neurogenic inflammation. Studies have indicated the following distribution of the VR1 receptor in humans: sensory neurons (dorsal root ganglia and trigeminal ganglia), pancreas, brain, spinal cord, bladder, kidney, liver, spleen, testis, lung, and bowel. Capsaicin-induced stimulation of prostaglandin biosynthesis has been shown using bull seminal vesicles and rheumatoid arthritis synoviocytes. It has also been shown that Capsaicin inhibits protein synthesis in Vero kidney cells and human neuroblastoma SHSY-5Y cells in vitro.

LD_{50} values of 161.2 mg/kg (rats) and 118.8 mg/kg (mice) have been reported for Capsaicin in an acute oral toxicity studies. Hemorrhage of the gastric fundus was observed in some of the animals that died. Slight erosion or ulceration of the gastric fundus was observed at microscopic examination. In another acute oral toxicity study involving mice, an LD_{50} of 190 mg/kg was reported for Capsaicin. Desquamatic necrosis of the gastric mucosa was noted at microscopic examination. An LD_{50} of >512 mg/kg for Capsaicin was reported in an acute dermal toxicity study involving mice. Signs of toxicity were not observed. Mean acute intravenous toxicity LD_{50} values of 0.56 mg/kg and 0.4 mg/kg for Capsaicin were reported in studies involving mice. The following acute intraperitoneal LD_{50} values for Capsaicin were reported in various animal experiments: weanling rats (13.20 mg/kg), adult rats (10.40 mg/kg), mice (7.65 mg/kg), hamsters (>120 mg/kg), guinea pigs (1.10 mg/kg), and rabbits (>50 mg/kg). An LD_{50} of 9.0 mg/kg (mice) was reported in an acute subcutaneous toxicity study. In 2-day-old rats injected subcutaneously with Capsaicin, a significant reduction in thymus weight and thymus cell numbers was noted. There was no evidence of any toxic effects. In two groups of guinea pigs injected subcutaneously with Capsaicin (50 mg/kg), 6 of 20 and 5 of 21 guinea pigs died, respectively. Exerted respiration, decreased breathing frequency, and itching were noted prior to death. Acute gastric erosions were observed in rats that received four equally divided oral doses (100 mg/kg, 1 h apart) of Capsaicin. Evidence of toxicity in the duodenal absorptive cells was noted following the administration of six oral doses of Capsaicin (1 mg/kg) to rats at various intervals over a period of 1 h.

No signs of toxicity were observed in groups of mice injected intraperitoneally with Capsaicin at doses up to 1.6 mg/kg body weight per day for 5 consecutive days. In another study, lesions of the alveolar cell lining and widening of the alveolar lumen were observed in two groups of rats dosed intraperitoneally with Capsaicin (1.68 mg/kg) for 1 and 3 days, respectively. None of the animals showed signs of morbidity or mortality. A significant increase in urinary bladder weight was observed in rats dosed subcutaneously with Capsaicin (total dose of 125 mg/kg at end of 2 days). None of the rats died.

In a subchronic oral toxicity study (12 weeks), differences in the growth rate between control groups and groups dosed with Capsaicin (1 and 2 mg/kg doses) were statistically significant. The most striking ultrastructural changes in intestinal absorptive cells were observed in mice dosed with 2 mg/kg. Thiamine absorption was also significantly inhibited at this dose. The ultrastructural changes may have been associated with inhibition of thiamine absorption.

A significant decrease in blood urea nitrogen, total cholesterol, and other blood plasma parameters was noted in rats dosed with Capsaicin (50 mg/kg day^{-1}) for 60 days. No significant gross pathological changes were observed. In another short-term oral toxicity study, significant reductions in weight gain and total cholesterol in the serum were noted in rats that received Capsaicin (up to 15 mg %) in the diet for 8 weeks. No significant gross pathology was noted.

The subchronic oral toxicity study of a mixture of capsaicinoids (64.5% Capsaicin and 32.6% dihydrocapsaicin) was evaluated using groups of mice fed diets containing 0.125% to 1.0% capsaicinoids for 13 weeks. A significant increase in the liver/body weight ratio was reported for the following dose groups: 0.0625% (females), 0.125% (males), 0.25% (males and females), and 1% (males and females). Focal tubular dilatation of the kidney was reported for males dosed with 1% capsaicinoids, but not for males of other dose groups.

Gastric administration of Capsaicin to rats resulted in an 89% increase in the ulcer index. Intraduodenal administration of Capsaicin did not affect the ulcer index, but induced a significant increase in total acidity.

Following inhalation exposure to 0.001% Capsaicin aerosol, statistically significant increases in eosinophils and neutrophils were noted in the bronchoalveolar lavage fluid.

Corneal lesions were observed in neonatal mice injected subcutaneously (doses down to 12.5 mg/kg) or intraperitoneally with Capsaicin. In adult rabbits, retrobulbar injection of Capsaicin induced a prompt inflammatory reaction, with conjunctival hyperemia, chemosis, and a dense aqueous flare.

Dose-related edema was observed in rats receiving Capsaicin injections (0.84 to 84 μg/kg) into the hindpaw. In another study, similar degrees of ear edema were observed in mice following 30, 40, and 50 μg applications of Capsaicin to the ear. Topical application of Capsaicin in acetone (12.5 mg/ml) to the ears of mice induced erythema and edema.

In guinea pigs, dinitrochlorobenzene (DNCB)-induced contact dermatitis was enhanced in the presence of Capsaicin, injected subcutaneously. Dermal application of Capsaicin inhibited DNCB-induced sensitization in mice. The results of a study using mice indicated that neuropeptide denervation by neonatal

administration of Capsaicin altered the induction and elicitation phases of contact hypersensitivity. A slight, significant decrease in IgG levels, but not IgA or IgE, was observed in adult rats injected subcutaneously with Capsaicin after aerosol immunization with ovalbumin. The following immune system effects have been observed in neonatal rats injected subcutaneously with Capsaicin: increased and decreased serum levels of IgE after subcutaneous and aerosol immunization with ovalbumin, marked inhibition of natural killer cell- and antibody-dependent cytotoxic functions, strong correlation between inhibition of cell proliferation and decreased numbers of CD5+ and CD4+ T lymphocytes, and strong decrease in mitogen-induced thymocyte proliferative response. In another study, an increase in the number of splenocytes and antibody-producing cells and enhanced T-cell mitogen-induced lymphocyte proliferation were noted in mice fed Capsaicin (20 ppm) in the diet for 3 weeks. Increased serum immunoglobulin concentrations (IgG and IgM) were observed in groups of mice fed 20 and 50 ppm Capsaicin in the diet for 3 weeks.

Capsaicin inhibited growth of *Escherichia coli*, *Pseudomonas solanacearum*, and *Bacillus subtilis* bacterial cultures, but had no long-term effect on the growth of *Saccharomyces cerevisiae*. In two in vitro cytotoxicity assays using baby hamster kidney fibroblasts, Capsaicin induced mild to moderate growth inhibition and cell detachment, respectively.

In both in vitro and in vivo experiments, Capsaicin had no effect on the osmotic fragility of rat red blood cells. In the in vivo experiment, rats were dosed orally with 5 or 50 mg/kg Capsaicin. In the in vitro experiment, red blood cells were incubated with Capsaicin concentrations ranging from 10^{-4} to 10^{-2} M. Mean corpuscular fragility was determined graphically and expressed as the sodium chloride concentration at which 50% of the red blood cells hemolyze. A dose-related increase in the hemolysis of human red blood cells in vitro was noted over the range of Capsaicin concentrations tested (1 to 100 nm). However, in another study, Capsaicin had a protective effect on the osmotic fragility of human erythrocytes. Compared to the control (normal blood), a significant decrease in mean erythrocyte fragility (MEF) was observed when erythrocytes were incubated with 10^{-4} and 10^{-5} M Capsaicin, but not 10^{-6} or 10^{-7} M Capsaicin. Concentration-dependent (0.025% to 0.2% weight/volume), Capsaicin-induced cytotoxicity was noted in human fibroblast and keratinocyte cultures.

The administration of Capsaicin to neonatal or adult animals destroys primary affarent neurons and depletes the neurochemical markers of these neurons. In one study, the dosing of neonatal rats with Capsaicin (50 mg/kg) caused a marked decrease in the density of corticotropin-releasing factor–, substance P–, vasoactive intestinal peptide–, and cholecystokinin-containing neurons in the dorsal spinal cord, the substantia gelatinosa, and the spinal trigeminal nucleus. Significant depletion of substance P from dorsal root ganglia and a nonsignificant, slight decrease in substance P in the dorsal cord were noted in adult guinea pigs that received subcutaneous doses of Capsaicin up to 500 mg/kg.

In one *Salmonella*/mammalian microsome mutagenicity assay, Capsaicin (0.04 mg/plate) was mutagenic, with metabolic activation, to *S. typhimurium* strains TA98, TA100, and TA1535, and results were negative (without metabolic activation) for these three strains as well as strains TA1537 and TA1538. In another test (same assay), Capsaicin (0.5 to 5000 μg/plate) was not mutagenic to the following strains with or without metabolic activation: TA98, TA100, TA1535, and TA1538. Capsaicin also induced either positive or negative results in micronucleus tests and sister-chromatid exchange assays, depending on the dose that was tested. Positive results for Capsaicin were also reported in DNA damage assays, even when Capsaicin dosing in vivo was not followed by dosing with cyclophosphamide. In another study, Capsaicin (injected intraperitoneally) significantly inhibited testicular DNA biosynthesis.

Concentrations of c-*fos* and c-*jun* mRNA in the adrenal gland increased after rats were dosed subcutaneously with Capsaicin. c-*fos* and c-*jun* genes are two genes that encode DNA binding transcription factors whose protein products interact with specific nucleotide sequence elements in gene promoter regions, thereby regulating mRNA synthesis. The results of another study suggested that the expression of β/γ-preprotachynin, calcitonin gene–related peptide, and trkA messenger RNAs is depressed after systemic (subcutaneous injection) administration of Capsaicin to adult rats, and that dosing with Capsaicin induces expression of vasoactive intestinal peptide and galanin messenger RNAs in sensory neurons. Compared to the saline control, a significant decrease in the frequency of mitotic cells and proliferating cell nuclear antigen (PCNA)-positive cells was noted after Capsaicin injection (injected into gingival mucosa) as well as after injection with Freund's complete adjuvant.

Carcinogenic, cocarcinogenic, anticarcinogenic, antitumorigenic, tumor promotion, and anti–tumor promotion effects of Capsaicin have been reported in animal studies.

No signs of skeletal or soft tissue malformations were observed in the litters of eight pregnant rats injected subcutaneously with Capsaicin (50 mg/kg) on gestation days 14, 16, 18, or 20. Compared to control fetuses, fetal weight, the average number of fetuses per dam, and the incidence of resorptions were not significantly different. However, crown-rump length was significantly reduced in litters of dams injected on day 18. In a study involving pregnant mice, dosed subcutaneously with Capsaicin, the test substance crossed the placenta and induced a neurotoxic effect, i.e., depletion of substance P in the spinal cord and peripheral nerves of pregnant females and fetuses. Epididymal sperm counts were similar to control values in groups of male mice that received intraperitoneal injections of Capsaicin, up to 1.6 mg/kg body weight per day, for 5 consecutive days. Additionally, there were no treatment-related changes in the percentage of abnormal sperms. Abnormal testicular descent was noted in 13 of 40 testes of newborn rats injected subcutaneously with Capsaicin. Abnormal testicular descent was not observed in any of the control newborn rats.

Nerve degeneration of intracutaneous nerve fibers and a dramatic decrease in pain sensation induced by heat and mechanical stimuli were evident in human subjects injected intradermally with Capsaicin (doses up to 20 μg in vehicle). In another study, topical application of 0.075% Capsaicin cream to human subjects resulted in transient sensations of burning and itching. A striking loss of nerve fibers, most pronounced in the epidermis, was noted.

An increase in mean inspiratory flow was reported for eight normal subjects who inhaled nebulized 10^{-7} M Capsaicin (particle size = 1 to 2 μm, mass median diameter).

In a percutaneous absorption study, a gel formulation containing 0.075% Capsaicin was applied to human subjects (forearm) according to an occlusive dressing procedure. At 8 h, the amount of Capsaicin absorbed was 22.65 ± 3.73 μg/cm^2 (flux = 2.28 ± 0.39 μg/cm^2/h).

The results of provocative and predictive tests involving human subjects indicated that Capsaicin is a skin irritant. Flare responses were common.

DISCUSSION

The Cosmetic Ingredient Review (CIR) Expert Panel recognizes that Capsaicin is the major pungent compound in chili peppers (from *Capsicum annuum* and *Capsicum frutescens* plants), and all of the Capsicum ingredients that are included in this safety assessment are derived from the Capsicum plant. For example, Capsicum Annuum Fruit Extract, as supplied, contains 0.5% Capsaicin and Capsicum Frutescens Fruit Extract and Capsicum Frutescens Resin trade name mixtures contain up to 5% and 21% Capsaicin, respectively. These data, together with the available cosmetic use concentration data on Capsicum Annuum Fruit Extract (up to 1%) and Capsicum Frutescens Fruit Extract (up to 1%), translate into effective cosmetic use concentrations of 0.005% Capsaicin (from Capsicum Annuum Fruit Extract) and 0.05% Capsaicin (from Capsicum Frutescens Fruit Extract). Although use concentration data on Capsicum Frutescens Resin were not available, the Panel expects that this ingredient would be in the same range of Capsaicin concentrations.

The low effective use concentrations of Capsaicin in cosmetic products (up to 0.05%), the generally recognized as safe status of the spices Capsicum (plant source: *Capsicum frutescens L.* or *Capsicum annuum L.*) and paprika (plant source: *Capsicum annuum L.*) for their intended use in food, and use concentrations of Capsaicin in over-the-counter topical drug products (0.025% and 0.075%) were taken into consideration. However, the Panel cited studies in which creams containing 0.025% and 0.1% Capsaicin induced skin irritation in human provocative and predictive skin irritation tests, respectively.

The genotoxicity, carcinogenicity, and tumor promotion potential of Capsaicin and studies indicating opposite effects (e.g., anticarcinogenicity), respectively, have also been demonstrated. In the view of the CIR Expert Panel, skin irritation and other tumor-promoting effects of Capsaicin are mediated through interaction of this chemical with the same vanilloid receptor. Given this mechanism of action (vanilloid receptor) and the observation that many tumor promoters are irritating to the skin, the Panel considers it likely that a potent tumor promoter may also be a moderate to severe skin irritant. Thus, a limitation on Capsaicin content that would significantly reduce its skin irritation potential is expected to, in effect, lessen any concerns relating to tumor promotion potential.

The Panel acknowledges the large amount of available data on Capsaicin and also the limited data on botanical ingredients derived from *Capsicum annuum* and *Capsicum frutescens*. However, the available UV absorption spectra on Capsaicin and tradename materials under which Capsicum Annuum Fruit Extract are marketed (Capsicum Tincture and Capsicum Tincture SH) are similar, and do not indicate significant absorbance in the UVB or UVA region. Furthermore, the absorption spectra do not support the presence of any organic component or impurity in Capsicum Annuum Fruit Extract in quantities that would be of phototoxicological significance. In light of these data, it is possible that similarities between the UV spectra on Capsaicin and other botanical ingredients and their trade name materials exist, suggesting similarities in composition. Thus, data on Capsaicin may be useful in terms of evaluating the safety of Capsicum-derived ingredients.

Because Capsaicin enhanced the penetration of an antiinflammatory agent through human skin, the Panel recommends that care should be exercised in using ingredients that contain Capsaicin in cosmetic products.

After considering that pesticide impurities may form part of the composition of these plant-derived ingredients, the Panel advises industry that the total PCB/pesticide contamination should be limited to not more than 40 ppm, with not more than 10 ppm for any specific residue, and agreed on the following limitations for other impurities: arsenic (3 mg/kg maximum), heavy metals (0.002% maximum), and lead (5 mg/kg maximum). Although aflatoxin has been detected in red chili grown in Africa, India, and Japan, the Panel believes that aflatoxin should not be present in Capsaicin and botanical ingredients that are derived from *Capsicum annuum* and *Capsicum frutescens*; the Panel adopted the U.S. Department of Agriculture (USDA) designation of $\leq$15 ppb as corresponding to "negative" aflatoxin content.

Furthermore, after considering that the *N*-nitroso compounds *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine have been detected in chili samples from different suppliers in West Germany, the Panel determined that ingredients derived from *Capsicum annuum* and *Capsicum frutescens* plant species should not be used in products where *N*-nitroso compounds may be formed.

CONCLUSION

On the basis of the available data included in this report, the CIR Expert Panel concludes that Capsaicin and Capsicum Annuum Extract, Capsicum Annuum Fruit Extract,

Capsicum Annuum Resin, Capsicum Annuum Fruit Powder, Capsicum Frutescens Fruit, Capsicum Frutescens Fruit Extract, and Capsicum Frutescens Resin are safe as cosmetics in the practices of use and concentration as described in the safety assessment, when formulated not to be irritating.

REFERENCES

Adegoke, G. O., A. R. E. Allamu, J. O. Akingbala, and A. O. Akanni. 1996. Influence of sundrying on the chemical composition, aflatoxin content and fungal counts of two pepper varieties—*Capsicum annuum* and *Capsicum frutescens*. *Plant Foods Hum. Nutr.* 49:113–117.

Agrawal, R. C., and S. V. Bhide. 1988. Histopathological studies on toxicity of chili (capsaicin) in Syrian Golden Hamsters. *Indian J. Exp. Biol.* 26:377–382.

Agrawal, R. C., M. Wiessler, E. Hecker, and S. V. Bhide. 1986. Tumour-promoting effect of chili extract in BALB/c mice. *Int. J. Cancer* 38:689–695.

Akagi, A., N. Sano, H. Uehara, T. Minami, H. Otsuka, and K. Izumi. 1998. Non-carcinogenicity of capsaicinoids in $B6C3F_1$ mice. *Food Chem. Toxicol.* 36:1065–1071.

Anand, P., S. R. Bloom, and G. P. McGregor. 1983. Topical capsaicin pretreatment inhibits axon reflex vasodilatation caused by somatostatin and vasoactive intestinal peptide in human skin. *Br. J. Pharmacol.* 78:665–669.

Anonymous. 1992. Capsaicin—A topical analgesic. *Med. Lett. Drugs Ther.* 34:62–63.

Arceo, S. D. B., E. Madrigal-Bujaidar, E. C. Montellano, L. R. Herrera, and B. D. D. García. 1995. Genotoxic effects produced by capsaicin in mouse during subchronic treatment. *Mutat. Res.* 345:105–109.

Archer, V. E., and D. W. Jones. 2002. Capsaicin pepper, cancer and ethnicity. *Med. Hypotheses* 59:450–457.

Anand, P., S. R. Bloom, and G. P. McGregor. 1983. Topical capsaicin pretreatment inhibits axon reflex vasodilation caused by somatostatin and vasoactive intestinal polypeptide in human skin. *Br. J. Pharmacol.* 78:665–669.

Atkinson, M. E. and J. S. Chaggar. 1983. The effects of prenatal capsaicin on the distribution of substance P in developing primary afferent neurons. *Neurosci. Lett.* 35:25–29.

Azizan, A., and R. D. Blevins. 1995. Mutagenicity and antimutagenicity testing of six chemicals associated with the pungent properties of specific spices as revealed by the Ames *Salmonella*/microsomal assay. *Arch. Environ. Contam. Toxicol.* 28:248–258.

Babish, J. C., B. Johnson, and D. Lisk. 1983. Mutagenicity of municipal sewage sludges of American cities. *Environ. Sci. Technol.* 17:272–277.

Balachandran, B., and V. M. Sivaramkrishnan. 1995. Induction of tumors by Indian dietary constituents. *Indian J. Cancer* 32:104–109.

Balsam, M. S., and E. Sagarin, eds. 1974. *Cosmetics. Science and Technology*, Vols 1–3, 2nd ed., 129. New York:Wiley-Interscience.

Barber, L. A., and M. R..Vasko. 1996. Activation of protein kinase C augments peptide release from rat sensory neurons. *J. Neurochem.* 67:72–80.

Barnes, P. J., M. J. Brown, C. T. Dollery, R. W. Fuller, D. J. Heavey, and P. W. Ind. 1986. Histamine is released from skin by substance P but does not act as the final vasodilator in the axon reflex. *Br. J. Pharmacol.* 88:741–745.

Batzinger, R. P., S. Y. Ou, and E. Beuding. 1979. Antimutagenic effects of 2(3)-tert-butyl-4-hydroxyanisole and antimicrobial agents. *Cancer Res.* 38:4478–4485.

Bauer, M. B., S. Murphy, and G. F. Gebhart. 1995. Stimulation of cyclic GMP production via a nitrosyl factor in sensory neuronal cultures by algesic or inflammatory agents. *J. Neurochem.* 65:363–372.

Bauer, M. B., M. L. Simmons, S. Murphy, and G. F. Gebhart. 1993. Bradykinin and capsaicin stimulate cyclic GMP production in cultured rat dorsal root gangion neurons via a nitrosyl intermediate. *J. Neurosci. Res.* 36:280–289.

Bennett, D. J., and G. W. Kirby. 1968. Constitution and biosynthesis of capsaicin. *J. Chem. Soc.* 4:442–446.

Bergren, D. R. 1988. Capsaicin challenge, reflex bronchoconstriction, and local action of substance P. *Am. J. Physiol.* 254:R845–R852.

Bernal, M. A., A. A. Calderón, M. A. Pedreño, M. A. Ferrer, F. Merino de Cáceres, and A. R. Barceló. 1994. Purification of a basic peroxidase isoenzyme from *Capsicum* fruits and the immunoinhibition of its capsaicin oxidation capacity by antibodies raised against horseradish peroxidase. *Z. Lebensm. Unters. Forsch* 199:240–242.

Bernstein, I. L. 1996. Neural mediation of food aversions and anorexia induced by tumor necrosis factor and tumors. *Neurosci. Biobehav. Rev.* 20:177–181.

Bernstein, J. E., D. R. Bickers, M. R. Dahl, and J. Y. Roshal. 1987. Treatment of chronic postherpetic neuralgia with topical capsaicin. A preliminary study. *J. Am Acad. Dermatol.* 17:93–96.

Bernstein, J. E., N. J. Korman, D. R. Bickers, M. V. Dahl, and L. E. Millikan. 1989. Topical capsaicin treatment of chronic postherpetic neuralgia. *J. Am. Acad. Dermatol.* 21:265–270.

Bertrand, C., J. A. Nadel, P. D. Graf, and P. Geppetti. 1993. Capsaicin increases airflow resistance in guinea pigs in vivo by activating both NK2 and NK1 tachykinin receptors. *Am. Rev. Respir. Dis.* 148:909–914.

Bervoets, K., and F. C. Colpaert. 1984. Respiratory effects of intrathecal capsaicin in arthritic and non-arthritic rats. *Life Sci*. 34:2477–2483.

Bevan, S., S. K. Hothi, G. Hughes, et al. 1992. Capsazepine: A competitive antagonist of the sensory neurone excitant capsaicin. *Br. J. Pharmacol*. 107:544–552.

Biggs, D. F., and V. Goel. 1985. Does capsaicin cause reflex bronchospasm in guinea pigs. *Eur. J. Pharmacol.* 115:71–80.

Birder, L. A., A. J. Kanai, W. C. Groat, et al. 2001. Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. *Proc. Natl. Acad. Sci. U. S. A.* 98:13396–13401.

Birnboim, H. C., and J. J. Jereak. 1981. Fluorometric method for rapid detection of DNA strand breaks in human white blood cells produced by low dose of radiation. *Cancer Res*. 41:1889–1892.

Biro, T., G. Acs, P. Acs, S. Modarres, and P. M. Blumberg. 1997. Recent advances in understanding of vanilloid receptors: A therapeutic target for treatment of pain and inflammation in skin. *J. Investig. Dermatol. Symp. Proc.* 2:56–60.

Blazsó, G., and M. Gábor. 1995. Effects of prostaglandin antagonist phloretin derivatives on mouse ear edema induced with different skin irritants. *Prostaglandins* 50:161–168.

Boersch, A. B. A., B. A. Callingham, F. Lembeck, and D. F. Sharman. 1991. Enzymic oxidation of capsaicin. *Biochem. Pharmacol.* 41:1863–1869.

Bolser, D. C., F. C. DeGennaro, S. O'Reilly, J. A. Hey, and R. W. Chapman. 1995. Pharmacological studies of allergic cough in the guinea pigs. *Eur. J. Pharmacol.* 277:159–164.

Bond, S. M., F. Cervero, and D. S. McQueen. 1982. Influence of neonatally administered capsaicin on baroreceptor and chemoreceptor reflexes in the adult rat. *Br. J.Pharmacol.* 77:517–524.

Brender, D., and M. M. Webb-Peploe. 1969. Vascular responses to stimulation of pulmonary and carotid baroreceptors by capsaicin. *Am. J. Physiol.* 217:1837–1845.

Brown, L., D. Takeuchi, and K. Challoner. 2000. Brief Reports. Corneal abrasions associated with pepper spray exposure. *Am. J. Emerg. Med.* 18:271–272.

Brzozowski, T., D. Drozdowicz, A. Szlachcic, J. Pytko-Polonczyk, J. Majka, and S. J. Konturek. 1993. Role of nitric oxide and prostaglandins in gastroprotection induced by capsaicin and papaverine. *Digestion* 54:16–23.

Buchanan, R. L., S. Goldstein, and J. D. Budroe. 1981. Examination of chili pepper and nutmeg oleoresins using the *Salmonella*/mammalian microsome mutagenicity assay. *J. Food Sci.* 47:330–333.

Buck, S., and T. Burks. 1986. The neuropharmacology of capsaicin: review of some recent observations. *Pharmacol. Rev.* 38:180–226.

Buck, S. H., P. P. Desmukyh, H. I. Yamamura, and T. F. Burks. 1981. Thermal analgesia and substance P depletion induced by capsaicin in guinea pigs. *Neuroscience* 6:2217–2222.

Buckley, T. L., S. D. Brain, P. F. Jose, and T. J. Williams. 1992. The partial inhibition of inflammatory responses induced by capsaicin using the FAB

fragment of a selective calcitonin gene-related peptide antiserum in rabbit skin. *Neuroscience* 48:963–968.

Budavari, S., ed. 1989. *The Merck Index. An encyclopedia of chemicals, drugs, and biologicals*, 11th ed., 266–267. Rahway, NJ: Merck & Co.

Buiatti, E., D. Palli, A. Decarli, D. Amadori, and C. Avellini. 1989. A case-control study of gastric cancer and diet in Italy. *Int. J. Cancer* 44:611–616.

Bunker, C. B., R. Cerio, H. A. Bull, J. Evans, P. M. Dowd, and J. C. Foreman. 1991. 6. Histamine and clinical studies. The effect of capsaicin application on mast cells in normal human skin. *Agents Actions* 33:195–196.

Bynke, G. 1983. Capsaicin pretreatment prevents disruption of the blood-aqueous barrier in the rabbit eye. *Invest. Ophthalmol.* 24:744–748.

Bynke, G., R. Håkanson, and J. Hörig. 1983. Ocular responses evoked by capsaicin and prostaglandin E_2 are inhibited by a substance P antagonist. *Experientia* 39:996–998.

Carpenter, S. E., and B. Lynn. 1981. Vascular and sensory responses of human skin to mild injury after topical treatment with capsaicin. *Br. J. Pharmacol.* 73:755–758.

Carter, R. B. 1991. Current trends review. Topical capsaicin in the treatment of cutaneous disorders. *Drug Dev. Res.* 22:109–123.

Castle, N. A. 1992. Differential inhibition of potassium currents in rat ventricular myocytes by capsaicin. *Cardiovasc. Res.* 26:1137–1144.

Castonguay, A., H. Tjalve, N. Trushin, and S. S. Hecht. 1984. Perinatal metabolism of the tobacco-specific nitrosamine 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone in C57BL mice. *J. Natl. Cancer Inst.* 72:1117–1124.

Caterina, M. J., M. A. Schumacher, M. Tominaga, et al. 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 389:816–824.

Chad, D., C. Rasool, W. G. Bradley, P. Good, and S. Reichlin. 1983. Unmyelinated axon subpopulations in the rat peripheral nervous system. *Neurology* 33:848–852.

Chahl, L. A., and A. M. Lynch. 1987. The acute effects of capsaicin on the cardiovascular system. *Acta Physiol. Hung.* 69:413–419.

Chan, T. C., G. M. Vilke, J. Clausen, et al. 2002. The effect of oleoresin capsicum "pepper" spray inhalation on respiratory function. *J. Forensic. Sci.* 47:299–304.

Chard, P. S., D. Bleakman, R. Savidge, and R. J. Miller. 1995. Capsaicin-induced neurotoxicity in cultured dorsal root ganglion neurons: Involvement of calcium-activated proteases. *Neuroscience* 65:1099–1108.

Cheshire, W. P., and C. R. Snyder. 1990. Treatment of reflex sympathetic dystrophy with topical capsaicin. *Pain* 42:307–311.

Chitra, S., P. Viswanathan, N. Nalini, K. Sabitha, and V. P. Menon. 1997. Role of Redchili (Capsaicin) in the formation of colonic carcinoma. *Indian J. Pathol. Microbiol.* 40:21–25.

Choi, S. Y., and K. T. Kim. 1999. Capsaicin inhibits phospholipase C-mediated Ca^{2+} increase by blocking thapsigargin-sensitive store-operated Ca^{2+} entry in PC12 cells. *J. Pharmacol. Exp. Ther.* 291:107–114.

Chudapongse, P., and W. Janthasoot. 1976. Studies on the effect of capsaicin on metabolic reactions of isolated rat liver mitochondria. *Toxicol. Appl. Pharmacol.* 37:263–270.

Chudapongse, P., and W. Janthasoot. 1981. Mechanism of the inhibitory action of capsaicin on energy metabolism by rat liver mitochondria. *Biochem. Pharmacol*. 30:735–740.

Cochereau, C., D. Sanchez, A. Bourhaoui, and E. E. Creppy. 1996. Capsaicin, a structural analog of tyrosine, inhibits the aminoacylation of tRNA. *Toxicol. Appl. Pharmacol.* 141:133–137.

Cochereau, C., D. Sanchez, and E. E. Creppy. 1997. Tyrosine prevents capsaicin-induced protein synthesis inhibition in cultured cells. *Toxicology* 117:133–139.

Coleridge, H. M., J. C. G. Coleridge, and C. Kidd. 1964. Role of the pulmonary arterial baroreceptors in the effects produced by capsaicin in the dog. *J. Physiol.* 170:272–285.

Collier, H. O., W. J. McDonald-Gibson, and S. A. Saeed. 1975. Letter: Stimulation of prostaglandin biosynthesis by capsaicin, ethanol, and tyramine. *Lancet* 1:702.

Colpaert, F. C., J. Donnerer, and F. Lembeck. 1983. Effects of capsaicin on inflammation and on the substance P content of nervous tissues in rats with adjuvant arthritis. *Life Sci*. 32:1827–1834.

Committee of Revision of the the United States Pharmacopeial Convention. 2000. The *United States Pharmacopoeia*, 24th ed. Rockville, MD: United States Pharmacopeial Convention.

Cooper, T. H., J. A. Guzinski, and C. Fisher. 1991. Improved high-performance liquid chromatography method for the determination of major capsaicinoids in capsicum oleoresins. *J. Agric. Food Chem*. 39:2253–2256.

Cordell, G. A., and O. E. Araujo. 1993. Capsaicin: Identification, nomenclature, and pharmacotherapy. *Ann. Pharmacother*. 27:330–336.

Cosmetic, Toiletry, and Fragrance Association (CTFA). 2001. Use concentration data on capsicum annuum fruit extract, capsicum annuum fruit powder, and capsicum frutescens fruit extract from industry survey. Unpublished data submitted by CTFA. 1 page.[2]

CTFA. 2002a. Cosmetic ingredient chemical description on capsicum annuum fruit extract. Unpublished data submitted by CTFA, December 4, 2002. 2 pages.[2]

CTFA. 2002b. Memorandum on Capsicum frutescens fruit extract. Composition of the trade name mixture, vegetol capsicum LC 481 Huiluex. Unpublished data submitted by CTFA, December 18, 2002. 1 page.[2]

CTFA 2002c. Cosmetic ingredient chemical description on capsicum frutescens resin. Unpublished data submitted by CTFA, December 19, 2002. 2 pages.[2]

CTFA. 2003a. Ingredient database on Capsicum annuum fruit powder. http://www.ctfa-online.org (accessed June, 2003).

CTFA. 2003b. Cosmetic ingredient chemical description on capsicum frutescens fruit extract. Unpublished data submitted by CTFA, January 8, 2003. 3 pages.[2]

CTFA. 2003c. Memorandum on capsicum frutescens fruit extract, relating to human repeated insult patch test of a product ("Pulse Point Gel") containing capsicum frutescens fruit extract. Statement on content of capsicum frutescens fruit extract in "Pulse Point Gel". Unpublisdhed data submitted by CTFA, January 9. 2003. 1 page.[2]

Cremer, D. R., and K. Eichner. 2000. Formation of volatile compounds during heating of spice paprika (*Capsicum annuum*) powder. *J. Agric. Food Chem*. 48:2454–2460.

Creppy, E. E., F. Richeux, M.-R. Carratú, V. Cuomo, C. Cochereau, R. Ennamany, and D. Saboureau. 2000. Cytotoxicity of capsaicin in monkey kidney cells: lack of antagonistic effects of capsazepine and ruthenium red. *Arch. Toxicol*. 73:40–47.

Crespi, C. L., B. W. Penman, H. V. Gelboin, and F. J. Gonzalez. 1991. A tobacco smoke derived nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, is activated by multiple human cytochrome P450s including polymorphic human cytochrome P450 2D6. *Carcinogenesis* 12:1197–1201.

Croft, D. N., D. J. Pollock, and N. F. Coghill. 1966. Cell loss from human gastric mucosa measured by the estimation of deoxyribonucleic acid (DNA) in gastric washings. *Gut* 7:333–343.

Cruwys, S. C., N. E. Garrett, and B. L. Kidd. 1995. Sensory denervation with capsaicin attenuates inflammation and nociception in arthritic rats. *Neurosci. Lett.* 193:205–207.

Cuello, A. C., R. Gamse, P. Holzer, and F. Lembeck. 1981. Substance P immunoreactive neurons following neonatal administration of capsaicin. *Naunyn Schmiedebergs Arch. Pharmakol.* 315:185–194.

Dailey, G. E. 1992. Effect of treatment with capsaicin on daily activities of patients with diabetic neuropathy. *Diabetes Care* 15:159–165.

D'Alonzo, A. J., G. J. Grover, R. B. Darbenzio, T. A. Hess, P. G. Sleph, S. Dzwonczyk, J. L. Zhu, and J. C. Sewter. 1995. In vitro effects of capsaicin: antiarrhythmic and antiischemic activity. *Eur. J. Pharmacol*. 272:269–278.

D'Amelio, F. S., Sr., ed. 1999. *Botanicals. A phytocosmetic desk reference*, 72–73. Boca Raton, FL: CRC Press.

[2]Available from the Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036, USA.

Damhoeri, A., A. Hosono, T. Itoh, and A. Matsuyama. 1985. *In vitro* mutagenicity tests on capsicum pepper, shallot and nutmeg oleoresins. *Agric. Biol. Chem.* 49:1519–1520.

Dasgupta, P., V. Chandiramani, M. C. Parkinson, A. Beckett, and C. J. Fowler.1998. Treating the human bladder with capsaicin: Is it safe? *Eur. Urol.* 33:28–31.

Davies, D., B. A. Spicer, H. Smith, and L. W. Haynes. 1994. Effect of neonatal capsaicin on peptide-containing primary afferent fibres, eosinophil distribution and hyperresponsiveness in rat lung tissue following experimentally induced eosinophilia. *Neuroimmunomodulation* 1:308–314.

Dawbarn, D., A. J. Harmar, and C. J. Pycock. 1981. Intranigral injection of capsaicin enhances motor activity and depletes nigral 5-hydroxytryptamine but not substance P. *Neuropharmacology* 20:341–346.

Dayer, J. M., S. M. Krane, R. G. G. Russel and D. R. Robinson. 1976. Production of collagenase and prostaglandins by isolated adherent rheumatoid synovial cells. *Proc. Natl. Acad. Sci.U. S. A.* 73:945–949.

De, A. K., K. Agrawal, A. Mukherjee, and D. Sengupta. 1995. Inhibition by capsaicin against cyclophosphamide-induced clastogenicity and DNA damage in mice. *Mutat. Res.* 335:253–258.

De, A. K., P. K. Das, and J. J. Ghosh. 1992. Dual effects of capsaicin treatment on macrophage lysosomal enzymes. *Med. Sci. Res.* 20:493–494.

De, A. K. and J. J. Ghosh. 1988a. Acute capsaicin treatment causes alteration in rat lung lipids. *Med. Sci. Res.* 16:929–930.

De, A. K. and J. J. Ghosh. 1988b. Inflammatory effects of acute and chronic capsaicin treatment on rat paw. *Phytother. Res.* 2:175–179.

De, A. K. and J. J. Ghosh. 1989. Short- and long-term effects of capsaicin on the pulmonary anti-oxidant enzyme system in rats. *Phytother. Res.* 3:184–187.

De, A. K., and J. J. Ghosh. 1990. Comparative studies on the involvement of histamine and substance P in the inflammatory response of capsaicin in rat paw. *Phytother. Res.* 4:42–44.

De, A. K. and J. J. Ghosh. 1993. Capsaicin and its protective effect against UV-induced lipid peroxidation on liposomal preparations. *Dev. Oncol.* 71:377–380.

De, A. K., J. J. Ghosh, and T. K. Mandal. 1993. Ultraviolet radiation-induced lipid peroxidation in liposomal membrane: Modification by capsaicin. *Phytother. Res.* 7:87–89.

De, A. K., D. Sengupta, and J. J. Ghosh. 1993. Interaction of capsaicin with human erythrocyte membranes. *Phytother. Res.* 7:310–313.

Deal, C. L., T. J. Schnitzer, E. Lipstein, et al. 1991. Treatment of arthritis with topical capsaicin: a double-blind trial. *Clin. Ther.* 13:383–395.

Debarre, S., L. Karinthi, S. Delamanche, P. Fuché Desforges, and J.-H. Calvet. 1999. Comparative acute toxicity of *o*-chlorobenzylidene malononitrile (CS) and oleoresin capsicum (OC) in awake rats. *Hum. Exp. Toxicol.* 18:724–730.

Degim, I. T., A. Uslu, J. Hadgraft, T. Atay, C. Akay, and S. Cevheroglu. 1999. The effects of Azone and capsaicin on the permeation of naproxen through human skin. *Int. J. Pharm.* 179:21–25.

Delaunois, A., P. Gustin, and M. Ansay. 1993. Effects of capsaicin on the endothelial permeability in isolated and perfused rabbit lungs. *Fundam Clin. Pharmacol.* 7:81–91.

Desai, H. G., K. Venugopalan, and F. P. Antia. 1976. The effect of capsaicin on the DNA content of gastric aspirate. *Indian J. Med. Res.* 64:163–167.

DiCecco, J. J. 1976. Flavors and nonalcoholic beverages. Gas-liquid chromatographic determination of capsaicin. *J. Assoc. Off. Anal. Chem.* 59:1–4.

Donnerer, J., R. Amann, R. Schuligoi, and F. Lembeck. 1990. Absorption and metabolism of capsaicinoids following intragastric administration in rats. *Nauyn-Schmiedebergs Arch. Pharmacol.* 342:357–361.

Donnerer, J., and Lembeck F. 1982. Analysis of the effects of intravenously injected capsaicin in the rat. *Naunyn Schmiedebergs Arch. Pharmakol.* 320:54–57.

Donofrio, P., F. Walker, V. Hunt, et al. 1991. Treatment of painful diabetic neuropathy with topical capsaicin. *Arch. Intern. Med.* 151:2225–2229.

Duckles, S. P. 1986. Effects of capsaicin on vascular muscle. *Naunyn Schmiedebergs Arch. Pharmakol.* 333:59–64.

El-kady, I. A., S. S. M. El-maraghy, and M. E. Mostafa. 1995. Natural occurrence of mycotoxins in different spices in Egypt. *Folia Microbiol.* 40:297–300.

Ellison, N., C. L. Loprinz, J. Kugler, et al. 1997. Phase III placebo-controlled trial of capsaicin cream in the management of surgical neuropathic pain in cancer patients. *J. Clin. Oncol.* 15:2974–2980.

Environmental Protection Agency (EPA). 1992. EPA reregistration eligibility document (RED). Capsaicin. NTIS Report No. PB93–101434.

European Economic Community. 1999. EEC Cosmetics Directive 76/768/EEC, as amended through the 26th Adapting Commission Directive 2002/34/EC, Annexes I–VII. Brussels: EEC.

Facial Neuralgia Resources. 2003. Capsaicin. Alternative treatments for facial neuralgia. http://facial-neuralgia.org (accessed June, 2003).

Fang, J., M. Tsai, Y. Huang, P. Wu, and Y. Tsai. 1997. Percutaneous absorption and skin erythema: quantification of capsaicin and its synthetic derivatives from gels incorporated with benzalkonium chloride by using non-invasive bioengineering methods. *Drug Dev. Res.* 40:56–67.

Fang, J.-Y., P.-C. Wu, Y.-B. Huang, and Y.-H. Tsai. 1995. In vitro permeation study of capsaicin and its synthetic derivatives from ointment bases using various skin types. *Int. J. Pharm.* 126:119–128.

Fang, J, P. Wu, Y. Huang, and Y. Tsai. 1996. Percutaneous absorption of capsaicin, nonivamide and sodium nonivamide acetate from gel and ointment bases: in vitro formulation evaluations in pigs and in vivo bioengineering methods in humans. *Int. J. Pharm.* 130:121–135.

Figini, M., P. Javdan, F. Cioncolini, and P. Geppetti. 1995. Involvement of tachykinins in plasma extravasation induced by bradykinin and low pH medium in the guinea-pig conjunctiva. *Br. J. Pharmacol.* 115:128–132.

Flynn D. L., M. F. Rafferty, and A. M. Boctor. 1986. Inhibition of human neutrophil 5-lipoxygenase activity by gingerdione, shogaol, capsaicin, and related pungent compounds. *Prostaglandins Leukotrienes Med.* 24:195–198.

Food and Drug Administration (FDA). 1993. Status of certain additional over-the-counter drug category II and III active ingredients. Final rule. *Fed. Register* 58:27636–27644.

FDA. 1994. *OTC Drug Review Ingredient Status Report*. Washington, DC: FDA.

FDA. 2002. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.

Fischer, B. S., D. Qin, K. Kim, and T. V. McDonald. 2001. Capsaicin inhibits Jurkat T-cell activation by blocking calcium entry current I_{CRAC}. *J. Pharmacol. Exp. Ther.* 299:238–246.

Foti, C., M. Carino, N. Cassano, R. Panebianco, G. A. Veña, and L. Ambrosi. 1997. Occupational contact urticaria from paprika. *Contact Dermatitis* 37:135.

Franco-Cereceda, A., L. Bengtsson, and J. M. Lundberg. 1987. Inotropic effects of calcitonin gene-related peptide, vasoactive intestinal polypeptide and somatostatin on the human right atrium in vitro. *Eur. J. Pharmacol.* 134:69–76.

Franco-Cereceda, A., and A. Rudehill. 1989. Capsaicin-induced vasodilatation of human coronary arteries in vitro is mediated by calcitonin gene-related peptide rather than substance P or neurokinin A. *Acta Physiol. Scand.* 136:575–580.

Fufa, H. and K. Urga. 1996. Screening of aflatoxins in Shiro and ground red pepper in Addis Ababa. *Ethiop. Med. J.* 34:243–249.

Fujita, S., Y. Miyazono, and N. Ohba. 1987. Capsaicin-induced corneal changes associated with sensory denervation in neonatal rat. *Jpn. J. Ophthalmol.* 31:412–424.

Fujita, S., M. Sameshima, N. Ohba, T. Shimizu, K. Izumi, and T. Fukuda. 1984. A corneal opacity induced by a single subcutaneous administration of capsaicin in the neonatal mouse. *Nippon Ganka Gakkai Zasshi* 88:381–383.

Fujita, S., T. Shimizu, K. Izumi, T. Fukuda, M. Sameshima, and N. Ohba. 1984. Capsaicin-induced neuroparalytic keratitis-like corneal changes in the mouse. *Exp. Eye Res.* 38:165–175.

Fukuda, N., and M. Fujiwara. 1969. Effect of capsaicin on the guinea-pig isolated atrium. *J. Physiol.* 179:622–623.

Fuller, R. W. 1990. The human pharmacology of capsaicin. *Arch. Int. Pharmacodyn*. 303:147–156.

Fuller, R. W., C. M. S. Dixon, and P. J. Barnes. 1985. Bronchoconstrictor response to inhaled capsaicin in humans. *J. Appl. Physiol.* 58:1080–1084.

Gábor, M., and Z. Rázga. 1992. Development and inhibition of mouse ear edema induced with capsaicin. *Agents Actions* 36:83–86.

Gallico, L., A. Ferrario, L. Piazzoni, and R. Ceserani. 1993. Capsaicin induces differing microvascular leakage in left and right main bronchus of guinea pigs. *Med. Sci. Res.* 21:755–756.

Games, D. E., N. J. Alcock, J. Van der Greef, et al. 1984. Analysis of pepper and capsicum oleoresins by high-performance liquid chromatography-mass spectrometry and field desorption mass spectrometry. *J. Chromatogr*. 294:269–279.

Gamse, R., S. E. Leeman, P. Holzer, and F. Lembeck. 1981. Differential effects of capsaicin on the content of somatostatin, substance P, and neurotensin in the nervous system of the rat. *Naunyn Schmiedebergs Arch. Pharmakol.* 317:140–148.

Gamse, R., U. Petsche, F. Lembeck, and G. Janscò. 1982. Capsaicin applied to peripheral nerve inhibits axoplasmic transport of substance P and somatostatin. *Brain Res.* 239:447–462.

Gamse, R., A. Saria, J. M. Lundberg, and E. Theodorsson-Norheim. 1986. Behavioral and neurochemical changes after intracisternal capsaicin treatment of the guinea pig. *Neurosci. Lett.* 64:287–292.

Gannett, P. M., P. Iverson, and T. Lawson. 1990. The mechanism of inhibition of cytochrome P450IIE1 by dihydrocapsaicin. *Bioorganic Chem.* 18:185–198.

Gannett, P. M., D. L. Nagel, P. J. Reilly, T. Lawson, J. Sharpe, and B. Toth. 1988. The capsaicinoids: Their separation, synthesis, and mutagenicity. *J. Org. Chem.* 53:1064–1071.

Gattafossé, S. A. 1998. Data sheet. Vegetol capsicum LC 481 huileux. Unpublished data submitted by CTFA, December 18, 2002. 2 pages.[2]

Gennaro, A. R. 1990. *Remington's pharmaceutical sciences*, 18th ed., 764. Easton, PA: Mack Publishing.

Glinski, W., M. Glinska-Ferenz, and M. Pierozynska-Dubowska. 1991. Neurogenic inflammation induced by capsaicin in patients with psoriasis. *Acta Derm. Venereol.* 71:51–54.

Glinsukon T., V. Stitmunnaithum, C. Toskulkao, T. Buranawuti, and V. Tangkrisanavinont. 1980. Acute toxicity of capsaicin in several animal species *Toxicon.* 18:215–220.

Godfraind, J. M., T. M. Jessell, J. S. Kelly, R. N. McBurney, A. W. Mudge, and M. Yamamoto. 1980. Capsaicin prolongs action potential duration in cultured sensory neurones. Meeting of the Physiological Society, November 7–8, 1980. *J. Physiol.* 312:23–33.

Godlewski, A., and M. Kozakiewicz. 1999. Estimation of early G1 phase keratinocyte frequencies in gingival epithelium as a tool for studies of gingival epithelium proliferation. *Folia Histochem. Cytobiol*. 37:155–156.

González de Mejía, E., J. Alfredo, J. A. Quintanar-Hernández, and G. Loarca-Piña. 1998. Antimutagenic activity of carotenoids in green peppers against some nitroarenes. *Mutat. Res.* 416:11–19.

Goodpasture, C. E., and F. E. Arrighi. 1976. Effects of food seasonings on the cell cycle and chromosome morphology of mammalian cells *in vitro* with special reference to turmeric. *Food Chem. Toxicol.* 14:9–14.

Goso, C., S. Evangelista, M. Tramontana, S. Manzini, P. M. Blumberg, and A. Szallasi. 1993. Topical capsaicin administration protects against trinitrobenzene sulfonic acid-induced colitis in the rat. *Eur. J. Pharmacol.* 249:185–190.

Govindarajan, V. S., and S. M. Ananthakrishna. 1974. Paper chromatographic determination of capsaicin. *Flavour Indust*. 5:176–178.

Grant, J., ed. 1972. *Hackh's chemical dictionary*, 4th ed., 130. New York, NY: McGraw-Hill.

Grasselli, J. G., ed. 1975. *Atlas of spectral data and physical constants for organic compounds*. Cleveland, OH: CRC Press,

Gunthorpe, M. J., C. D. Benham, A. Randall, and J. B. Davis. 2002. The diversity in the vanilloid (TRPV) receptor family of ion channels. *Trends Pharmacol. Sci.* 23:183–191.

Haas, J. S., R. E. Whipple, P. M. Grant, and B. D. Andresen. 1997. Chemical and elemental comparison of two formulations of oleoresin capsicum. *Sci. Justice* 37:15–24.

Hallagan, J. B., D. C. Allen, and J. F. Borzelleca. 1995. The safety and regulatory status of food, drug and cosmetics color additives exempt from certification. *Food Chem. Toxicol.* 33:515–528.

Hadjokas, N., and T. Goodfriend. 1991. Inhibition of aldosterone production and angiotensin action by drugs affecting potassium channels. *Pharmacol.* 43:141–150.

Hadžćovic, S., S. Huković, and P. Stern. 1964. A contribution to the pharmacology of capsaicin (English translation). *Veterinaria* 13:449–452.

Hamid, M. R., and U. A. Boelsterli. 1989. Effect of capsaicin on osmotic fragility of red blood cells in rats. *J. Drug Res.* 18:169–176.

Hamid, M. R., and M. Flury-Rowersi. 1984. Effect of capsaicin on bleeding time in rats. *J. Drug Res.* 15:207–209.

Hamid, M. R., U. Friedich, and G. Zbinden. 1989. Evaluation of capsaicin mutagenicity by the Salmonella/mammalian-microsome testing system. *J. Drug Res.* 18:153–160.

Hamid, M. R., and C. A. Reinhardt. 1989. Cytotoxicty of capsaicin. *J. Drug Res.* 18:161–168.

Han, S.-S., Y.-S. Keum, K.-S. Chun, and Y.-J. Surh. 2002. Suppression of phorbol ester-induced NF-κB activation by capsaicin in cultured human promyelocytic leukemia cells. *Arch. Pharm. Res.* 25:475–479.

Harris, E. D., Jr. 1985. Pathogenesis of rheumatoid arthritis. In *Textbook of rheumatology*, ed. W. N. Kelley, E. D. Harris Jr, S. Ruddy, and C. B. Sledge, 896–927. Philadelphia: W. B. Saunders.

Harvey, J. S., C. Davis, I. F. James, and G. M. Burgess. 1995. Activation of protein kinase C by the Capsaicin analogue resiniferatoxin in sensory neurones. *J. Neurochem.* 65:1309–1317.

Hayes, A. G., A. B. Hawcock, and R. G. Hill. 1984. The depolarising action of capsaicin on rat isolated sciatic nerve. *Life Sci.* 8:1561–1568.

Hayes, P., H. J. Meadows, M. J. Gunthorpe, et al. 2000. Cloning and functional expression of a human orthologue of rat vanilloid receptor-1. *Pain* 88:205–215.

Hecht, S. S., and D. Hoffmann. 1988. Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis* 9:875–884.

Helme, R. D., A. Eglezos, G. W. Dandie, P. V. Andrews, and R. L. Boyd. 1987. The effect of substance P on the regional lymph node antibody response to antigenic stimulation in capsaicin-pretreated rats. *J. Immunol*. 139:3470–3473.

Helme, R. D. and S. McKernan.1984. Flare responses in man following topical application of capsaicin. In *Antidromic Vasodilation and Neurogenic Inflammation. Satellite Symposium of the 29*th *International Congress of Physiological Science, Newcastle, Australia*, ed. L. A. Chahl, J. Szolcsányi, and F. Lembeck, 303–312. Budapest: Kultura Hungarian Foreign Trading Company.

Helme, R. D., and S. McKernan.1985. Neurogenic flare responses following topical application of capsaicin in humans. *Ann. Neurol.* 18:505–509.

Herbert, M. K., and S. Hering. 1995. Tumor necrosis factor-α prevents interleukin-1β from augmenting capsaicin-induced vasodilation in the rat skin. *Eur. J. Pharmacol*. 286:273–279.

Herbert, M. K., and P. Holzer. 1994. Nitric oxide mediates the amplification by interleukin-1β of neurogenic vasodilation in the rat skin. *Eur. J. Pharmacol.* 260:89–93.

Hoch-Ligeti, C. 1951. Production of liver tumors by dietary means: Effect of feeding chilies [Capsicum Frutescens and Annuum (Linn.)] to rats. *Acta Unio Int. Contra. Cancrum*. 7:606–611.

Hogaboam, C. M., and J. L. Wallace. 1991. Inhibition of platelet aggregation by capsaicin. An effect unrelated to actions on sensory afferent neurons. *Eur. J. Pharmacol.* 202:129–131.

Holzer, P. 1991. Capsaicin: Cellular targets, mechanisms of action, and selectivity for thin sensory neurons. *Pharmacol. Rev.* 43:143–200.

Holzer, P., A. Saria, G. Skofitsch, and F. Lembeck. 1981. Increase in tissue concentrations of histamine and 5-hydroxytryptamine following capsaicin treatment of newborn rats. *Life Sci.* 29:1099–1105.

Honda, I., H. Kohrogi, T. Yamaguchi, M. Ando, and S. Araki. 1991. Enkephalinase inhibitor potentiates substance P- and capsaicin-induced bronchial smooth muscle contractions in humans. *Am. Rev. Respir. Dis.* 143:1416–1418.

Hoyes A. D., and P. Barber. 1981. Degeneration of axons in the ureteric and duodenal nerve plexuses of the adult rat following in vivo treatment with capsaicin. *Neurosci. Lett.* 7:19–24.

Hoyes, A. D., P. Barber, and H. Jagessar. 1981. Effect of capsaicin on the intraperitoneal axons of the rat trachea. *Neurosci. Lett.* 26:329–334.

Hsiue, T.-R., A. R. Leff, A. Garland, B. Hershenson, D. W. Ray, and J. Solway. 1993. Impaired sensorineural function after allergen-induced mediator release. *Am. Rev. Respir. Dis*. 148:447–454.

Ibeh, I. N., N. Uraih, and J. I. Ogonor. 1991. Dietary exposure to aflatoxin in Benin City, Nigeria: a possible public health concern. *Int. J. Food Microbiol.* 14:171–174.

Imai S. 1991. Effect of topically applied capsaicin on the cutaneous reaction to histamine. *Arch. Dermatol. Res.* 283:414–416.

Inoue, H., N. Nagata, and Y. Koshihara. 1995. Involvement of substance P as a mediator in capsaicin-induced mouse ear edema. *Inflamm. Res.* 44:470–474.

Institut D'Expertise Clinique. 1995. Clinical study for the verification of the good epicutaneous local tolerance of a cosmetic test article, after a single application to the skin of the back and under occlusive patch for 48 hours, in 10 healthy adult volunteers: "single patch test". Unpublished data submitted by CTFA, December 18, 2002.[2]

Iwama, M., T. Tojima, Y. Iioti, N. Takahashi, and Y. Kanke. 1990. Effects of capsaicin and ethanol on hepatic drug-metabolizing enzymes in rat. *Int. J. Vit. Nutr. Res.* 60:100–103.

Jancsó, G. 1981. Intracisternal capsaicin: Selective degeneration of chemosensitive primary sensory afferents in the adult rat. *Neurosci. Lett.* 27:41–45.

Jancsó, G., M. Ferencsik, G. Such, E. Király, A. Nagy, and M. Bujudosó. 1985. Morphological effects of capsaicin and its analogues in newborn and adult mammals. *Fernstroem. Found. Ser.* 6:35–44.

Jancsó, G., E. Király, G. Joó, G. Such, and A. Nagy. 1985. Selective degeneration by capsaicin of a subpopulation of primary sensory neurons in the adult rat. *Neurosci. Lett.* 59:209–214.

Jancsó, G., E. Király, G. Such, S. F. Joó, and A. Nagy. 1987. Neurotoxic effect of capsaicin in mammals *Acta Physiol. Hung.* 69:295–313.

Jancsó, G., and M. Wollemann. 1977. The effect of capsaicin on the adenylate cyclase activity of rat brain. *Brain Res.* 123:323–329.

Jancsó-Gabór A., J. Szolcsányi, and N. Jancsó. 1970. Irreversible impairment of thermoregulation induced by capsaicin and similar pungent substances in rats and guinea-pigs. *J. Physiol.* 206:495–507.

Jang, J. J., K. J. Cho, Y. S. Lee, and J. H. Bae. 1991. Different modifying responses of capsaicin in a wide-spectrum initiation model of F344 rat. *J. Korean Med. Sci.* 6:31–36.

Jang, J.-J., D. E. Devor, D. L. Logsdon, and J. M. Ward. 1992. A 4-week feeding study of ground red chili (*Capsicum annuum*) in male $B6C3F_1$ mice. *Food Chem. Toxicol.* 30:783–787.

Jang, J. J., S. H. Kim, and T. K. Yun. 1989. Inhibitory effect of capsaicin on mouse lung tumor development. *In Vivo* 3:49–54.

Jarrah, S., M. Eran, S. Freier, and E. Yefenof. 1995. Cholecystokinin-octapeptide (CCK-OP) and substance P (SP) influence immune response to cholera toxin in live animals. *Adv. Exp. Med. Biol.* 371A:563–566.

Jhamandas, K., T. L. Yaksh, G. Harty, J. Szolcsanyi, and V. L. W. Go. 1984. Action of intrathecal capsaicin and its structural analogues on the content and release of spinal substance P: Selectivity of action and relationship to analgesis. *Brain Res.* 306:215–225.

John, A. T., and S. Abraham. 1994. Cytological abnormalities induced by red pepper in mouse bone marrow cells *in vivo*. *Caryologia* 47:53–58.

Joó, F., J. Szolcsányi, and A. Jancsó-Gábor. 1969. Mitochondrial alterations in the spinal ganglion cells of the rat accompanying the long-lasting sensory disturbance induced by capsaicin. *Life Sci.* 8:621–626.

Joe, B., and B. R. Lokesh. 1994. Role of capsaicin, curcumin and dietary n-3 fatty acids in lowering the generation of reactive oxygen species in rat peritoneal macrophages. *Biochim. Biophys. Acta* 1224:255–263.

Joe, B., and B. R. Lokesh. 1997. Effect of curcumin and capsaicin on arachidonic acid metabolism and lysosomal enzyme secretion by rat peritoneal macrophages. *Lipids* 32:1173–1180.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 1970. *Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives. Oleoresins of Paprika.* JECFA Monograph 208. FAO Nutrition Meetings Report Series 48a. WHO/Food Add/70.39. Geneva:World Health Organization.

Jung, M.-Y., H.-J. Kang, and A. Moon. 2001. Capsaicin-induced apoptosis in SK-Hep-1 hepatocarcinoma cells involves Bcl-2 downregulation and caspase-3 activation. *Cancer Lett*. 165:139–145.

Kanerva, L., T. Estlander, and R. Jolanki. 1996. Occupational allergic contact dermatitis from spices. *Contact Dermatitis* 35:157–162.

Kang, H.-J., Y. Soh, M.-S. Kim, et al. 2003. Roles of JNK-1 and p38 in selective induction of apoptosis by capsaicin in ras-transformed human breast epithelial cells. *Int. J. Cancer* 103:475–482.

Karawya, M. S., S. I. Balbaa, and A. N. Girgis. 1968. Estimation of capsaicine in certain pharmaceutical preparations. *J. Pharm. Sci.* 9:27–33.

Kashiba, H., E. Senba, Y. Ueda, and M. Tohyama. 1990. Relative sparing of calcitonin gene-related peptide-containing primary sensory neurons following neonatal capsaicin treatment in the rat. *Peptides* 11:491–496.

Kashiba, H., Y. Ueda, and E. Senba. 1997. Systemic capsaicin in the adult rat differentially affects gene expression for neuropeptides and neurotrophin receptors in primary sensory neurons. *Neuroscience* 76:299–312.

Katakami C., and M. Yamamoto. 1996. Capsaicin-induced corneal epithelial changes in rats. *Nippon Ganka Gakkai Zasshi* 100:653–659.

Kawada, T., T. Suzuki, M. Takahashi, and K. Iwai. 1984. Gastrointestinal absorption and metabolism of capsaicin and dihydrocapsaicin in rats. *Toxicol. Appl. Pharmacol.* 72:449–456.

Kawada, T., T. Watanabe, K. Katsura, H. Takami, and K. Iwai. 1985. Formation and metabolism of pungent principle of capsaicin fruits. XV. Microdetermination of capsaicin by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr.* 329:99–105.

Kawada, T., T. Watanabe, T. Takaishi, T. Tanaka, and K. Iwai. 1986. Capsaicin-induced ß-adrenergic action on energy metabolism in rats: Influence of capsaicin on oxygen consumption, the respiratory quotient, and substrate utilization. *Proc. Soc. Exp. Biol. Med.* 183:250–256.

Khaled, M. Y., M. R. Anderson, and H. M. McNair. 1993. Micellar electrokinetic capillary chromatography of pungent compounds using simultaneous on-line ultraviolet and electrochemical detection. *J. Chromatogr. Sci.* 31:259–264.

Kim, J.-D., J.-M. Kim, J.-O. Pyo, S.-Y. Kim, B.-S. Kim, R. Yu, and I.-S. Han. 1997. Capsaicin can alter the expression of tumor forming-related genes which might be followed by induction of apoptosis of a Korean stomach cancer cell line, SNU-1. *Cancer Lett*. 120:235–241.

Kim, J.-M., J.-D. Kim, R. Yu, B.-S. Kim, M.-K. Shin, and I.-S. Han. 1999. Effects of capsaicin on induction of c-jun proto-oncogene expression in Fisher-344 rats by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. *Cancer Lett.* 142:155–160.

Kim, J.-P., J.-G. Park, M.-D. Lee, M.-D. Han, S.-T. Park, B.-H. Lee, and S.-E. Jung. 1985. Co-carcinogenic effects of several Korean foods on gastric cancer induced by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine in rats. *Jpn. J. Surg.* 15:427–437.

Kim, M. H., N. D. Kim, and S. S. Lee. 1979. Effects of capsaicin on liver cytochrome P450 in the rat. *Yakhak Hoe Chi* 23:111–118.

Kim, N. D., and C. Y. Park. 1981. Study on the absorption and excretion of capsaicin in rabbits. *Yakhak Hoe Chi* 25:101–108.

Kimura, S., M. Totani, and T. Murachi. 1973. Indices of viability of isolated intestines: glucose uptake and release of enzymes *Jpn. J. Exp. Med.* 43:163–177.

Kinnman, E., and J. D. Levine. 1995. Involvement of the sympathetic postganglionic neuron in capsaicin-induced secondary hyperalgesia in the rat. *Neuroscience* 65:283–291.

Király, E., G. Jancsó, and F. Joó. 1986. Changes in fibre populations of peripheral nerves after capsaicin treatment of newborn and adult rats. *Verh. Anat. Ges.* 80:269–270.

Kirby, M. L., T. F. Gale, and T. G. Mattio. 1982. Effects of prenatal capsaicin treatment on fetal spontaneous activity, opiate receptor binding, and acid phosphatase in the spinal cord. *Exp. Neurol.* 76:298–308.

Kishi, T., D. M. Morré, and D. J. Morré. 1999. The plasma membrane NADH oxidase of HeLa cells has hydroquinone oxidase activity. *Biochim. Biophys. Acta* 1412:66–77.

Klaassen, C. D., ed. 1996. *Casarett And Doull's toxicology. The basic science of poisons*, 5th ed., 57. New York: McGraw-Hill.

Ko, F., M. Diaz, P. Smith, E. Emerson, Y. J. Kim, T. J. Krizek, and M. C. Robson.1998. Toxic effects of capsaicin on keratinocytes and fibroblasts. *J. Burn Care Rehabil.* 19:409–413.

Kopanitsa, M. V., V. A. Panchenko, E. I. Magura, P. V. Lishko, and O. A. Krishtal. 1995. Capsaicin blocks Ca^{2+} channels in isolated rat trigeminal and hippocampal neurones. *NeuroReport* 6:2338–2340.

Kopczyńska, B., and M. Szereda-Przestaszewska. 1998. Apnoeic responses to pulmonary and systemic challenge of capsaicin in cats. *J. Physiol. Pharmacol*. 49:25–35.

Krajewska, A. M., and J. J. Powers. 1987. Gas chromatographic determination of capsaicinoids in green *Capsicum* fruits. *J. Assoc. Off. Anal. Chem.* 70:926–928.

Krogstad, A. L., P. Lönnroth, G. Larson, and B. G. Wallin. 1999. Capsaicin treatment induces histamine release and perfusion changes in psoriatic skin. *Br. J. Dermatol.* 141:87–93.

Kudlacz, E. M., and R. W. Knippenberg. 1994. In vitro and in vivo effects of tachykinins on immune cell function in guinea pig airways. *J. Neuroimmunol*. 50:119–125.

Kuo, H.-C. 1997. Inhibitory effect of capsaicin on detrusor contractility: Further study in the presence of ganglionic blocker and neurokinin receptor antagonist in the rat urinary bladder. *Urol. Int.* 59:95–101.

LaHann, T. R. 1986. Effect of capsaicin on croton oil and TPA induced tumorigenesis and inflammation. *Proc. Western Pharmacol. Soc.* 29:145–149.

Langenbach, R. L., H. J. Freed, and E. Huberman. 1978. Liver cell-mediated mutagenesis of mammalian cells. *Proc. Natl. Acad. Sci. U. S. A.* 75:2864–2867.

Lankatilake, K. N. and C. G. Uragoda. 1993. Respiratory function in chili grinders. *Occup. Med.* 43:139–142.

Law, M. W. 1983. High pressure liquid chromatographic determination of capsaicin in oleoresin and personal protection aerosols. *J. Assoc. Off. Anal. Chem.* 66:1304–1306.

Lawson, T., and P. Gannett. 1989. The mutagenicity of capsaicin and dihydrocapsaicin in V79 cells. *Cancer Lett.* 48:109–113.

Lee, K.-R., T. Suzuki, M. Kobashi, K. Hasegawa, and K. Kwai. 1976. Quantitative microanalysis of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin using mass fragmentography. *J. Chromatogr*. 123:119–128.

Lee, L.-Y., and J. M. Lundberg. 1994. Capsazepine abolishes pulmonary chemoreflexes induced by capsaicin in anesthetized rats. *J. Appl. Physiol.* 76:1848–1855.

Lee, S. O. 1963. Studies on the influence of diets and lipotropic substances upon the various organs and metabolic changes in rabbits on long term feeding with red pepper [English translation]. *Korean J. Internal Med.* 6:383–481.

Lee, S. S., and S. Kumar. 1980. Metabolism *in vitro* of capsaicin, a pungent principle of red pepper, with rat liver homogenates. In *Microsomes, drug oxidations, and chemical carcinogenesi*, ed. M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette, P. J. O'Brien, 1009–1012. New York: Academic Press.

Leelahuta, Y., T. Glinsukon, and W. Wangpanish. 1983. In vitro capsaicin metabolism in the rat, mouse, and hamster: A preliminary report. *Toxicon* 3:245–248.

Lide, D. R., ed. 1993. *CRC handbook of chemistry and physics*, 74th ed., 3–164. Boca Raton, FL: CRC Press.

López-Carrillo, L., M. H. Avila, and R. Dubrow. 1994. Chili pepper consumption and gastric cancer in Mexico: A case-control study. *Am. J. Epidemiol.* 122:51–65.

Lukovič, L., W. de Jong, and D. de Wied. 1987. Cardiovascular effects of substance P and capsaicin microinjected into the nucleus tractus solitarii of the rat. *Brain Res*. 422:312–318.

Lundberg, J. M., Y. Hua, and B. B. Fredholm. 1984. Capsaicin-induced stiumlation of the guinea-pig atrium. Involvement of a novel sensory transmitter or a direct action on myocytes. *Naunyn Schmiedebergs Arch. Pharmakol*. 325:176–182.

Lundberg, J. M., C.-R. Martling, and A. Saria. 1983. Substance P and capsaicin-induced contraction of human bronchi. *Acta Physiol. Scand*. 119:49–53.

Lundberg, J. M., and A. Saria. 1982. Bronchial smooth muscle contraction induced by stimulation of capsaicin sensitive sensory nerves. *Acta Physiol. Scand*. 115:521.

Lundblad, L., X.-Y. Hua, and J. M. Lundberg. 1984. Mechanisms for reflexive hypertension induced by local application of capsaicin and nicotine to the nasal mucosa. *Acta Physiol. Scand.* 121:277–282.

Lynn, B. 1990. Review article. Capsaicin: Actions on nociceptive C-fibres and therapeutic potential. *Pain* 41:61–69.

Macho, A., M. A. Calzado, J. Muñoz-Blanco, et al. 1999. Selective induction of apoptosis by capsaicin in transformed cells: The role of reactive oxygen species and calcium. *Cell Death Differ.* 6:155–165.

Maggi, C. A., S. Giuliani, P. Santicioli, and A. Meli. 1987. Capsaicin-induced inhibition of motility of the rat isolated vas deferens: Do multiple neuropeptides mediate the visceromotor effects of capsaicin? *J. Auton. Pharm.* 7:243–255.

Maggi, C. A., P. Santicioli, and A. Meli. 1984. The effects of topical capsaicin on rat urinary bladder motility in vivo. *Eur. J. Pharmacol*. 103:41–50.

Maggi, C. A., P. Santicioli, and A. Meli. 1986. Postnatal development of micturition reflex in rats. *Am. J. Physiol*. 250:926–931.

Maggi, C. A., P. Santicioli, D. Renzi, R. Patacchini, C. Surrenti, and A. Meli. 1989. Release of substance P- and calcitonin gene-related peptide-like immunoreactivity and motor response of the isolated guinea pig gallbladder to capsaicin. *Gastroenterology* 96:1093–1101.

Magnusson, B. M., and L.-O. D. Koskinen. 2000. In vitro percutaneous penetration of topically applied capsaicin in relation to in vivo sensation responses. *Int. J. Pharmaceut.* 195:55–62.

Makara, G. B., L. Csalay, R. Frenkl, Z. Somfai, and K. Szepesházi. 1965. Effect of capsaicin on experimental ulcer in the rat. *Acta Med.* 21:214–216.

Makara, G. B., L. György, and J. Molnár. 1967. Circulatory and respiratory responses to capsaicin, 5-hydroxytryptamine and histamine in rats pretreated with capsaicin. *Arch. Int. Pharmacodyn.* 17:39–45.

Malmgren, A., E. Ekblad, F. Sundler, K. E. Andersson, and P. O. Andersson. 1990. Muscarinic supersensitivity in the rat urinary bladder after capsaicin pretreatment. *Acta Physiol. Scand.* 138:377–387.

Mandal, T. K., A. K. De, and J. J. Ghosh. 1994. Histomorphological and biochemical changes of lung tissues following capsaicin treatment in the rat. *Phytother. Res.* 8:22–27.

Mann, N. S. 1977. Capsaicin induced acute erosive gastritis: its prevention by antacid metiamide and cimetidine. *J. Kentucky Med. Assoc*. 75:71–73.

Marciniak, B. H., B. Brown, B. Peterson, L. Boult, and D. Guay. 1995. Adverse consequences of capsaicin exposure in health care workers [letter]. *J. Am. Geriatr. Soc.* 43:1181–1182.

Marfurt, C. F., L. C. Ellis, and M. A. Jones. 1993. Sensory and sympathetic nerve sprouting in the rat cornea following neonatal administration of capsaicin. *Somatosens. Motor Res.* 10:377–398.

Marks, D. R., A. Rapoport, D. Padla, R. Weeks, R. Rosum, F. Shetfall, and F. Arrowsmith. 1993. A double-blind placebo-controlled trial of intranasal capsaicin for cluster headache. *Cephalalgia* 13:114–116.

Marques, S., N. G. Oliveira, T. Chaveca, and J. Rueff. 2002. Micronuclei and sister chromatid exchanges induced by capsaicin in human lymphocytes. *Mutat. Res.* 517:39–46.

Martins, M. A., M. Dolhnikoff, W. A. Zin, and H. N. Saldiva. 1993. Airway and pulmonary tissue responses to capsaicin in guinea pigs assessed with the alveolar capsule technique. *Am. Rev. Respir. Dis.* 147:466–470.

Maruzen Pharmaceuticals Co., Ltd. 2002. Data on Capsicum annuum fruit extract: UV absorption, concentration of use, and skin irritation (rabbits). Unpublished data submitted by CTFA. 2 pages.[2]

Mathew, A., P. Gangadharan, C. Varghese, and M. K. Nair. 2000. Diet and stomach cancer: a case-control study in South India. *Eur. J. Cancer Prev*. 9:89–97.

Matsumoto, J., K. Takeuchi, H. Niida, K. Ueshima, and S. Okabe. 1993. Gastric cytoprotection induced by capsaicin in the rat—relation to motility, blood flow, and HCO_3^- secretion. *Asia Pac. J. Pharmacol*. 8:45–53.

Matucci-Cerinic, M., S. Marabini, S. Jantsch, M. Cagnoni, and G. Partsch. 1990. Effects of capsaicin on the metabolism of rheumatoid arthritis synoviocytes in vitro. *Ann. Rheum. Dis.* 49:598–602.

Maxwell, D. L., R. W. Fuller, and C. M. S. Dixon. 1987. Ventilatory effects of inhaled capsaicin in man. *Eur. J. Clin. Pharmacol*. 31:715–717.

May, A., H. Kaube, C. Büchel, C. Eichten, M. Rijntjes, M. Jüptner, C. Weiller, and H. C. Diener. 1998. Experimental cranial pain elicited by capsaicin: a PET study. *Pain* 74:61–66.

McCarthy, G., and D. McCarty. 1992. Effect of topical capsaicin on osteoarthritis of the hands. *J Rheumatol* 19:604–607.

McCleane, G. J., and M. McLaughlin. 1998. Clinical note. The addition of GTN (glyceryl trinitrate) to capsaicin cream reduces the discomfort associated with application of capsaicin alone. *Pain* 78:149–151.

McCusker, M. T., F. K. Chung, N. M. Roberts, and P. J. Barnes. 1989. Effect of topical capsaicin on the cutaneous responses to inflammatory mediators and to antigen in man. *J. Allergy* 83:1118–1124.

Meddings, J. B., C. M. Hogaboam, K. Tran, J. D. Reynolds, and J. L. Wallace. 1991. Capsaicin effects on non-neuronal plasma membranes. *Biochim. Biophys. Acta* 1070:43–50.

Mende, P., M. Siddiqi, R. Preussmann, and B. Spiegelhalder. 1994. Identification of nitrophenols as nitrosation products from capsaicin and red chilies. *Cancer Lett.* 83:277–282.

Metwally, S. A., and A. M. Kandil. 1985. Effect of capsaicin on gastrointestinal absorption of acetaminophen. *J. Drug Res.* 16:1–6.

Mezey, É., Z. E. Tóth, D. N. Cortright, et al. 2000. Distribution of mRNA for vanilloid receptor subtype-1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. *Proc. Natl. Acad. Sci. U. S. A.* 97:3655–3660.

Miller, C. H., S. M. Hamilton, and R. W. Teel. 1994. Effects of compounds of plant origin on the mutagenicity and metabolism of the tobacco-specific nitrosamine NNK. *Phytother. Res.* 8:342–347.

Miller, M. S., K. Brendel, T. F. Burks, and I. G. Sipes. 1983. Interaction of capsaicinoids with drug-metabolizing systems. *Biochem. Pharmacol.* 32:547–551.

Miller, M. S., S. H. Buck, I. G. Sipes, and T. F. Burks. 1982. Altered axoplasmic transport of nerve growth factor by capsaicin. *Proc. Western Pharmacol. Soc.* 25:87–88.

Ministry of Health, Labor and Welfare (MHLW). June 2001a. Unofficial translation of MHW Ordinance No. 331, Attached Table 2 [Restricted list]. Ministry of Health, Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 2-2, 1-chome, Kasumigaseki,Chiyoda-ku, Tokyo 100-8045, Japan.

Ministry of Health, Labor and Welfare (MHLW). June 2001b. Unofficial translation of MHW Ordinance No. 332. Ingredients of quasi-drugs. Products to be used directly on the body. Ministry of Health, Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 2-2, 1-chome, Kasumigaseki,Chiyoda-ku, Tokyo 100-8045, Japan.

Mitchell, H. W., J. Tomlin, and R. J. Ward. 1984. Reflex changes in respiration and heart rate evoked by intravenous and left ventricular injection of 5-HT and capsaicin in anesthetized rats: a comparison of mechanisms. *Lung* 162:153–163.

Modly, C. E., M. Das, P. S. C. Don, C. L. Marcelo, H. Mukhtar, and D. R. Bickers.1986. Capsaicin as an in vitro inhibitor of benzo(a)pyrene metabolism and its DNA binding in human and murine keratinocytes. *Drug Metab. Dispos.* 14:413–416.

Mohammadian, P., O. K. Andersen, and L. Arendt-Nielsen. 1998. Research report. Correlation between local vascular and sensory changes following tissue inflammation induced by repetitive application of topical capsaicin. *Brain Res.* 792:1–9.

Mohr, W., G. Beneke, and W. Mohning. 1975. Proliferation of synovial lining cells and fibroblasts. *Ann. Rheum. Dis.* 34:219–224.

Molina-Torres, J., A. García-Chávez, and E. Ramírez-Chávez. 1999. Antimicrobial properties of alkamides present in flavouring plants traditionally used in Mesoamerica: affinin and capsaicin. *J. Ethnopharmacol.* 64:241–248.

Molnár J. No date. *The pharmacological effects of capsaicin, the pungent principle in paprika.* Budapest: Institute for Experimental Medical Research of the Hungarian Academy of Sciences, 38 pages.

Molnár, J., and L. György. 1967. Pulmonary hypertensive and other hemodynamic effects of capsaicin in the cat. *Eur. J. Pharmacol*. 1:86–92.

Molnár, J., L. György, G. Unyi, and J. Kenyers. 1969. Effect of capsaicin on the isolated ileum and auricle of the guinea pig. *Acta Physiol. Acad. Sci. Hung.* 35:369–374.

Molnár, J., G. Makara, L. György, and G. Unyi. 1969. The bronchoconstrictor action of capsaicin in the guinea pig. *Acta Physiol. Acad. Sci. Hung.* 36:413–420.

Monsereenusorn, Y. 1979. Effect of capsaicin on intestinal glucose metabolism in vitro. *Toxicol. Lett.* 3:279–283.

Monsereenusorn, Y. 1983. Subchronic toxicity studies of capsaicin and capsicum in rats. *Res. Commun. Chem. Pathol. Pharmacol.* 41:95–110.

Monsereenusorn, Y., and S. Kongsamut S. 1985. Inhibition of calcium uptake by capsaicin. *Res. Commun. Chem. Pathol. Pharmacol*. 47:453–456.

Morré, D. J., P.-J. Chueh, and D. M. Morré. 1995. Capsaicin inhibits preferentially the NADH oxidase and growth of transformed cells in culture. *Proc. Natl. Acad. Sci. U. S. A.* 92:1831–1835.

Morré, D. J., E. Sun, C. Geilen, et al. 1996. Capsaicin inhibits plasma membrane NADH oxidase and growth of human and mouse melanoma lines. *Eur. J. Cancer* 32A:1995–2003.

Muralidhara, N., and K. Narasimhamurthy. 1988. Non-mutagenicity of capsaicin in albino mice. *Food Chem. Toxicol.* 26:955–958.

Nagabhushan, M. and S. V. Bhide. 1985. Mutagenicity of chili extract and capsaicin in short-term tests *Environ. Mutagen.* 7:881–888.

Nalini, H. N., K. Sabitha, S. Chitra, P. Viswanathan, and V. P. Menon. 1997. Histopathological and lipid changes in experimental colon cancer: Effect of coconut kernal (Cocos nucifera Linn.) and (Capsicum annuum Linn) red chili powder. *Indian J. Exp. Biol.* 35:964–971.

Nalini, N., K. Sabitha, P. Viswanathan, and V. P. Menon. 1998. Influence of spices on the bacterial (enzyme) activity in experimental colon cancer. *J. Ethnopharmacol*. 62:15–24.

Nalono, M., D. A. Simone, G. Wendelschafer-Crabb, T. Johnson, E. Hazen, and W. R. Kennedy. 1999. Topical capsaicin in humans: Parallel loss of epidermal nerve fibers and pain sensation. *Pain* 81:135–145.

National Academy of Sciences (NAS). 1996. *Food Chemicals Codex*, 4th ed., 391–392. Washington, DC: National Academy Press.

Nilsson, G., and S. Ahlstedt. 1988. Altered lymphocyte proliferation of immunized rats after neurological manipulation with capsaicin. *Int. J. Immunopharmacol*. 10:747–752.

Nilsson, G., K. Alving, and S. Ahlstedt. 1991. Effects on immune responses in rats after neuromanipulation with capsaicin. *Int. J. Immunopharmac.* 13:21–26.

Nopanitaya, W. 1973. Long-term effects of capsaicin on fat absorption and the growth of the rat. *Growth* 37:269–279.

Nopanitaya, W., and S. W. Nye. 1974. Duodenal mucosal response to the pungent principle of hot pepper (capsaicin) in the rat: Light and electron microscopic study. *Toxicol. Appl. Pharmacol*. 30:149–161.

Northam, W. J., and D. J. Jones. 1984. Comparison of capsaicin and substance P induced cyclic AMP accumulation in spinal cord tissue slices. *Life Sci.* 35:293–302.

Notani, P. N., and K. Jayant. 1987. Role of diet in upper aerodigestive tract cancers. *Nutr. Cancer* 10:103–113.

Ogilvy, C. S., and L. F. Borges. 1990. Changes in corneal innervation during postnatal development in normal rats and in rats treated at birth with capsaicin. *Invest. Ophthalmol. Vis. Sci.* 31:1810–1815.

Osadchii, L. I., V. Balueva, and J. Molnár. 1967. Reflex changes in systemic vascular resistance following intracoronary injection of capsaicin and veratrine in the cat. *Acta Physiol. Acad. Sci. Hung.* 32:215–219.

Palecek, F., O. P. Matthew, F. B. Sant'Ambrogio, and G. Sant'Ambrogio. 1990. Cardiorespiratory responses to inhaled laryngeal irritants. *Inhalation Toxicol.* 2:93–104.

Pandey, M., and V. K. Shukla. 2002. Diet and gall bladder cancer: A case-control study. *Eur. J. Cancer Prev.* 11:365–368.

Pankar, D. S., and N. G. Magar. 1977. New method for the determination of capsaicin by using multi-band thin-layer chromatography. *J. Chromatogr.* 21:149–152.

Park, K.-H., and J.-P. Kim. 1993. Effect of red pepper and bracken fern on gastroduodenal carcinogenesis in Wistar rats induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. *Seoul J. Med.* 34:117–125.

Park, K.-K., K.-S. Chun, J. I. Yook, and Y.-J. Surh. 1998. Lack of tumor promoting activity of capsaicin, a principal pungent ingredient of red pepper, in mouse skin carcinogenesis. *Anticancer Res.* 18:4201–4206.

Park, K.-K., and Y.-J. Surh. 1997. Effects of capsaicin on chemically-induced two-stage mouse skin carcinogenesis. *Cancer Lett.* 114:183–184.

Parrish, M. 1996. Liquid chromatographic method for determining capsaicinoids in capsicums and their extractives: Collaborative study. *J. Assoc. Off. Analyt. Chem.* 79:738–745.

Partsch, G., and M. Matucci-Cerinic. 1993. Capsaicin stimulates the migration of human polymorphonuclear cells (PMN) in vitro. *Life Sci.* 53:PL309–PL314.

Pelto-Huikko, M., A. Dagerlind, S. Ceccatelli, and T. Hökfelt. 1991. The immediate-early genes c-*fos* and c-*jun* are differentially expressed in the rat adrenal gland after capsaicin treatment. *Neurosci. Lett*. 126:163–166.

Pérez, H., S. Ruiz, H. Inostroza, and M. Perretta. 1991. Neonatal capsaicin treatment impairs functional properties of primary olfactory afferents in the rat. *Neurosci. Lett.* 127:251–254.

Perfumi, M., and L. Sparapassi. 1999. Rat offspring treated prenatally with capsaicin do not show some of the irreversible effects induced by neonatal treatment with neurotoxin. *Pharmacol. Toxicol*. 84:66–71.

Perretti, M., A. Ahluwalia, R. J. Flower, and S. Manzini. 1993. Endogenous tachykinins play a role in IL-1-induced neutrophil accumulation: involvement of NK-1 receptors. *Immunology* 80:73–77.

Petersen, L. J., K. Winge, E. Brodin, and P. S. Skov. 1997. No release of histamine and substance P in capsaicin-induced neurogenic inflammation in intact human skin *in vivo*: A microdialysis study. *Clin. Exp. Allergy* 27:957–965.

Pisarri, T. E., J. C. G. Coleridge, and H. M. Coleridge. 1993. Capsaicin-induced bronchial vasodilation in dogs: central and peripheral neural mechanisms. *J. Appl. Physiol.* 74:259–266.

Podolsky, R. S., M. Grabowski, R. Milner, W. P. Ritchie Jr., and D. T. Dempsey. 1994. Capsaicin-induced gastric hyperemia and protection are NO^- dependent. *J. Surg. Res.* 57:438–442.

Pórszász, J., L. György, and K. Pórszász-Gibiszer. 1955. Cardiovascular and respiratory effects of capsaicin. *Acta Physiol. Acad. Sci. Hung.* 8:60–76.

Pórszász, R., and J. Szolcsányi. 1992. Circulatory and respiratory effects of capsaicin and resiniferatoxin on guinea pigs. *Acta Biochim. Biophys. Hung.* 26:131–138.

Pyo, C.-O., S.-A. Ju, S.-Y. Choe, R. Yu, I.-S. Han, and B.-S. Kim. 1998. Proceedings of the 1st ASIATOX Conference, June 29–July 2, 1997, Yokohama, Japan. Capsaicin produces apoptosis in Korean stomach cancer cell line, SNU-1. *J. Toxicol. Sci.* 23:328.

Qarawi, A. A., and S. E. I. Adam. 1999. Effects of red chili (*Capsicum frutescens* L.) on rats. *Vet. Human Toxicol.* 41:293–295.

Quest, J. A., J. Dias-Souza, W. P. Norman, J. R. Holtman Jr., and R. A. Gillis. 1984. Central nervous system site of action of capsaicin-induced cardiovascular changes in the cat. *J. Pharmacol. Exp. Ther.* 228:719–724.

Rabe, L. S., S. H. Buck, L. Moreno, T. F. Burks, and N. Dafny. 1980. Neurophysiological and thermoregulatory effects of capsaicin. *Brain Res. Bull.* 5:755–758.

Reilly, D. M., and M. R. Green. 1999. Eicosanoid and cytokine levels in acute skin irritation in response to tape stripping and capsaicin. *Acta Derm. Venereol*. 79:187–190.

Reinhardt, C. A., D. A. Peli, and G. Zbinden. 1985. Interpretation of cell toxicity data for the estimation of potential irritation. *Food Chem. Toxicol*. 23:247–252.

Reliance Clinical Testing Services, Inc. 2000. Final report. Clinical safety evaluation. Repeated insult patch test. Test article: 1913.02 (Amber Gel). Unpublished data submitted by CTFA, January 9, 2003. 9 pages.[2]

Reynier-Rebuffel, A.-M., P. Mathiau, J. Callebert, et al. 1994. Substance P, calcitonin gene-related peptide, and capsaicin release serotonin from cerebrovascular mast cells. *Am. J. Physiol.* 267:R1421–R1429.

Richeux, F., M. Cascante, R. Ennamany, D. Saboureau, and E. E. Creppy. 1999. Cytotoxicity and genotoxicity of capsaicin in human neuroblastoma cells SHSY-5Y. *Arch. Toxicol.* 73:403–409.

Rietschel, R. L., and J. F. Fowler. 1995. *Fisher's contact dermatitis*, 4th ed., 512, 628. Baltimore, MD: Williams & Wilkins.

Ritter, S., and T. T. Dinh. 1990. Capsaicin-induced neuronal degeneration in the brain and retina of preweanling rats. *J. Comp. Neurol.* 296:447–461.

Ritter, S., and T. T. Dinh. 1992. Age-related changes in capsaicin-induced degeneration in rat brain. *J. Comp. Neurol.* 318:103–116.

Rizvi, S. I., and P. P. Srivastava. 1999. Protective effect of capsaicin on the osmotic fragility of human erythrocytes. *Indian J. Exp. Biol.* 37:835–836.

Robineau, P., M. Lonchampt, N. Kucharczyk et al. 1995. In vitro and in vivo pharmacology of S 16474, a novel dual tachykinin NK_1 and NK_2 receptor agonist. *Eur. J. Pharmacol.* 294:677–684.

Roychowdhury, P., and P. Sukul. 1997. Residue behavior of fluvalinate in chili (*Capsicum annuum* L.) under Indian climatic condition. *Bull. Environ. Contam. Toxicol*. 59:723–727.

Sagara, K., S. Kakizawa, K. Kasuya, T. Misaki, and H. Yoshizawa. 1980. Simultaneous determination of capsaicin, dihydrocapsaicin and nordihydrocapsaicin in capsicum fruit by gas chromatography. *Chem. Pharm. Bull*. 28:2796–2799.

Saito, A., T. Masaki, T. J.-F. Lee, and K. Goto. 1988. Effects of capsaicin on the contractility and peptide-containing nerves of large cerebral arteries of the cat. *Arch. Int. Pharmacodyn.* 295:194–203.

Saito, A., and M. Yamamoto. 1996. Acute oral toxicity of capsaicin in mice and rats. *J. Toxicol. Sci.* 21:195–200.

Sambaiah, K., and M. N. Satyanarayana. 1987. Stimulation of liver triglyceride secretion in rats by capsaicin. *Curr. Sci*. 56:897–898.

Sambaiah, K., and M. N. Satyanarayana. 1980. Hypocholesterolemic effect of red pepper and capsaicin. *Indian J. Exp. Biol*. 18:898–899.

Sandberg, G., and A. Ljungdahl. 1986. Mitogenic responses of splenic B and T lymphocytes in neonatally capsaicin-treated mice. *Int. Arch. Allergy Appl. Immun.* 81:343–347.

Sandia National Labs. 1995. Oleoresin capsicum toxicology evaluation and hazard review. NTIS Report No. DE96001765.

Sann, H., E. Pinter, J. Szolcsányi, and F. K. Pierau. 1988. Peptidergic afferents might contribute to the regulation of skin blood flow. *Agents Actions* 23:14–15.

Santicioli, P., C. A. Maggi, and A. Meli. 1985. The effect of capsaicin pretreatment on the cystometrograms of urethane anesthetized rats. *J. Urol*. 133:700–703.

Santoni, G., M. Perfumi, P. Birarelli, A. Procaccini, and M. Piccoli. 1995. In vivo capsaicin treatment inhibits rat NK cell cytotoxic functions. *Immunopharmacol. Immunotoxicol.* 17:511–528.

Santoni, G., M. Perfumi, A. M. Bressan, and M. Piccoli. 1996. Capsaicin-induced inhibition of mitogen and interleukin-2-stimulated T cell proliferation: its reversal by in vivo substance P adminstration. *J. Neuroimmunol.* 68:131–138.

Santoni, G., M. C. Perfumi, P. Pompei, et al. 2000. Impairment of rat thymocyte differentiation and functions by neonatal capsaicin treatment is associated with induction of apoptosis. *J. Neuroimmunol*. 104:37–46.

Saria, A., F. Lembeck, and G. Skofitsch. 1981. Determination of capsaicin in tissues and separation of capsaicin analogues by high-performance liquid chromatography. *J Chromatogr*. 208:41–46.

Saria, A., J. M. Lundberg, G. Skofitsch, and F. Lembeck. 1983. Vascular protein leakage in various tissues induced by substance P, capsaicin, bradykinin, serotonin, histamine, and antigen challenge. *Naunyn Schmiedebergs Arch. Pharmakol.* 324:212–218.

Saria, A., G. Skofitsch, and F. J. Lembeck. 1982. Distribution of capsaicin in rat tissues after systemic administration. *J. Pharm. Pharmacol*. 34:273–275.

Sasajima, K., J. C. Willey, S. P. Banks-Schlegel, and C. C. Harris. 1987. Effects of tumor promoters and cocarcinogens on growth and differentiation of cultured human esophageal epithelial cells. *J. Natl. Cancer Inst.* 78:419–423.

Sastre, J., M. Olmo, A. Novalvos, D. Ibañez, and C. Lahoz. 1996. Case report. Occupational asthma due to different spices. *Allergy* 51:117–120.

Savitha, G., and B. P. Salimath. 1995. Effect of capsaicin on phospholipase A_2 activity and superoxide generation in macrophages. *Nutr. Res.* 15:1417–1427.

Sax, N. I. 1979 *Dangerous properties of industrial materials*, 5th ed., 467. New York, NY: Van Nostrand Reinhold.

Scadding, J. W. 1980. The permanent anatomical effects of neonatal capsaicin on somatosensory nerves. *J. Anat.* 131:473–484.

Scoville, W. L. 1912. Note on Capsicum. *J. Am. Pharm. Assoc.* 1:453–454.

Serra, J., M. Campero, and J. Ochoa. 1998. Flare and hyperalgesia after intradermal capsaicin injection in human skin. *J. Neurophysiol.* 80:2801–2810.

Shashikanth, K. N. and A. Hosono. 1987. Screening of streptomycin-dependent strains of *Salmonella typhimurium* and *Escherichia coli* for *in vitro* detection of spice-induced mutagenicity. *Lebensm. Wiss. Technol.* 20:91–94.

Shimizu, T. S. Fujita, K. Izumi, T. Koja, N. Ohba, and T. Fukuda. 1984. Corneal lesions induced by the systemic administration of capsaicin in neonatal mice and rats. *Naunyn Schmiedebergs Arch. Pharmakol*. 326:347–351.

Shimizu, T., K. Izumi, S. Fujita, T. Koja, M. Sorimachi, N. Ohba, and T. Fukuda. 1987. Capsaicin-induced corneal lesions in mice and the effects of chemical sympathectomy. *J. Pharmacol. Exp. Ther.* 243:690–695.

Shimomura, Y, T. Kawada, and M. Suzuki. 1989. Capsaicin and its analogs inhibit the activity of NADH-Coenzyme Q oxidoreductase of the mitochondrial respiratory chain. *Arch. Biochem. Biophys.* 270:573–577.

Shono, T. and J. M. Hutson. 1994. Capsaicin increases the frequency of cryptorchidism in flutamide-treated rats. *J. Urol*. 152:763–765.

Sicuteri, F., M. Fanciullacci, M. Nicolodi et al. 1990. Substance P theory: A unique focus on the painful and painless phenomena of cluster headache. *Headache* 30:69–79.

Sicuteri, F., B. M. Fusco, S. Marabini, et al. 1989. Beneficial effect of capsaicin application to the nasal mucosa in cluster headache. *Clin. J. Pathol*. 5:49–53.

Siris Impex. 2002. Specification: Capsicum annuum extract. http://www.sirisimpex.com/capsicum.htm.

Silva, H. M., E. B. Correa, R. A. D. Santos, S. B. Rascop, and S. T. Iaria. 1990. Microbiota Fúngica, espécies aflatoxigênicas do gênero aspergillus e detecção de aflatoxinas em pimenta verhelha (capsicum sp) comdrcializada no município de goiânia-go [Isolation of fungi, production of aflatoxins by Aspergillus sp., and aflatoxins in samples of red pepper (Capsicum sp.)]. *Rev. Microbiol.* 21:149–152.

Simone, D. A., T. K. Baumann, and R. H. LaMotte. 1989. Dose-dependent pain and mechanical hyperalgesia in humans after intradermal injection of capsaicin. *Pain* 38:99–108.

Simone, D. A., M. Nolano, T. Johnson, G. Wendcelschafer-Crabb, and W. R. Kennedy. 1998. Intradermal injection of capsaicin in humans produces degeneration and subsequent reinnervation of epidermal nerve fibers: Correlation with sensory function. *J. Neurosci*. 18:8947–8959.

Simone, D. A., and J. Ochoa. 1991. Early and late effects of prolonged topical capsaicin on cutaeous sensibility and neurogenic vasodilatation in humans. *Pain* 47:285–294.

Singh, S., S. F. Asad, A. Ahmad, N. U. Khan, and S. M. Hadi. 2001. Oxidative DNA damage by capsaicin and dihydrocapsaicin in the presence of Cu(II). *Cancer Lett*. 169:139–146.

Singh, S., K. Natarajan, and B. B. Aggarwal. 1996. Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is a potent inhibitor of nuclear transcription factor-κB activation by diverse agents. *J. Immunol*. 157:4412–4420.

Skofitsch, G., G. S. Hamill, and D. M. Jacobowitz. 1984. Capsaicin depletes corticotropin-releasing factor-like immunoreactive neurons in the rat spinal cord and medulla oblongata. *Neuroendocrinology* 38:514–517.

Skofitsch, G., N. Zamir, C. Helke, J. M. Savitt, and D. M. Jacobowitz. 1985. Corticotropin releasing factor-like immunoreactivity in sensory ganglia and capsaicin sensitive neurons of the rat central nervous system: Colocalization with other neuropeptides. *Peptides* 6:307–318.

Smith, J. G., Jr., R. G. Crounse, and D. Spence. 1970. The effect of capsaicin on human skin, liver, and epidermal lysosomes. *J. Invest. Dermatol.* 54:170–173.

Smith, T. J., Z. Guo, P. E. Thomas, F. L. Chung, M. A. Morse, K. Elkind, and C. S. Yang. 1990. Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in mouse lung microsomes and its inhibition by isothiocyanates. *Cancer Res.* 50:6817–6822.

Spanyár, P. and M. Blazovich. 1969. A thin-layer chromatographic method for the determination of capsaicin in ground paprika. *Analyst* 94:1084–1089.

Srinivasan, M. R., K. Sambaiah, M. N. Satyanarayna, and M. V. L. Rao. 1980. Influence of red pepper and capsaicin on growth, blood constituents, and nitrogen balance in rats. *Nutr. Rep. Int.* 21:455–467.

Srinivasan, M. R., and M. N. Satyanarayana. 1987. Influence of capsaicin, curcumin and ferulic acid in rats fed high fat diets. *J. Biosci.* 12:143–152.

Srinivasan, M. R., M. N. Satyanarayana, and M. V. L. Rao. 1981. A thin layer chromatographic method for the estimation of capsaicin and related compounds. *Res. Ind.* 26:180–183.

Staszewska-Woolley, J., D. E. Luk, and P. N. Nolan. 1986. Cardiovascular reflexes mediated by capsaicin sensitive cardiac afferent neurones in the dog. *Cardiovasc. Res.* 20:897–906.

Staszewska-Woolley, J., P. N. Nolan, and D. E. Luk. 1991. Studies of reflexogenic effects of capsaicin and neuropeptides on neural afferents in the dog parietal pericardium. *Neuropeptides* 20:191–200.

Steffee, C. H., P. E. Lantz, L. M. Flannagan, et al. 1995. Oleoresin capsicum (pepper) spray and "in custody deaths". *Am. J. Forensic Med. Pathol*. 16:185–192.

Sterner, O., and A. Szallasi. 1999. Novel natural vanilloid receptor agonists: New therapeutic targets for drug development. *Trends Pharmacol. Sci.* 20:459–465.

Sternini, C., J. R. Reeve Jr., and N. Brecha.1987. Distribution and characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats. *Gastroenterology* 93:852–862.

Sticher, O., F. Soldati, and R. K. Joshi. 1978. High-performance liquid chromatographic separation and quantitative determination of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, and homodihydrocapsaicin in natural capsaicinoid mixtures and Fructus capsici. *J. Chromatogr*. 166:221–231.

Sugimoto, T., C. Xiao, and H. Ichikawa. 1998. Neonatal primary neuronal death induced by capsaicin and axotomy involves an apoptotic mechanism. *Brain Res.* 807:147–154.

Surh, Y.-J., S. H. Ahn, K.-C. Kim, J.-B. Park, Y. W. Sohn, and S. S. Lee. 1995. Metabolism of capsaicinioids: Evidence for aliphatic hydroxylation and its pharmacological implications. *Life Sci.* 56:PL305-PL311.

Surh, Y.-J., and S. S. Lee. 1995. Capsaicin, a double-edged sword: Toxicity, metabolism, and chemopreventive potential. *Life Sci.* 56:1845–1855.

Surh, Y.-J., and S. S. Lee. 1996. Capsaicin in hot chili pepper: Carcinogen, co-carcinogen, or anticarcinogen. *Food Chem. Toxicol.* 34:313–316.

Surh, Y.-J., R. C.-J. Lee, K.-K. Park, S. T. Mayne, A. Liem, and J. A. Miller. 1995. Chemoprotective effects of capsaicin and diallyl sulfide against mutagenesis or tumorigenesis by vinyl carbamate and *N*-nitrosodimethylamine. *Carcinogenesis* 16:2467–2471.

Suzuki, T. 1980. Effective separation of capsaicin and its analogues by reversed phase high performance thin layer chromatography. *J. Chromatog*. 173:217–223.

Sylvester, D. M., and T. R. LaHann. 1989. Effects of capsaicinoids on platelet aggregation. *Proc. Western Pharmacol. Soc*. 32:95–100.

Szallasi, A., and P. M. Blumberg. 1999. Vanilloid (capsaicin) receptors and mechanisms. *Pharmacol. Rev*. 51:159–212.

Szeimies, R. M., W. Stolz, U. Wlotzke, H. C. Korting, and M. Landthaler. 1994. Successful treatment of hydroxyethyl starch-induced pruritus with topical capsaicin. *Br. J. Dermatol.* 131:380–382.

Szikszay, M., F. Obál Jr., and F. Obál. 1982. Dose-response relationships in the thermoregulatory effects of capsaicin. *Naunyn Schmiedebergs Arch. Pharmakol*. 320:97–100.

Szolcs'anyi, J., F. Jo'o, and A. Jancso-G'abor. 1971. Mitochondrial changes in preoptic neurons after capsaicin desensitization of the hypothalamic thermodetectors in rats. *Nature* 229:116–117.

Tabata, S., H. Kammura, A. Ibe, H. Hashimoto, M. Iida, Y. Tamura, and T. Nishima. 1993. Aflatoxin contamination in foods and foodstuffs in Tokyo: 1986–1990. *J. Assoc. Off. Anal.Chem.* 76:32–35.

Taguchi, S., S. Fukushima, T. Summoto, S. Yoshida, and T. Nishimune. 1995. Aflatoxins in foods collected in Osaka, Japan, from 1988 to 1992. *J. Assoc. Off. Anal.Chem.* 78:325–333.

Takahata, K., X. Chen, K. Monobe, and M. Tada. 1999. Growth inhibition of capsaicin on HeLa cells is not mediated by intracellular mobilization. *Life Sci*. 64:166–171.

Tandan, R., G. A. Lewis, P. B. Krusinski, G. B. Badger, and T. J. Fries. 1992. Topical 0.075% capsaicin in painful diabetic neuropathy. I: A controlled study with long-term follow-up. *Diabetes Care* 15:15–18.

Tátrai, E., and G. Ungváry. 1992. The aetiology of experimental fibrosing alveobronchiolitis induced in rats by paprika dust. *Br. J. Ind. Med*. 49:494–498.

Teel, R. W. 1991. Effects of capsaicin on rat liver S9-mediated metabolism and DNA binding of aflatoxin. *Nutr. Cancer* 15:27–32.

Teel, R. W. 1993. Effect of phytochemicals on the mutagenicity of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in Salmonella typhimurium strain TA1535. *Phytother. Res.* 7:248–251.

Teel, R. W., and H. T. Huynh. 1999. Lack of the inhibitory effect of intragastrically administered capsaicin on NNK-induced lung tumor formation in the A/J mouse. *In Vivo* 13:231–234.

Thatte, U., N. Pai, S. R. Naik, S. M. Karandikar, and S. A. Dahanukar. 1988. Mechanism of chili induced gastric vascular damage and protective effects of drugs. *Indian J. Pharmac.* 20:75–79.

Toda, N., H. Usui, N. Nishino, and M. Fujiwara. 1972. Cardiovascular effects of capsaicin in dogs and rabbits. *J. Pharmacol. Exp. Ther.* 181:512–521.

Todd, P., Jr., M. Bensinger, and T. Biftu. 1975. TLC screening techniques for the qualitative determination of natural and synthetic capsaicinoids. *J. Chromatogr. Sci.* 13:577–579.

Torebjörk, H. E., L. E. R. Lundberg, and R. H. LaMotte. 1992. Central changes in processing of mecha noreceptive input in capsaicin-induced secondary hyperalgesia in humans. *J. Physiol.* 448:765–780.

Toskulkao, C., and P. Hatthachote. 1992. Long-term effects of capsaicin on intestinal thiamine absorption in mice. *J. Clin. Biochem. Nutr.* 13:179–187.

Toskulkao, C., P. Hatthachote, P. Saitongdee, and T. Glinsukon. 1992. Inhibitory effect of capsaicin on intestinal thiamine absorption in mice. *J. Sci. Soc. Thailand* 18:129–140.

Toth, B., D. Nagel, and K. Patil. 1980. Tumorigenic action of N-n-butyl-N-formylhydrazine in mice. *Carcinogenesis* 1:589–593.

Toth, B., and P. Gannett. 1992. Carcinogenicity of lifelong administration of Capsaicin of hot pepper in mice. *In Vivo* 6:59–63.

Toth, B., E. Rogan, and B. Walke. 1984. Tumorigenicity and mutagenicity studies with capsaicin of hot peppers. *Anticancer Res.* 4:117–119.

Traurig, H. H., R. E. Papka, and M. E. Rush. 1988. Effects of capsaicin on reproductive function in the female rat: role peptide-containing primary afferent nerves innervating the uterine cervix in the neuroendocrine copulatory response. *Cell Tissue Res.* 253:573–581.

Traurig, H., R. E. Papka, A. Saria, and F. Lembeck. 1984. Substance P immunoreactivity in the rat mammary nipple and the effects of capsaicin treatment on lactation. *Naunyn Schmiedebergs Arch. Pharmakol.* 328:1–8.

Tricker, A. R., M. Siddiq, and R. Preussmann. 1988. Occurrence of volatile N-nitrosamines in dried chilies. *Cancer Lett*. 38:271–274.

Tsai, Y. H., Y. B. Huang, J. Y. Fang, and P. C. Wu. 1994. Percutaneous absorption of capsaicin and its derivatives. *Drug Dev. Ind. Pharm*. 20:719–730.

Ueda, N., I. Muramatsu, and M. Fujiwara. 1984. Capsaicin and bradykinin-induced substance P-ergic responses in the iris sphincter muscle of the rabbit. *J. Pharmacol. Exp. Ther.* 230:469–473.

Uhlén, S., and J. E. S. Wikberg. 1989. α2-Adrenoceptors mediate inhibition of cyclic AMP production in the spinal cord after stimulation of cyclic AMP with Forskolin but not after stimulation with capsaicin or vasoactive intestinal peptide. *J. Neurochem.* 52:761–767.

van Wyk, C. W., A. Olivier, C. de Miranda, P. van der Bijl, A. F. Grobler-Rabie, and D. O. Chalton. 1995. Effect of chili (*Capsicum frutescens*) extract on proliferation of oral mucosal fibroblasts. *Indian J. Exp. Biol.* 33:244–248.

Vega de la Osada, F., K. P. Esteve, E. Alonso Lebrero, I. Sandin, M. C. Munoz Martinez, and M. T. Laso Borrego. 1998. Sensitization to paprika: anaphylaxis after intake and rhinoconjunctivitis after contact through airways. *Med. Clin*. 111:263–266.

Veronesi, B., J. D. Carter, R. B. Devlin, S. A. Simon, and M. Oortgiesen. 1999. Neuropeptides and capsaicin stimulate the release of inflammatory cytokines in a human bronchial epithelial cell line. *Neuropeptides* 33:447–456.

Veronesi, B., D. M. Salistad, D. L. Doerfler, and M. J. Selgrade. 1995. Neuropeptide modulation of chemically induced skin irritation. *Toxicol. Appl. Pharmacol*. 135:258–267.

Veronesi, B., W. C. Williams, R. J. Smialowicz, D. M. Sailstad, D. Doerfler, and M. J. K. Selgrade. 1998. Neuropeptide denervation alters both the elicitation and induction phases of contact hypersensitivity in mice. *Toxicol. Appl. Pharmacol.* 153:243–249.

Vesaluoma, M., L. Müller, J. Gallar, A. Lambiase, J. Moilanen, T. Hack, C. Belmonte, and T. Tervo. 2000. Effects of oleoresin capsicum pepper spray on human corneal morphology and sensitivity. *Invest. Ophthalmol. Vis. Sci.* 41:2138–2147.

Villaseñor, I. M., and E. J. de Ocampo. 1994. Clastogenicity of red pepper (*Capsicum frutescens* L.) extracts. *Mutat. Res*. 312:151–155.

Villaseñor, I. M., E. de Ocampo, and J. B. Bremner. 1993. Effect of natural capsaicin on bone marrow cells of mice. *Philipp. J. Sci.* 122:397–402.

Villaseñor, I. M., E. de Ocampo, and J. B. Bremner. 1995. Genotoxic acetamide(s) from capsicum frutescens fruits. *Nat. Prod. Lett.* 6:247–253.

Virus, R. M., M. M. Knuepfer, D. Q. McManus, M. J. Brody, and G. F. Gebhart. 1981. Capsaicin treatment in adult Wistar-Kyoto and spontaneously hypertensive rats: Effects on nociceptive behavior and cardiovascular regulation. *Eur. J. Pharmacol.* 72:209–217.

Wahlestedt, C., G. Bynke, and R. Håkanson. 1984. Pupillary constriction by bradykinin and capsaicin: Mode of action. *Eur. J. Pharmacol.* 27:577–583.

Wallace, J. L., G. W. McKnight, and A. D. Befus. 1992. Capsaicin-induced hyperemia in the stomach: possible contribution of mast cells. *Am. J. Physiol.* 263:209–214.

Wallengren, J., R. Ekman, and H. Möller. 1991. Capsaicin enhances allergic contact dermatitis in the guinea pig. *Contact Dermatitis* 24:30–34.

Wallengren, J., and M. Klinker. 1995. Successful treatment of notalgia paresthetica with topical capsaicin: Vehicle-controlled, double-blind, crossover study. *J. Am. Acad. Dermatol*. 32:287–289.

Wang, J.-P., M.-F. Hsu, and C.-M. Teng. 1984. Antiplatelet effect of capsaicin. *Thromb. Res.* 36:497–507.

Wanichanon, C., P. Hatthachote, C. Toskulkao, and P. Saitongdee. 1996. Ultrastructural changes in the intestinal absorptive cells of the mouse induced by capsaicin in relation to thiamine absorption. *Phytother. Res.* 10:136–139.

Watanabe, T., T. Kawada, M. Kurosawa, A. Sato, and K. Iwai. 1988. Adrenal sympathetic efferent nerve and catecholamine secretion excitation caused by capsaicin in rats. *Am. J. Physiol.* 255:E23-E27.

Watson, C. P.N., R. J. Evans, and V. R. Watt. 1988. Post-herpetic neuralgia and topical capsaicin. *Pain* 33:333–340.

Watson, C. P.N., R. J. Evans, and V. R. Watt. 1989. The postmastectomy pain syndrome and the effect of topical capsaicin. *Pain* 38:177–186.

Watson, W. A., K. R. Stremel, and E. J. Westdorp. 1996. Oleoresin Capsicum (Cap-Stun) toxicity from aerosol exposure. *Ann. Pharmacother.* 30:733–735.

Weisshaar, E., G. Heyer, C. Forster, and H. O. Handwerker. 1998. Effect of topical capsaicin on the cutaneous reactions and itching to histamine in atopic eczema compared to healthy skin. *Arch. Dermatol. Res.* 290:306–311.

Wenninger, J. A., and G. N. McEwen, Jr., eds. 2000. *International cosmetic ingredient dictionary and handbook*, 8th ed., Vols. 1–3. Washington, DC: CTFA.

Wijsman, J. H., R. R. Jonker, R. Keijzer, C. J. H. Van De Velde, C. J. Cornelisse, and J. H. Van Dierendonck. 1993. A new method to detect apoptosis in paraffin sections: In situ end-labelling of fragmented DNA. *J. Histochem. Cytochem*. 41:7–12.

Wilkins, J. P. G. 1993. Determination of residues of the insecticide diflubenzuron in foodstuffs using liquid chromatography-thermospray mass spectrometry and identification of some natural components found in chilies. *Anal. Proc.* 30:396–397.

Wilkinson, J., and R. Moore, eds.1982. *Harry's cosmeticology*, 7th ed., 503. New York: Chemical Publishing.

Wille, J. J., A. F. Kydonieus, and G. F. Murphy. 1999. *cis*-Urocanic Acid induces mast cell degranulation and release of preformed TNF-α: A possible mechanism linking UVB and *cis*-Urocanic Acid to immunosuppression of contact hypersensitivity. *Skin Pharmacol. Appl. Skin Physiol* 12:18–27.

Williams, S. R., R. F. Clark, and J. V. Dunford. 1995. Contact dermatitis associated with capsaicin: Hunan hand syndrome. *Ann. Emerg. Med.* 25:713–715.

Wolvetang, E. J., J. A. Larm, P. Moutsoulas, and A. Lawen. 1996. Apoptosis induced by inhibitors of the plasma membrane NADH-oxidase involves Bcl-2 and calcineurin. *Cell Growth Differ*. 7:1315–1325.

Wood, J. N., J. Winter, I. F. James, et al. 1988. Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. *J. Neurosci.* 8:3208–3220.

Yiangou, Y., P. Facer, N. H. C. Dyer, et al. 2001. Vanilloid receptor-1 immunoreactivity in inflamed human bowel. *Lancet* 357:1338–1339.

Yamato, T., M. Aomine, M. Ikeda, H. Noto, and C. Ohta. 1996. Inhibition of contractile tension by capsaicin in isolated rat papillary muscle. *Gen. Pharmacol*. 27:129–132.

Yang, J.-M., S.-N. Wu, and I.-J. Chen. 1993. Spinal adenosine modulates capsaicin-induced depressor reflex: Involvement of adenosine A_2 receptor. *Gen. Pharmacol.* 24:961–970.

Yu, R., J.-W. Park, T. Kurata, and K. L. Erickson. 1998. Modulation of select immune responses by dietary capsaicin. *Internat. J. Vit. Nutr. Res.* 68:114–119.

Zhang, Z., S. M. Hamilton, C. Stewart, A. Strother, and R. W. Teel. 1993. Inhibition of liver microsomal cytochrome P450 activity and metabolism of the tobacco-specific nitrosamine NNK by capsaicin and ellagic acid. *Anticancer Res.* 13:2341–2346.

Zhang, Z., H. Huynh, and R. W. Teel. 1997. Effects of orally administered capsaicin, the principal component of capsicum fruits, on the in vitro metabolism of the tobacco-specific nitrosamine NNK in hamster lung and liver microsomes. *Anticancer Res.* 17:1093–1098.

Zhukova, E. M., O. P. Makarova. 2002. Effect of capsaicin on dynamics of neutrophil functional activity in Wistar rat venous blood. *Bull. Exp. Biol. Med*. 134:233–235.