Final Report on the Safety Assessment of Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water[1]

Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, Mentha Piperita (Peppermint) Leaf Water are obtained from the *Mentha piperita* plant. The oil is currently used in cosmetic formulations as a fragrance component, but previously had been also described as a denaturant. The extract and leaves are described as biological additives, but only the extract is reported to be used. Peppermint Water is described as a flavoring agent or fragrance component, but is not currently in use. Peppermint Oil is used at a concentration of ≤3% in rinse-off formulations and ≤0.2% in leave-on formulations. Peppermint Oil is composed primarily of menthol and menthone. Other possible constituents include pulegone, menthofuran, and limone. Most of the safety test data concern Peppermint Oil. The oil is considered to present the "worst case scenario" because of its many constituents, so data on the oil were considered relevant to the entire group of ingredients. Peppermint Oil was minimally toxic in acute oral studies. Short-term and subchronic oral studies reported cystlike lesions in the cerebellum in rats that were given doses of Peppermint Oil containing pulegone, pulegone alone, or large amounts (>200 mg/kg/day) of menthone. Pulegone is also a recognized hepatotoxin. Repeated intradermal dosing with Peppermint Oil produced moderate and severe reactions in rabbits, although Peppermint Oil did not appear to be phototoxic. Peppermint Oil was negative in the Ames test and a mouse lymphoma mutagenesis assay but gave equivocal results in a Chinese hamster fibroblast cell chromosome aberration assay. In a carcinogenicity study of toothpaste and its components, no apparent differences were noted between mice treated with Peppermint Oil and those treated with the toothpaste base. Isolated clinical cases of irritation and/or sensitization to Peppermint Oil and/or its constituents have been reported, but Peppermint Oil (8%) was not a sensitizer when tested using a maximization protocol. It was expected that dermal absorption of Peppermint Oil would be rapid, following that of menthol, a major component, but in no case would be greater than absorption through the gastrointestinal tract. Because of the toxicity of pulegone, the safe concentration of this constituent was limited to ≤1%. This concentration was achievable both by controlling the time of harvest and processing technique. There is evidence that menthol can enhance penetration of other agents. Formulators were cautioned that this enhanced penetration can affect the use of other ingredients whose safety assessment was based on their lack of absorption. With the limitation that the concentration of pulegone in these ingredients should not exceed 1%, it was concluded that Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Extract, Mentha Piperita (Peppermint) Leaves, Mentha Piperita (Peppermint) Water are safe as used in cosmetic formulations.

INTRODUCTION

The following is a compilation of studies concerning Mentha Piperita (Peppermint) Oil (CAS No. 8006-90-4), Mentha Piperita (Peppermint) Leaf Extract (CAS No. 84082-70-2), Mentha Piperita (Peppermint) Leaf, Mentha Piperita (Peppermint) Leaf Water. (Note: recently, cosmetic ingredient terminology for these ingredients has changed. For example, Mentha Piperita [Peppermint] Oil previously was called Peppermint [Mentha Piperita] Oil (Wenninger and McEwen 1997].) For brevity, these ingredients are identified as Peppermint Oil, Peppermint Extract, Peppermint Leaves, and Peppermint Water in this report. Most of the information concerns Peppermint Oil. Data on the Oil were considered relevant to the entire group of ingredients.

CHEMISTRY

Definition and Structure

Peppermint United States Pharmacopeia, (USP) is defined as the "dried leaves and flowering tops of *Mentha piperita* L. (Labiatae), having carminative, gastric stimulant, and counter-irritant properties; used as an oil, spirit, or water extract as a flavored vehicles for drugs" (Taylor 1988).

Mentha Piperita (Peppermint) Oil is a volatile oil obtained from the plant *Mentha piperita*. Synonyms include Mentha Oil, Mentha Piperita Oil, Oil of Peppermint, and Peppermint Oil (Wenninger, Canterbery, and McEwen 2000).

Mentha Piperita (Peppermint) Leaf Extract is an extract of the leaves of the peppermint, *Mentha piperita*. Synonyms include Extract of Mentha Piperita, Extract of Peppermint, Extract of Peppermint Leaves, Mentha Piperita Extract, Peppermint

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Extract, and Peppermint Leaf Extract (Wenninger, Canterbery, and McEwen 2000).

Mentha Piperita (Peppermint) Leaf is the cosmetic ingredient made from the dried leaves and tops of the peppermint, *Mentha piperita*. Synonyms include Leaf, Mentha Piperita; Leaf, Peppermint; Mentha Piperita Leaves; Peppermint Leaf, and Peppermint Leaves (Wenninger, Canterbery, and McEwen 2000).

Mentha Piperita (Peppermint) Leaf Water is an aqueous solution of the odoriferous principles of the leaves of *Mentha piperita*. Synonyms include Peppermint Water and Peppermint Leaf Water (Wenninger, Canterbery, and McEwen 2000).

Physical and Chemical Properties

One supplier noted that cosmetic Peppermint Oil is natural and of food grade quality (Ungerer and Company 1997).

Peppermint Oil is described as a colorless or pale yellow liquid having a strong, penetrating odor of peppermint and a pungent taste, followed by the sensation of coldness when air is drawn into the mouth (National Academy of Sciences 1981). Table 1 lists some properties and specifications.

Peppermint Oil has over 30 known components. lt is comprised mainly of menthol (35% to 60%), and menthone (15% to 30%) (Sang 1982; Thorup et al. 1983a; Madsen, Würtzen, and Carstensen 1986; Saito and Oka 1990; Bowen and Cubbin 1992). Because it is the primary component, menthol is further examined in the next section of this report.

Menthyl acetate (4% to 14%) and small amounts of cineole and other terpenes are also found in Peppermint Oil. Other identified components are acetaldehyde, amyl alcohol, menthyl esters, limone, pinene, phellandrene, cadinene, and dimethyl sulfide (Dooms-Goossens et al. 1977; Andersen 1978). In addition, Lawrence (1972) and Baslas, Singh, and Baslas (1973) identified several trace constituents including α-pinene, *p*-menthane, sabinene, terpinolene, ocimene, gamma-terpinene, fenchene, α-thujone, β-thujone, citronellol, α-cadinene, α-amorphene, α-gurjunene, and β-copaene.

TABLE 1
Chemical/physical properties and specifications of Peppermint Oil (National Academy of Sciences 1981; National Formulary 1995)

Property	
Angular rotation	Between −18°C and 32°C
Refractive index	Between 1.459 and 1.465 at 20°
Specific gravity	Between 0.896 and 0.908
Assay for total esters	Not less than 5.0% of esters, calculated as menthyl acetate
Assay for total menthol	Not less than 50.0% of menthol
Dimethyl sulfide	Passes test (rectified); fails test (natural)
Heavy metals (as Pb)	Passes test (limit of 0.004%)
Solubility in alcohol	Passes test (1 volume dissolves in 3 volumes of 70% alcohol)

Sullivan et al. (1979) stated that pulegone is found in young peppermint leaves, and is metabolized to menthol as the leaves mature. Similarly, in their review, Bowen and Cubbin (1992) stressed that the pulegone is found only in Peppermint Oil made from young plants and in trace amounts in "inferior" oils; pulegone is absent from "good-quality" Peppermint Oil. However, a supplier of cosmetic Peppermint Oil reported pulegone concentrations of 1% to 4% depending on the origin of the oil (Ungerer and Company 1997). Published studies that investigated the pulegone content of Peppermint Oil also reported a range of <1% to 4% for Oils from a North American origin (Lawrence 1993; Ravid, Putievsky, and Katzir 1994). Several studies cited in this literature review used Peppermint Oil containing pulegone. Because of the extensive study done on this component, pulegone is further examined in the next section of this report.

Components

Menthol

Menthol, the primary component of Peppermint Oil, exists as four pairs of optical isomers: (−)- and (+)-menthol, (−)- and (+)-isomenthol, (−)- and (+)-neomenthol, and (−)- and (+)-isoneomenthol. (−)-Menthol is the isomer that is most commonly found in nature (Eccles 1994). Menthol conforms to the structure shown in Figure 1.

Method of Manufacture

European and American Peppermint Oil is distilled with steam from the fresh, above-ground parts of the flowering plant *Mentha piperita* Linne, rectified by distillation and not dementholized (Dooms-Goossens et al. 1977). Fehr (1984) reported that the menthone content decreases while the menthol content increases in peppermint leaves upon storage for 1 to 2 months, at 22°C to 24°C. However, the relative menthone to menthol proportion remained practically constant during the total storage time.

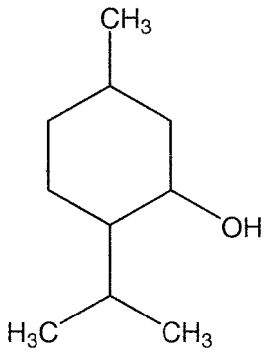

FIGURE 1
Menthol.

A patented technique is available to reduce the pulegone content of a Peppermint Oil preparation by stereospecifically reducing it with Na_2SO_3 in the presence of water (as a hydrogen ion source) at neutral pH. The process stabilizes menthofuran against oxidative breakdown and the final product has an increased menthone and menthol content. A sample containing 2.35% pulegone was reduced to 0.34% pulegone (Spencer 1989).

Analytical Methods

Several components of Peppermint Oil can be estimated by capillary gas chromatography (Sang 1982).

USE

Cosmetic

Peppermint Oil is used in cosmetic formulations as a fragrance component and skin-conditioning agent—miscellaneous (Wenninger, Canterbery, and McEwen 2000) and had previously been used as a denaturant (Wenninger and McEwen 1995). Table 2 presents the 102 reported uses of Peppermint Oil in cosmetics as a function of product type (FDA 1998).

Concentrations of use are no longer reported to the Food and Drug Administration (FDA) (FDA 1992). However, data submitted by the Cosmetic, Toiletry, and Fragrance Association (CTFA) directly to Cosmetic Ingredient Review (CIR) indicated use of Peppermint Oil at the following concentrations: 0.02% in a medicated face mask; 0.1% in a facial cleanser; 0.2% in a lipstick, a body splash, a foot refresher/lotion, and a body refresher; 0.5% in a toothpaste; 0.9% in a fluoride toothpaste; 1.2% in a mouthwash; 2.0% in a lip balm; and 3.0% in a hair lotion (CTFA 1997a).

In addition, two denatured alcohol formulas contained a maximum of 0.4% and 1.5% Peppermint Oil, respectively (CTFA 1997b).

Peppermint Extract is used in cosmetic formulations as a fragrance ingredient, skin-conditioning agent—miscellanous and skin-conditioning agent—occlusive (Wenninger, Canterbery, and McEwen 2000). As of January 1998, it was reported to be used in 35 formulations (Table 2).

Peppermint Leaves can be used in cosmetic formulations as fragrance ingredients (Wenninger, Canterbery, and McEwen 2000). Peppermint Water can be used as a flavoring agent or fragrance component (Wenninger, Canterbery, and McEwen 2000). While neither was reported in use, the FDA listed two uses of the ingredient "Peppermint" in January 1998 (Table 2).

International

All of these ingredients are included in the single name, Mentha Piperita, in the European Union (Wenninger, Canterbery, and McEwen 2000).

Mentha Piperita (Peppermint) Leaf Extract, as Peppermint Extract, and Mentha Piperita (Peppermint) Leaf Water, as Peppermint Distillate, are listed in the *Japanese Comprehensive Licensing Standards of Cosmetics by Category (CLS)* with precedent for use without restriction in all *CLS* categories except eyeliner preparations, for which there is no precedent (Santucci 1999). According to Notification 990 of the Pharmaceutical and Medical Safety Bureau of the Japan Ministry of Health and Welfare, issued September 29, 2000, none of these ingredients are prohibited or restricted in its use beyond a basic obligation of manufacturers to use all ingredients in a manner that guarantees safety (Japan Ministry of Health and Welfare 2000).

Noncosmetic

Peppermint Oil is a generally recognized as safe (GRAS) ingredient for use in dietary supplements (Rothschild 1990). It is described as a naturally occurring carminative that relaxes gastrointestinal smooth muscle (Gosselin, Smith, and Hodge 1984; Kaffenberger and Doyle 1990). See General Biology for more details.

A final ruling by the FDA labeled Peppermint Oil as safe and effective as an antitussive (topical/inhalant). Final rulings cautioned that Peppermint Oil is not safe and effective for use as an expectorant in either topical/inhalant or lozenge form, or for use as a nasal decongestant, mouthwash, or digestive aid (FDA 1991).

Exposure Assessment

The FDA calculated an estimated human exposure from cosmetic use based on the concentration of use information supplied by industry. Using a body splash product containing 0.2% Peppermint Oil and assuming 100% absorption over a body surface of 17,000 cm^2 and a daily application of 1 mg/cm^2 (~17 ml of the product), the FDA estimated an exposure of 34 mg/day. For a 60-kg person, this amounted to an estimated daily dose of 0.6 mg/kg/day (FDA 1997).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, Excretion

Dermal Administration

Eserine in a Peppermint Oil vehicle was applied to a 2.2-cm^2 shaved area on the abdomen of mice. The absorption rate for Peppermint Oil was measured as the latent period between application and appearance of Eserine-induced signs. Peppermint Oil had a latent period of 58 minutes (Meyer and Meyer 1959).

Oral Delivery

The rate of Peppermint Oil absorption and excretion following oral administration was determined by measuring urinary menthol glucuronide. Four male volunteers ingested 180 mg of an enteric-coated Peppermint Oil capsule following a 16-hour fast. The panelists were instructed to increase water intake throughout the day. Total urine output was collected every 2 hours for up to 14 hours post ingestion. Menthol was liberated

TABLE 2
Frequency of use of Peppermint Oil (FDA 1998)

Product category	No. of formulations in category	No. containing Peppermint Oil
Peppermint Oil		
Bath oils, tablets, and salts	124	1
Bubble baths	200	2
Other bath preparations	159	3
Hair conditioners	636	1
Lipstick	790	2
Makeup bases	132	1
Cuticle softeners	19	1
Nail creams and lotions	17	1
Dentrifices	38	11
Mouthwashes and breath fresheners	49	10
Other oral hygiene products	6	1
Shaving cream	139	2
Cleansing	653	13
Face and neck skin care (excluding shaving)	263	3
Body and hand skin care (excluding shaving)	796	6
Foot powders and sprays	35	4
Moisturizing skin care	769	3
Night skin care	188	1
Paste masks (mud packs)	255	6
Skin fresheners	184	9
Other skin care preparations	692	21
1998 total for Peppermint Oil		**102**
Peppermint Extract		
Bubble baths	200	1
Hair conditioners	636	7
Rinses (noncoloring)	40	1
Shampoos (noncoloring)	860	4
Tonics, dressings, and other hair-grooming aids	549	1
Bath soaps and detergents	385	2
Other personal cleanliness products	291	3
Other shaving preparation products	60	1
Cleansing skin care preparations	653	1
Face and neck skin care (excluding shaving)	263	2
Body and hand skin care (excluding shaving)	796	4
Paste masks (mud packs)	255	5
Skin fresheners	184	1
Other skin care preparations	692	2
1998 total for Peppermint Extract		**35**
Peppermint (no further description)		
Other bath preparations	159	1
Moisturizing	769	1
1998 total for Peppermint		**2**

from its glucuronide metabolite by treating the urine with β-D-glucuronidase and quantitated with capillary gas chromatography. A significant amount of variance was found among individuals. It was estimated that between 37 and 116 mg of menthol corresponding to an average 40% recovery of the administered menthol dose was excreted by each panelist within 14 hours (Kaffenberger and Doyle 1990).

Menthol

In a review, Eccles (1994) noted that some phase 1 metabolism of menthol can occur in the skin and gut following dermal, oral, or inhalation exposure; most of the absorbed compound is transported to the liver. Yamaguchi, Caldwell, and Farmer (1994) administered ^{3}H-(−)-menthol (500 mg/kg) to intact and bile duct–cannulated male Fischer 344 rats. In intact rats, ~71% of the dose was recovered within 48 hours, with almost equal amounts in the urine and feces. In bile duct–cannulated rats, 74% of the dose was recovered with 67% in the bile and 7% in the urine. The major biliary metabolite was menthol glucuronide. Madyastha and Srivatsan (1988) reported that oral administration of (−)-menthol to rats (800 mg/kg/day for 20 days) resulted in two major urinary metabolites: *p*-menthane-3,8-diol and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid and two minor metabolites: *p*-menthane-3,9-diol and 3,8-oxy-*p*-menthane-7-carboxylic acid. An in vitro study by these investigators noted that oral administration for up to 7 days induced hepatic microsomal enzymes cytochrome P450 and NADPH-cytochrome *c* (P450) reductase.

Smooth Muscle–Relaxing Effects

An investigation that used isolated pharmacological preparations from guinea pig large intestine and patch clamp electrophysiology techniques on rabbit jejunum concluded that Peppermint Oil relaxed gastrointestinal smooth muscle by reducing calcium influx (Hills and Aaronson 1991). Similar findings were reported earlier by Hawthorn et al. (1988) and confirmed by Dalvi et al. (1991). Researchers have reported on the use of Peppermint Oil capsules to treat spastic colon and irritable bowel syndrome (Somerville, Richmond, and Bell 1984; Friedman 1991).

Peppermint Oil, in combination with eucalyptus oil and ethanol, increased cognitive performance, and possessed muscle-relaxing properties when applied to large areas of the forehead and temples of 32 healthy human subjects. The mixture did not influence pain sensitivity. A significant reduction in sensitivity to headache was produced by a combination of Peppermint Oil and ethanol (Gobel, Schmidt, and Soyka 1994).

Effect on Virus

Herrmann and Kucera (1967) reported that Peppermint Extract had protective activity against Newcastle disease and herpes simplex, vaccinia, Semliki forest, and West Nile viruses in embryonated chicken egg and chick embryo fibroblast cell-culture systems. The effect was noted in eggs only when the Peppermint Oil preparation was injected into the allantoic sac at 3 to 24 hours prior to inoculation with virus; no effect was observed in influenza virus–infected eggs. The protective activity against most of the viruses was attributed to a tannin, consisting of trimers of caffeic acid, that has an affinity for many viruses and is contained in mint plants. A nontannin fraction was considered active against herpes simplex virus.

Acceptable Daily Intake

In 1976, the Food and Agriculture Organization of the United Nations World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA) established an acceptable daily intake (ADI) of 0.2 mg/kg body weight/day for menthol (FAO/WHO 1976, 1994). The JECFA comments were as follows:

> Evidence from human studies suggest that menthol is well absorbed from the gut. A large proportion is excreted in urine as glucuronides but the metabolic rate of the remainder has not been elucidated. No long-term studies have been carried out but a 24 week lung adenoma (i.p. dosing) study and extensive mutagenicity studies gave negative results. The results of one study suggested that adverse effects may occur in man ingesting about 2 mg menthol/kg/day. Other evidence from human exposure shows that adverse effects are unlikely to occur when 0.2 mg menthol/kg/day is ingested. The Committee agreed, however, that further information on human menthol intake from food and medicine, and if possible, observations on a group of people with a higher than average intake would need to be carried out if any increase in the ADI is to be contemplated (FAO/WHO 1976).

The evaluation section of the report cited an unpublished oral-dose study in which groups of 80 rats (40 each sex) received 0, 100, or 200 mg/kg of either *l* or *d*,*l*-menthol for $5^{1}/_{2}$ weeks. No adverse effect was observed in weight gain or excretion of glucuronide, water, and electrolytes; central nervous system reactions to cardrazol or electric shock and intravenous (IV) hexobarbital sleeping time were not affected. Using the 200-mg/kg/body weight level for rats, the Committee estimated an acceptable ADI for humans of 0 to 0.2 mg menthol/kg/body weight.

The Committee listed the following additional studies needed to increase the ADI: long-term toxicity and carcinogenicity in rats, information regarding average and maximum likely intake of menthol, clinical observation following a higher than average intake, and metabolic studies (FAO/WHO 1976).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Peppermint Oil USP had a 24-hour oral LD_{50} of 4441 mg/kg in fasted Wistar rats; the 48-hour LD_{50} was 2426 mg/kg (Eickholt and Box 1965). Ohsumi et al. (1984) reported an oral LD_{50} of 2410 mg/kg in fasted mice for Peppermint Oil diluted in olive oil.

Short-Term Oral Toxicity

Studies using Wistar rats described in this section are summarized in Table 3.

Thorup et al. (1983a) investigated the toxicity of Peppermint Oil administered per os (PO) to groups of 20 Wistar rats at doses of 10, 40, and 100 mg/kg body weight/day for 28 days. The Peppermint Oil sample contained 38.1% menthol, 33.7% menthone, and 1.7% pulegone; the remaining components could not be identified. The sample was diluted in a soybean oil vehicle. Rats were inspected twice daily, and body weight and feed and water consumption were recorded weekly. On day 21, blood samples were taken from 16 animals (8 of each sex) for analysis. Animals were killed at the end of week 4 and necropsied. Organ samples were prepared for light microscopy. Frozen sections of brain were stained with Luxol fast blue. No difference in body weight or feed consumption was observed between groups. A nonsignificant increase (of 10%) in water consumption was noted in dosed groups. All hematological and biochemical parameters were within the normal range. No significant differences were found in absolute and relative organ weights. Cystlike spaces scattered in the white matter of the cerebellum were noted in animals of the 40- and 100-mg/kg/day groups, but no clinical signs of encephalopathy were observed. A non–dose-related dissociation and vacuolization of the hepatocytes, mainly around the central vein, were noted in some rats of the 40- and 100-mg/kg/day groups. The vacuoles did not contain fat.

TABLE 3
Oral dosing studies of Peppermint Oil and components using Wistar rats

Test material	Protocol	Finding (specifically cystlike spaces in the cerebellum)	NOAEL	Reference
Peppermint Oil (38% menthol, 1.7% pulegone)	10, 40, or 100 mg/kg/day for 28 days (20 rats per group, 10 each sex)	Lesions noted in 2/10 males and 2/10 females of 40-mg/kg group and in 7/10 males and 4/10 females in 100-mg/kg group	10 mg/kg/day	Thorup et al. 1983a
Peppermint Oil (content not specified)	20, 150, or 500 mg/kg for 5 weeks (12 rats per group)	No lesions noted		Mengs and Stotzen 1989
Peppermint Oil (content not specified)	10, 40, or 100 mg/kg/day for 28 days (20 rats per group, 10 each sex)	Lesions noted in 4/20 rats of 40-mg/kg group and in 11/20 rats of 100-mg/kg group	10 mg/kg/day	Olsen and Thorup 1984
Peppermint Oil (42% menthol, 1.1% pulegone)	10, 40, or 100 mg/kg/day for 90 days (28 rats per group, 14 each sex)	Lesions noted in all groups, significant in highest dose group	40 mg/kg/day	Spindler and Madsen 1992
Components				
Pulegone	20, 80, or 160 mg/kg/day for 28 days (20 rats per group, 10 each sex)	Lesions noted in 4/20 rats of 80-mg/kg group and in 13/17 rats of 160-mg/kg group	20 mg/kg/day	Olsen and Thorup 1984
Pulegone	20, 80, or 160 mg/kg/day for 28 days (20 rats per group, 10 each sex)	Lesions observed in 4/20 rats of 80-mg/kg group and in 13/19 rats of 160-mg/kg group	20 mg/kg/day	Thorup et al. 1983b
Menthol	200, 400, or 800 mg/kg/day for 28 days (20 rats per group, 10 each sex)	No lesions noted	<200 mg/kg/day (because of hepatocyte vacuolization observed in all groups)	Thorup et al. 1983b
Menthone	200, 400, or 800 mg/kg/day for 28 days; highest dose reduced to 400 mg/kg/day for females on day 19 (20 rats per group, 10 each sex)	Lesions noted in 1/8 females and 3/9 males of 400-mg/kg group and in 7/10 females 5and /10 males of the 800-mg/kg group	<200 mg/kg/day	Madsen, Würtzen, and Carstensen 1986

The researchers determined the no-effect level for Peppermint Oil was 10 mg/kg body weight/day and noted that consumption of a 28-g box of mint lozenges containing 0.4% Peppermint Oil was an intake close to the observed no effect level.

In a 5-week gavage study by Mengs and Stotzen (1989), 12 male Wistar rats received daily doses of either 20, 150, or 500 mg Peppermint Oil/kg body weight. Mean body weight was between 184 and 214 g. A control group received 4 ml/kg olive oil. The animals were inspected daily and weighed weekly. Blood samples were taken prior to and at the termination of dosing. Rats were killed at the end of dosing and organs were weighed and examined microscopically.

No changes in general condition, behavior, or body weight were observed in treated rats as compared to control rats. All hematological and urine parameters were comparable among treated and control rats. Plasma triglyceride concentrations were lower in rats of the high-dose group at week 5 as compared to the control group; the change was attributed to lower feed consumption by the high-dose group. A non–dose-dependent increase in alkaline phosphatase activity was noted in rats of all treated groups. Relative mean weights of the liver and kidneys were greater in high-dose rats than in controls. These changes were not of "toxicological relevance." No specific lesions of toxicity were noted in the cerebellum, liver, or kidneys. In contrast to the studies of Thorup et al. (1983a), Mengs and Stotzen did not observe cystlike spaces in the white matter of the cerebellum.

Mengs and Stotzen (1989) also conducted a 5-week oral study using dogs. Three dogs received daily oral doses of 25 or 125 mg Peppermint Oil/kg body weight. The doses were contained in enteric-coated gelatin capsules. The animals were inspected daily and weighed weekly. Blood samples were taken prior to and at the termination of dosing.

No effects were noted on body weight or on hematological or urinary parameters. High-dose males had slightly increased plasma alkaline phosphatase activity and urea concentrations during week 5 but the values remained within normal range. No lesions were noted at microscopic examination.

In a study by Olsen and Thorup (1984), groups of 20 rats (10 of each sex) received either 10, 40, or 100 mg/kg/day Peppermint Oil or 20, 80, or 160 mg/kg/day pulegone for 28 days. Encephalopathy developed in rats of the high-dose groups. Cystlike spaces were observed scattered in the white matter of rats treated with either Peppermint Oil or pulegone. The lesions were noted especially in the cerebellum; neither cellular reaction in the surrounding tissue nor demyelination was observed. The changes were dose-related and were not observed in rats that were given the lowest doses of either Peppermint Oil or pulegone.

Thorup et al. (1983b) investigated the toxic effects of pulegone and menthol, two components of Peppermint Oil. Groups of 20 rats (10 of each sex) received either 20, 80, or 160 mg pulegone/kg body weight/day or 200, 400, or 800 mg menthol/kg body weight/day, by gavage for 28 days. The test materials were diluted in soybean oil. Blood and urine samples were obtained. Animals were killed at the end of week 4 and necropsied. Organ samples were stained for histopathological examination.

Rats treated with pulegone developed dose-dependent atonia shortly after dosing was initiated. Both compounds significantly increased water consumption at the highest dose. Renal function was normal in pulegone-treated rats. Weight gains were significantly reduced in the high-dose (by 20%) and mid-dose (by 10%) pulegone groups. A pulegone dose–dependent decrease in blood creatinine was observed; the value was significant at the highest dose. An increased number of neutrophilic granulocytes was observed in rats of the high-dose group. At necropsy, rats of the high-dose pulegone group had markedly distended and atonic stomachs packed with feed. A significant decrease in terminal body and organ weights was observed in pulegone-dosed rats. Dose-related vacuolization of hepatocytes in the zone around the central vein was observed in 10 of 20 rats of the mid-dose group and in 15 of 19 rats of the high-dose group. Cystlike spaces were observed in the white matter of the cerebellum of pulegone-treated rats from the mid- (4 of 20 rats) and high-dose groups (13 of 19 rats).

In the menthol group, a significant increase in absolute and relative liver weights and vacuolization of hepatocytes were noted in all animals. Neutrophilic granulocytes were increased in animals of the highest dose group. No sign of encephalopathy was observed in rats given menthol. The no effect concentration for pulegone was 20 mg/kg body weight/day and for menthol it was <200 mg/kg body weight/day.

A subsequent study by Madsen, Würtzen, and Carstensen (1986) tested the toxicity of menthone, another component of Peppermint Oil. Groups of 20 rats (10 of each sex) received either 200, 400, or 800 mg/kg body weight/day of menthone by gavage for 28 days. However, after 19 days, females of the highest dose group had pale mucous membranes and signs of pain and their dose was reduced to 400 mg/kg/day. Rats were killed at the end of the study for necropsy.

Dose-dependent decreases in creatinine content and increases in alkaline phosphatase activity and bilirubin were noted in blood samples obtained on the last day of the dosing period. Relative spleen and liver weights were increased. At microscopic examination, cystlike spaces were found in the white matter of the cerebellum in rats of the two highest dose groups. No clinical signs were observed. The no-effect level for menthone was <200 mg/kg body weight/day.

Subchronic Oral Toxicity

Spindler and Madsen (1992) treated groups of 28 Wistar rats with Peppermint Oil (diluted with soybean oil) at oral doses of 10, 40, and 100 mg/kg body weight/day for 90 days. Gas chromatography analysis determined that the Peppermint Oil sample contained 42% menthol, 25% menthone, 7% iso-menthone, 1.5% limonene, 1.4% cineole, and 1.1% pulegone; the remaining 22% could not be identified. Rats were inspected twice daily,

and body weight and feed and water consumption were recorded weekly. On days 30 and 86, blood samples were taken from 20 animals (10 of each sex) for analysis. Animals were killed on day 90 and necropsied. Organ samples were fixed in formaldehyde, sectioned, and stained for histopathologic examination.

No difference in body weight or feed consumption was observed between groups. All hematological and biochemical parameters were within the normal range. No significant differences were observed in absolute and relative organ weights. Cystlike spaces were scattered in the white matter of the cerebellum of rats from all groups and were significant in high-dose rats. The researchers stated that similar to the results of the 28-day study by Thorup et al. (1983a), the spongiform lesions were unaccompanied by cellular reaction in the adjacent tissues, were not surrounded by a membrane, and did not appear to occur intracellularly. No other lesions of encephalopathy were observed. Nephropathy as evidenced by hyaline droplets was observed in males rats of the highest dose group with no epithelial degeneration. A no-observed-adverse-effec t level (NOAEL) of 40 mg/kg body weight/day was determined.

Acute Parenteral Toxicity

The 24-hour intraperitoneal LD_{50} for Peppermint Oil USP was 819 mg/kg in Wistar male rats (Eickholt and Box 1965).

Immunotoxicity

Basophil cell suspensions, obtained from the blood of factory workers exposed to additive containing penicillin, were incubated with 10^{-1} to 10^{-3} mg/ml Peppermint (dry aroma). A dose-dependent increase in histamine release was noted. This increase was also noted in control samples obtained from nonfactory employees. The response curve did not change when cells deprived of basophil surface immunoglobins were used, indicating the reaction was not a type I allergy. It was suggested that the histamine release was caused by nonimmunological mechanisms (Moller, Skov, and Norn 1984).

Gaworski et al. (1994) used a rapid screening protocol incorporating elements of the National Toxicology Program's (NTP) immunotoxicity tier testing strategy and FDA testing guidelines to test several GRAS flavoring ingredients for their immunotoxic potential. Groups of 30 female CD-1 mice received either 313, 625, or 1250 mg Peppermint Oil/kg body weight/day in the feed for 5 days. (The high dose was selected to produce minimal toxicity based on early acute toxicity studies; the mid and low dose were one half and one quarter the high dose, respectively.) The Peppermint Oil was diluted in corn oil. Ten animals of each group were used in the plaque-forming cell assay (PFC) and the remaining 20 animals of each group were used in the host resistance assay.

Animals in the PFC assay group were injected with 2×10^8 sheep red blood cells (SRBCs) at the end of the 5-day dosing period. Three days after the SRBC injection, a positive-control group (10 mice) was injected (intraperitoneally, IP) with cyclophosphamide, while an untreated control group (10 mice) received an equivalent amount of saline. Four days after SRBC injection, all mice were killed and single spleen cell suspensions were prepared. The suspensions were mixed with a SRBC–guinea pig complement mixture and incubated. The resulting immunoglobulin M (IgM) anti-SRBC plaques were counted. Peppermint Oil did not significantly alter the PFC response as compared to the nontreated control. The response in the positive-control group was 10% less than that in the nontreated control group, thus validating the test (Gaworski et al. 1994).

Animals of the host-resistance assay group were injected on the third day of Peppermint Oil dosing with *Listeria monocytogenes* isolated from a clinical case of meningitis. Inoculum equivalent to the LD_{20} was injected into vehicle control animals. The average survival time for vehicle-control mice was 9.7 days. In contrast, survival times decreased in mice treated with 625 or 1250 mg Peppermint Oil/kg to 7.5 days and 2.8 days, respectively. The decreases were significant ($p \leq .05$) and suggested an increased susceptibility to bacterial-induced deaths and/or immunosuppression (Gaworski et al. 1994).

Phototoxicity

Undiluted Peppermint Oil was applied to the back of six Skh:hairless mice. Thirty minutes later, mice were irradiated for either 1 hour with light from a fluorescent blacklight at an integrated UVA of 3 W/m^2, or for 40 minutes with light from a Xenon Lamp at a weighted erythema energy of 0.1667 W/m^2. Mice were examined at 4, 24, 48, 72, and 96 hours following radiation treatment. No effects were noted. In a second experiment using two miniature swine and following the same protocol, no effect was produced by 100% Peppermint Oil (Research Institute for Fragrance Materials 1996).

Dermal Irritation

Hairless sites on five white rabbits were injected intradermally with 0.05 ml Peppermint Oil. Gross examinations were made at 24 and 48 hours, at 1 and 2 weeks, and in some cases, 1 month following dosing. The dosing was repeated between 5 and 10 times. Microscopic examination of skin samples followed. Moderate reactions characterized by polymorphonuclear leukocytes, lymphocytes, and plasma cells (without necrosis) were noted in three rabbits. Severe reactions, which were marked by the above as well as necrosis, were noted in the other two rabbits (Grossman and Lally 1982).

Pulegone Hepatotoxicity

As noted earlier, pulegone is found in young peppermint leaves (Sullivan et al. 1979). (*R*)-(+)-Pulegone (CAS No. 15932-80-6; 89-82-7) is described in the literature as a hepatotoxin that is the main constituent of the abortifacient pennyroyal oil (Sullivan et al. 1979; Gordon et al. 1987; Thomassen, Slattery, and Nelson 1990). It is a monoterpene that conforms to the structure shown in Figure 2 (McClanahan et al. 1989).

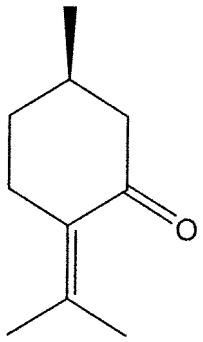

FIGURE 2
Pulegone.

Pulegone is oxidized by cytochrome P450 to reactive metabolites such as menthofuran that are partly responsible for the toxicity observed in mice, rats, and humans (Mizutani et al. 1987; Madyastha and Moorthy 1989; McClanahan et al. 1989; Nelson et al. 1992). Thomassen, Slattery, and Nelson (1990) reported a depletion of both serum and hepatic concentrations of reduced glutathione following intraperitoneal administration of 150 mg/kg pulegone to rats. Oral administration of pulegone (400 mg/ kg/day for 5 days) produced significant decreases in activities of hepatic cytochrome P450 and values of heme but did not affect activities of cytochrome b5 or NADPH-cytochrome *c* reductase (Moorthy, Madyastha, and Madyastha 1989).

Menthol

Two Tiger Balm formulations containing 8% and 10% menthol were applied for 23 hours under occlusive patches to abraded and intact sites on New Zealand white rabbits. A total of 21 patches were applied. A third group was treated with a control wax (a mixture of hard and soft waxes). Untreated sites on each rabbit served as negative controls. Irritation was scored using the Draize scale. Dermal irritation was noted in all treated animals with the following severity scale: 8% menthol balm < control wax <10% menthol balm. The 8% menthol balm was almost innocuous in male rabbits. The irritation was not progressive and tolerance developed within 10 days. No severe damage was noted at microscopic examination of the skin (increased hyperkeratosis was noted at treated sites) and no evidence of systemic toxicity was noted. The investigators noted that the balm contained "irritants" such as clove oil, camphor, and menthol and remarked that the irritation was not unexpected (Guppy, Lowes, and Walker 1982).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Menthol

Groups of 15 to 23 pregnant animals were dosed by oral intubation with natural Brazilian menthol. Mice were dosed with up to 185 mg/kg body weight on gestation days (GDs) 6 to 15; pregnant rats were given doses up to 218 mg/kg on GDs 6 to 15; pregnant hamsters were dosed up to 405 mg/kg on GDs 6 to 10; and artificially inseminated rabbits were given doses up to 425 mg/kg on GDs 6 to 18. Maternal body weight was recorded regularly. Caesarean sections were performed on all dams. No teratogenic effect was noted (Food and Drug Research Labs, Inc. 1973).

GENOTOXICITY

Bacterial Assays

Andersen and Jensen (1984) investigated the mutagenic potential of Peppermint Oil and some of its components in the *Salmonella*/mammalian microsome test. *Salmonella* strains used were TA1535, TA100, TA1537, and TA98. The sample tested contained 38.1% menthol, 33.7% menthone, and 1.7% pulegone; the remaining components were not identified. Peppermint Oil, menthol, and pulegone, all tested at doses of 6.4, 32, and 160 μg/plate, produced the same number of revertants as the negative control. Toxicity was noted at the next (and maximum) dose of 800 μg/plate. Addition of S9 appeared to make the compounds less toxic to the bacteria. In contrast, menthone induced a statistically significant number of revertants in TA1537 without S9 activation at doses of 6.4 and 32 μg/plate. Menthone was further tested using the more sensitive TA97 strain. Statistically significant increases in the number of revertants were noted at all doses tested without S9 activation; the results were dose related (though toxicity was observed at 800 μg/plate). The researchers remarked on the unexpected results—menthone was mutagenic, but Peppermint Oil, which contained 33.7% menthone, was not.

Mammalian Cell Assays

In an in vitro chromosomal aberration test using a Chinese hamster fibroblast cell line, Peppermint Oil, at a maximum concentration of 0.25 mg/ml (in an ethanol solvent), produced polyploidism in 3.0% and structural aberrations in 7.0% of the cells at 48 hours after treatment. The results were considered equivocal as scores of either $\geq$10.0% or $\leq$4.9% were necessary for classification as either positive or negative, respectively (Ishidate et al. 1984).

Peppermint Oil (150 μg/ml) was negative in a mouse lymphoma L5178Y TK +/− cell mutagenesis assay. It was also negative (at 155 μg) in an unscheduled DNA synthesis assay using rat hepatocytes (Heck et al. 1989).

Menthol

The mutagenic potential of natural Brazilian menthol was tested in the cytogenetic assay (rats), the host-mediated assay (mice), and the dominant lethal assay (rats). The assays were done with menthol doses of 1.45, 14.5, and 145.0 mg/kg and, in some instances, subacute and acute studies were done with doses of 500, 1150, and 3000, or 5000 mg/kg. In the host-mediated assay, a weakly positive but significant response was noted with

the acute high dose against *Salmonella typhimurium* TA 1530, and elevated recombinant frequencies were noted with the subacute doses against *Saccharomyces* D3. All other assays were negative (Litton Bionetics, Inc. 1975).

CARCINOGENICITY

In a carcinogenicity study of toothpaste and its components, groups of 52 male pathogen-free CFLP (ICI-redefined) mice were dosed by gavage with 4 or 16 mg Peppermint Oil/kg/day, 6 days a week for 80 weeks. A 16- to 24-week observation period followed treatment. An untreated group of 52 male mice and a vehicle group of 260 male mice that received the toothpaste base (which did not contain chloroform, eucalyptol, or Peppermint Oil) were maintained as controls.

Body weight gain was reduced initially in animals of the 16-mg/kg/day group. At least one neoplasm at any site was observed in 73%, 69%, 65%, and 71% of mice of the low-dose, high-dose, untreated-control, and vehicle-control groups, respectively. Malignant neoplasms were noted in 39%, 35%, 23%, and 31% of mice of the low-dose, high-dose, untreated-control, and vehicle-control groups, respectively. The incidence of neoplasms of the lungs and kidneys were comparable among mice of the treated and nontreated groups. Hepatic cell tumor incidence for Peppermint Oil–dosed mice (25%) was comparable to the incidence for mice of the vehicle-control group (27%); the incidence for the untreated group was 19%. Malignant lymphoma was found in 25%, 21%, 10%, and 14% of mice of the low-dose, high-dose, untreated, and vehicle-control groups, respectively. The researchers did not discuss if the difference in the incidence rate was significant (Roe et al. 1979).

A review of the study by the British Industrial Biological Research Association (1992) noted that it was not designed to examine the carcinogenic potential of Peppermint Oil and thus "would have had only a very limited sensitivity to this particular component."

Menthol

A 2-year oral dosing study by the National Cancer Institute (NCI 1979) found no evidence of carcinogenicity following dosing of Fischer 344 rats with 3750 or 7500 ppm or dosing of $B6C3F_1$ mice with 2000 or 4000 ppm *d*,*l*-menthol. A dose-related trend in increased number of deaths was noted in female mice. A negative trend in fibroadenomas of the mammary gland was observed in female rats (20 of 50 control; 10 of 49 low-dose; 7 of 49 high-dose).

Russin et al. (1989) reported a significant inhibition ($p < .001$) of 7,12-dimethylbenz[a]anthracene (DMBA)-induced rat mammary gland carcinogenesis following 20 weeks of oral dosing with 1% (−)-menthol. Dosing with menthol began 2 weeks prior to DMBA tumor induction. A chemopreventive effect was noted when rats were dosed with 0.5% menthol for 2 weeks prior to and 1 week after DMBA induction.

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation and Sensitization

The International Contact Dermatitis Research Group recommends patch testing with 2% Peppermint Oil in petrolatum. Peppermint Oil is recognized to produce immediate contact reactions (urticaria) (DeGroot 1994).

Clinical studies are cited in the Eccles review (1994) that described the coolant action of 0.2% and 2% menthol following dermal application. However, 5% and 10% menthol produced a strong burning sensation. Menthol also possessed irritant properties. It was suggested that in addition to stimulating cold receptors, menthol also stimulated nociceptors that subsequently released vasodilator peptides. The increased penetration of topically applied drugs in menthol (studies in hairless mice with 1% to 5% menthol) could have resulted from a combination of the vasodilation and the lipophilic nature of menthol.

Following the Kligman maximization protocol, 25 healthy male panelists received five occlusive induction patches containing 8% Peppermint Oil (in petrolatum) for 48 hours. Pretreatment was for 24 hours with an occlusive patch containing 5% sodium lauryl sulfate (SLS) prior to each exposure. After a 10-day nontreatment period, subjects were challenged on the back with a 48-hour patch (also preceded by SLS treatment). No evidence of sensitization was found (Research Institute for Fragrance Materials 1996).

Positive reactions were noted in 7 of 450 dermatitic patients who were patch tested with 2% Peppermint Oil in yellow soft paraffin (Rudzki and Grzywa 1977). In another study, positive reactions to 2% Peppermint Oil were noted in 6 of 86 dermatitic patients. The 86 patients were selected because they had previously responded to the ICDRG perfume mixture (Rudzki and Grzywa 1986).

An A1 patch containing 1% Peppermint Oil (unknown vehicle) was applied to the back of 56 patients with chronic urticaria. No reactions were noted after a 1- or 48-hour exposure (Warin and Smith 1982).

Positive reactions were noted in 3 of 1200 dermatitis patients patch tested with 2% Peppermint Oil in petrolatum (Santucci et al. 1987).

No reactions were noted in 25 spice factory workers who were patch tested with 2% Peppermint Oil in petrolatum. The workers were selected because they had reported signs and symptoms of dry skin, pruritus, and eczema (Meding 1993).

Saito and Oka (1990) reported that patch testing of individual components of Peppermint Oil using three patients with allergic contact dermatitis established that the allergens were menthol and trace components such as piperitone or pulegone.

Rudzki and Kleniewska (1970) tested 5% menthol in yellow paraffin in 877 people with primary contact, atopic, nummular, and stasis dermatitis and eczema. Reactions were noted in 1% of the panelists within 96 hours.

Others have reported isolated cases of irritation and/or sensitization to Peppermint Oil and/or its components (Smith 1968;

Dooms-Goossens et al. 1977; Andersen 1978; DeGroot 1987; Basto and Azenha 1991; Abifadel et al. 1992; Wilkinson and Beck 1994; Lewis, Shah, and Gawkrodger 1995; Morton et al. 1995).

SUMMARY

Peppermint Oil, Peppermint Extract, and Peppermint Leaves are obtained from the *Mentha piperita* plant. In 1998, Peppermint Oil was used in 102 cosmetic formulations as a fragrance component. Peppermint Extract was used in 35 formulations as a flavoring agent and fragrance component. Peppermint was used in two formulations.

Peppermint Oil is composed primarily of menthol and menthone. Numerous other possible constituents include pulegone, menthofuran, and limone; some components have yet to be identified.

The 24-hour oral LD_{50} for Peppermint Oil in fasted mice and rats was 2410 and 4441 mg/kg, respectively. Several (but not all) short-term and subchronic oral studies noted cystlike lesions in the cerebellum in rats that were given doses of Peppermint Oil containing pulegone, pulegone alone, or large amounts ($>$200 mg/ kg/day) of menthone.

Results of a host-resistance assay suggested immunosuppression and/or increased susceptibility to bacterial-induced mortality. Studies on human basophil suspensions suggested that Peppermint Oil induced histamine release by nonimmunological mechanisms. It was negative in a plaque-forming assay.

Repeated intradermal dosing with Peppermint Oil produced moderate and severe reactions in rabbits. Peppermint Oil did not appear to be phototoxic.

Peppermint Oil was negative in the Ames test and a mouse lymphoma mutagenesis assay but gave equivocal results in a Chinese hamster fibroblast cell chromosome aberration assay. In a carcinogenicity study of toothpaste, mice treated with Peppermint Oil developed neoplasms at the same rate as those treated with the toothpaste base. In some instances, the rates were comparable to those in mice of the untreated control group.

Isolated clinical cases of irritation and/or sensitization to Peppermint Oil and/or its constituents have been reported, but Peppermint Oil (8%) was not a sensitizer when tested using the Kligman maximization protocol.

DISCUSSION

In assessing the safety of Peppermint (Mentha Piperita) Oil, Peppermint (Mentha Piperita) Extract, Peppermint (Mentha Piperita) Leaves, and Peppermint (Mentha Piperita) Water, the CIR Expert Panel was concerned about oral-dosing studies that reported cystlike spaces in the cerebellum of rats. The results of these studies were difficult to interpret. The findings were not consistent among studies (lesions were noted in some studies but not others), and though the lesions appeared to depend on the pulegone content, no definitive conclusion could be made (a greater NOAEL was reported in a 90-day study using a Peppermint Oil containing 1.1% pulegone versus a 28-day study that tested a Peppermint Oil containing 1.7% pulegone). The Panel also noted that the large differences between doses within each study made it impossible to pinpoint exactly the dose at which changes first appeared.

Noting the lack of dermal exposure studies on Peppermint Oil, the Panel expected its absorption would be rapid, following that of menthol, a major component. Dermal absorption, however, was not expected to be greater than absorption through the gastrointestinal tract. Metabolism from either route of exposure would be similar—phase 1 metabolism followed by transport to the liver. The Panel was of the opinion that the oral-dose data contained in this report were sufficient to address concerns resulting from the expected rapid absorption. However, the Panel noted the evidence that menthol can enhance penetration. Formulators are cautioned that this enhanced penetration can affect the use of other ingredients whose safety assessment was based on their lack of absorption.

Clinical dermal testing demonstrated that 8% Peppermint Oil was not a sensitizer, and that 2% Peppermint Oil produced a small number of positive reactions in dermatitic patients.

Because pulegone is toxic, the Panel limited it to $\leq$1% in cosmetic grade Peppermint (Mentha Piperita) Oil, Peppermint (Mentha Piperita) Extract, Peppermint (Mentha Piperita) Leaves, and Peppermint (Mentha Piperita) Water. The Panel was confident that this concentration was achievable both by controlling the time of harvest, and through the patented technique described in this report. Recent data reported that Peppermint (Mentha Piperita) Oil is used at a concentration of $\leq$3% in rinse-off formulations and $\leq$0.2% in leave-on formulations. This concentration of use data coupled with the $\leq$1% restriction on pulegone suggested to the Panel that pulegone toxicity would not be seen with cosmetic use.

CONCLUSION

On the basis of the available data, the CIR Expert Panel concludes that Peppermint (Mentha Piperita) Oil, Peppermint (Mentha Piperita) Extract, Peppermint (Mentha Piperita) Leaves, and Peppermint (Mentha Piperita) Water are safe as used in cosmetic formulations. The concentration of pulegone in these ingredients should not exceed 1%.

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