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Amended Safety Assessment of *Mentha piperita* (Peppermint)–Derived Ingredients as Used in Cosmetics

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Abstract

The Expert Panel for Cosmetic Ingredient Safety (Panel) reviewed the safety of *M piperita* (peppermint)–derived ingredients. The Panel reviewed data relevant to the safety of these ingredients. Because final product formulations may contain multiple botanicals, each containing the same constituent(s) of concern, formulators are advised to be aware of these constituents and avoid reaching levels that may be hazardous to consumers. Industry should continue to use good manufacturing practices to limit impurities that could be present in botanical ingredients. The Panel concluded that *M piperita* (Peppermint) Oil, Extract, Leaf, and leaf-derived ingredients are safe in cosmetics in the present practices of use and concentration when formulated to be non-sensitizing, and that the available data are insufficient for determining that *M piperita* (Peppermint) Flower/Leaf/Stem Extract, *M piperita* (Peppermint) Flower/Leaf/Stem Water, and *M piperita* (Peppermint) Meristem Cell Culture are safe under the intended conditions of use in cosmetic formulations.

Keywords

Safety, Cosmetics, Mentha Piperita (Peppermint) Extract, Mentha Piperita (Peppermint) Flower/Leaf/Stem Extract, Mentha Piperita (Peppermint) Flower/Leaf/Stem Water, Mentha Piperita (Peppermint) Leaf, Mentha Piperita (Peppermint) Leaf Cell Extract, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf Juice, Mentha Piperita (Peppermint) Leaf Water, Mentha Piperita (Peppermint) Meristem Cell Culture, Mentha Piperita (Peppermint) Oil

Introduction

The safety of four of the cosmetic ingredients named in this safety assessment has been previously reviewed by the Panel; in 1998, the Panel issued a final report (published in 2001) with a conclusion stating that Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water are safe as used in cosmetic formulations.[1] The conclusion also stated that the concentration of pulegone, a constituent of these botanical ingredients, should not exceed 1%. In accordance with the Cosmetic Ingredient Review (CIR) Procedures, the Panel evaluates the conclusions of previously issued reports every 15 yr, and therefore a re-review was initiated. The new conclusion reached in this re-review supersedes the original conclusion. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), most of these ingredients are reported to function as fragrance ingredients and/or skin conditioning agents in cosmetic products.[2]

In addition to the four *M piperita* (peppermint)–derived ingredients that are mentioned above, this re-review included 6 related, previously unreviewed ingredients. The complete list of ingredients included in this assessment is:

Mentha Piperita (Peppermint) Extract
Mentha Piperita (Peppermint) Flower/Leaf/Stem Extract
Mentha Piperita (Peppermint) Flower/Leaf/Stem Water
Mentha Piperita (Peppermint) Leaf
Mentha Piperita (Peppermint) Leaf Cell Extract
Mentha Piperita (Peppermint) Leaf Extract
Mentha Piperita (Peppermint) Leaf Juice

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Mentha Piperita (Peppermint) Leaf Water
Mentha Piperita (Peppermint) Meristem Cell Culture
Mentha Piperita (Peppermint) Oil

*Previously reviewed ingredients are indicated in italics.

The cosmetic ingredient names, according to the *Dictionary*, are written as above, that is, capitalized, without italics, and unabbreviated. When referring to the plant from which these ingredients are derived, the traditional taxonomic nomenclature practice of using italics to identify genus and species will be followed (e.g., *M piperita*).

This safety assessment includes relevant published and unpublished data for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A list of the typical search engines and websites used, sources explored, and endpoints that Panel evaluates is available on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Safety test data that have been found in the published literature or those provided by the Personal Care Products Council (Council) as unpublished data since the original report was issued are included. Some safety test data on menthol, menthone, and pulegone are also included in the original published final report; these data and additional data are presented in Table 1. Considering that a limitation on pulegone is mentioned in the original conclusion, it should be noted that a National Toxicology Program (NTP) oral carcinogenicity study with positive results on pulegone was published in 2011[3] and that a 1998 publication on the absence of test substance-related histological cerebellar changes in Wistar rats dosed orally with pulegone is available.[4] These studies are also presented in Table 1.

Excerpts from the 2001 safety assessment on the previously reviewed ingredients are disseminated throughout the text of this re-review document, as appropriate, and are identified by italicized text. For complete and detailed information, please refer to the original report, which is available on the CIR website (https://www.cir-safety.org/ingredients).

Chemistry

Definition and General Characterization

Collectively, the botanical ingredients reviewed in this safety assessment are derivatives of the leaf, stem root, and whole *M piperita* herb. The definitions of *M piperita* (peppermint)–derived ingredients are stated in Table 2.[2]

Chemical and Physical Properties

Properties/specifications relating to *M piperita* (peppermint)–derived ingredients are presented in Table 3.[1,5-7]

Method of Manufacture

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

According to one source, Mentha Piperita (Peppermint) Oil has been extracted (distilled; water solvent) from the leaves of *M piperita* harvested (first in July and second harvest in September) in Washington state.[8]

M piperita *(Peppermint) Extract.* According to one source, the main steps in the process of manufacturing a trade name mixture defined as an aqueous solution containing 7.5% Mentha Piperita (Peppermint) Extract are: solubilization of *M piperita* in water, separation of soluble and insoluble phases, and filtration and sterilizing filtration.[9]

M Piperita *(Peppermint) Leaf Extract.* The following method relates to preparation of the butylene glycol/water extract of Mentha Piperita (Peppermint) Leaf Extract.[10] Dried raw material is extracted with 50 vol% 1,3-butylene glycolic solution. After extraction, the additional steps in the production process include: filtrate → sedimentation → filtrate → adjustment → packaging.

In another method, the preparation of a water/ethanol extract is described.[11] Dried raw material is extracted with 30 vol% ethanol solution. After extraction, the additional steps in the production process include: filtrate → concentration → adjustment → sedimentation → filtrate → adjustment → packaging.

The production method described herein relates to preparation of Mentha Piperita (Peppermint) Leaf Extract (powder form).[10] According to the method of production, dried raw material is extracted with 30 vol% ethanol solution. After extraction, the additional steps in the production process include: filtrate → concentration → add exsiccated sodium sulfate as vehicle → drying → packaging.

In the supercritical fluid extraction with natural carbon dioxide production method for Mentha Piperita (Peppermint) Leaf Extract from the dried leaves of *M piperita*, neither additives nor other technical adjuncts are introduced during the production process.[12]

M Piperita *(Peppermint) Leaf Water.* In the preparation of Mentha Piperita (Peppermint) Leaf Water, dried raw material is subjected to steam distillation.[10] After distillation, the remaining steps in the production process are: water soluble fraction obtained → adjustment → filtrate → packaging.

Composition and Impurities

Pulegone is found in young peppermint leaves, and is metabolized to menthol as the leaves mature. It has also been

Table 1. Toxicity Data on Components of *M piperita* (Peppermint)–Derived Ingredients.

Study Type/Protocol	Results
Short-term oral toxicity	
Menthone: 28-day study on groups of 20 rats. Doses up to 800 mg/kg/day	Brain lesions (cyst-like spaces in the cerebellum)[1]
Menthol: 28-day study on groups of 20 rats. Doses up to 800 mg/kg/day	No brain lesions[1]
Pulegone: 28-day study on groups of 20 rats. Doses up to 160 mg/kg/day	Brain lesions (cyst-like spaces in the cerebellum)[1]
Pulegone: 28-day study on groups of 28 female rats (CrL: (WI)BR, SPF strain). Doses (in soybean oil) of 160 mg/kg/day. Untreated control	Alopecia. Statistically significant, test substance-related decrease in body weight and food consumption ($P < .001$) observed throughout t study. Statistically significant reduction in plasma creatinine. Statistically significant increase in plasma alkaline phosphatase and non-statistically significant increase in alanine aminotransferase. Increased plasma concentrations of alkaline phosphatase and alanine aminotransferase taken together with increased absolute liver weight was non-statistically significant, but increased relative liver weight ($P < .05$) was indicative of adverse effect on the liver. However, no significant histopathology of liver observed. Number and severity of cyst-like spaces in cerebellum considered comparable to observations normally present in historical control Wistar rats at laboratory where study was performed. Authors noted that dose-dependent cyst-like spaces in cerebellum observed in previous studies could have been due to interaction between impurities in test substance and fixation agent used[4]
Developmental and reproductive toxicity	
Menthol: Groups of 15 to 23 pregnant animals dosed with Brazilian menthol via oral intubation. Mice received doses up to 185 mg/kg/day on gestation days (GD) 6 to 15. Rats received doses up to 218 mg/kg on GDs 6 to 15. Hamsters received doses up to 405 mg/kg/day on GDs 6 to 10. Artificially inseminated rabbits received doses up to 425 mg/kg/day on GDs 6 to 18. Caesarean sections were performed on all dams	No teratogenic effects[1]
Genotoxicity	
Menthol: *Salmonella typhimurium* strains: TA1535, TA100, TA1537, and TA98. Doses of 6.4, 32, 160, and 800 μg/plate in Ames test with or without metabolic activation	Toxicity at highest dosed; less toxicity with metabolic activation. Same number of revertants reported for test and control cultures exposed to lower doses[1]
Pulegone: *S typhimurium* strains: TA1535, TA100, TA1537, and TA98. Doses of 6.4, 32, 160, and 800 μg/plate in Ames test with or without metabolic activation	Toxicity at highest dose; less toxicity with metabolic activation. Same number of revertants reported for test and control culture exposed to lower doses[1]
Menthone: *S typhimurium* strains: TA1535, TA100, TA1537, TA98, and TA97. Doses of 6.4, 32, 160, and 800 μg/plate in Ames test with or without metabolic activation	Toxicity at highest dose. Statistically significant number of revertants in strain TA1537 at doses of 6.4 and 32 μg/plate without metabolic activation. Further testing with a more sensitive strain (TA98) yielded statistically significant increases in the number of revertants at all doses tested without metabolic activation; results were dose-related[1]
Menthol (Brazilian): Cytogenetic assay (rats). Doses of 1.45, 14.5, and 145 mg/kg, and, in some instances, doses of 500, 1150, and 3000 mg/kg, or 5000 mg/kg	Non-genotoxic[1]
Menthol (Brazilian): Dominant lethal assay (rats). Doses of 1.45, 14.5, and 145 mg/kg, and, in some instances, doses of 500, 1150, and 3000 mg/kg, or 5000 mg/kg	Non-genotoxic[1]
Menthol (Brazilian): Host-mediated assay (mice). Doses of 1.45, 14.5, and 145 mg/kg, and, in some instances, doses of 500, 1150, and 3000 mg/kg, or 5000 mg/kg. *S typhimurium* strain TA1530 and *Saccharomyces* strain D3 tested *in vitro*	Weakly positive but significant response at high dose in *S typhimurium* TA1530, and elevated recombinant frequencies noted in *Saccharomyces* strain D3[1]

(continued)

Table 1. (continued)

Study Type/Protocol	Results
Carcinogenicity	
Menthol: National Cancer Institute (NCI) 2-year bioassay. *Dl*-menthol administered orally to Fischer 344 rats (3750 ppm or 7500 ppm) or to $B6C3F_1$ mice (2000 ppm or 4000 ppm)	Negative trend in fibroadenomas of the mammary gland observed in female rats (20 of 50 control; 10 of 49 low-dose; 7 of 49 high-dose). No evidence of carcinogenicity in rats or mice or either sex[1]
Carcinogenicity	
Pulegone: National Toxicology Program (NTP) 2-year bioassay. Pulegone (in corn oil) administered to groups of 50 male and 50 female F344/N rats and groups of 50 male and 50 female B6C3F1 mice by gavage (5 days/week) for 105 weeks. Male rats received doses of 18.75, 37.5, or 75 mg/kg; female rats and male and female mice received doses of 37.5, 75, or 150 mg/kg	Effects in the kidneys (hyaline glomerulopathy and nephropathy), liver (oval cell hyperplasia, bile duct hyperplasia, hypertrophy, hepatocyte necrosis, and portal fibrosis), nose (olfactory epithelium degeneration, inflammation, and metaplasia), and forestomach (inflammation, hyperplasia, and ulcer) reported. Increased incidences of liver neoplasms in male and female B6C3F1 mice in the study led to the conclusion that there was clear evidence of carcinogenic activity in mice. For female F344/N rats, it was concluded that there was some evidence of carcinogenicity based on an increased incidence of urinary bladder neoplasms. Five of 47 rats in 150 mg/kg female stop-exposure group (gavage with pulegone stopped at week 60 because of severely reduced body weights) diagnosed with papilloma and carcinoma, combined. Male rats did not show increased incidences of bladder tumors or neoplasms of other organs.[3] The International Agency for Research on Cancer (IARC) concluded that there is sufficient evidence in experimental animals for the carcinogenicity of pulegone, and that there is inadequate evidence in humans for the carcinogenicity of pulegone.[78] IARC's conclusions were based on positive oral carcinogenicity data in male and female mice and in female rats
Pulegone: Female rats dosed orally (gavage) with 75 or 150 mg/kg for 4 and 6 weeks	Results supported hypothesis that cytotoxicity followed by regenerative cell proliferation is mode-of-action for pulegone-induced urothelial tumors in female rats[79]
Anticarcinogenicity	
(-)-Menthol: Rats dosed orally with 1% or .5% (-)-menthol for 20 weeks. Dosing initiated 2 weeks prior to dimethylbenzanthracene (DMBA) tumor induction	Significant inhibition ($P < .001$) of DMBA-induced rat mammary gland carcinogenesis following 20 weeks of oral dosing with 1% (-)-menthol. Chemopreventive effect noted when rats dosed with .5% menthol for 2 weeks prior to and 1 week after DMBA induction[1]
Skin irritation	
Menthol: Two Tiger Balm™ formulations containing 8% and 10% menthol applied for 23 h, under occlusive patches, to abraded and intact sites on New Zealand white rabbits. Total number of patches applied was 21. A third group was treated with control wax (mixture of hard and soft waxes)	Dermal irritation observed in all treated animals, with the following severity scale: 8% menthol balm < control wax <10% menthol balm. 8% menthol balm almost innocuous in male rabbits. Irritation observed was not progressive and tolerance developed within 10 days. No severe damage noted at microscopic examination of skin (increased hyperkeratosis noted at treated sites), and no evidence of systemic toxicity[1]
Skin sensitization	
Menthol	Because menthol is a predominant component of Mentha Piperita (Peppermint) Oil and Mentha Piperita (Peppermint) Leaf Extract, it should be noted that a review article on the sensitization potential of menthol is available. After performing an extensive review of the reported cases of sensitization to menthol, it was concluded that the literature does not adequately document the clinical relevance of the patch test reactions nor the impact of irritant reactions[80]
Other clinical reports	
Menthol: 877 patients with primary contact, atopic, nummular, and stasis dermatitis, and eczema were tested with 5% menthol in yellow paraffin	Reactions observed in 1% of the panelists within 96 h[1]

Table 2. Definitions and Functions of the Ingredients in This Safety Assessment.[2]

Ingredient CAS No.	Definition and Idealized Structures	Function
Mentha Piperita Peppermint) Oil 8006-90-4 84082-70-2	Mentha Piperita (Peppermint) Oil is a volatile oil obtained from the whole plant *Mentha piperita*. The accepted scientific name for *Mentha piperita* is *Mentha x piperita*	Fragrance Ingredients; Skin-Conditioning Agents – Miscellaneous
Mentha Piperita (Peppermint) Leaf Extract 84082-70-2	Mentha Piperita (Peppermint) Leaf Extract is the extract of the leaves of the peppermint, *Mentha piperita*. The accepted scientific name for *Mentha piperita* is *Mentha x piperita*	Fragrance Ingredients; Skin-Conditioning Agents – Miscellaneous; Skin-Conditioning Agents – Occlusive
Mentha Piperita (Peppermint) Leaf	Mentha Piperita (Peppermint) Leaf is the dried leaves of *Mentha piperita*. The accepted scientific name for *Mentha piperita* is *Mentha x piperita*	Fragrance Ingredients
Mentha Piperita (Peppermint) Leaf Water 84082-70-2	Mentha Piperita (Peppermint) Leaf Water is an aqueous solution of the steam distillate obtained from the leaves of *Mentha piperita*. The accepted scientific name for *Mentha piperita* is *Mentha x piperita*	Flavoring Agents; Fragrance Ingredients; Skin-Conditioning Agents – Miscellaneous
Mentha Piperita (Peppermint) Extract 84082-70-2	Mentha Piperita (Peppermint) Extract is the extract of the whole plant, *Mentha piperita*. The accepted scientific name for *Mentha piperita* is *Mentha x piperita*	Skin-Conditioning Agents – Miscellaneous
Mentha Piperita (Peppermint) Flower/Leaf/Stem Extract 84082-70-2	Mentha Piperita (Peppermint) Flower/Leaf/Stem Extract is the extract of the flowers, leaves, and stems of *M piperita*. The accepted scientific name for *Mentha piperita* is *Mentha x piperita*	Flavoring Agents; Fragrance Ingredients; Skin-Conditioning Agents – Miscellaneous
Mentha Piperita (Peppermint) Flower/Leaf/Stem Water 84082-70-2	Mentha Piperita (Peppermint) Flower/Leaf/Stem Water is the aqueous solution of the steam distillates obtained from the flowers, leaves and stems of *Mentha piperita*. The accepted scientific name for *Mentha piperita* is *Mentha x piperita*	Fragrance Ingredients
Mentha Piperita (Peppermint) Leaf Cell Extract	Mentha Piperita (Peppermint) Leaf Cell Extract is the extract of a culture of the leaf cells of *Mentha piperita*. The accepted scientific name for *Mentha piperita* is *Mentha x piperita*	Antioxidants; Skin Protectants
Mentha Piperita (Peppermint) Leaf Juice 84082-70-2	Mentha Piperita (Peppermint) Leaf Juice is the juice expressed from the leaves of *Mentha piperita*. The accepted scientific name for *Mentha piperita* is *Mentha x piperita*	Skin-Conditioning Agents – Miscellaneous
Mentha Piperita (Peppermint) Meristem Cell Culture	Mentha Piperita (Peppermint) Meristem Cell Culture is a suspension of the cultured meristem cells of *Mentha piperita*. The accepted scientific name for *Mentha piperita* is *Mentha x piperita*	Skin-Conditioning Agents – Miscellaneous

reported that pulegone is found only in Mentha Piperita (Peppermint) Oil from young plants and in trace amounts in "inferior" oils; pulegone is absent from "good quality" Mentha Piperita (Peppermint) Oil.[1] However, a supplier of Mentha Piperita (Peppermint) Oil reported pulegone concentrations of 1 to 4%, depending on the origin of the oil. Published studies that have investigated the pulegone content of Mentha Piperita (Peppermint) Oil also reported a range of <1 to 4% for Mentha Piperita (Peppermint) Oils of a North American origin.

M Piperita (Peppermint) Oil. The major constituents of Mentha Piperita (Peppermint) Oil include the terpenes: (−)-menthol (30%–55%), (−)-menthone (14%–32%), (+)-isomenthone (1.5%–10%), (−)-menthyl acetate (2.8%–10%), (+)-menthofuran (1.0%–9.0%), and 1,8-cineol (3.5%–14%).[13]

Certain trends were observed between oil extracted from first and second harvest leaves, and oil extracted from fresh leaves vs dried leaves.[8] When compared to the second harvest, oils from the first harvest were generally higher in (*Z*)-3-hexenol, 1,8-cineol, α-pinene, β-pinene, sabinene hydrate, isomenthone, menthofuran, pulegone, β-caryophyllene, and germacrene D, but lower in limonene, menthol, and menthone. When compared to oils from dried leaves, oils from fresh leaves were higher in 1,8-cineol, α-pinene, limonene, isomenthone, menthofuran, menthone, and pulegone and lower in β-caryophyllene, germacrene D, and menthol. Menthyl

Table 3. Physical and Chemical Properties of *Mentha piperita* (Peppermint)–Derived Ingredients.

Property	Value	Reference
Mentha Piperita (Peppermint) Oil		
Form	Colorless or pale yellow liquid	1
Angular rotation (°)	Between −18 and 32	1
Refractive index (at 20°C)	Between 1.459 and 1.465	1
Specific gravity	Between .896 and .908	1
Assay for total esters	Not less than 5% of esters, calculated as menthyl acetate	1
Assay for total menthol	Not less than 50% of menthol	1
Dimethyl sulfide	Passes test (rectified); fails test (natural)	1
Heavy metals (as Pb)	Passes test (limit of .004%)	1
Solubility	Soluble in alcohol: Passes test (1 vol dissolves in 3 vol of 70% alcohol)	1
	Soluble in most vegetable oils; insoluble in propylene glycol	7
Mentha Piperita (Peppermint) Leaf Extract		
Form	Yellow to light brown, clear oil	5
Refractive index (at 20°C)	1459-1469	5
Density (at 20°C)	.890-.910	5
Mentha Piperita (Peppermint) Extract (2.5% in tradename mixture)		
Refractive index	1.4498	6
Specific gravity	1.031	6

formate was found in all of the *M piperita* (peppermint) oils (from leaf extraction) that were analyzed.

Major components of the essential oil of *M piperita* adult plants from Poland include menthone, menthol, menthyl acetate, carvone, piperitone, 1,8-cineol, and pulegone.[14]

According to the United States Pharmacopeial Convention's (USP) Food Ingredients Expert Committee, the acceptance criteria for Mentha Piperita (Peppermint) Oil include not less than 5% total esters (calculated as menthyl acetate) and not less than 50% menthol.[7]

The international standard for Mentha Piperita (Peppermint) Oil, published by the International Organization for Standardization, contains the chromatographic profile for this ingredient that is presented in Table 4.[15] Pulegone and menthofuran were among the chemicals detected. A public statement from the European Medicines Agency on the use of herbal medicinal products containing pulegone and menthofuran indicated that Mentha Piperita (Peppermint) Oil contains a maximum of 4% pulegone and between 1% and 9% menthofuran.[16] It was also noted that the Scientific Committee on Food (SCF) has concluded that pulegone is mainly metabolized through pathways involving menthofuran and that these two substances show similar toxicity.

M Piperita *(Peppermint) Extract.* Mentha Piperita (Peppermint) Extract (combined in a trade name mixture) is an aqueous solution composed of 7.5% (maximum percentage) Mentha Piperita (Peppermint) Extract, with <40 ppm pulegone and <50 ppm menthol.[9] The following statement relating to composition was also provided: "Our active can be divided in sugars (47%), mineral ashes (38%), proteins (13%), and polyphenols (2%)." Composition data on this trade name mixture also include the following impurities: alkaloids (<.05 g/L; assay of alkaloids performed with Dragendorff reagent), copper (.23 ppm), iron (3.76 ppm), manganese (21 ppm), nickel (.19 ppm), and zinc (3.14 ppm). An assay of allergens was performed to characterize and quantify 26 allergen compounds in this trade name mixture in order to comply with the requirements of European Regulation 12234/2009. Allergens were not detected. Thus, the concentrations of the 26 allergens were less than the sensitivity of the method (<1 ppm).

The following information relating to impurities is included in a certificate of analysis for a trade name mixture containing 2.5% Mentha Piperita (Peppermint) Extract: lead (<10 ppm), arsenic (<3 ppm), mercury (<1 ppm), and pesticide residues (meets USP specification).[6]

M Piperita *(Peppermint) Leaf.* The major monoterpene constituents of Mentha Piperita (Peppermint) Leaf are: (−)-limonene; 1,8-cineol; (+)-pulegone; (−)-menthone; (+)-isomenthone; (+)-menthofuran; (−)-menthol; and (+)-neomenthol.[17] Mentha Piperita (Peppermint) Leaf also contains caffeic acid, rosmarinic acid, and the following flavonoids: apigenin-, diosmetin-, and luteolin-glycosides, and free lipophile methoxylized flavones such as xanthomicrol and gardenine D.[18]

The following elemental contaminants have been detected in *M piperita* herbal tea (tea leaves) samples (n = 3) from Serbia: manganese (111.97 mg/kg dry weight), iron (443.90 mg/kg), copper (17.15 mg/kg), and zinc (26.86 mg/kg), molybdenum (2.695 mg/kg), cobalt (.161 mg/kg), nickel (1.882 mg/kg), selenium (.107 mg/kg), aluminum (554 mg/kg), and tin (3.66 mg/kg).[19]

Table 4. Chromatographic Profiles for Mentha Piperita (Peppermint) Oil[15] and Mentha Piperita (Peppermint) Leaf Extract[a].[5,23]

	Origins Other Than U.S.		U.S. Type	
Components	Min. (%)	Max. (%)	Min. (%)	Max. (%)
Mentha Piperita (Peppermint) Oil				
3-Octanol	0.1	0.5	0.1	0.4
1,8-Cineol	3.0	8.0	4.0	6.0
Limonene[b]	1.0	3.0	1.0	2.5
trans-Sabinene Hydrate	0.5	2.0	0.5	2.3
Menthone	13.0	28.0	15.0	25.0
Isomenthone	2.0	8.0	2.0	4.5
Menthofuran	1.0	8.0	1.5	6.0
Neomenthol	2.0	6.0	2.5	4.5
Menthol	32.0	49.0	36.0	46.0
Pulegone	0.5	3.0	0.5	2.5
Menthyl Acetate[c]	2.0	8.0	3.0	6.5
β-Caryophyllene	1.0	3.5	1.0	2.5
	Origin Not Stated			
Mentha Piperita (Peppermint) Leaf Extract				
Essential Oil[d]	80	90		
Essential Oil[e]		>65		
Water		Not specified		
Volatile Compounds[f]				
Limonene		<2		
1,8-Cineol		Not specified		
L-menthone	20	40		
Menthofuran		<2		
Isomenthone		Not specified		
Isomenthol		Not specified		
Neomenthol		Not specified		
L-menthol	25	40		
Pulegone		<3		
Menthyl Acetate	5	15		
Beta Caryophyllene		Not specified		
Germacrene		Not specified		
Allergen Compounds[g]				
Eugenol		<.05		
Linalool		<0.3		
D-Limonene		<1		

[a]Mentha Piperita (Peppermint) Leaf Extract (supercritical CO_2 extract).
[b]The limonene is regarded to be predominantly L-limonene based on physical tests. It is believed that there might be a small amount of D-limonene present, but the exact quantity is unknown.
[c]The menthyl acetate is regarded to be predominantly L-menthyl acetate based on the physical tests. It is believed that there might be a small amount of D-menthyl acetate present, but the exact quantity is unknown.
[d]Gravimetric distillation detection method used.
[e]Volumetric distillation detection method used.
[f]Gas chromatography-mass spectrometry detection method used.
[g]Allergen compounds present are subject to declaration if the concentration exceeds .001% in leave-on and .01% in rinse-off products. However, values <.01% are not mentioned.

It is possible that pesticide residues may be present as impurities in the leaves of *M piperita*. In a study in which Mentha Piperita (Peppermint) Leaves were soaked in pesticides, the dissipation rate of pesticide residues during the drying process was said to have been satisfactory, except for the pirimiphos-ethyl pesticide, because of its high octanol-water partition coefficient and low vapor pressure.[20]

M piperita *(Peppermint) Leaf Extract.* An analysis of Mentha Piperita (Peppermint) Leaf Extract indicated that the leaves

principally contained cinnamic acid, caffeic acid, rosmarinic acid, and various flavonoids (flavones and flavanones).[21] The following solvents were used to extract the peppermint leaves: light petroleum, dichloromethane, acetonitrile, ethyl acetate, methanol, *n*-butanol, and water. Eriocitrin (383.3 ± 2.2 mg/g extract) and rosmarinic acid (381.2 ± 1.9 mg/g extract) were the most abundant components identified within the leaves, while naringenin-7-*O*-glucoside (.8 ± .01 mg/g extract) was the least abundant component identified. Kynurenic acid (3.82 ± .46 µg/g) has also been detected in Mentha Piperita (Peppermint) Leaf Extract.[22] It should be noted that kynurenic acid is a constituent of human synovial fluid.

Composition data provided by the Council indicate that Mentha Piperita (Peppermint) Leaf Extract (butylene glycol/water extract) contains tannin and terpenoid (which contains 2.8 ppm pulegone) and that Mentha Piperita (Peppermint) Leaf Extract (water/ethanol extract) contains essential oil, tannin, and terpenoid.[10]

Data on impurities provided by the Council indicate that Mentha Piperita (Peppermint) Leaf Extract (butylene glycol/water extract) contains not more than 10 ppm heavy metals and not more than 2 ppm arsenic.[10] Mentha Piperita (Peppermint) Leaf Extract (water/ethanol extract) contains not more than 10 ppm heavy metals and not more than 1 ppm arsenic.

The supercritical fluid extraction with natural carbon dioxide production method for *M piperita* (Peppermint) Leaf Extract from the dried leaves of *M piperita* results in no solvent residues, no inorganic salts, no heavy metals, and no reproducible microorganisms.[12]

According to data provided by the Council, *M piperita* (peppermint) leaf extract powder contains not more than 10 ppm heavy metals and not more than 2 ppm arsenic.[10]

A chromatographic profile for Mentha Piperita (Peppermint) Leaf Extract (supercritical CO_2 extract) is presented in Table 4.[5,23]

M Piperita *(Peppermint) Leaf Water.* Composition data provided by the Council indicate that Mentha Piperita (Peppermint) Leaf Water contains essential oil (menthol).[10] The data also included specifications that indicate that Mentha Piperita (Peppermint) Leaf Water contains not more than 10 ppm heavy metals and not more than 1 ppm arsenic.[10]

Use

Cosmetic

The safety of *M piperita* (peppermint)–derived ingredients is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database.[24] Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Council, of maximum reported use concentrations by product category.[25]

According to 2018 VCRP data, the greatest use frequency is being reported for Mentha Piperita (Peppermint) Oil, which is being used in 815 cosmetic products (432 leave-on products +349 rinse-off products +34 products diluted for bath use).[24] The results of a concentration of use survey conducted in 2016 indicate that Mentha Piperita (Peppermint) Leaf Water is being used at concentrations up to 40% in leave-on products (face and neck products [not spray]), which is the greatest use concentration that is being reported for *M piperita* (peppermint)–derived ingredients reviewed in this safety assessment.[25] Current and historical use frequency and concentration of use data are presented in Table 5. These data indicate that the highest maximum use concentration of *M piperita* (Peppermint) Oil in cosmetics increased from 3% in 1997 to 5% (in face and neck leave-on products) in 2017. Because 1997 use concentration data on the remaining 3 ingredients that were reviewed in the original safety assessment, Mentha Piperita (Peppermint) Leaf Extract, *M piperita* (Peppermint) Leaf, and Mentha Piperita (Peppermint) were not provided, a comparison of 1997 vs 2017 use concentration data cannot be made.

According to VCRP and Council survey data, the following *M piperita* (peppermint)–derived ingredients are not being used in cosmetic products: *M piperita* (Peppermint) Flower/Leaf/Stem Water, *M piperita* (Peppermint) Flower/Leaf/Stem Extract, *M piperita* (Peppermint) Leaf Cell Extract, *M piperita* (Peppermint) Leaf Juice, and *M piperita* (Peppermint) Meristem Cell Culture.

Cosmetic products containing *M piperita* (peppermint)–derived ingredients may be applied to the skin and hair or, incidentally, may come in contact with the eyes (at maximum use concentrations up to .0018% *M piperita* (Peppermint) Leaf Extract in eye lotions) and mucous membranes (at maximum use concentrations up to 3.9% *M piperita* (Peppermint) Oil in bath oils, tablets, and salts). Additionally, use in lipstick products (at maximum use concentrations up to 2.9% *M piperita* (Peppermint) Oil) is being reported, the application of which may result in incidental ingestion. Products containing *M piperita* (peppermint)–derived ingredients may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

M piperita (Peppermint) Oil is being used in both pump hair sprays (maximum use concentrations up to .02%) and aerosol hair sprays (maximum use concentrations up to .017%) which may result in incidental inhalation exposure. Additionally, use of this ingredient in foot sprays at maximum use concentrations up to .5% is being reported. *M piperita* (Peppermint) Leaf Extract is also being used in pump and aerosol hair sprays, but at lower maximum use concentrations, and in face and neck/body and hand spray products at

Table 5. Frequency and Concentration of Use of *Mentha piperita* (Peppermint)–Derived Ingredients According to Duration and Exposure.[1,24,25]

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	Mentha Piperita (Peppermint) Oil				Mentha Piperita (Peppermint) Leaf Extract			
	2018	**1998**	**2017**	**1997**	**2018**	**1998**	**2017**	**1997**
Totals[a]	**815**	**102**	**.0001-5**	**.1-3**	**205**	**35**	**.000075-0.5**	**NR**
Duration of use								
Leave-on	*432*	*52*	*.0006-5*	*.2-2*	*126*	*10*	*.000075-0.5*	*NR*
Rinse-off	*349*	*44*	*.0001-1.9*	*.1-3*	*78*	*24*	*.0001-0.2*	*NR*
Diluted for (bath) use	*34*	*6*	*.018-3.9*	*NR*	*1*	*1*	*NR*	*NR*
Exposure type								
Eye area	4	NR	.00094	NR	5	NR	.0018	NR
Incidental ingestion	207	24	.05-2.9	.2-1.2	8	NR	.00075-0.5	NR
Incidental inhalation-spray	22; 114[b]	NR; 23[b]	.017-1; 0.002-1.1[b]	NR	5; 36[b]	NR; 2[b]	.00041-.0046; 0.00057-.026[b]	NR
Incidental inhalation-powder	1	NR	.01-1	NR	3	NR	.0018	NR
Dermal contact	457	75	.0006-5	.1-2	147	22	.000075-0.2	NR
Deodorant (underarm)	4	NR	NR	NR	NR	NR	.0018	NR
Hair – non-coloring	144	1	.0001-.96	3	48	13	.00018-0.2	NR
Hair-coloring	NR	NR	.0024	NR	NR	NR	.032	NR
Nail	7	2	.00064-1.5	NR	1	NR	NR	NR
Mucous membrane	310	30	.0025-3.9	.2-1.2	7	6	.00075-0.5	NR
Baby products	1	NR	0.2	NR	NR	NR	NR	NR

	Mentha Piperita (Peppermint) Leaf				Mentha Piperita (Peppermint) Leaf Water			
	2018	**1998**	**2017**	**1997**	**2018**	**1998**	**2017**	**1997**
Totals[a]	**NR**	**NR**	**.0002-1.6**	**NR**	**16**	**NR**	**.00013-40**	**NR**
Duration of use								
Leave-on	*NR*	*NR*	*.001*	*NR*	*9*	*NR*	*.00013-40*	*NR*
Rinse-off	*NR*	*NR*	*.0002-1.6*	*NR*	*7*	*NR*	*.00021-.00067*	*NR*
Diluted for (bath) use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	***NR***	*NR*
Exposure type								
Eye area	NR	NR	NR	NR	2	NR	NR	NR
Incidental ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental inhalation-spray	NR	NR	NR; 0.001[b]	NR	NR; 4[b]	NR	NR; 0.00043-10[b]	NR
Incidental inhalation-powder	NR	NR	NR	NR	NR	NR	NR	NR
Dermal contact	NR	NR	.001-1.6	NR	13	NR	.00013-40	NR
Deodorant (underarm)	NR	NR	NR	NR	1	NR	15	NR
Hair – non-coloring	NR	NR	.0002-.023	NR	1	NR	NR	NR
Hair-coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous membrane	NR	NR	.001-.16	NR	NR	NR	NR	NR
Baby products	NR	NR	NR	NR	NR	NR	NR	NR

	Mentha Piperita (Peppermint) Extract		
	2018	**2017**	
Totals[a]	**82**	**.00006-7.9**	
Duration of use			
Leave-on	*49*	*.00006-7.9*	*NR*
Rinse-off	*32*	*.0001-1*	*NR*
Diluted for (bath) use	*1*	*1*	*NR*
Exposure type			
Eye area	NR	NR	NR

(continued)

Table 5. (continued)

Incidental ingestion	4	.0099-3.4	NR
Incidental inhalation-spray	10; 22[b]	1.3; .005-1[b]	NR
Incidental inhalation-powder	NR	NR	NR
Dermal contact	73	.00006-7.9	NR
Deodorant (underarm)	2	1	NR
Hair – non-coloring	5	.0004-1	NR
Hair-coloring	NR	NR	NR
Nail	NR	NR	NR
Mucous membrane	7	.0099-3.4	NR
Baby products	NR	NR	NR

[a]Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
[b]It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
NR, no reported use.

maximum use concentrations up to .001%. *M piperita* (Peppermint) Extract is being used in face and neck sprays at a highest maximum use concentration of 1.3%. In practice, most droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm, compared with pump sprays.[26-29] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[26,27]

M piperita (Peppermint) Oil is being used in foot powders at maximum use concentrations up to 1%, and *M piperita* (Peppermint) Leaf Extract is being used in face powders at maximum use concentrations up to .0018%. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[30-32]

Noncosmetic

Mentha Piperita (Peppermint) Oil is a generally recognized as safe (GRAS) ingredient according to the FDA for use in dietary supplements.[1] It is described as a naturally occurring carminative that relaxes gastrointestinal smooth muscle. A final ruling by the FDA labeled Mentha Piperita (Peppermint) Oil as safe and effective as an antitussive (topical/inhalant). Final rulings cautioned that Mentha Piperita (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.

M piperita (Peppermint) Oil, *M piperita* (Peppermint) Extract, *M piperita* (Peppermint) Flower/Leaf/Stem Extract, and Mentha Piperita (Peppermint) Leaf Juice are generally recognized as safe (GRAS) by the US FDA for use in food for human consumption (21CFR182.20). *M piperita* (Peppermint) Oil is an inactive ingredient in drug products that have been approved by the U.S. FDA.[33] A number of active ingredients, *M piperita* (Peppermint) Oil included, have been present in over-the-counter (*OTC*) drug products for various uses.[34] However, the FDA has determined that, based on evidence currently available, there are inadequate data to establish general recognition of the safety and effectiveness of Mentha Piperita (Peppermint) Oil as an active ingredient in the following drug products: nasal decongestant drug products, digestive aid drug products, insect bite and sting drug products, and astringent drug products.

M piperita (Peppermint) Oil is on the U.S. Environmental Protection Agency (EPA) list of active ingredients eligible for minimum risk pesticide products.[35]

According to the European Medicines Agency Committee on Herbal Medicinal Products (HMPC) community herbal monograph on *Mentha x piperita* L., aetheroleum (i.e., peppermint oil; aetheroleum is a term used to describe a preparation made from the above-ground parts of a plant), this herbal medicine is administered orally for the symptomatic relief of minor spasms of the gastrointestinal tract, flatulence, and abdominal pain, especially in patients with irritable bowel syndrome.[36] It is also an herbal medicine that is administered cutaneously for the symptomatic relief of mild tension-type headache. These uses have been identified as well-established uses by the HMPC. The highest recommended daily dose in the European Union is 1.2 mL peppermint oil (i.e., 1,080 mg peppermint oil, which contains maximum 140 mg pulegone + menthofuran). For a 60 kg person, this would correspond to a daily intake of 2.3 mg/kg body weight.

Toxicokinetic Studies

Dermal Penetration

M piperita *(Peppermint) Oil.* Eserine in a Mentha Piperita (Peppermint) Oil vehicle was applied to a 2.2 cm^2 shaved area on the abdomen of mice. The absorption rate for Mentha Piperita (Peppermint) Oil was measured as the latent period between application and appearance of eserine-induced signs of cholinergic excess.[1] Mentha Piperita (Peppermint) Oil had a latent period of 58 min.

Penetration Enhancement

M piperita *(Peppermint) Leaf Extract.* The skin penetration enhancement potential of Mentha Piperita (Peppermint) Leaf Extract (aqueous ethanol extract) was evaluated using dorsal porcine skin (dermatomed to thickness of 500 μm).[37] A square section of skin was cut to provide a dose area of 1 cm^2 and placed in a flow-through diffusion cell. [^{14}C]Caffeine (hydrophilic) or [^{14}C]salicylic acid (hydrophobic) was applied topically with 10% Mentha Piperita (Peppermint) Leaf Extract to porcine skin. The receptor fluid for the diffusion cell was "a Krebs-Ringer bicarbonate buffer spiked with dextrose and bovine serum albumin (BSA)." Ethanol alone served as the control. When compared to [^{14}C]caffeine in the presence of ethanol (control), the dermal absorption of [^{14}C]caffeine was significantly greater ($P > .05$; flux and permeability of caffeine increased by over 3-fold) in the presence of Mentha Piperita (Peppermint) Leaf Extract. However, this was not true for [^{14}C]salicylic acid.

Penetration Inhibition

M piperita *(Peppermint) Oil.* Mentha Piperita (Peppermint) Oil and ring-UL-[^{14}C]benzoic acid were applied to full-thickness human skin (breast or abdominal) samples in a static diffusion cell.[38] The receptor fluid for the diffusion cell was "0.9% sodium chloride and 1% Tween in water." As the concentration of *M piperita* (Peppermint) Oil increased from zero to 5% in the donor phase, the maximal flux of benzoic acid decreased. The differences were significant at 1.0% and 5.0% *M piperita* (Peppermint) Oil, where the maximal fluxes were reduced to 81% and 52% of the control, respectively.

Absorption, Distribution, Metabolism, and Excretion

Oral

M piperita *(Peppermint) Oil.* The rate of Mentha Piperita (Peppermint) Oil absorption and excretion following oral administration was determined by measuring urinary menthol glucuronide.[1] Four male volunteers ingested 180 mg of an enteric-coated Mentha Piperita (Peppermint) Oil-coated capsule following a 16-h fast. Menthol was liberated from its glucuronide metabolite by treating the urine with β-D-glucuronidase. 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 h.

Mentha Piperita (Peppermint) Oil is relatively rapidly absorbed after oral administration and eliminated mainly via the bile.[13] The major biliary metabolite is menthol glucuronide, which undergoes enterohepatic circulation. The urinary metabolites result from hydroxylation at the C-7 methyl group at C-8 and C-9 of the isopropyl moiety, forming a series of mono- and dihydroxymenthols and carboxylic acids, some of which are excreted, in part, as glucuronic acid conjugates. Studies with tritiated L-menthol in rats indicated approximately equal excretion in the feces and urine. The main metabolite identified was menthol-glucuronide. Additional metabolites are mono- or di-hydroxylated menthol derivatives.

Toxicological studies

Acute Toxicity Studies

Oral

M piperita *(Peppermint) Oil.* Mentha Piperita (Peppermint) Oil had a 24-h oral LD_{50} of 4441 mg/kg in fasted Wistar rats; the 48-h LD_{50} was 2426 mg/kg.[1] In a study involving fasted mice, an LD_{50} of 2410 mg/kg was reported for Mentha Piperita (Peppermint) Oil diluted in olive oil.

Short-Term Toxicity Studies

Oral

M piperita *(Peppermint) Oil.* In 3 of 4 short-term oral toxicity studies (28-d or 5-wk studies) involving 20 to 28 rats per group, brain lesions (specifically, cyst-like spaces in the cerebellum) were observed at Mentha Piperita (Peppermint) Oil doses up to 100 mg/kg/d.[1] In the remaining study (12 rats per group), these lesions were not observed in rats dosed with *M piperita* (Peppermint) Oil at doses of 20, 150, or 500 mg/kg/d for 5 wk.

Subchronic Toxicity Studies

Oral

M piperita *(Peppermint) Oil.* Groups of 28 Wistar rats were given oral doses of 10, 40, and 100 mg/kg *M piperita* (Peppermint) Oil (diluted with soybean oil) daily for 90 d.[1] All hematological and biochemical parameters were within normal range, and there were no significant differences in absolute and relative organ weights. Brain lesions (specifically, cyst-like spaces in the cerebellum) were observed in all dose groups, but these results were classified as significant only for animals of the 100 mg/kg/d dose group. No other lesions of encephalopathy were observed. Nephropathy (hyaline droplet formation) was observed only in male rats of the 100 mg/kg/d dose group, and there was no evidence of epithelial degeneration. The no-observed-adverse-effect level (NOAEL) for Mentha Piperita (Peppermint) Oil was 40 mg/kg/d in this study.

Genotoxicity Studies

In Vitro

M piperita *(Peppermint) Oil.* The mutagenic potential of Mentha Piperita (Peppermint) Oil was investigated using the *Salmonella*/mammalian microsome test.[1] The following *Salmonella typhimurium* strains were used: 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. Mentha Piperita (Peppermint) Oil, 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. Metabolic activation appeared to have made the oil less toxic to the bacteria. Mentha Piperita (Peppermint) Oil was not genotoxic.

In an in vitro chromosomal aberration test using a Chinese hamster fibroblast cell line, Mentha Piperita (Peppermint) Oil, at a maximum concentration of .25 mg/mL (in ethanol), produced polyploidism in 3% of the cells and structural aberrations in 7% of the cells at 48 h after treatment. The results were considered equivocal, as scores of either ≥10% or ≤4.9% were necessary for classification as either positive or negative, respectively. The results for Mentha Piperita (Peppermint) Oil (150 µg/ml) were negative in a mouse lymphoma L5178Y TK +/− cell mutagenesis assay. Results were also negative for this ingredient (at 155 µg/ml) in an unscheduled DNA synthesis assay using rat hepatocytes.[1]

The genotoxicity of Mentha Piperita (Peppermint) Oil was evaluated in a chromosome aberration test using human peripheral blood lymphocytes.[39] Lymphocyte cultures were incubated for 24 h with test substance concentrations up to .30 µl/ml. When chromosome aberrations (chromatid breaks, chromatid exchanges, chromosome breaks, and chromosome exchanges) were scored, not less than 100 metaphases per culture were analyzed. Mentha Piperita (Peppermint) Oil was the most clastogenic at a concentration of .20 µl/ml (8-fold increase over acetone solvent control); the number of aberrant cells decreased at higher concentrations. The authors noted that the dose-response curve for *M Piperita* (Peppermint) Oil was complicated, with a clear peak response at a concentration of .20 µl/ml.

M piperita (Peppermint) Oil was tested at concentrations up to .30 µl/ml in the sister chromatid exchange (SCE) test involving human lymphocytes.[39] The test conditions were essentially the same as those in the preceding chromosome aberration test, with the exception that 5-bromo-2'-deoxyuridine was added (10 µg/ml) to cultures initially. To determine the replicative index, 200 cells were scored. *M piperita* (Peppermint) Oil induced SCEs in a dose-independent manner. The authors noted that, seemingly, the saturation of SCE-inducing capacity occurred at high concentrations of Mentha Piperita (Peppermint) Oil. Results also indicated that Mentha Piperita (Peppermint) Oil inhibited cell replicative kinetics, some signs of which were observed at a concentration of .15 µl/ml. At concentrations ≥.20 µl/ml, statistically significant inhibition of cell replicative kinetics was evident.

M piperita *(Peppermint) Extract.* The genotoxicity of a trade name mixture containing 2.5% Mentha Piperita (Peppermint) Extract was evaluated in the Ames test using the following *Salmonella typhimurium* strains, with and without metabolic activation: TA97a, TA98, TA100, TA102, and TA1535.[40] The test substance was diluted with sterile distilled water to a concentration of 10% (effective concentration of extract = .25%) prior to testing each strain. Sterile deionized water served as the solvent control and positive controls (not stated) were also used. The test substance was not cytotoxic to the test system and was not genotoxic to any of the strains tested, either with or without metabolic activation. The bacterial strains tested were sensitive to the positive control mutagens and had a spontaneous reversion rate that was well within the accepted values for each strain.

Antigenotoxicity Studies

M piperita *(Peppermint) Leaf Extract*

Oral pretreatment with Mentha Piperita (Peppermint) Leaf Extract (aqueous extract) (1 g/kg/d for 3 consecutive days) before exposure to gamma radiation was found to be effective in protecting against chromosomal damage in the bone marrow of Swiss albino mice (number tested not stated).[41] The exposure of mice to 8 gray (Gy) ionizing radiation (1 Gy is equivalent to the absorption of one joule of radiation energy per kilogram of matter) only resulted in chromosomal aberrations in the form of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges, and acentric fragments. In mice pretreated with Mentha Piperita (Peppermint) Leaf Extract, there was a significant decrease in the frequency of aberrant cells when compared to the irradiated control. A significant increase in the percentage of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges, acentric fragments, total aberrations, and aberrations/damaged cell was observed at 12 h post-irradiation necropsy time in control animals. However, a significant decrease in the percentage of aberrations of this type was observed in mice pretreated with *M piperita* (Peppermint) Leaf Extract.

The modulatory effects of *M piperita* (Peppermint) Leaf Extract (aqueous extract) on genotoxicity and lung tumor incidence were evaluated using 4 groups of 30 to 76 Swiss albino mice.[42] Beginning at 3 wk of age (weaning), the mice received a single subcutaneous injection of benzo[a]pyrene and were then dosed orally (by gavage) with either water (group of 53 mice) or *M piperita* (Peppermint) Leaf Extract (1 g/kg; group of 76 mice). The remaining 2 groups of mice in the study were identified as no benzo[a]pyrene or *M piperita* (Peppermint) Leaf Extract dosing (30 mice) and *M piperita* (Peppermint) Leaf Extract alone (30 mice). When compared to mice in the benzo[a]pyrene only group, *M piperita* (Peppermint) Leaf Extract reduced the frequency of chromosomal aberrations and micronuclei in bone marrow cells. *M piperita* (Peppermint) Leaf Extract had an antigenotoxic effect in this study. Results relating to the modulatory effect of *M piperita* (Peppermint) Leaf Extract on lung tumor formation are included in the Anticarcinogenicity section of the report text.

Carcinogenicity Studies

Oral

M piperita *(Peppermint) Oil.* 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 *M piperita* (Peppermint) Oil/kg/d, 6 d/wk for 80 wk. Treatment was followed by a 16- to 24-wk observation period. An untreated group of 52 male mice and a vehicle control group of 260 male mice that received the toothpaste base (which did not contain chloroform, eucalyptol, or *M piperita* (Peppermint) Oil) were maintained as controls. 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. The incidence of neoplasms of the lungs and kidneys was comparable among mice of the treated and nontreated groups. Hepatic cell tumor incidence for *M piperita* (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 whether the differences in tumor incidence were significant.[1]

Anticarcinogenicity Studies

M piperita *(Peppermint) Leaf Extract*

The modulatory effects of *M piperita* (Peppermint) Leaf Extract (aqueous extract) on genotoxicity and lung tumor incidence were evaluated using 4 groups of 30 to 76 Swiss albino mice.[42] The dosing procedure (including groups tested) was identical to that stated for Swiss mice in the second study of the Antigenotoxicity section in this report. The mice were killed at 9 wk of age and evaluated for lung tumor incidence. Dosing with *M piperita* (Peppermint) Leaf Extract caused a significant reduction in the number of lung adenomas from an incidence of 67.92% in mice given benzo[a]pyrene only to 26.31%, which amounted to 61.26% inhibition. Tumor multiplicity was 1.22 in the benzo[a]pyrene only group and 1.15 in the benzo[a]pyrene + *M piperita* (Peppermint) Leaf Extract group. *M piperita* (Peppermint) Leaf Extract had an inhibitory effect on lung tumor formation in this study. Results relating to the modulation of genotoxicity are included in the Antigenotoxicity section of the report text.

The anticancer potential of *M piperita* (Peppermint) Leaf Extract (double-distilled; water extract) was studied using Swiss albino mice (number not stated).[43] Two stage mouse skin carcinogenesis was initiated by 7,12-dimethyl-benz[a] anthracene (DMBA). Two weeks later, croton oil (promoter) was applied 3 times per week for 14 wk. The mice were dosed orally with *M piperita* (Peppermint) Leaf Extract (800 mg/kg/d) for the same period. At the end of the dosing period, average latent period, tumor incidence, size, burden, weight and cumulative number of papillomas were assessed. Dosing with *M piperita* (Peppermint) Leaf Extract caused inhibition of skin papilloma formation induced by DMBA and the application of croton oil, in terms of a significant decrease in the cumulative number of papillomas, tumor burden, and tumor incidence. In the control group, the tumor incidence was 100%. However, after dosing with the test substance for 15 d, the tumor incidence was reduced to 64%. There was a significant increase in the latency period for the appearance of papillomas in test animals (11 wk in control group; 13 wk in test group).

The possible molecular mechanisms underlying the cytotoxicity and anticarcinogenic potential of *M piperita* (Peppermint) Leaf Extract (petroleum ether, benzene, chloroform, ethyl acetate, methanol, or water extract) on 6 human cancer (HeLa, MCF-7, Jurkat, T24, HT-29, and MIAPaCa-2) and normal (IMR-90 and HEK-293) cell lines were evaluated.[44] In the human cancer cell lines tested with doses of 1, 10, and 100 µg/ml for 6 h, the number of apoptotic cells was incremental with an increase in the dose of *M piperita* extracts. However, of all the extracts tested, the chloroform and ethyl acetate extracts resulted in a significantly higher apoptotic index after 6 h, and the results were dose-dependent. When compared to the cancer cell lines, no significant changes were observed in normal cells. Similarly, of all of the extracts tested, the chloroform and ethyl acetate extracts of *M piperita* had significant dose- and time-dependent anticarcinogenic activity, leading to G1 cell cycle arrest and mitochondrial-mediated apoptosis, perturbation of oxidative balance, upregulation of Bax gene, elevated expression of p53 and p21 in the treated cells, and acquisition of senescence phenotype, while inducing pro-inflammatory cytokines response.

A study was performed to evaluate the antitumor activity of *M piperita* (Peppermint) Leaf Extract (methanol extract), using SW-480 human colon adenocarcinoma cells in a relevant cell anti-proliferation assay.[45] Statistically significant ($\alpha = .05$) growth inhibition was observed at a concentration of 31 µg/ml. An IC_{50} (concentration required for 50% inhibition, µg/ml) of 92.3 µg/ml was reported for *M piperita* (Peppermint) Leaf Extract.

Other Relevant Studies

Cytotoxicity

M piperita *(Peppermint) Oil.* The cytotoxicity of Mentha Piperita (Peppermint) Oil was evaluated in the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using 2 human cancer cell lines, MCF-7 and LNCaP.[46] Mentha Piperita (Peppermint) Oil from plants that were harvested during the summer and winter was tested. The following IC_{50} values (µg/ml) were reported: MCF-7 cell line (75.2 ± 2.9 [summer]; 80.8 ± 3.2 [winter]) and LNCaP cell line (90.4 ± 3.7 [summer]; 95.7 ± 4.5 [winter]). IC_{50} values in the 10 to 100 µg/ml range represented a potentially toxic chemical, and IC_{50} values <10 µg/ml represented a potentially very toxic chemical.

In another study, essential oil was extracted from the leaves of *M piperita*.[47] The oil essential oil was found to be cytotoxic in the following 4 human cancer cell lines: human lung carcinoma SPC-A1 cells (IC_{50} = 10.89 µg/ml), human leukemia K562 cells (IC_{50} = 16.16 µg/ml) and human gastric

cancer SGC-7901 cells (IC_{50} = 38.76 μg/ml). The essential oil was inactive against human hepatocellular carcinoma BEL-7402 cells.

M piperita. The inhibitory effect of polysaccharides extracted from *M piperita* on A549 non-small cell lung adenocarcinoma cells was investigated using the MTT assay.[48] The results indicated that polysaccharides extracted from *M piperita* had a moderate toxic effect on the A549 cell line (IC_{50} = 879.52 ± 22.55 μg/mL). The growth of A549 cells was inhibited by *M piperita* in a dose-dependent manner. The inhibitory rate was 54.54% ± 1.38% at the highest concentration tested (1 mg/mL).

Hepatotoxicity

M piperita *(Peppermint) Leaf Extract.* *M piperita* (Peppermint) Leaf Extract (methanol extract) and other botanical extracts were tested on both human (HepG2/C3A) and rat (MH1C1) hepatoma cells, using a battery of toxicity endpoints.[49] The extract was dissolved in dimethyl sulfoxide (DMSO) and then diluted in culture medium to a final concentration of 1000 μg/ml. The following 8 endpoints covering a variety of biological activities relevant to hepatotoxicity were used for hepatotoxicity evaluation: oxidative stress, mitochondrial membrane permeability, cellular neutral and polar lipid accumulation, CYP1A, 2B, 3A activities, albumin excretion, and total DNA content. Cluster analysis was used to group the phenolics into 4 clusters for each cell type. Two of the clusters were cluster 1 (compounds clustering with the solvent control (DMSO)) and cluster 2 (compounds with reported in vivo liver toxicity). Overall and individual liver activity of the phenolics on both human and rat hepatoma cell lines were compared. For HepG2/C3A cells, 100% of the observations for *M piperita* (Peppermint) Leaf Extract and thyme extract, 92% for cinnamon extract, and 89% for juniper berry extract were assigned to cluster 1 (control group). For rat MH1C1 cells, 100% of the juniper berry extract and *M piperita* (Peppermint) Leaf Extract observations were assigned to cluster 1. The authors noted that because there are currently no reports of liver toxicity associated with peppermint, *M piperita* (Peppermint) Leaf Extract is useful as a negative control.

Nephrotoxicity

M piperita *(Peppermint) Leaf Extract.* The effects of *M piperita* (Peppermint) Leaf Extract ("*M piperita* tea" (i.e., aqueous extract)) on rat kidney tissue were evaluated. The tea (prepared daily) was made by pouring 250 mL of boiling water over one heaped teaspoon (5 g) of the dried leaves of *M piperita* L (grown in Turkey) and steeping for 5 to 10 min. Groups of 12 male Wistar albino rats were used. Test animals received *M piperita* tea (20 g/L) in drinking water for 30 d. Control rats were given commercial drinking water during the study. The following histopathological changes, described as slight, were reported for the group dosed with *M piperita* tea: hydropic degeneration of tubular epithelial cells, epithelial cells with pyknotic nuclei and eosinophilic cytoplasm, tubular dilatation and enlargements in Bowman capsules. In conclusion, the results indicate that *M piperita* is not nephrotoxic to rats.[50]

Effect on Histamine Release

M piperita *(Peppermint) Leaf Extract.* The 50% ethanol extract of peppermint leaves and stems significantly inhibited histamine release from rat peritoneal mast cells that was induced by compound 48/80 (polymer produced by the condensation of *N*-methyl-*p*-methoxyphenethylamine with formaldehyde) *in vitro*.[51]

M piperita. In a study involving human basophil cell suspensions, obtained from workers who were exposed to an additive containing penicillin, the cell suspensions were incubated with .1 to .001 mg/mL Peppermint (dry aroma). A dose-dependent increase in histamine release was noted, and it was concluded that this release was due to nonimmunological mechanisms.[1]

Immune System Effects

M piperita *(Peppermint) Oil.* The results of a host-resistance assay involving groups of 20 mice that had been dosed orally with *M piperita* (Peppermint) Oil (up to 1250 mg/kg/d for 5 d) suggested immunosuppression and/or increased susceptibility to bacterial-induced mortality. The results of a plaque-forming assay involving groups of 10 mice that received the same oral doses were negative.[1]

Effect on Hair Growth

In a study involving C57BL/6 mice, the data suggest that 3% *M piperita* (Peppermint) Oil (diluted in jojoba oil) facilitates hair growth by promoting the conservation of vascularization of hair dermal papilla, which may contribute to the induction of early anagen stage.[52]

Dermal Irritation and Sensitization Studies

Dermal irritation and sensitization studies are summarized in Table 6.

Irritation

In Vitro

M Piperita *(Peppermint) Leaf Extract.* The skin irritation potential of *M Piperita* (Peppermint) Leaf Extract (water/ethanol extract) at concentrations of 10% and 100% was evaluated using the in vitro reconstructed human epidermis test method, and results were negative.

Table 6. Dermal Irritation and Sensitization Data.

Ingredient	Subjects/Cell type	Protocol	Results
Irritation studies			
In Vitro			
Mentha Piperita (Peppermint) Leaf Extract (water/ethanol extract) (10% and 100%)	Reconstructed human epidermis	*In vitro* reconstructed human epidermis test method. In this test, the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in test material-treated tissues is expressed as a percentage relative to negative control-treated cultures	Negative (MTT >50%)[11]
Animal			
Trade name mixture containing 7.5% Mentha Piperita (Peppermint) Extract	3 rabbits (strain not stated)	Mixture (.5 mL) applied under semi-occlusive dressing to intact skin for 4 h. Area of application (cm^2) not stated	No cutaneous reactions observed. Classified as non-irritant[9]
Human			
Lipstick containing .2961% Mentha Piperita (Peppermint) Leaf Extract	50 subjects, described as follows: Normal (25), with eczema (4 subjects), with allergy (4 subjects) and with sensitive skin (17 subjects)	In occlusive patch test, product (~20 mg) applied in a square test chamber (8 mm × 8 mm) to the back of each subject for 48 h. Reactions scored 30 minutes after patch removal and at 72 h post-application. Sodium dodecyl sulfate (1% in water) and water served as the positive and negative controls, respectively	Product and negative control did not cause skin irritation in any of the subjects tested and product was classified as harmless. Positive control caused positive reactions in 12 subjects[53]
50% Mentha Piperita (Peppermint) Leaf Water in a cleansing gel	50 subjects	Subjects applied product twice per day for 4 weeks, and were instructed to record any signs felt or observed during product use	Slight erythema was observed in one subject[54]
50% Mentha Piperita (Peppermint) Leaf Water in a cleansing gel (10% aqueous dilution [effective concentration = 5%])	52 subjects	Diluted product applied, under occlusive patch, to the skin for 48 h. Reactions scored up to 48 ± 4 h after patch removal (day 4)	A score of 1 (mild erythema) was reported for 12 subjects on day 2 and for 18 subjects on day 3. A score of 2 (moderate erythema) was reported for 2 subjects on day 2 and for 3 subjects on day 3. On day 4, 46 subjects had a score of zero and 6 had a score of 1. Skin compatibility of diluted product considered good[55]

(continued)

Table 6. (continued)

Ingredient	Subjects/Cell type	Protocol	Results
Sensitization studies			
Animal			
Trade name material containing 7.5% Mentha Piperita (Peppermint) Extract	10 albino guinea pigs	Maximization test. 1st induction: 2 intradermal injections of trade name material, 2 injections of Freund's complete adjuvant (FCA), and 2 injections of FCA and material mixture. 2nd induction: Topical application of material 24 h after brushing with 10% sodium lauryl sulfate (SLS). After 19-day non-treatment period, challenge phase involved topical applications of material (undiluted and at concentration of 50% (effective concentration of extract = 3.75%)) under an occlusive dressing for 24 h	No macroscopic cutaneous reactions attributable to allergy associated with application of material. No cutaneous intolerance reactions in animals of the negative control group (further details not provided)[9]
Human			
Trade name mixture containing 2.5% Mentha Piperita (Peppermint) Extract. Prior to application, mixture prepared as a 10% dilution using distilled water (effective concentration of extract = .25%)	52 male and female subjects	Human repeated insult patch test (HRIPT). 1" × 1" semi-occlusive patch containing the diluted mixture (.2 mL) applied for 24 h to upper back (between the scapulae) 3 times per week for total of 9 applications. After a 2-week non-treatment period, diluted mixture applied to new test site adjacent to original site. Reactions scored at 24 h and 72-h post-application	No evidence of dermal irritation or allergic contact sensitization in any of the subjects tested. Also, no adverse events were identified during the study[58]
Cosmetic product (off-white cream) containing .00554% Mentha Piperita (Peppermint) Extract	51 male and female subjects	HRIPT. Cream (~.23 g) applied for 24 h to upper back (between the scapulae; area (cm^2) not stated). Application procedure (induction and challenge) same as reported in preceding study. Challenge reactions scored at same intervals	No dermal irritation or allergic contact dermatitis in subjects tested[59]
Cosmetic product (off-white cream) containing .00554% Mentha Piperita (Peppermint) Extract	26 male and female subjects	Maximization test. The test site (upper outer arm; area not stated) pre-treated with .25% aqueous SLS, applied under occlusive patch for 24 h. Product (.05 mL) then applied, under occlusive patch, to same site for 48 h (or for 72 h, if placed over weekend). Product application followed by re-application of SLS patch for 24 h. Sequence repeated for total of 5 induction exposures. After 10-day non-treatment period, new test site (on opposite arm) pre-treated with SLS prior to application of challenge patch. At challenge, product (.05 mL) applied for 48 h, under an occlusive patch, to same site. Reactions scored at time of patch removal and 48 h later	No evidence of contact sensitization[60]
100% Mentha Piperita (Peppermint) Leaf Extract (water/ethanol extract)	52 subjects	HRIPT (protocol details not included)	Negative results[11]

(continued)

Table 6. (continued)

Ingredient	Subjects/Cell type	Protocol	Results
20% Mentha Piperita (Peppermint) Leaf Water in a face cream	107 subjects (96 women, 11 men) with no history of atopy	In HRIPT, product applied (.02 mL) to the back using occlusive patch (small Finn chamber), and 9 induction applications made over 3-week period. For 1st, 2nd, 5th, 7th, and 8th applications, duration of exposure was 48±4 h. Duration of exposure was 72±4 h for 3rd, 6th, and 9th applications. Challenge phase consisted of single application to new site and a previously treated site for 48±4 h	Product did not induce cumulative irritation or sensitization[61]
20% Mentha Piperita (Peppermint) Oil	104 male and female subjects	In HRIPT, test substance applied (.2 mL) for 24 h to upper back (between scapulae) using 3/4" x 3/4" semi-occlusive patch. Application repeated 3 times per week for total of 9 induction applications. 24-h Challenge patch applied after 2-week non-treatment period, and reactions scored at 24 h and 72 h	No evidence of irritation or sensitization[56]
20% Mentha Piperita (Peppermint) Oil	101 male and female subjects	In HRIPT, nine 24-h applications of test substance (.2 mL) made to back using semi-occlusive patches (dimensions not stated). 24-h Challenge patch applied after 10- to 15-day non-treatment period	No evidence of sensitization[57]
Multicenter studies			
Mentha Piperita (Peppermint) Oil (concentration not stated)	73 patients	Patients patch tested according to International Contact Dermatitis Research Group (ICDRG) patch test procedures during 1994 to 1998	Neither irritant nor allergic reactions reported[63]
Mentha Piperita (Peppermint) Oil (2% in petrolatum)	13,398 patients in study (71 patch tested with ingredient)	Patients patch tested during years 2009 to 2014 to determine frequency of positive patch test reactions to essential oils. Study used a retrospective analysis of patch test results and relevant demographical/clinical data that were collected electronically by the US/Canadian North American Contact Dermatitis Group (NACDG) and other networks	Positive reaction prevalence rate of .53% reported for Mentha Piperita (Peppermint) Oil[64]
Case reports			
Mentha Piperita (Peppermint) Oil (2% in petrolatum)	Male patient with orofacial granulomatosis mainly affecting lower lip	Patch test	Allergic positive reaction[65]
Mentha Piperita (Peppermint) Oil (concentration not stated)	Female patient with lichenoid eruption on oral mucosa	Patch and prick tests	Positive patch test reaction, i.e., itching, erythema, and swelling, beginning at day 5; ++ reaction by day 7. Prick test results negative[66]

(continued)

Table 6. (continued)

Ingredient	Subjects/Cell type	Protocol	Results
Mentha Piperita (Peppermint) Oil (2% in petrolatum)	Male patient with history of hand eczema and sensitization to tixocortol pivalate. Presented with severe eczematous contact dermatitis after repeated applications of a local action transcutaneous (LAT) patch for lumbar pain containing Mentha Piperita (Peppermint) Oil	Patch test	Strong positive reactions to LAT patch and to Mentha Piperita (Peppermint) oil (2% in petrolatum)[67]
Mentha Piperita (Peppermint) Oil (2% in petrolatum)	Female patient with allergic contact dermatitis after consuming herbal tea containing Mentha Piperita (Peppermint) Oil	Patch test. Reactions evaluated at 2, 3, and 7 days according to ICDRG procedures	Positive patch test reaction (+reaction) observed on days 2 and 3[68]
Mentha Piperita (Peppermint) Oil (concentration not stated)	4 patients with allergic contact cheilitis (lips and perioral skin), secondary to exposure to lip balm that contained Mentha Piperita (Peppermint) Oil	Patch test. Reactions evaluated at 48 h and 96 h	Positive patch test reactions to lip balm and to Mentha Piperita (Peppermint) Oil[69]
Mentha Piperita (Peppermint) Leaf and Peppermint (concentration not stated)	Male patient with IgE-mediated anaphylaxis to peppermint (*Mentha piperita*) after sucking on peppermint candy. 5 healthy controls	Skin prick and prick-to-prick tests	Patient had strongly positive prick test reaction to slurry of peppermint candy and fresh peppermint leaf. Prick testing of patient with saline slurry of peppermint candy caused wheal and flare, with largest diameters of 10 mm and 35 mm (W10/*F*25), respectively. Prick-to-prick test with fresh peppermint leaf revealed skin test response of W25/*F*50. All prick tests on 5 controls negative[72]
Mentha Piperita (Peppermint) Leaf Extract (concentration not stated)	Female patient became symptomatic with dyspnea when near peppermint (*Mentha piperita*) scent	Skin prick test	Positive skin prick test reaction to commercial Mentha Piperita (Peppermint) Leaf Extract[73]
Mentha Piperita (Peppermint) (concentration not stated)	Male patient with severe cheilitis	Patch test	Negative reaction[70]
Mentha Piperita (Peppermint) fragrance (1:50, 2% in petrolatum)	Female patient with recurrent irritant rash after applying a Mentha Piperita (Peppermint) foot spray	Patch test	+ reaction on days 2 and 4[71]

Animal

M Piperita *(Peppermint) Oil.* Hairless sites on 5 white rabbits were injected intradermally with .05 mL *M Piperita* (Peppermint) Oil. Gross examinations were performed at 24 h and 48 h, at 1 and 2 wk, and, in some cases, at 1 mo after dosing. Dosing was repeated between 5 and 10 times. At microscopic examination of skin samples, moderate reactions characterized by polymorphonuclear leucocytes, lymphocytes, and plasma cells (without necrosis) were observed in 3 rabbits. Severe reactions, which were marked by the above as well as necrosis, were observed in the other 2 rabbits.[1]

M Piperita *(Peppermint) Extract.* The skin irritation potential of a trade name mixture containing 7.5% Mentha Piperita (Peppermint) Extract was evaluated using 3 rabbits (strain not stated).[9] No cutaneous reactions were observed, and the authors concluded that the mixture was a non-irritant.

Human

M Piperita *(Peppermint) Oil,* M Piperita *(Peppermint) Leaf Extract, and* M Piperita *(Peppermint) Leaf Water.* The skin irritation potential of a lipstick containing .2961% *M Piperita* (Peppermint) Leaf Extract was evaluated in a 48-h occlusive patch test using the following group of 50 subjects: normal (25), with eczema (4 subjects), with allergy (4 subjects) and with sensitive skin (17 subjects). Results were classified as negative.[53] Slight erythema was observed in 1 of 50 subjects after repeated applications of a cleaning gel containing 50% *M Piperita* (Peppermint) Leaf Water in a product use study.[54] Mild and moderate erythema were observed in 12 and 6 subjects, respectively, patch tested with 50% *M Piperita* (Peppermint) Leaf Water (10% aqueous solution dilution; effective concentration = 5% *M Piperita* (Peppermint) Leaf Water).[55] In one of the skin sensitization studies on 20% *M Piperita* (Peppermint) Oil that is summarized in the following section, it was reported that there was no evidence of skin irritation in the 104 subjects tested.[56]

Sensitization

Animal

M Piperita *(Peppermint) Extract.* The skin sensitization potential of a trade name material containing 7.5% Mentha Piperita (Peppermint) Extract was evaluated in the maximization test using 10 albino guinea pigs.[9] No macroscopic cutaneous reactions attributable to allergy were associated with application of the trade name material. There also were no cutaneous intolerance reactions in animals of the negative control group (further details not provided).

Human

Mentha Piperita (Peppermint) Oil. In the maximization test, 25 healthy male panelists received five 48-h occlusive induction patch (containing 8% *M Piperita* (Peppermint) Oil) applications.[1] Pre-treatment was for 24 h with an occlusive patch containing 5% sodium lauryl sulfate (SLS) prior to each exposure. After a 10-d non-treatment period, the subjects were challenged on the back with a 48-h patch (also preceded by SLS treatment). No evidence of sensitization was found.

M Piperita *(Peppermint) Oil,* M Piperita *(Peppermint) Extract,* M Piperita *(Peppermint) Leaf Extract, and* M Piperita *(Peppermint) Leaf Water.* In a human repeated insult patch test (HRIPT) on 20% *M Piperita* (Peppermint) Oil involving 104 subjects, results were negative for skin irritation and sensitization.[56] Skin sensitization also was not observed in another HRIPT on 20% *M Piperita* (Peppermint) Oil involving 101 subjects.[57] An HRIPT on a trade name mixture containing 2.5% *M Piperita* (Peppermint) Extract was performed using 52 male and female subjects, and results were negative for dermal irritation and allergic contact sensitization.[58] Results were also negative in an HRIPT evaluating the cumulative irritation and/or allergic contact sensitization potential of a cosmetic product containing .00554% *M Piperita* (Peppermint) Extract in 51 subjects.[59] In a maximization test on a cosmetic product containing .00554% *M Piperita* (Peppermint) Extract involving 26 subjects, there was no evidence of contact allergy.[60] HRIPT results for undiluted *M Piperita* (Peppermint) Leaf Extract (water/ethanol extract) in 52 subjects were also negative.[11] A face cream containing 20% *M Piperita* (Peppermint) Leaf Water did not induce cumulative skin irritation or sensitization in an HRIPT involving 107 subjects.[61]

Photosensitization/Phototoxicity

Animal

M Piperita *(Peppermint) Oil.* Undiluted *M Piperita* (Peppermint) Oil was applied to the backs of 6 Skh: hairless mice. Thirty minutes later, the mice were irradiated for either 1 h 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 .1667 W/m^2. The mice were examined at 4, 24, 48, 72, and 96 h after radiation treatment. No effects were noted. In a second experiment, using 2 miniature swine and following the same protocol, no effect was produced by 100% *M Piperita* (Peppermint) Oil.[1]

Ocular Irritation Studies

In Vitro

M Piperita *(Peppermint) Extract.* The ocular irritation potential of a trade name mixture containing 2.5% *M Piperita* (Peppermint) Extract was evaluated using an in vitro toxicity testing system consisting of normal, human-derived epidermal keratinocytes.[62] The cells had been cultured to form a stratified squamous epithelium that is similar to that found in the cornea. The procedure utilized a tetrazolium salt (3-[4,5-

dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT)) that is reduced by succinate dehydrogenase (in viable mitochondria of viable cells) to a formazan derivative. The amount of MTT that is reduced by a culture is proportional to the number of viable cells. The trade name mixture, at a concentration of 10% in corn oil (effective concentration of extract = .25%) and a volume of 100 µl, was added to cell cultures; the incubation periods were 1, 4, and 24 h. Corn oil served as the negative control. An ET_{50} (time of exposure needed for a test material to reduce the viability of treated tissues to 50% of control tissues) was calculated. Values for % viability were: 108% (at 1 h), 100% (at 4 h), and 34% (at 24 h). Results indicated that the trade name mixture at a concentration of 10% (ET_{50} = 15.5 h (non-irritating, minimal)) had an ocular irritation potential that was somewhat less that sodium dodecyl sulfate at a concentration of .3% (ET_{50} = 740 min (12.3 h)).

Animal

M Piperita *(Peppermint) Extract.* A trade name mixture containing 7.5% *M Piperita* (Peppermint) Extract was instilled (.1 mL) into 1 eye of each of 3 New Zealand rabbits.[9] Slight conjunctival redness was observed in 2 animals and lacrimation was observed in 1 animal. The trade name mixture was classified as a slight ocular irritant.

Human

M Piperita *(Peppermint) Leaf Water.* The ocular irritation potential of a cleansing gel containing 50% Mentha Piperita (Peppermint) Leaf Water was studied using 50 subjects.[54] The subjects applied the product twice per day for 4 wk, and were instructed to record any signs felt or observed during product use. Product use did not cause any signs of ocular or palpebral irritation.

Clinical Studies

Multicenter Studies

M Piperita *(Peppermint) Oil.* Data from multicenter studies evaluating the skin irritation/sensitization potential of *M Piperita* (Peppermint) Oil in patients are summarized in Table 6.[63,64]

A multicenter study involving 13,398 patients was performed by the US/Canadian North American Contact Dermatitis Group (NACDG), whereby 71 patients were patch tested with *M Piperita* (Peppermint) Oil (2% in petrolatum). A positive reaction prevalence rate of .53% was reported for this ingredient.[64] In another multicenter study, neither irritant nor allergic reactions were observed in 73 patients patch tested with *M Piperita* (Peppermint) Oil according to International Contact Dermatitis Research Group (ICDRG) patch test procedures.[63]

Case Reports

Case reports are summarized in Table 6.

Positive patch test reactions to *M Piperita* (Peppermint) Oil and Mentha Piperita were reported in 8 of 9 case reports on patients with diseased skin.[65-71] Though positive patch test results were reported in one of the studies on *M Piperita* (Peppermint) Oil, prick test results in that study were negative. In patients without skin disease, but with peppermint sensitivity, positive prick test reactions to *M Piperita* (Peppermint) Leaf, *M Piperita* (Peppermint) Leaf Extract, and *M Piperita* were reported.[72,73]

Other Clinical Reports

M Piperita *(Peppermint) Oil.* Positive reactions were observed in 7 of 450 dermatitic patients who were patch tested with 2% *M Piperita* (Peppermint) Oil in yellow soft paraffin.[1] In another study, positive reactions to 2% *M Piperita* (Peppermint) Oil were observed in 6 of 86 dermatitic patients. A patch containing 1% *M Piperita* (Peppermint) Oil (vehicle unknown) was applied to the backs of 56 patients with chronic urticaria. No reactions were noted after a 1-h or 48-h exposure.

No reactions were observed in 25 spice factory workers who were patch tested with 2% *M Piperita* (Peppermint) Oil in petrolatum. It has been reported that the patch testing of individual components of *M Piperita* (Peppermint) Oil using 3 patients with allergic contact dermatitis established that the allergens were menthol and trace components such as piperitone or pulegone.[1]

Dermal. A triple-blind clinical trial involved 96 randomly selected subjects (47 cases and 49 controls; all pregnant women) with a diagnosis of pruritus gravidarum.[74] The case and control subjects were instructed to apply .5% peppermint oil in sesame oil (from 60 mL bottle of the solution) and sesame oil (from 60 mL bottle), respectively, twice per day for 2 wk. Applications (volume per application not stated) were made to the area of the itch. The authors noted that *M Piperita* (Peppermint) Oil did not cause any special side effects in any of the subjects tested. Itch severity in the group treated with *M Piperita* (Peppermint) Oil in comparison with the group treated with sesame oil was statistically significant (P = .003). The authors stated that, based on the results of this study, *M Piperita* (Peppermint) Oil can be used for symptomatic treatment of skin itching in pregnant women.

Oral. Each of 6 pediatric patients with irritable bowel syndrome received a single oral dose of *M Piperita* (Peppermint) Oil (187 mg).[75] Each capsule contained 83 mg of menthol as a constituent of *M Piperita* (Peppermint) Oil. Each patient drank 125 mL of water after ingestion of the capsule. No adverse events were reported. The delayed appearance of menthol in the plasma was reported; a substantial lag time (range 1 to 4 h) was observed in all subjects. Thus, an apparent prolonged

absorption time was demonstrated. The authors noted that reasons for the delayed time of peak (T_{max}) likely related to formulation-specific factors (i.e., delayed release) and, potentially, enterohepatic recirculation.

Exposure Assessment

Dermal

M Piperita *(Peppermint) Oil.* The FDA calculated an estimated human exposure from cosmetic use based on the concentration of use information supplied by industry.[1] Using a body splash product containing .2% *M Piperita* (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/d. For a 60-kg person, this amounted to an estimated daily dose of .6 mg/kg/day.

Oral

M Piperita *(Peppermint) Oil.* In the European Union, the highest recommended daily dose of *M Piperita* (Peppermint) Oil is 1.2 mL, that is, 1080 mg *M Piperita* (Peppermint) Oil (contains a maximum of 140 mg pulegone + menthofuran).[16] For a 60 kg person, this would correspond to a daily intake of 2.3 mg/kg body weight. This recommended daily dose of *M Piperita* (Peppermint) Oil in medicinal products results in pulegone/menthofuran that exceeds the tolerated daily intake (TDI) (.1 mg/kg) that was established for food by the Committee of Experts on Flavoring Substances (CEFS).

Risk Assessment

Dermal

M Piperita *(Peppermint) Oil.* A maximum dermal use level of 5.4% has been recommended for *M Piperita* (Peppermint) Oil.[76] This dermal restriction is based on 8% menthofuran (pulegone metabolite) and 3% pulegone content, with limits of .5% for menthofuran and of 1.2% for pulegone. The authors also recommended that *M Piperita* (Peppermint) Oil, due to menthol content, should be avoided altogether in cases of cardiac fibrillation and in individuals with a glucose-6-phosphate dehydrogenase deficiency. No further information relating to these recommendations is provided.

Oral

M Piperita *(Peppermint) Oil and Pulegone (a component of* M Piperita *(Peppermint) Oil).* Based on 8% menthofuran and 3% pulegone content, with limits of .2 mg/kg/d for menthofuran and .5 mg/kg/d for pulegone, the authors of one study recommended a maximum daily oral dose of 152 mg Mentha Piperita (Peppermint) Oil.[76]

M piperita. A study was performed to characterize data on dietary botanical supplement (DBSs) associated with adverse event reports submitted to the FDA Center for Food Safety and Applied Nutrition's Adverse Event Reporting System (CAERS).[77] FDA obtained CAERS data from 1999 to 2003 involving adverse effects associated with the 6 most frequently used DBSs, including peppermint. No adverse events were reported for single-ingredient peppermint supplements during the study period.

Summary

The safety of the following ingredients in cosmetics was previously reviewed by the Panel, and a final report with a conclusion stating that these ingredients are safe as used in cosmetic formulations was published in 2001: *M Piperita* (Peppermint) Oil, *M Piperita* (Peppermint) Leaf Extract, *M Piperita* (Peppermint) Leaf, and *M Piperita* (Peppermint) Leaf Water. The conclusion also stated that the concentration of pulegone, a constituent of these botanical ingredients, should not exceed 1% in the finished product. The current safety assessment is, in part, a re-review of the 4 *M piperita* (peppermint)–derived ingredients, and is inclusive of safety test data that have become available since the final report was issued.

The current safety assessment is also a first review of the following 6 *M piperita* (peppermint)–derived ingredients: *M Piperita* (Peppermint) Extract, *M Piperita* (Peppermint) Flower/Leaf/Stem Extract, *M Piperita* (Peppermint) Flower/Leaf/Stem Water, *M Piperita* (Peppermint) Leaf Cell Extract, *M Piperita* (Peppermint) Leaf Juice, and *M Piperita* (Peppermint) Meristem Cell Culture.

According to 2018 VCRP data, the greatest use frequency is being reported for *M Piperita* (Peppermint) Oil, which is being used in 815 cosmetic products, mostly leave-on products. The results of a concentration of use survey provided in 2016 indicate that *M Piperita* (Peppermint) Leaf Water is being used at a concentration up to 40% in leave-on products, which is the greatest use concentration that is being reported for *M piperita* (peppermint)–derived ingredients reviewed in this safety assessment.

In the US, *M Piperita* (Peppermint) Oil is GRAS for use in food for human consumption. It is also an inactive ingredient in drug products that have been approved by the FDA and is on the EPA list of active ingredients eligible for minimum risk pesticide products.

M Piperita (Peppermint) Leaf Extract (10% aqueous ethanol extract) caused a statistically significant increase in the penetration of caffeine, but not salicylic acid, through porcine skin. *M Piperita* (Peppermint) Oil inhibited the penetration of benzoic acid through human skin.

Following oral administration, *M Piperita* (Peppermint) Oil is relatively rapidly absorbed and eliminated mainly via the bile. The major biliary metabolite is menthol glucuronide. Additional metabolites are mono- or di-hydroxylated menthol derivatives.

M Piperita (Peppermint) Leaf Extract (aqueous extract) was not nephrotoxic to rats when administered (20 g/L) in drinking water daily for 30 d.

No adverse events were reported for single-ingredient peppermint supplements in a study that was performed to characterize data on dietary botanical supplement associated with adverse event reports submitted to the FDA Center for Food Safety and Applied Nutrition's Adverse Event Reporting System (CaliforniaERS).

M Piperita (Peppermint) Oil was clastogenic in a chromosome aberration test involving peripheral blood lymphocytes. The authors noted that the dose-response curve for *M Piperita* (Peppermint) Oil was complicated, with a clear peak response at a concentration of .20 µl/ml; the number of aberrant cells decreased at higher concentrations. In a genotoxicity assay involving human lymphocytes, *M Piperita* (Peppermint) Oil induced sister chromatid exchanges in a dose-dependent manner. A trade name mixture containing 2.5% *M Piperita* (Peppermint) Extract (diluted to a concentration of 10% (effective concentration of extract = .25%)) was not cytotoxic or genotoxic in the Ames test, with or without metabolic activation. Numerous genotoxicity studies were evaluated, and the preponderance of the data was negative.

Oral pretreatment with *M Piperita* (Peppermint) Leaf Extract (aqueous extract) before exposure to gamma radiation was found to be effective in protecting against chromosomal damage in the bone marrow of Swiss albino mice. In another study, the oral administration of *M Piperita* (Peppermint) Leaf Extract had an antigenotoxic (i.e., reduced the frequency of chromosomal aberrations and micronuclei in bone marrow cells) in Swiss albino mice intraperitoneally injected with benzo[a]pyrene.

Oral dosing with *M Piperita* (Peppermint) Leaf Extract caused a significant reduction in the number of lung adenomas from an incidence of 67.92% in Swiss albino mice intraperitoneally injected with benzo[a]pyrene to 26.31%. Oral dosing with *M Piperita* (Peppermint) Leaf Extract also caused inhibition of skin papilloma formation induced by DMBA and the application of croton oil, in terms of a significant decrease in the cumulative number of papillomas, tumor burden, and tumor incidence.

In the human cancer cell lines tested with *M Piperita* (Peppermint) Leaf Extract (various extractants used), the number of apoptotic cells was incremental with an increase in the dose of *M piperita* extracts. *M Piperita* (Peppermint) Leaf Extract (only from chloroform and ethyl acetate extractants) had significant dose- and time-dependent anticarcinogenic activity, leading to G1 cell cycle arrest and mitochondrial-mediated apoptosis, perturbation of oxidative balance, upregulation of Bax gene, and elevated expression of p53 and p21 in the treated cells. In a study that was performed to evaluate the antitumor activity of *M Piperita* (Peppermint) Leaf Extract (methanol extract) in an anti-proliferation assay involving SW-480 human colon adenocarcinoma cells, statistically significant growth inhibition was observed.

Results were positive for *M Piperita* (Peppermint) Oil (from plants harvested during seasons of the year) in cytotoxicity assays involving human cancer cell lines: The following IC_{50} values (µg/ml) were reported: MCF-7 cell line (75.2 ± 2.9 [summer]; 80.8 ± 3.2 [winter]) and LNCaP cell line (90.4 ± 3.7 [summer]; 95.7 ± 4.5 [winter]). In another study, *M Piperita* (Peppermint) Oil was cytotoxic to the following human cancer cell lines: human lung carcinoma SPC-A1 cells (IC_{50} = 10.89 µg/ml), human leukemia K562 cells (IC_{50} = 16.16 µg/ml) and human gastric cancer SGC-7901 cells (IC_{50} = 38.76 µg/ml). The essential oil was inactive against human hepatocellular carcinoma BEL-7402 cells.

M Piperita (Peppermint) Leaf Extract (1000 µg/ml, methanol extract) did not induce hepatotoxicity in vitro assays involving human (HepG2/C3A) and rat (MH1C1) hepatoma cells.

The 50% ethanol extract of peppermint leaves and stems significantly inhibited compound 48/80-induced histamine release from rat peritoneal mast cells *in vitro*.

In a study involving C57BL/6 mice, it was concluded that 3% *M Piperita* (Peppermint) Oil (diluted in jojoba oil) facilitated hair growth by promoting the conservation of vascularization of hair dermal papilla.

A trade name mixture containing 7.5% *M Piperita* (Peppermint) Extract was non-irritating to the skin of 3 rabbits. No macroscopic cutaneous reactions attributable to allergy were observed in a maximization test in which 10 albino guinea pigs were patch tested with a trade name material containing 7.5% *M Piperita* (Peppermint) Extract during induction and challenged with the undiluted material and the material at a concentration of 50% (effective concentration of extract = 3.75%)

In a 48-h occlusive patch test, a lipstick product containing .2961% *M Piperita* (Peppermint) Leaf Extract did not cause skin irritation in any of the 50 subjects tested. In an *in vitro* skin irritation study on *M Piperita* (Peppermint) Leaf Extract (water/ethanol extract) involving reconstructed human epidermis, results were negative (MTT >50%).

There was no evidence of dermal irritation or allergic contact sensitization in an HRIPT in which 52 male and female subjects were patch tested with a 10% dilution of a trade name mixture containing 2.5% *M Piperita* (Peppermint) Extract (effective concentration of extract = .25%). A cosmetic product containing .00554% *M Piperita* (Peppermint) Extract did not cause dermal irritation or allergic contact dermatitis in 51 male and female subjects patch tested with a cosmetic product (an off-white cream) containing .00554% *M Piperita* (Peppermint) Extract. In the maximization test, a cosmetic product (off-white cream) containing .00554% *M Piperita* (Peppermint) did not induce contact sensitization in the 26 male and female subjects tested.

A face cream containing 20% *M Piperita* (Peppermint) Leaf Water did not induce cumulative skin irritation or sensitization in an HRIPT involving 107 subjects. Negative results were also reported for a lipstick product containing .2961% *M Piperita* (Peppermint) Leaf Extract in a 48-h occlusive patch test (skin irritation test) involving 50 subjects. Slight erythema was observed in 1 of 50 subjects who applied

a cleansing gel containing 50% *M Piperita* (Peppermint) Leaf Water twice per day for 4 wk; there were no signs of ocular or palpebral irritation in any of the subjects. In a 48-h, single-application patch test, the skin irritation potential of a cleansing gel containing 50% *M Piperita* (Peppermint) Leaf Water (10% aqueous dilution [effective concentration = 5%]) was evaluated using 52 subjects. A score of 1 (mild erythema) was reported for 12 subjects on day 2 and for 18 subjects on day 3. A score of 2 (moderate erythema) was reported for 2 subjects on day 2 and for 3 subjects on day 3. On day 4, 46 subjects had a score of zero and 6 had a score of 1. The authors concluded that the skin compatibility of the diluted product was considered good.

The skin irritation and sensitization potential of 20% *M Piperita* (Peppermint) Oil was evaluated in an HRIPT involving 104 subjects and results were negative. Similarly, there was no evidence of sensitization to 20% *M Piperita* (Peppermint) Oil in an HRIPT involving 101 subjects. HRIPT results for undiluted *M Piperita* (Peppermint) Leaf Extract (water/ethanol extract) in 52 subjects were also negative.

A trade name mixture containing 2.5% *M Piperita* (Peppermint) Extract was classified as non-irritating in an in vitro toxicity testing system, consisting of normal, human-derived epidermal keratinocytes, for evaluating ocular irritation potential. Slight ocular irritation was observed in a study in which 3 rabbits were tested with a trade name mixture containing 7.5% *M Piperita* (Peppermint) Extract.

In a multicenter study, neither irritant nor allergic reactions were observed in 73 patients patch tested with *M Piperita* (Peppermint) Oil according to International Contact Dermatitis Research Group (ICDRG) patch test procedures. Another multicenter study involving 13,398 patients was performed by the US/Canadian North American Contact Dermatitis Group (NACDG), whereby 71 patients were tested with Piperita (Peppermint) Oil (2% in petrolatum). The prevalence rate for this ingredient was .53%.

Mostly positive patch test reactions to *M Piperita* (Peppermint) Oil (2% in petrolatum) and *M Piperita* were reported in case reports on patients with diseased skin. In patients without skin disease, but with peppermint sensitivity, positive prick test reactions to *M Piperita* (Peppermint) Leaf, *M Piperita* (Peppermint) Leaf Extract, and *M Piperita* were reported. In other clinical reports, dermal application of *M Piperita* (Peppermint) Oil (.5% in sesame oil) did not cause any side effects in 47 pregnant female patients, and a single oral dose of *M Piperita* (Peppermint) Oil did not cause any adverse events in 6 pediatric patients.

Discussion

The safety of the following cosmetic ingredients was reviewed previously by the Panel, and a final report with a conclusion stating that these ingredients are safe as used in cosmetic formulations was published in 2001: *M Piperita* (Peppermint) Oil, *M Piperita* (Peppermint) Leaf Extract, *M Piperita* (Peppermint) Leaf, and *M Piperita* (Peppermint) Leaf Water. The conclusion also states that the concentration of pulegone, a constituent of these botanical ingredients, should not exceed 1% in the finished product. The current safety assessment is a re-review of the safety of these 4 ingredients, and is also an initial safety evaluation of the following 6 related ingredients that were not listed as cosmetic ingredients prior to development of the published safety assessment: *M Piperita* (Peppermint) Extract, *M Piperita* (Peppermint) Flower/Leaf/Stem Extract, *M Piperita* (Peppermint) Flower/Leaf/Stem Water, *M Piperita* (Peppermint) Leaf Cell Extract, *M Piperita* (Peppermint) Leaf Juice, and *M Piperita* (Peppermint) Meristem Cell Culture.

In the 2001 published final safety assessment on *M piperita* (peppermint)–derived ingredients, the Panel expressed concern that rat oral-dosing studies on *M Piperita* (Peppermint) Oil and pulegone reported cyst-like spaces in the cerebellum that were attributed to pulegone. Therefore, the Panel established a 1% concentration limit on pulegone in *M piperita* (peppermint)–derived ingredients due to toxicity concerns. In these studies, brain sections were fixed by immersion using 4% neutral buffered formaldehyde. Because immersion fixation of nervous tissue might cause artifacts observed as vacuolar retraction spaces around neurons, a study involving rats was performed, using both immersion and perfusion tissue fixation methods, to determine whether the cerebellar lesions observed in earlier studies were caused by dosing with pulegone. Study results for rats dosed with pulegone did not reveal the occurrence of test substance-related, cyst-like spaces in the white matter of the cerebellum using either perfusion or immersion tissue fixation techniques. A possible explanation for the observed dose-dependent cyst-like spaces seen in previous studies could have been due to an interaction between impurities in the test substance and the fixation agent used therein. Given this possibility, the Panel agreed that the brain lesions may have been an artifact of the fixation method, and that the 1% limitation on pulegone is no longer warranted.

The panel also considered the carcinogenic effects of pulegone in female rats, and in male and female mice, in the 2011 National Toxicology Program (NTP) oral carcinogenicity study. However, the Panel did not feel that the cytotoxic dose-response relationship (renal and liver toxicity) associated with cancer development would be relevant to pulegone exposure from a cosmetic product containing *M piperita* (Peppermint) Oil at current use concentrations.

The Panel expressed concern about pesticide residues, heavy metals, and other plant species that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities.

Additionally, the Panel recognized that *M Piperita* (Peppermint) Leaf Extract can enhance the penetration of other ingredients through the skin. The Panel cautioned that care should be taken in formulating cosmetic products that may contain *M Piperita* (Peppermint) Leaf Extract in combination with any ingredients whose safety was based on their lack of dermal absorption data, or when dermal absorption was a concern.

The issue of incidental inhalation exposure was discussed by the Panel, as *M piperita* (peppermint)–derived ingredients are being used in products that could possibly be inhaled. For example, *M Piperita* (Peppermint) Leaf Extract is used in face and neck sprays at maximum use concentrations up to 1.3%. Another example relating to inhalation exposure from products that are sprayed is the use of *M Piperita* (Peppermint) Oil in both pump hair sprays (maximum use concentrations up to .02%) and aerosol hair sprays (maximum use concentrations up to .017%) which may result in incidental inhalation exposure. The use of *M Piperita* (Peppermint) Leaf Extract at maximum use concentrations up to .0018% in face powders may also result in incidental inhalation exposure. Additionally, the Panel noted that droplets/particles from spray cosmetic products would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

Finally, the Panel agreed that the data relating to composition of *M Piperita* (Peppermint) Flower/Leaf/Stem Extract, *M Piperita* (Peppermint) Flower/Leaf/Stem/Water, and *M Piperita* (Peppermint) Meristem Cell Culture are insufficient. Also, after considering the available skin irritation and sensitization data, the Panel determined that skin sensitization data on these three ingredients are also insufficient. Thus, the Panel determined that the following additional data are needed in order to evaluate the safety of these three ingredients:

- Composition data on *M Piperita* (Peppermint) Flower/Leaf/Stem Extract, *M Piperita* (Peppermint) Flower/Leaf/Stem/Water and *M Piperita* (Peppermint) Meristem Cell Culture.
  - Depending on the composition data that are received, other toxicological endpoints may be needed.
- Skin irritation and sensitization data on *M Piperita* (Peppermint) Flower/Leaf/Stem Extract, *M Piperita* (Peppermint) Flower/Leaf/Stem/Water and *M Piperita* (Peppermint) Meristem Cell Culture.

The Panel also agreed that the available data are sufficient for determining that *M Piperita* (Peppermint) Oil, Leaf, and leaf-derived ingredients are safe in cosmetics in the present practices of use and concentration, when formulated to be non-sensitizing. Because final product formulations may contain multiple botanicals, each possibly containing the same constituent(s) of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. For *M piperita* (peppermint)–derived ingredients, the Panel was concerned about the presence of terpenes (e.g., limonene) and terpenoids (e.g., menthol) in cosmetics, which could result in sensitization. Thus, this non-sensitizing caveat relates to the avoidance of a cumulative effect of multiple botanicals, in a single formulation, that share one or more constituents in common.

Conclusion

The Expert Panel for Cosmetic Ingredient Safety concluded that the following 7 *M piperita* (peppermint)–derived ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment, when formulated to be non-sensitizing:

- *M Piperita* (Peppermint) Oil
- *M Piperita* (Peppermint) Extract
- *M Piperita* (Peppermint) Leaf
- *M Piperita* (Peppermint) Leaf Cell Extract*
- *M Piperita* (Peppermint) Leaf Extract
- *M Piperita* (Peppermint) Leaf Juice*
- *M Piperita* (Peppermint) Leaf Water

**Not reported to be in use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

The Panel also concluded that the available data are insufficient to make a determination that the following 3 *M piperita* (peppermint)–derived ingredients are safe under the intended conditions of use in cosmetic formulations:

- *M Piperita* (Peppermint) Flower/Leaf/Stem Extract**
- *M Piperita* (Peppermint) Flower/Leaf/Stem Water**
- *M Piperita* (Peppermint) Meristem Cell Culture**

**Not reported to be in use. These ingredients are thus categorized as Insufficient Data—No Reported Use.

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