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Safety Assessment of Hops as Used in Cosmetics

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Abstract

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Humulus Lupulus (Hops) Extract (reported functions include antimicrobial agent and hair conditioning agent) and Humulus Lupulus (Hops) Oil (reported function is fragrance). The Panel reviewed the relevant data related to these ingredients. Because final product formulations may contain multiple botanicals, each containing the same constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. For these ingredients, the Panel was concerned about the presence of 8-prenylnaringenin, β-myrcene, and quercetin in cosmetics, which could result in estrogenic effects, dermal irritation, and genotoxicity, respectively. Industry should use current good manufacturing practices to limit impurities and constituents of concern. The Panel concluded that Humulus Lupulus (Hops) Extract and Humulus Lupulus (Hops) Oil are safe in cosmetics in the present practices of use and concentration when formulated to be non-sensitizing.

Keywords

Cosmetics, Safety, Hops, Humulus Lupulus (Hops) Extract, Humulus Lupulus (Hops) Oil

Introduction

This is a safety assessment of Humulus Lupulus (Hops) Extract and Humulus Lupulus (Hops) Oil as used in cosmetics. According to the web-based *Ingredient Dictionary* (wINCI; *Dictionary*), the functions of Humulus Lupulus (Hops) Extract in cosmetics include antimicrobial agent, hair conditioning agent, and skin-conditioning agent – miscellaneous; Humulus Lupulus (Hops) Oil is reported to function as a fragrance ingredient (Table 1).[1] Antiperspirant agent is also listed as a function of Humulus Lupulus (Hops) Extract; however, antiperspirant agent is not a cosmetic function and is not evaluated in this safety assessment. Both of these ingredients are derived from the strobile of the *Humulus lupulus* (commonly called hops) plant. The strobiles of this plant are generally known as an ingredient in the brewing of beer.[2,3]

Previously, the *Dictionary* listed four other *Humulus lupulus* (hops)-derived ingredients: Humulus Lupulus (Hops) Cone Extract, Humulus Lupulus (Hops) Flower Extract, Humulus Lupulus (Hops) Stem Extract, and Humulus Lupulus (Hops) Strobile (Table 1).[4] It was determined by the International Nomenclature Committee (INC) that these ingredients were all extracts of the inflorescence (hops cone) of the *Humulus lupulus* (hops) plant. It was also determined that the previous definition of Humulus Lupulus (Hops) Extract, i.e., the extract of the whole plant, was erroneous and that this ingredient is also the extract of the inflorescence (hops cone). To correct these errors, these five ingredients were deemed synonymous and the single name Humulus Lupulus (Hops) Extract is now the official INCI name. Additionally, Humulus Lupulus (Hops) Cone Oil (the volatile oil obtained from the cones of *Humulus lupulus*), has been changed to Humulus Lupulus (Hops) Oil and the definition is now "the volatile oil obtained from the inflorescence (hops cone) of *Humulus*

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Table 1. Current and Revised INCI Names, Definitions, and Functions of the *Humulus lupulus* (Hops)-Derived Ingredients in This Safety Assessment.[1,4]

Ingredient	Definition	Functions
Humulus Lupulus (Hops) Extract 8016-25-9 8060-28-4	Humulus Lupulus (Hops) Extract is the extract obtained from the inflorescence (hops cone) of *Humulus lupulus*. In 2017, the definition of Humulus Lupulus (Hops) Extract was changed from "the extract of the whole plant, *Humulus lupulus*."	Antimicrobial agent; fragrance ingredient; hair conditioning agent; skin-conditioning agent – miscellaneous; skin protectant; skin-conditioning agent – miscellaneous
Humulus Lupulus (Hops) Cone Extract (deleted monograph)	In 2017, the INCI name Humulus Lupulus (Hops) Cone Extract was revised and the name will be retained in the monograph as a technical name of Humulus Lupulus (Hops) Extract.	Antimicrobial agent; antiperspirant agent; hair conditioning agent
Humulus Lupulus (Hops) Flower Extract (deleted monograph)	In 2017, the INCI name Humulus Lupulus (Hops) Flower Extract was revised and the name will be retained in the monograph as a technical name Humulus Lupulus (Hops) Extract.	Skin-conditioning agent – miscellaneous
Humulus Lupulus (Hops) Stem Extract (deleted monograph)	In 2017, the INCI name Humulus Lupulus (Hops) Stem Extract was revised and the name will be retained in the monograph as a technical name Humulus Lupulus (Hops) Extract.	Skin protectant
Humulus Lupulus (Hops) Strobile (deleted monograph)	In 2017, the INCI name Humulus Lupulus (Hops) Strobile was revised and the name will be retained in the monograph as a technical name Humulus Lupulus (Hops) Extract.	None reported
Humulus Lupulus (Hops) Oil 8007-04-3	Humulus Lupulus (Hops) Oil is the volatile oil obtained from the inflorescence (hops cone) of *Humulus lupulus*.	Fragrance ingredient
Humulus Lupulus (Hops) Cone Oil (deleted monograph) 8007-04-3	In 2017, the INCI name Humulus Lupulus (Hops) Cone Oil was revised and the name will be retained in the monograph as a technical name Humulus Lupulus (Hops) Oil	Fragrance ingredient

lupulus". Data have been submitted under the revised names; with the exception of the cosmetic use data, these data are presented under the corrected names, Humulus Lupulus (Hops) Extract and Humulus Lupulus (Hops) Oil.

The terms "inflorescence," "cone," and "strobile" refer, synonymously, to the structures formed by the female *Humulus lupulus* (hops) plant after the flowers have bloomed, whether or not they have been fertilized. Thus, for example, "cone oil" is the same as "strobile oil. Both "cone" and "strobile" are used in the literature. Also, "hops" is used commonly for the plant name as well as for the harvested strobiles; in the literature, it is not always clear if authors are referring to the whole plant or just the strobiles. In this report, it is assumed that the authors are referring solely to the strobiles, unless otherwise indicated.

Most of the parts of *Humulus lupulus* (hops) (i.e., shoots, leaves, flowers, seeds, rhizomes, and essential oils) are edible; the strobile is the most commonly consumed in food (mostly in beer).[5,6] The U.S. Food and Drug Administration (FDA) has determined that essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of *Humulus lupulus* (hops) are generally recognized as safe (GRAS) for human consumption. [21CFR182.20] Consumption of these *Humulus lupulus* (hops)-derived foods would result in much larger systemic exposures than what is expected from use of ingredients in cosmetic products, even if there was 100% dermal absorption. Thus, the systemic toxicity potential of *Humulus lupulus* (hops)-derived ingredients is not the focus of this safety assessment (although such information may be included). The primary focus of this safety assessment is the review of safety based on local effects (e.g., topical exposures).

Botanical cosmetic ingredients, such as *Humulus lupulus* (hops)-derived ingredients, may contain hundreds of constituents, some of which have the potential to cause toxic effects. For example, sesquiterpene lactones, which are present in the *Humulus lupulus* (hops) plant, may cause Type IV allergic reactions (cell-mediated, delayed-type hypersensitivity) and other toxicity when present in sufficient amounts. Another example, β-myrcene, is reported to be a dermal irritant and a possible carcinogen (including in a National Toxicology Program (NTP) report).[7-11] In this safety assessment, the Panel is reviewing information available

to evaluate the potential toxicity of each of the *Humulus lupulus* (hops)-derived ingredients as whole, complex ingredients. Except for specific constituents of concern, the Panel is not reviewing information that may be available to assess the potential toxicity of the individual constituents derived from the *Humulus lupulus* (hops) plant.

The Panel has reported on related ingredients that can be used to support the safety of the *Humulus lupulus* (hops)-derived ingredients. The information on these related ingredients may be relevant for this safety assessment. The Panel reviewed the safety of phytosterols, which are plant-derived sterols that are likely constituents of most of the *Humulus lupulus* (hops)-derived ingredients[12] in 2013, and concluded that the phytosterols are safe as used.[13]

The names of the ingredients in this report are written in accordance with the *Dictionary*, as shown above, capitalized without italics and without abbreviations. When referring to the plant from which these ingredients are derived, the standard taxonomic practice of using *italics* will be followed (e.g., *Humulus lupulus*).

Often in the published literature, the information provided is not sufficient to determine how well the tested substance represents the cosmetic ingredient; the taxonomic name is used, unless it is clear that the test substance is similar to the cosmetic ingredient. If it is similar to the cosmetic ingredients, then the INCI name is used.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (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.

Chemistry

Definition

The definitions of these *Humulus lupulus* (hops)-derived ingredients (with technical names, included for cross reference) are provided in Table 1.

The terms "cone" and "strobile" are synonymous and refer to the structures formed by the female *Humulus lupulus* (hops) plant after the flowers have bloomed, whether or not they have been fertilized.

Plant Identification

The genus *Humulus* consists of dioecious, perennial, climbing vines and bines (i.e., a twining plant stem or flexible shoot).[14] This genus belongs to the Cannabaceae family of the Urticales order which, in 2003, was incorporated into the natural order of Rosales.[15] The plant, which is native to Europe and western Asia, is now cultivated in North and South America, Africa, Asia and Australia and is invasive in many areas.[3] Cultivation is predominately in the northwestern United States and Germany.[2] Within *Humulus lupulus*, there are five taxonomic subspecies based on morphological properties and geographical location: *Humulus lupulus* var. lupulus, *Humulus lupulus* var. cordifolius, *Humulus lupulus* var. neomexicanus, *Humulus lupulus* var. pubescens, and *Humulus lupulus* var. lupuloides.[16] Over 100 cultivars have been named.[17] It is not known whether a single or multiple varieties are used in cosmetics.

While the *Humulus lupulus* (hops) plant is typically dioecious (i.e., the male and female flowers usually develop on separate plants), occasional fertile monoecious individual plants have been reported.[18] When grown for beer, viable seeds are undesirable; therefore, only female plants are grown in hops fields to prevent pollination. Female plants are propagated vegetatively, and male plants are culled if plants are grown from seeds. Under natural conditions, the flowers are wind pollinated and the female inflorescence develops to form a strobile (or cone). Only the strobiles of the female plants are able to develop the lupulin glands that secrete a fine yellow resinous powder.[2] These glands secrete predominantly bitter acids and hop oil, the constituents of which include phytoestrogens such as 8-prenylnaringenin (8-PN) and other prenylflavonoids.[19,20]

Humulus lupulus (hops) is a climbing perennial bine, which means that it grows in a helix around a support and uses downward-facing bristles/hooked hairs for grip instead of tendrils or suckers as would a vine.[3,6,21] Generally, the bines are trained to 25 ft (7.6 m) or higher on a trellis. Lateral arms develop at the nodes, producing flowers at their terminal buds. The green to yellowish-brown leaves have three or five lobes, depending on the variety, and are hairy on both sides; the margins of the cordate (heart-shaped) leaves are serrated and the petioles are slightly fleshy with stout hooked hairs. *Humulus lupulus* (hops) is a perennial plant that regrows each spring from the rhizomes of an underground rootstock in commercial hops production.

Physical and Chemical Properties

The chemical and physical properties for Humulus Lupulus (Hops) Extract are presented in Table 2.

Once harvested, *Humulus lupulus* (hops) strobiles deteriorate upon aging and exposure to the atmosphere.[3] The stability of stored strobiles and ethanol extracts, as measured by humulones, lupulones, and xanthohumol content, is optimal in 70% ethanol.[22]

Green *Humulus lupulus* (hops) strobiles have a variety of odors including: citrus, tropical fruit, stone fruit, pine, cedar, floral, spicy, herbal, earthy, tobacco, onion/garlic and grassy.[23]

Table 2. Chemical and Physical Properties of Humulus Lupulus (Hops) Extract.

Property	Value	Reference
Physical Form	Oil	57
	Viscous liquid[a]	107
Color	Greenish-yellow-reddish brown	57
	Yellow/orange to brown/green[a]	107
Odor	Harsh and bitter	57
	Citrus, tropical fruit, stone fruit, pine, cedar, floral, spicy, herbal, earthy, tobacco, onion/garlic and/or grassy	23
Specific Gravity (@ 20°C)	.883–.900	57
	850–1100[a]	107
Vapor Density (mmHg)	>1	57
Melting Point (°C)	40–60[a]	107
Water Solubility	Negligible	57
	Insoluble[a]	107

[a]A CO_2 extract of dried cones manufactured for use as a food ingredient.

Preparation/Extraction

Humulus lupulus (hops). In general, when the strobiles are harvested from *Humulus lupulus* (hops) bines for beer production, the strobiles are immediately dried by forced hot air, and are often pressed into dense cylindrical pellets, 5 to 8 mm in diameter and up to 25 mm long.[17] The pelletization reduces the overall surface area and therefore reduces the rate of chemical oxidation/degradation, and provides a more compact product for shipping. Not all suppliers dry the strobiles; the strobiles may be harvested fresh as whole, wet cones at the farm, and shipped for immediate use within 36 h of harvest.[23]

Sometimes, hops are treated with sulfur dioxide to improve the color and prevent change of active constituents.[3]

Methylene chloride is the most common solvent used for the extraction of *Humulus lupulus* (hops) for beer brewing; hexane and methanol are also employed.[24] Typically, at least 95% of the available α-acids (source of the bitter flavorings) can be extracted from fresh *Humulus lupulus* (hops) strobiles. Methanol is the most efficient solvent for the extraction of α-acids (approximately 25%), followed by methylene chloride (approximately 20%) and hexane (approximately 18%).

Supercritical fluid extraction (SFE) with carbon dioxide is also used to collect the extract for beer brewing.[25-28] Another modern method is pressurized fluid extraction (PFE), which is employed for extracting different polyphenols.[29-33] PFE has been developed to extract bitter acids.

FDA regulations on the method of manufacture of "modified hop extract," as a food additive intended for use as a flavoring agent in the brewing of beer, are provided in Table 3. Six of the eight listed methods follow extraction with isomerization of the extracted substance. The allowed solvents include benzene, light petroleum spirits, methyl alcohol, *n*-butyl alcohol, and ethyl acetate. [21CFR172.560]

In general, oils are extracted from *Humulus lupulus* (hops) by steam distillation at 100°C.[34]

Humulus Lupulus (Hops) Extract. One manufacturer reported that the method of manufacture of Humulus Lupulus (Hops) Extract for use in cosmetics begins with extraction with water and propylene glycol.[35] The extract solution is then pressed, clarified, and decontaminated. Further details were not provided.

Another manufacturer reports that Humulus Lupulus (Hops) Extract may be extracted with either a 50% volume ethanol solution or a 50% volume butylene glycol solution.[36] After the dried raw material (the flower head) is extracted with the solution, the extract is filtered and concentrated. After sedimentation, the filtrate is "adjusted" and packaged.

To manufacture a product mixture containing Humulus Lupulus (Hops) Extract, dried whole strobiles of *Humulus lupulus* (hops) are dispersed and solubilized with stirring in caprylic/capric triglyceride.[37] The solution is then filtered to obtain the desired mixture.

Composition

Humulus lupulus *(hops).* The components of fresh and dried *Humulus lupulus* (hops) strobiles/cones are listed in Table 4 and Table 5, respectivly.[2,38] Analysis of dried food grade *Humulus lupulus* (hops) strobiles grown for beer production showed α-acids at 3.0 to 15.5% (w/w) and β-acids at 3.0 to 5.5% (w/w).[23] *Humulus lupulus* (hops) bitter acids are classified as either "α-acids" or "β-acids" that are, respectively, di- or tri-prenylated phloroglucinol derivatives. In addition, they each contain a 3-, 4-, 5-, or 6-carbon oxoalkyl side chain.[6,39] Historically, the α-acids were distinguished because they precipitated from a crude extract of hops with the addition of lead acetate. The β-acids, by definition, would remain in solution. The α-acids, particularly humulone (35 to 70% of total α-acids), cohumulone (20 to 65%), and adhumulone (10 to 15%) are regarded as the most important constituents determining the quality of hops.[6,39]

Table 3. FDA Regulations on the Method of Manufacture and Residual Solvents in "Modified Hop Extract," as a Food Additive Used or Intended for Use as a Flavoring Agent in the Brewing of Beer [21CFR172.560].

Method of Manufacture	Solvent/Impurities restrictions
(b)(1) The additive is manufactured from a hexane extract of hops by simultaneous isomerization and selective reduction in an alkaline aqueous medium with sodium borohydride, whereby the additive meets the following specifications:	The boron content of the food additive does not exceed 310 ppm (.0310%), calculated as boron.
A solution of the food additive solids is made up in approximately .012 *n* alkaline methyl alcohol (6 mL of 1 *n* sodium hydroxide diluted to 500 mL with methyl alcohol) to show an absorbance at 253 μm of .6 to .9/cm. (This absorbance is obtained by approximately .03 mg solids/mL.) The ultraviolet absorption spectrum of this solution exhibits the following characteristics: An absorption peak at 253 mμ; no absorption peak at 325 to 330 mμ; the absorbance at 268 mμ does not exceed the absorbance at 272 mμ.	
(b)(2) The additive is manufactured from hops by a sequence of extractions and fractionations, using benzene, light petroleum spirits, and methyl alcohol as solvents, followed by isomerization by potassium carbonate treatment. The light petroleum spirits and benzene solvents shall comply with the specifications in §172.250 except that the boiling point range for light petroleum spirits is 150°F to 300°F.	Residues of solvents in the modified hop extract shall not exceed 1.0 ppm of benzene, 1.0 ppm of light petroleum spirits, and 250 ppm of methyl alcohol.
(b)(3) The additive is manufactured from hops by a sequence of extractions and fractionations, using methylene chloride, hexane, and methyl alcohol as solvents, followed by isomerization by sodium hydroxide treatment.	Residues of the solvents in the modified hop extract shall not exceed 5 ppm of methylene chloride, 25 ppm of hexane, and 100 ppm of methyl alcohol.
(b)(4) The additive is manufactured from hops by a sequence of extractions and fractionations, using benzene, light petroleum spirits, methyl alcohol, *n*-butyl alcohol, and ethyl acetate as solvents, followed by isomerization by potassium carbonate treatment. The light petroleum spirits and benzene solvents shall comply with the specifications in §172.250 except that the boiling point range for light petroleum spirits is 150°F to 300°F.	Residues of solvents in the modified hop extract shall not exceed 1.0 ppm of benzene, 1.0 ppm of light petroleum spirits, 50 ppm of methyl alcohol, 50 ppm of *n*-butyl alcohol, and 1 ppm of ethyl acetate.
(b)(5) The additive is manufactured from hops by an initial extraction and fractionation using one or more of the following solvents: ethylene dichloride, hexane, isopropyl alcohol, methyl alcohol, methylene chloride, trichloroethylene, and water; followed by isomerization by calcium chloride or magnesium chloride treatment in ethylene dichloride, methylene chloride, or trichloroethylene and a further sequence of extractions and fractionations using one or more of the solvents set forth in this paragraph.	Residues of the solvents in the modified hop extract shall not exceed 125 ppm of hexane; 150 ppm of ethylene dichloride, methylene chloride, or trichloroethylene; or 250 ppm of isopropyl alcohol or methyl alcohol.
(b)(6) The additive is manufactured from hops by an initial extraction and fractionation using one or more of the solvents listed in paragraph (b)(5) of this section followed by: hydrogenation using palladium as a catalyst in methyl alcohol, ethyl alcohol, or isopropyl alcohol acidified with hydrochloric or sulfuric acid; oxidation with peracetic acid; isomerization by calcium chloride or magnesium chloride treatment in ethylene dichloride, methylene chloride, or trichloroethylene (alternatively, the hydrogenation and isomerization steps may be performed in reverse order); and a further sequence of extractions and fractionations using one or more of the solvents listed in paragraph (b)(5) of this section.	The additive shall meet the residue limitations as prescribed in paragraph (b)(5) of this section.
(b)(7) The additive is manufactured from hops as set forth in paragraph (b)(6) of this section followed by reduction with sodium borohydride in aqueous alkaline methyl alcohol, and a sequence of extractions and fractionations using one or more of the solvents listed in paragraph (b)(5) of this section.	The additive shall meet the residue limitations as prescribed in paragraph (b)(5) of this section, and a boron content level not in excess of 300 ppm (.0300%), calculated as boron.

(continued)

Table 3. (continued)

Method of Manufacture	Solvent/Impurities restrictions
(b)(8) The additive is manufactured from hops as a nonisomerizable nonvolatile hop resin by an initial extraction and fractionation using one or more of the solvents listed in paragraph (b)(5) of this section followed by a sequence of aqueous extractions. The additive is added to the wort before or during cooking in the manufacture of beer.	Removal of nonaqueous solvents to less than .5%.

Table 4. Fresh *Humulus lupulus* (Hops) Strobile Composition.[38]

Principle Components	Concentration (%w/w)
Cellulose+lignin	40.0–50.0
Protein	15.0
α-Acids	2.0–17.0
β-Acids	2.0–10.0
Water	8.0–12.0
Minerals	8.0
Polyphenols and tannins	3.0–6.0
Lipids and fatty acids	1.0–5.0
Hop oil	.5–3.0
Monosaccharides	2.0
Pectin	2.0

Table 5. Dried *Humulus lupulus* (Hops) Strobile Composition.[2]

Principle Components	Concentration (%)
Cellulose, etc.	43
Proteins	15
Amino acids	.1
Moisture	10
Ash	8
Polyphenols (tannins)	4
Essential oil	.5–3
Waxes and steroids	Trace–25
Monosaccharides	2
Pectins	2
Total resins	15–30

Table 6. Composition of Polyphenols and Their Concentrations in *Humulus lupulus* (Hops).[2]

Polyphenols and Polyphenol Groups	Concentration (%)
Phenolic carboxylic acids	
Benzoic acid derivatives	<.01
Cinnamic acid derivatives	.01–.03
Flavonoids	
8- or 6-Prenylnaringenin	< .01
Acylphloroglucinol derivatives (multifidols)	.05–.50
Catechins and epicatechins	.03–.30
Kaempferol	.02–.24
Oligomeric proanthocyanidins	.20–.50
Quercetin	.05–.23
Xanthohumol	.20–1.70
Higher molecular substances	
Catechin tanning agents and tannins	2.00–7.00

In an analysis of *Humulus lupulus* (hops) samples by high-performance liquid chromatography (HPLC)-diode array detection, over 100 compounds were in the polyphenol fraction of *Humulus lupulus* (hops).[40] The composition of the polyphenols in *Humulus lupulus* (hops) is provided in Table 6.

Flavonoids are composed of different chemical classes such as flavones, isoflavones, flavonols, flavanols, flavanones, and chalcones. These compounds differ in the level of oxidation of the flavane nucleus and in the number and position of hydroxyl, methyl, and methoxyl substituents.[41] Flavonoids, which make up .5% to 1.5% of the dried strobile, include quercetin and kaempferol glycosides.[42,43] Thirty prenylated, geranylated, oxidized and/or cyclized chalcones have been isolated from the secretions of the lupulin glands.[17] The prenylated, geranylated flavonoids constitute up to 1% of the dried strobile and 80% to 90% of total flavonoids.[19,20,44-46] The chalcone xanthohumol is the most abundant prenylated flavonoid in fresh and properly preserved strobiles (approximately .01 to .5%); desmethylxanthohumol, dehydrocycloxanthohumol, and the flavanones isoxanthohumol, 8-PN (25 to 60 mg/kg) and 6-prenylnaringenin are also found in the strobiles.[19,20,44-47] A majority of the known flavonoids from *Humulus lupulus* (hops) strobiles can be considered to be derivatives of the compound 2',4,4',6'-tetrahydroxy-3'-prenylchalcone (chalcone numbering), commonly known as desmethylxanthohumol.

The constituents and their ranges of concentration of aqueous (hydrodistilled) *Humulus Lupulus* (hops) extracts from 10 different cultivars are provided in Table 7.[48]

Table 7. Constituents Found in the Aqueous Extract[a] of 10 Cultivars of "Aroma"-Type *Humulus lupulus* (Hops) Extracts Analyzed by GC/MS.[48]

Constituent	Composition range (%)[b]
(2*E*)-Dodecen-1-ol	ND–.2
(2*E*)-Hexenal	0–.1
(2*Z*,6*E*)-Farnesol	.2–.9
(6*Z*)-Pentadecen-2-one	ND–2.5
(*E*)-Caryophyllene	4.1–11.3
(*E*)-Nerolidol	ND–.3
(*E*)-β-Farnesene	ND–8.1
(*E*)-β-Ocimene	0–.2
(*E*,*E*)-α-Farnesene	.1–.9
(*Z*)-Caryophyllene	ND–.1
14-Hydroxy-(*E*)-caryophyllene	ND–.8
1-*epi*-Cubenol	ND–.7
1-Octen-3-ol	0–.2
2-Decanone	0–.5
2-Dodecanone	0–.4
2-Methylbutanoic acid	0–.6
2-Methylbutyl 2-methylbutyrate	0–.3
2-Methylbutyl isovalerate	0–.1
2-Nonanone	0–.5
2-Pentadecanone	ND–.8
2-Tridecanone	ND–1.6
2-Undecanone	.1–1.6
3-Methyl-2-buten-1-ol	0–.5
3-Methyl-2-butenal	0–.9
3-Methyl-2-pentanone	0–1.0
4-Methyl-2-pentanone	0–3.4
4-Methyl-2-pentenolide	.1–1.5
6,7-Epoxymyrcene	0–.3
6-Methyl-5-hepten-2-one	0–.4
9-Decenoic acid	0–.5
ar-Curcumene	.2–1.2
Cadalene	ND–.4
Caryophylla-4(12),8(13)-dien-5-ol	ND–.7
Caryophyllene Oxide	.6–3.0
cis-Linalool oxide (furanoid)	0–.2
cis-Linalool oxide (furanoid)	0–.6
Decanoic acid	0–trace
Dendrolasin	ND–.1
Furfural	Trace
Geranial	0–.2
Geraniol	0–1.1
Geranyl acetate	0–1.8
Geranyl isobutyrate	ND–2.1
Geranyl propionate	ND–1.7
Heptyl isobutanoate	0–trace
Hexanal	0–.2
Humulene epoxide II	1.4–7.9
Isoamyl isobutyrate	.3–1.6
Isoamyl propionate	0–.3
Isobutyl isobutyrate	0–.3
Isobutyl isopentanoate	0–.8
Isovaleric acid	0–3.8
Limonene/β-Phellandrene	0–1.2
Linalool	.2–3.2
Methyl (4*Z*)-decenoate	.4–2.2
Methyl decanoate	0–1.0
Methyl geranate	0–.7
Methyl heptanoate	0–1.1
Methyl nonanoate	0–.5
Methyl nonenoate	0–.3
Methyl octanoate	0–.1
Myrcene	8–52.4
Neral	0–trace
Neryl acetate	0–.1
Neryl isobutyrate	ND–trace
n-Nonanal	0–.5
Nonanoic acid	0–.4
Octanoic acid	0–.1
Palmitic acid	ND–.5
Perilla alcohol	0–.2
Phenylacetaldehyde	0–.1
Prenyl isobutyrate	0–trace
Tetradecane	ND–trace
trans-Cadina-1(6),4-diene	ND–.1
trans-Cadina-1,4-diene	ND–.1
trans-Calamenene	ND–1.2
Unidentified	0–1.5
Unidentified	0–.7
Unidentified	.4–9.7
Unidentified	.1–1.8
Unidentified	.2–3.5
α-Amorphene	ND–.1
α-Cadinene	Trace–.2
α-Cadinol	.1–1.2
α-Calacorene	Trace–.4
α-Copaene	.3–.9
α-Humulene	12.6–51.2
α-Humulene hydrate	.3–2.9
α-Muurolene	.3–.6
α-Muurolol	ND–.1
α-Pinene	0–.4
α-Selinene	ND–1.8
α-Terpineol	0–.2
α-*trans*-Bergamotene	ND–1.7
α-Ylangene	Trace–.3
β-Copaene	.1–.2
β-Pinene	.2–1.5
β-Selinene	.2–1.2
γ-Cadinene	ND–1.5
γ-Muurolene	ND–1.7
δ-Cadinene	1.1–2.4
δ-Selinene	ND–1.2
τ-Cadinol	.2–1.1

(continued)

Table 7. (continued)

(continued)

Table 7. (continued)

Constituent Groups	
Aldehydes	ND–1.3
Aliphatic alcohols	ND–1.1
Aliphatic ketones	.4–8.9
Carboxylic acids	ND–6.4
Carboxylic esters	1.2–9.1
Monoterpene hydrocarbons	9.4–54.5
Oxygenated monoterpenoids	.4–3.5
Oxygenated sesquiterpenoids	3.3–18.4
Sesquiterpene hydrocarbons	.4–3.5

GC/MS = gas chromatography – mass spectrometry.
[a]Hydrodistilled for 4 h with continuous extraction with dichloromethane.
[b]Percent composition determined from total ion current count without correction.

Humulus lupulus *(hops) oil.* The cone oil of *Humulus lupulus* (hops) contains secondary metabolites of the plant, which are secreted in the lupulin glands (located on the female flower cones/strobiles).[2] The strobile oil makes up .5% to 1.5% of the dried inflorescence of *Humulus lupulus* (hops) strobile.[49] The strobile oil contains many volatile constituents, including simple oxidized alkanes, monoterpenes, and sesquiterpenes.[17,50-52] It is possible that the strobile oil contains over 1000 compounds. The primary volatile constituents are the monoterpene β-myrcene, and the sesquiterpenes β-caryophyllene and humulene, which together were shown to comprise between 57% and 82% of the volatile oil, depending on the cultivar and the method of detection. There are only traces of 2-methyl-3-buten-2-ol found in freshly harvested strobile; after drying and storing, the amount is higher, increasing to a maximum of approximately .15% of the dry weight (up to 20% of the volatile constituents) after 2 years due to degradation of humulones and lupulones.[49] The constituents in *Humulus lupulus* (hops) oil are not consistent across years or cultivars and not all of the constituents are detectable in every essential oil sample; β-myrcene, linalool, α-humulene, β-caryophyllene, undecanone-2, geranyl acetate, humulene epoxide-2, and α-selinene are always present.[53] Lists of some of the compounds in strobile oil are provided in Table 8 and Table 9.

Humulus lupulus (hops) extract. The ethanol extract of Humulus Lupulus (Hops) Extract is reported to contain flavonoid and tannin.[36] The butylene glycol extract of Humulus Lupulus (Hops) Extract is reported to contain tannin and amino acid.

Table 8. Compounds in *Humulus Lupulus* (Hops) Strobile Oil.[52]

Classification	Compound
Hydrocarbon, Monoterpene	α-Pinene
	β-Pinene
	β-Myrcene
	Limonene
	ρ-Cymene
Hydrocarbon, Sesquiterpene	Caryophyllene
	E, β-Farnesene
	Humulene
Oxygenated, Ester	Methyl Heptonoate
Oxygenated, Monoterpene Alcohol	Geraniol
	Linalool
Oxygenated, Monoterpene	Citronellol
Oxygenated, Sesquiterpene Alcohol	Farnesol
Oxygenated, Other	Citral
Oxygenated, Monoterpene or Ester	Geranyl Acetate
Oxygenated, Epoxide	Humulene Epoxide 1
	Humulene Epoxide 2

Table 9. Composition and Concentrations of Compounds in *Humulus lupulus* (Hops) Strobile Oil Extracted by Steam Distillation for Constituents ≥1%.[108,109]

Compound	Percentage[a]
α-Caryophyllene	36.7
β-Myrcene	25.4
β-Caryophyllene	9.8
γ-Cadinene	5.5
δ-Cadinene	4.1
α-Muurolene	3.0
α-Copaene	1.5
Geraniol	1.5
Sabinene	1.4
β-Selinene	1.2
Linalool	1.1
α-Selinene	1.0
(*E*)-β-Ocimene	1.0

[a]This reference lists constituents at 1% or greater unless there is a safety concern.

Constituents of Concern

Humulus Lupulus (hops)

Humulus lupulus (hops) plants are reported to contain linalool, quercetin, β-myrcene, 8-PN (or hopein), and other prenylated flavonoids (Figure 1).[42,43,52,54-56] *Humulus lupulus* (hops) oil is reported to contain sesquiterpene lactones.[17,50-52] The potential adverse effects of exposures to these constituents are summarized in Table 10.

The International Fragrance Association (IFRA) publishes restrictions for fragrance ingredients. Constituents of *Humulus lupulus* (hops) that have restrictions established by IFRA are listed in Table 11.

Humulus Lupulus (Hops) Extract. According to one supplier, the butylene glycol extract of Humulus Lupulus (Hops) Extract does not contain β-myrcene (detection limit .01 mg/100 g).[36]

A product mixture that contains approximately .18% Humulus Lupulus (Hops) Extract (extracted with caprylic/capric

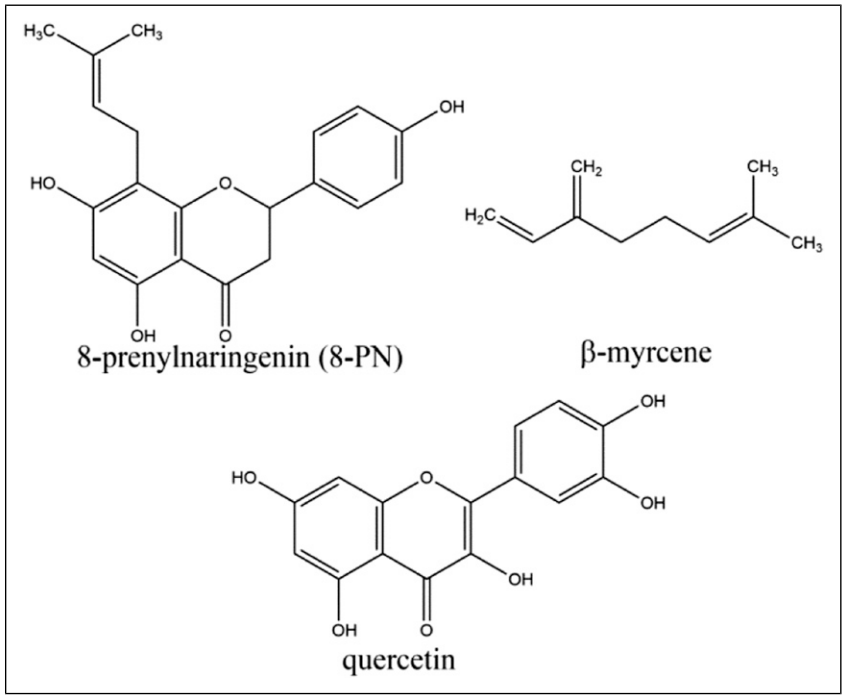

Figure 1. Constituents of concern of *Humulus lupulus* (hops) plants.

Table 10. Constituents of Concern Found in *Humulus lupulus* (Hops).

Constituent	Concern	Reference
8-Prenylnaringenin (8-PN), 6-prenylnaringenin (6-PN), 8-geranylnaringenin (8-GN) and 6,8-diprenylnaringenin (6,8-PN)	Estrogenic activity 8-PN is found in the strobiles of *Humulus lupulus* (hops) and has been proposed as a possible treatment for menopausal hot flashes Subcutaneous administration of 8-PN to rats has estrogenic activity (as measured by the effect on uterine and vaginal weights), but is 20,000-fold less potent than estradiol In vitro studies showed that 8-PN generally mimicked the action of 17β-estradiol with a lesser (10- to 20,000-fold) potency	44,56,84-90,110
Geraniol	Potential dermal sensitizer	109,111-113
Limonene	Hydroperoxides of limonene are potential dermal sensitizers	109,114
Linalool	Hydroperoxides of linalool are potential dermal sensitizers. Safe at up to 4.3% (20% in a consumer fragrance)	115
β-Myrcene	Potential dermal irritant; dermatitis, conjunctivitis, somnolence, and asthma-like symptoms Oral dosing for 2 years caused kidney cancers in male rats (.25 g/kg) and liver cancer in male mice (.25 g/kg); may be related to the occurrence of kidney tumors in female rats and liver tumors in female rats. Associated with other lesions of the kidney in rats, the liver in mice, and the nose in male rats	7,11,54
Quercetin	Positive genotoxic effect in an Ames assay Consistently genotoxic in in vitro tests and in some in vivo studies of i.p. exposures, but was consistently nongenotoxic in oral exposure studies	116,117
Sesquiterpene lactones	Potential dermal sensitizers	8,10

Table 11. Constituents of *Humulus lupulus* (Hops) that Have IFRA Standards.[118]

Constituent	Standard Limits
Citral	Limited to .04–5%, depending on use category[a] due to sensitization
Citronellol	Limited to .8–21.4%, depending on use category due to sensitization
Farnesol	Limited to .08–5%, depending on use category due to sensitization
Furfural	Skin contact – .001%; non-skin contact – .05% due to carcinogenicity
Geranial	Limited to .04–5%, depending on use category due to sensitization
Geraniol	Limited to .03–8.6%, depending on use category due to sensitization
(2*E*)-Hexenal	Limited to .01–02%, depending on use category due to sensitization
Limonene	*d*-, *l*-and *dl*-Limonene and natural products containing substantial amounts of it, should only be used when the level of peroxides is kept to the lowest practical level, for instance by adding antioxidants at the time of production. Such products should have a peroxide value of less than 20 millimoles peroxides per liter due to sensitization
Linalool	Limit peroxide level to 20 mmol/L due to sensitization Linalool and natural products known to be rich in linalool, such as bois de rose, coriander or ho wood oil, should only be used when the level of peroxides is kept to the lowest practical level. It is recommended to add antioxidants at the time of production of the raw material. The addition of .1% BHT or alpha-tocopherol for example has shown great efficiency. The maximum peroxide level for products in use should be 20 mmol/L
Neral (citral)	Limited to .04–5%, depending on use category due to sensitization
Phenylacetaldehyde	Limited to .02–3%, depending on use category due to sensitization

IFRA – International Fragrance Association.
[a]Use categories are based on types of skin contact (e.g., skin, lips), length of contact (e.g., leave-on, rinse-off), or type of use (e.g., mouthwash).

triglyceride) reports a theoretical content of β-myrcene of 22 ppm based on the content of the starting materials.[37] However, it is noted that the method of manufacture does not favor β-myrcene retention. The HPLC profile of this mixture shows a peak at xanthohumol and no other prenylflavonoids.

Humulus Lupulus (Hops) Oil. Humulus Lupulus (Hops) Oil is reported to contain geraniol (.2%), limonene (1%), and linalool (.6%).[57]

Impurities

Humulus lupulus *(hops).* Multiple fungi and bacteria may be found on *Humulus lupulus* (hops) plants.[3] Analysis of the dust in air samples collected during harvest showed that total concentrations of microorganisms ranged between 2.08 and 129.58×10^3 cfu/m^3; the concentrations of endotoxin ranged between 26 and 6250 ng/m^3.[58] In samples of the settled dust after harvest, the concentrations of total microorganisms ranged from $.25 \times 10^6$ to 2.87×10^8 cfu/g; the concentrations of endotoxin ranged between 312.5 and 6250 µg/g (median 6250 µg/g).

FDA regulations restrict the amounts of residuals from solvents in the manufacture of "modified hop extract" as a food additive (Table 3). [21CFR172.560] These restrictions include: boron, 310 ppm; benzene, 1.0 ppm; light petroleum spirits, 1.0 ppm; methyl alcohol, 250 ppm; hexane, 125 ppm; ethylene dichloride, 150 ppm; methylene chloride, 250 ppm; trichloroethylene, 250 ppm; and isopropyl alcohol, 250 ppm.

Analysis of dried food grade *Humulus lupulus* (hops) strobiles (possible source material for Humulus Lupulus (Hops) Extract) produced for beer production had the following results: lead <1.0 ppm, arsenic <.5 ppm, cadmium <.03 ppm, and total heavy metals <10 ppm.[23] Heavy metals, pesticides, herbicides, fungicides, nitrates, and radioactivity are reported to be below tolerance levels. Another analyses of leaves and strobiles of *Humulus lupulus* (hops) plants had the following results: copper, 102.3 and 81.1 ppm; vanadium, .07 and .05 ppm; molybdenum, .07 and .12 ppm; iron, 49.3 and 54.2 ppm; tin, 1.4 and 1.2 ppm; lead, 3.1 and 2.3 ppm, and nickel, 7.9 and 5.5 ppm, respectively.[59]

The levels of residual solvent present in commercial hop extracts used for brewing beer are reported to be <100 ppm.[60]

Humulus Lupulus (Hops) Extract. Authors of an analysis of a product mixture that contained Humulus Lupulus (Hops) Extract (.6 to 1.2%) reported that heavy metals were certified to be present at ≤5 ppm, microbes at <100 cfu/mL, yeasts and molds at < 100 cfu/mL, and enterobacteria absent.[61]

Use

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the US FDA and the cosmetic 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. Use concentration data are submitted by the cosmetic industry in response to surveys, conducted by the Personal Care Products

Council (Council), of maximum reported use concentration by product category.

The *Humulus lupulus* (hops)-derived ingredients were reported to the 2017 VCRP and surveyed by the Council in 2015 and 2016 under the former INCI names, and that is how they are reported here.[62,63]

According to VCRP data received in 2017, Humulus Lupulus (Hops) Extract was reported to be used in 375 formulations, including 317 leave-on formulations and 54 rinse-off formulations (Table 12).[62]

The results of the concentration of use survey conducted by the Council in 2015 (and updated in 2016) indicate that Humulus Lupulus (Hops) Extract is used at up to .2% in hair conditioners.[63,64] The highest reported maximum concentration of use with dermal contact was reported to be .13% in eye lotion and in the category of other skin care preparations.

Humulus Lupulus (Hops) Cone Oil is not in use according to the VCRP and the industry survey results.

Humulus Lupulus (Hops) Extract is reported to be used in formulations that are used around the eyes at up to .13% and in formulations that come in contact with mucus membranes at up to .084% (e.g., bath soaps and detergents, bubble baths).

Humulus Lupulus (Hops) Extract is used in cosmetic sprays and could possibly be inhaled; for example, this ingredient is reported to be used at up to .0002% in hair sprays. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μm, with propellant sprays yielding a greater fraction of droplets/particles <10 μm compared with pump sprays.[65,66] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[67,68] There is a reported use in face powders at up to .00055%. 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.[69-71]

Neither of the *Humulus lupulus* (hops)-derived ingredients named in the report (under the new or revised names) are restricted from use in any way under the rules governing cosmetic products in the European Union.[72]

Non-Cosmetic

Humulus lupulus (hops) strobiles are predominantly used to make beer.[2,3] They were originally added to beer for their antimicrobial properties. Brewers then began using strobiles (and their extracts) to add bitterness, flavor, and aroma.[24]

FDA determined that essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of *Humulus lupulus* L. (hops) are GRAS for human consumption. [21CFR182.20] Modified *Humulus lupulus* (hops) extract may be safely used in beer in accordance with the following prescribed conditions: (a) the food additive is used or intended for use as a flavoring agent in the brewing of beer, and (b) the food additive is manufactured by one of the prescribed processes (Table 3). [21CFR172.560]

Most parts of the *Humulus lupulus* (hops) plant (shoots, leaves, flowers, seeds, rhizomes, and essential oils) are edible.[5,6] The shoots are consumed as a delicacy and resemble asparagus.

In Europe, *Humulus lupulus* (hops) is administered as an herbal supplement in the form of powders, liquid extracts (ethanol extract drug ratio/dry extract ratio [DER] 1:1; sweet wine extract DER 1:10), tinctures (ethanol extract DER 1:5), and dry extracts (50% methanol extract DER 4 to 5:1) of the inflorescence of the plant.[49,73] It is also administered as a tea. *Humulus lupulus* (hops) strobiles are used in European, Indian-Ayurvedic, and Native American traditional medicines for the relief of insomnia, excitability, and specifically for restlessness associated with nervous tension, headache and/or indigestion.

It has been shown that *Humulus lupulus* (hops) byproducts, after harvesting of the strobiles, can be used to absorb lead from contaminated waters.[74]

Toxicokinetic Studies

Obtaining data on the toxicokinetics of *Humulus lupulus* (hops)-derived ingredients would not be practical because these ingredients are complex mixtures. Exposure to the components of these ingredients in cosmetics is expected to be lower than that from dietary exposure because these ingredients are incorporated into cosmetic products only at very low concentrations.

Toxicological Studies

Acute Toxicological Studies

Acute toxicity data on *Humulus lupulus* (hops)-derived ingredients were not found in the published literature and no unpublished data were submitted.

Short-Term Toxicity Studies

Oral. Wistar rats (n = 7/group) were fed a low-fat diet, a high-fat diet, or high-fat diet supplemented with 1% xanthohumol-rich *Humulus lupulus* (hops) extract for 41 days.[75] There were no mortalities or other adverse effects observed in any of these groups. The addition of the extract reduced the effects of the high-fat diet on weight gain from days 21 to 41 of the study. The weights of livers of rats fed the supplemented high-fat diets were similar to the controls, as were the plasma glucose levels, at the end of the test period.

Table 12. Frequency of use according to duration and exposure of *Humulus lupulus* (hops) Extract. Data reported under the technical names of this ingredient are also reported, but not included in the total of the named ingredient.[61,62]

Humulus Lupulus (Hops) Extract			
Use type	**VCRP Reported Name**	**Uses**	**Maximum Concentration (%)**
Total/range	**Humulus Lupulus (Hops) Extract**	**375**[a]	**0.000006-0.2**[a]
	Humulus Lupulus (Hops) Cone Extract[b]	17	0.00055
	Humulus Lupulus (Hops) Flower Extract[b]	3	0.000055-0.001
	Humulus Lupulus (Hops) Strobile[b]	12	NR
Duration of use[c]			
Leave-on	**Humulus Lupulus (Hops) Extract**	**317**	**0.00005-0.13**
	Humulus Lupulus (Hops) Cone Extract	8	0.00055
	Humulus Lupulus (Hops) Flower Extract	1	0.001
	Humulus Lupulus (Hops) Strobile	9	NR
Rinse-off	**Humulus Lupulus (Hops) Extract**	**54**	**0.000006-0.2**
	Humulus Lupulus (Hops) Cone Extract	1	NR
	Humulus Lupulus (Hops) Flower Extract	1	0.000055-0.001
	Humulus Lupulus (Hops) Strobile	3	NR
Diluted for (bath) use	**Humulus Lupulus (Hops) Extract**	4	NR
	Humulus Lupulus (Hops) Cone Extract	**8**	NR
	Humulus Lupulus (Hops) Flower Extract	1	NR
	Humulus Lupulus (Hops) Strobile	NR	NR
Exposure type			
Eye area	**Humulus Lupulus (Hops) Extract**	**9**	**0.0024-0.13**
	Humulus Lupulus (Hops) Cone Extract	1	NR
	Humulus Lupulus (Hops) Flower Extract	NR	NR
	Humulus Lupulus (Hops) Strobile	2	NR
Incidental ingestion	**Humulus Lupulus (Hops) Extract**	NR	NR
	Humulus Lupulus (Hops) Cone Extract	NR	NR
	Humulus Lupulus (Hops) Flower Extract	NR	NR
	Humulus Lupulus (Hops) Strobile	NR	NR
Incidental Inhalation-sprays	**Humulus Lupulus (Hops) Extract**	**2; 184**[d]**; 89**[e]	**0.00008-0.0002; 0.00005-0.1**[d]
	Humulus Lupulus (Hops) Cone Extract	3[d]; 4[e]	NR
	Humulus Lupulus (Hops) Flower Extract	1[b]	NR
	Humulus Lupulus (Hops) Strobile	2[b]	NR
Incidental inhalation-powders	**Humulus Lupulus (Hops) Extract**	**89**[e]	**0.0003-0.084**[f]
	Humulus Lupulus (Hops) Cone Extract	4[e]	0.00055
	Humulus Lupulus (Hops) Flower Extract	NR	NR
	Humulus Lupulus (Hops) Strobile	NR	NR
Dermal contact	**Humulus Lupulus (Hops) Extract**	**310**	**0.0001-0.13**
	Humulus Lupulus (Hops) Cone Extract	17	0.00055
	Humulus Lupulus (Hops) Flower Extract	3	0.000055
	Humulus Lupulus (Hops) Strobile	9	NR
Deodorant (underarm)	**Humulus Lupulus (Hops) Extract**	**8**[e]	**NR**
	Humulus Lupulus (Hops) Cone Extract	0.13[g]	NR
	Humulus Lupulus (Hops) Flower Extract	NR	NR
	Humulus Lupulus (Hops) Strobile	NR	NR
Hair-noncoloring	**Humulus Lupulus (Hops) Extract**	**65**	**0.00005-0.2**
	Humulus Lupulus (Hops) Cone Extract	NR	NR
	Humulus Lupulus (Hops) Flower Extract	NR	0.001
	Humulus Lupulus (Hops) Strobile	2	NR
Hair-coloring	**Humulus Lupulus (Hops) Extract**	NR	**0.000006-0.00001**
	Humulus Lupulus (Hops) Cone Extract	NR	NR
	Humulus Lupulus (Hops) Flower Extract	NR	NR
	Humulus Lupulus (Hops) Strobile	NR	NR
Nail	**Humulus Lupulus (Hops) Extract**	NR	NR
	Humulus Lupulus (Hops) Cone Extract	NR	NR
	Humulus Lupulus (Hops) Flower Extract	NR	NR
	Humulus Lupulus (Hops) Strobile	NR	NR
Mucous Membrane	**Humulus Lupulus (Hops) Extract**	7	**0.0003-0.084**
	Humulus Lupulus (Hops) Cone Extract	**9**	NR
	Humulus Lupulus (Hops) Flower Extract	1	0.000055
	Humulus Lupulus (Hops) Strobile	NR	NR
Baby	**Humulus Lupulus (Hops) Extract**	NR	NR
	Humulus Lupulus (Hops) Cone Extract	NR	NR
	Humulus Lupulus (Hops) Flower Extract	NR	NR
	Humulus Lupulus (Hops) Strobile	NR	NR

NR = Not Reported; Totals = Rinse-off + Leave-on Product +Diluted for (Bath) Uses.

Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

[a] Highest number of reported uses or concentration of use is in bold.

[b] Technical name for Humulus Lupulus (Hops) Extract.

[c] 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.

[d] It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.

[e] Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

[f] It is possible these products may be powders, but it is not specified whether the reported uses are powders.

[g] Not a spray

Subchronic Toxicity Studies

Oral. In a study on the effects of *Humulus lupulus* (hops) extract on high-fat diets, male C57BL/6J mice (n = 10/group) were fed a normal diet, a high-fat diet, or a high-fat diet supplemented with 2 or 5% of various *Humulus lupulus* (hops) extracts for 20 weeks.[76] The high-fat diet was supplemented with one of the following: aqueous *Humulus lupulus* (hops) extract, ethyl acetate-soluble fraction of the aqueous *Humulus lupulus* (hops) extract, ethyl acetate-insoluble fraction of the aqueous *Humulus lupulus* (hops) extract, methanol-soluble fraction of the ethyl acetate-insoluble fraction of the aqueous *Humulus lupulus* (hops) extract, or methanol-insoluble fraction of the ethyl acetate-insoluble fraction of the aqueous *Humulus lupulus* (hops) extract. There were no mortalities or adverse effects reported for any group. The addition of any *Humulus lupulus* (hops) extract reduced the effects of the high-fat diet on weight gain. The weights of livers and mesenteric and epididymal adipose tissues of mice fed the supplemented high-fat diets were similar to that of the controls, as were plasma glucose levels, at the end of the test period; the extract had no additional effect on the effects of the high-fat diets.

Developmental and Reproductive Toxicity (Dart) Studies

Developmental and reproductive toxicity data on *Humulus lupulus* (hops)-derived ingredients were not found in the published literature and no unpublished data were submitted.

Genotoxicity Studies

In Vitro

Humulus Lupulus (Hops) Extract. An aqueous Humulus Lupulus (Hops) Extract (10 to 400 mg/μL in ethanol) was weakly mutagenic (a 2- to 4-fold increase in induced revertants compared with controls) in *Salmonella typhimurium* (strains TA98 and TA100), with or without metabolic activation.[77] No further details were provided.

A Humulus Lupulus (Hops) Extract (0, 1000, 2500, 5000, 7500, and 10,000 μg/plate; extract solvent not specified; water control) was not mutagenic in *S. typhimurium* (strains TA98 and TA100) or *Escherichia coli* (strain pKM101), with or without metabolic activation.[78] The positive and negative controls yielded the expected results.

An Ames test was performed on a product mixture containing 5% Humulus Lupulus (Hops) Extract (extracted in water/glycerin 50/50) at 10% in deionized water (effective concentration of .5% hops) with and without metabolic activation using *S. typhimurium* (strains TA97a, TA98, TA100, TA201, and TA1535).[79] The test substance was not mutagenic in this assay with or without metabolic activation.

Carcinogenicity Studies

Carcinogenicity data on *Humulus lupulus* (hops)-derived ingredients were not found in the published literature and no unpublished data were submitted.

Other Relevant Studies

Estrogenic Activity

Historically, there is circumstantial evidence of potential estrogenic activity connected to *Humulus lupulus* (hops) exposure, including menstrual disturbances reported to be common among female *Humulus lupulus* (hops) harvesters.[80,81] In an investigation of the reported observation that women who normally live "a distance" from hop gardens regularly begin to menstruate two days after arriving to pick hops, it was reported that hops contain "the equivalent of 20 to 300 μg estradiol/g".[49] *Humulus lupulus* (hops) extracts have been reported to reduce hot flashes in menopausal women and, in Germany, hops baths containing approximately 30% *Humulus lupulus* (hops) extracts (which have been discontinued) were used to treat gynecological disorders.[49,82] However, early studies to confirm this activity experimentally were inconclusive or contradictory because of inadequate sensitivity of the methods used.[80,83]

More recently, 8-PN has been shown to be the source of the estrogenic activity of *Humulus lupulus* (hops). 8-PN mimics the action of 17β-estradiol, albeit with less (10- to 20,000-fold) potency.[84-88] It is a potent ligand for the α-estrogen receptor (ER) with an IC_{50} value in the nanomolar range; it stimulates the production of alkaline phosphatase in Ishikawa cells, and stimulates the growth of estrogen-dependent MCF7 breast cancer cells.[44,89] It was reported that 8-PN has a greater affinity for the ERα (where it is 70-fold less potent than estradiol) than for ERβ (reported to be 20,000-fold less potent than estradiol).[90]

In a screening for drugs derived from plants for estrogenic activity, an ethanolic *Humulus lupulus* (hops) extract (50%; .2 g/mL) exhibited binding to ERs in intact, estrogen-dependent [ER(+)], human breast cancer MCF-7 cells with a potency equivalent to .5 μg of estradiol per 2 g of dried *Humulus lupulus* (hops) strobile (for comparison, the potencies of 2 g of thyme or red clover were equivalent to .5 or 3 μg of estradiol, respectively).[91] *Humulus lupulus* (hops) extract also showed significant ability to stimulate cell proliferation in ER (+) T47D, but not in ER(−) MDA 468, breast cancer cells.[91] In contrast, in a different series of experiments, a similarly prepared *Humulus lupulus* (hops) extract at concentrations of .01–1.0% v/v was found to inhibit serum-stimulated growth of ER(+)T47D breast cancer cells.[92] Ovarian cells isolated from immature female rats, which 48 h previously had been injected (primed) with pregnant mare's serum gonadotropin, were incubated with follicle-stimulating hormone to induce estradiol secretion. The

addition of purified water-soluble fractions from defatted *Humulus lupulus* (hops) extract to the culture medium reduced the estrogen E_2 released from the ovarian cells with a probably related decrease in cyclic adenosine monophosphate (cAMP) release.[49]

A *Humulus lupulus* (hops) extract activated the estrogen response element (ERE) in Ishikawa cells and induced ERE-luciferase expression in MCF-7 cells. In the MCF-7 cell line, progesterone receptor (PR) mRNA was significantly upregulated by *Humulus lupulus* (hops) extract with an EC_{50} of 1.1 µg/mL.[93] *Humulus lupulus* (hops) consisted of a chloroform partition of a methanolic extract from a previously SFE-CO_2-extracted Nugget *Humulus lupulus* (hops) cultivar; the individual constituents included prenylated flavanones and isoflavonoids. The estrogenic activity proved to be considerably greater than that of established phytoestrogens such as coumestrol (present in red clover) and genistein and daidzein (present in soy).

Dermal Irritation and Sensitization Studies

Irritation

Human

Humulus Lupulus (Hops) Extract. In a 2-week cumulative irritation test (n = 26) of a formulation containing Humulus Lupulus (Hops) Extract (.125%), the test formulation did not demonstrate a significant irritation potential in human subjects.[94]

A formulation containing Humulus Lupulus (Hops) Extract (.6 to 1.2%; 20 µL) was patch tested (n = 12) at 10% (final concentration .06 to .12%) using 8-mm aluminum cups covering 50 mm^2 skin.[95,96] The patches were administered to the upper back for 24 h. Controls were water and sodium lauryl sulfate (1%). The test sites were examined 30 min to 1 h and 24 h after patch removal. One subject had a reaction at the site of the negative control and was not included in the final analysis of the results. No reactions to the test material were observed in 9 subjects; two subjects showed very slight erythema. The controls had the expected results in the rest of the subjects. The irritation index was .04 (out of 5) and the test article was rated a non-irritant.

A patch test (n = 12) was conducted on a product mixture that contained Humulus Lupulus (Hops) Extract (approximately .18% in caprylic/capric triglyceride).[37] The test substance was applied to the skin on the back under a patch for 48 h. No adverse reactions were observed. It was concluded that the test substance had good cutaneous compatibility.

Sensitization

Humulus lupulus (hops) extract. In a human maximization test (n = 26) of a product containing Humulus Lupulus (Hops) Extract (.125%), the test product did not demonstrate contact sensitization potential.[94] No further information was provided.

A human repeated insult patch test (HRIPT; n = 52) of Humulus Lupulus (Hops) Extract (10%; extracted with butylene glycol) had negative results.[36] No further information was provided.

An HRIPT (n = 102) was conducted of a product mixture that contained Humulus Lupulus (Hops) Extract (approximately .18% in caprylic/capric triglyceride).[37] The test substance was applied to the same site three times per week for 9 applications. The challenge was applied two weeks after the last induction application. All patches were in place for 48 h. Patch sites were examined for reactions when patches were removed and at 72 and 96 h. No reactions were observed.

An HRIPT (n = 102) was conducted of a product mixture that contained Humulus Lupulus (Hops) Extract (approximately 5% in glycerin/water).[97] The induction and challenge phases were conducted at 10% (.5% Humulus Lupulus (Hops) Extract). Induction and challenge patches were in place for 24 h. The test sites were observed before the application of the next patch and at 24 and 72 h after the application of the challenge patch. There were no signs of irritation or sensitization at any time during the test period; there was no indication of a potential to cause dermal irritation or contact sensitization.

Ocular Irritation Studies

In Vitro

Products and product mixtures containing Humulus Lupulus (Hops) Extract were assayed at up to .5% (Table 13). Humulus Lupulus (Hops) Extract was predicted to be a slight ocular irritant in hen's egg test-chorion-allantoic membrane (HET-CAM) and cornea fibroblast (CFIO) assays and a non-irritant in an EpiOcular assay.[95,96,98] Another Humulus Lupulus (Hops) Extract was predicted to be non-irritating in an HET-CAM assay and to SIRC fibroblastic cells.[37]

Human

In a 4-week use study (n = 48) of an eye cream that contained Humulus Lupulus (Hops) Extract (.125%), the test material did not demonstrate a potential to cause eye irritation.[94]

Clinical Studies

Occupational Exposure

Dermal. The causative agents of *Humulus lupulus* (hops) plant-induced contact skin reactions have not been established.[99,100] Both irritant and allergic effects have been described. In *Humulus lupulus* (hops) harvesters, dermatitis has been attributed to mechanical abrasion by the rough hairs on the climbing stem. It has also been suggested that lupulin, the yellow powdery secretion of the glandular hairs on the scales of the strobiles, may be responsible for the irritation.

Table 13. In vitro Ocular Assays of Humulus Lupulus (Hops) Extract.

Concentration (%)	Assay and Ingredient	Results	Reference
(.06–.12)	HET-CAM. A product containing Humulus Lupulus (Hops) Extract (.6% to 1.2%) tested at 10%	There were no signs of potential irritation in any form of hyper-anemia, hemorrhage, or coagulation. The IP-CAM score was .00 and the product was rated a practically non-irritating under these conditions	95,96
(.06–.12)	CFIO. A product containing Humulus Lupulus (Hops) Extract (.6% to 1.2%) at 10%	The IOeq was 2.8 with a MCI of .0; the test substance was rated at the lowest irritation level (IOeq = 0 to 15) as a slight ocular irritant When combining the results of these two in vitro HET-CAM/CFIO assays, the author concluded that this cosmetic formulation containing Humulus Lupulus (Hops) Extract (.6% to 1.2%) was a slight ocular irritant	95,96
.5	EpiOcular. A product mixture containing Humulus Lupulus (Hops) Extract at 5% in glycerin/water (50/50) tested at 10%	Viability at 1, 4, and 24 h was 114%, 90%, and 79%. The ET_{50} was > 1440 min. The test substance has virtually no ocular irritation potential	98
Approximately .18	HET-CAM. A product mixture containing Humulus Lupulus (Hops) Extract (approximately .18% in caprylic/capric triglyceride)	Predicted that the test substance was practically non-irritating. No further details were provided	37
Approximately	A product mixture that contains Humulus Lupulus (Hops) Extract at 5%, 15%, 25%, and 50% in paraffin oil was administered to SIRC fibroblastic cells and evaluated for cytotoxicity by the use of the Neutral Red Release method	Cytotoxicity was not observed at any concentration. Predicted to be non-irritating	37
.009, .027, .045, and .09	Paraffin oil was the negative control and sodium dodecyl sulfate (.01–.2%) was the positive control		

CFIO – cornea fibroblast; ET_{50} – time until viability reaches 50%; HET-CAM – hen's egg test-chorion-allantoic membrane; IOeq – ocular irritation index; IP-CAM – primary irritancy index; MCI – mean cytotoxicity index.

Farmers (n = 73) who cultivated *Humulus lupulus* (hops) plants and other crops from 18 randomly selected farms filled out a questionnaire on their skin diseases and were administered skin prick tests (SPT) for allergens of *Humulus lupulus* (hops) as well as grain dust, straw dust, hay dust, storage mites, and antigens of microorganisms typical of farm environments.[101] Only the results of the *Humulus lupulus* (hops) are reported here. Fresh strobiles and leaves were cut into small pieces, and extracted with glycerol and saline at 1:2 (w/w) for 48 h at 4°C. The extracts were centrifuged and clear supernatants were used in the testing. The subjects consisted of 42 males and 31 females, aged 16 to 84 (median 46) years, with duration of employment resulting in exposure ranging from 2 to 73 (median 31) years.

The questionnaire showed that *Humulus lupulus* (hops) was reported to cause the greatest number of skin problems; 14 farmers (19.2%) reported work-related skin symptoms, 2 (11%) of which were caused by *Humulus lupulus* (hops). There were no reported skin problems associated with working with *Humulus lupulus* (hops) by 65 subjects. The reported skin symptoms of the subjects with skin problems were mostly mild: four reported rashes on uncovered skin (the description of which was sufficient to diagnose airborne dermatitis), two subjects reported hand dermatitis, and two reported pruritus without visible skin changes. One case of airborne dermatitis to *Humulus lupulus* (hops) was severe enough to be classified as debilitating.

Positive skin reactions to four *Humulus lupulus* (hops) allergen preparations, i.e., cone extract in glycerol, cone extract in saline, leaf extract in glycerol, and leaf extract in saline, were found in one, two, three, and four of 65 subjects, respectively. In all, six subjects (8.2%) reacted to at least one extract. Among the subjects reporting skin problems related to *Humulus lupulus* (hops), SPTs gave positive results in two subjects, and the tests were negative in six subjects. The tests were also positive in four persons who did not report any *Humulus lupulus* (hops)-related skin problems. The predictive values for SPT of the extracts (skin reaction to at least one of the preparations) were: positive predictive value (PPV) = .33 and negative predictive value (NPV) = .91.[101]

Inhalation. Washington State Workers' Compensation claims filed by *Humulus lupulus* (hops) workers for respiratory disease between 1995 and 2011 were systematically identified and reviewed in a study of occupational respiratory disease in *Humulus lupulus* (hops) workers.[102] Incidences of respiratory disease in *Humulus lupulus* (hops) workers were compared

with rates in field vegetable crop farm workers. A total of 57 cases of respiratory disease associated with *Humulus lupulus* (hops) dust inhalation were reported. The attending health care practitioner diagnosed 61% of these cases as having work-related asthma. Chronic obstructive pulmonary disease was diagnosed in 7% of these cases; the remaining cases were diagnosed as allergic respiratory disorders (e.g., allergic rhinitis [18%] or asthma-associated symptoms [e.g., dyspnea; 14%]). All cases were associated with *Humulus lupulus* (hops) harvesting, secondary hops processing, or indirect exposure. The incidence of respiratory disease in *Humulus lupulus* (hops) workers was 15 cases per 10,000 full-time workers, which was 30 times greater than the incidence for field vegetable crop workers. A strong temporal association between *Humulus lupulus* (hops) dust exposure and respiratory symptoms and a clear association between an increase in *Humulus lupulus* (hops) dust concentrations and the clinical onset of symptoms were apparent in 3 cases. The authors concluded that occupational exposure to *Humulus lupulus* (hops) dust is associated with respiratory disease; respiratory disease rates were higher in *Humulus lupulus* (hops) workers than in a comparison group of agricultural workers.

In a study of occupational exposure of brewery workers to organic dusts such as *Humulus lupulus* (hops), barley, and brewery yeast, the potential to affect respiratory function and immunological status was examined.[103] Male subjects (n = 97) employed in a brewery plant had a mean age of 40 years, and the mean duration of employment was 16 years. The control group consisted of unexposed workers (n = 76). Respiratory symptoms were recorded. Lung function was measured by recording maximum expiratory flow-volume (MEFV) curves. Immunological testing was performed on all brewery workers and 37 of the control volunteers using SPTs with *Humulus lupulus* (hops), barley, and yeast antigens as well as other non-occupational allergens, and by determining total serum IgE levels. There was a higher prevalence of most of the chronic respiratory symptoms in brewery workers compared to controls. Occupational asthma was recorded in only 2 (2.1%) of the brewery workers; smoking was reported to be the major factor (that was examined in this study) responsible for the high prevalence of chronic respiratory symptoms in workers, not exposure to dust of the brewing ingredients, including *Humulus lupulus* (hops). A large number of brewery workers complained of acute symptoms that developed during the work shift. Lung function test scores were decreased compared to predicted levels. Multivariate analysis of these respiratory function parameters suggested the importance of workplace exposure in explaining lung function abnormalities. There was a greater instance of positive SPTs in brewery workers for *Humulus lupulus* (hops) than in controls (15% vs 3%). There were increased serum levels of total IgE in 34 out of 97 (45.1%) brewery workers compared to controls, 1 out of 76 (2.7%). However, workers with positive SPTs had a prevalence of chronic respiratory symptoms and lung function changes similar to those of workers with negative SPTs. The authors concluded that the data suggest that both smoking and dust exposure in the brewery industry may be responsible for the development of respiratory impairment and immunological reactions.

Case Reports

Case reports of irritation and sensitization to *Humulus lupulus* (hops) or the constituent β-myrcene while working with the plant on farms, laboratories, and in breweries are recited in Table 14.[11,54,104-106]

Summary

This is a safety assessment of Humulus Lupulus (Hops) Extract and Humulus Lupulus (Hops) Oil as used in cosmetics. Both of these ingredients are derived from the strobile of the *Humulus lupulus* plant, commonly called hops. The reported functions of Humulus Lupulus (Hops) Extract in cosmetics include antimicrobial agent, hair conditioning agent, and skin-conditioning agent – miscellaneous; Humulus Lupulus (Hops) Oil is reported to function as a fragrance ingredient.

Previously, the wINCI listed four other names for Humulus Lupulus (Hops) Extract. Data submitted under those deleted names have been assigned to Humulus Lupulus (Hops) Extract and the deleted names are now technical names for Humulus Lupulus (Hops) Extract.

Humulus lupulus (hops) strobile is an ingredient in food (most commonly in beer) and most of the other parts of this plant (shoots, leaves, flowers, seeds, rhizomes, and essential oils) are edible. The FDA determined that essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of *Humulus lupulus* L. (hops) are GRAS for human consumption.

Humulus lupulus (hops) plants are reported to contain several constituents of concern, including 8-PN, β-myrcene, and quercetin; these constituents could result in estrogenic activity, dermal irritation, and genotoxicity, respectively, if concentrations were high enough. Geraniol, limonene, linalool, and sesquiterpene lactones are potential dermal sensitizers. Humulus Lupulus (Hops) Oil is reported to contain sesquiterpene lactones.

The butylene glycol extract of Humulus Lupulus (Hops) Extract is reported to contain no detectable β-myrcene. A product mixture that contains approximately .18% Humulus Lupulus (Hops) Extract is reported to have a theoretical content of β-myrcene of .0022% based on the content of the starting materials; it is noted that the method of manufacture does not favor β-myrcene retention.

The *Humulus lupulus* (hops)-derived ingredients were reported to the VCRP database and surveyed by the Council in

Table 14. Case Reports of Sensitization to *Humulus lupulus* (Hops).

Presentation	Data, Tests, and Results	Reference
A 57-years-old female farmer presented with occupational airborne dermatitis and hand dermatitis from *Humulus lupulus* (hops)	Disease appeared at the age of 46, after 30 years of working with *Humulus lupulus* (hops) without any health problems. Patient had erythema of the skin of face, neck and upper chest, edema of eyelids, conjunctivitis, as well as acute dermatitis of hands. Symptoms were provoked by exposure to fresh or dried *Humulus lupulus* (hops). Symptoms appeared after 30 min of work and persisted over 1 to 2 days. There were no other skin or allergic problems. Skin tests were conducted with *Humulus lupulus* (hops) leaves (saline extract: prick positive, patch negative; glycerol extract: prick positive, patch negative) and hop strobiles (saline extract: prick positive, patch negative; glycerol extract: prick negative, patch positive after 48 and 72 h). Despite discontinuing work, patient experienced several relapses of her dermatitis. A cream and an herbal sedative, both containing *Humulus lupulus* (hops) extract, were identified as causing her dermatitis. During next *Humulus lupulus* (hops) cultivation period it also turned out that physical proximity to her husband was provoking relapses of the patient's dermatitis. Husband said that sometimes he did not wash thoroughly after working with the plant.	[104]
An atopic 35-year-old male brewery worker with rhinoconjunctivitis diagnosis due to grass and olea pollen presented with occupational rhinoconjunctivitis after 3 years of exposure to *Humulus lupulus* (hops).	Subject had no symptoms when he was away from his workplace. He was able to drink beer without symptoms. An SPT with a common commercial inhalants battery including pollens, mites, animal dander, molds, and latex was performed. Additionally, SPTs were performed using *Humulus lupulus* (hops) and barley extracts. Assays for specific IgE to barley, malt, corn, wheat and hops were also carried out. To demonstrate the patient's symptoms, a nasal challenge with *Humulus lupulus* (hops) extract was performed. SPTs were positive to grass, olive pollen, and *Humulus lupulus* (hops). Specific IgE was positive only to *Humulus lupulus* (hops). Nasal challenge with *Humulus lupulus* (hops) extract reproduced an immediate nasal response. SPTs with *Humulus lupulus* (hops) controls subjects (n = 10) were negative.	[105]
A 29-year- old male subject, who had three episodes of urticaria–angioedema immediately after ingestion of peanuts, chestnuts, and banana over the last 4 years, the latter requiring emergency treatment. Subject presented with urticaria on both hands while working with ripe dried *Humulus lupulus* (hops), though not with fresh *Humulus lupulus* (hops).	SPTs were negative for common aeroallergens (soy, latex, rapeseed, and the fish nematode *Anisakis simplex*) and positive for wheat and corn; controls (histamine and normal saline) had expected results. SPTs for banana, chestnut, walnut, almond, kiwi, avocado, and ripe dried *Humulus lupulus* (hops) were positive. Two additional atopic subjects served as controls; they had negative results for ripe dried *Humulus lupulus* (hops). Specific IgE to chestnut was 1.69kU/L, and ripe dried hops: 1.00 kU/L: total IgE: 64 IU/mL. Authors commented that *Humulus lupulus* (hops) rash as skin irritation has been known for several decades, but this subject could not be included in this category. A diagnosis of immunological contact urticaria due to dried *Humulus lupulus* (hops) by was made because: 1) work-related symptoms from handling dried *Humulus lupulus* (hops), but not fresh, 2) positive SPT, and 3) positive specific IgE. Authors hypothesized that *Humulus lupulus* (hops) drying process may transform certain proteins into allergens. Authors were not able to conclude if cross-reactivity between various fruits and dried *Humulus lupulus* (hops) was feasible.	[54]

(continued)

Table 14. (continued)

Presentation	Data, Tests, and Results	Reference
A 43-year-old female subject who worked in a laboratory that investigated *Humulus lupulus* (hops), presented with conjunctivitis, rhinitis, bronchitis, and dermatitis of the face.	She had no history of asthma or hay fever or previous dermatitis or inflammation of the mucous membranes. In her workplace, the dried plant strobiles were pulverized in a mill, some of which became airborne; she had no exposure to *Humulus lupulus* (hops) pollen. The results of a patch test of the *Humulus lupulus* (hops) dust was ++ and an intradermal test of .1 mL aqueous *Humulus lupulus* (hops) extract was +++. She was able to drink beer with no symptoms.	106
A 28-year-old male subject who was a chemist for a brewery presented with sneezing, itching, hives, closed feeling in his throat, wheezing, shortness of breath, abdominal bloating, watering eyes, and irregular heartbeat.	His job required exposure to *Humulus lupulus* (hops) plants in the field and laboratory. In laboratory, he crushed and rubbed strobiles in his hands and inhaled for aroma. Laboratory also used pure β-myrcene, which is a volatile oil. He had never had hay fever or asthma, but had a former allergy (watering eyes) to Siamese cats. As a child, milk ingestion would produce hives. Walnuts induced a burning feeling in his throat and stomach. Patch tests with crushed, dried *Humulus lupulus* (hops) flowers (two varieties) were negative. A patch test of β-myrcene was positive after 4 h and strongly positive after 48 h. Strongly positive reactions were observed in scratch and intradermal tests to most pollens, house dust, pyrethrum, orris root, and grain dust; moderate reactions were observed to some molds and horse dander. Tests were negative for other molds and other animals. Drinking a beer did not produce symptoms unless he had been exposed to β-myrcene.	11

2015(and updated in 2016) under the revised INCI names, and that is how they are reported herein.

According to VCRP data received in 2017, Humulus Lupulus (Hops) Extract was reported to be used in 375 formulations, including 317 leave-on formulations and 54 rinse-off formulations. The results of the concentration of use survey conducted by the Council in 2015 (and updated in 2016) indicate that the highest reported maximum concentration of use of Humulus Lupulus (Hops) Extract is up to .2% in hair conditioners.

Humulus Lupulus (Hops) Oil is not in use according to the VCRP and the industry survey.

Rats fed a low-fat diet, a high-fat diet, or high-fat diet supplemented with 1% xanthohumol-rich extract of *Humulus lupulus* (hops) extracts for 41 days and male mice fed a normal diet, a high-fat diet, or high-fat diets supplemented with 2% or 5% of various *Humulus lupulus* (hops) extracts for 20 weeks had no mortalities or adverse effects reported for any group. The additions of any of the *Humulus lupulus* (hops) extracts reduced the effects of the high-fat diet on weight gain. The weights of livers and mesenteric and epididymal adipose tissues of mice fed the supplemented high-fat diets were similar to those of the controls, as were the plasma glucose levels at the end of the test period.

An aqueous Humulus Lupulus (Hops) Extract (10 to 400 mg/μL in ethanol) was weakly mutagenic (an increase in induced revertants 2 to 4 times the controls) to *S. typhimurium* with and without metabolic activation. In another assay, a Humulus Lupulus (Hops) Extract was not mutagenic to *S. typhimurium* and *E. coli* at up to 10,000 μg/plate, with or without metabolic activation. A product mixture containing 5% Humulus Lupulus (Hops) Extract at 10% (.05% hops in deionized water) was not mutagenic to *S. typhimurium* with or without metabolic activation.

Historically, there is circumstantial evidence of potential estrogenic activity connected to *Humulus lupulus* (hops) exposure, including menstrual disturbances reported to be common among female *Humulus lupulus* (hops) harvesters. 8-PN has been shown to be the source of the estrogenic activity of *Humulus lupulus* (hops) plants. It mimics the action of 17β-estradiol, albeit with a lesser (10- to 20,000-fold) potency.

In a 2-week cumulative irritation test of a product containing Humulus Lupulus (Hops) Extract (.125%), the test product did not demonstrate a significant irritation potential in human skin. In a human patch test of a product containing Humulus Lupulus (Hops) Extract at .06 to .12%, the irritation index was .04 (out of 5) and the test article was rated a non-irritant. No adverse reactions were observed in a patch test of a product mixture that contains Humulus Lupulus (Hops) Extract (approximately .18%).

The causative agents of *Humulus lupulus* (hops) plant-induced contact skin reactions have not been established. In

Humulus lupulus (hops) harvesters, dermatitis has been attributed to mechanical abrasion by the rough hairs on the climbing stem. It has also been suggested that lupulin, the yellow powdery secretion of the glandular hairs on the scales of the strobiles, may be responsible for the irritation.

In a human maximization test of a product containing Humulus Lupulus (Hops) Extract (.125%), the test product did not demonstrate contact sensitization potential. An HRIPT of Humulus Lupulus (Hops) Extract (10%; extracted with butylene glycol) gave negative results. No reactions were observed in an HRIPT that was conducted of a product mixture that contains Humulus Lupulus (Hops) Extract (approximately .18%). There were no signs of irritation or sensitization in an HRIPT of a product mixture that contained Humulus Lupulus (Hops) Extract (approximately 5%).

In vitro assays showed that products and product mixtures containing Humulus Lupulus (Hops) Extract up to .5% were predicted to be either non-irritating or slight ocular irritants. Humulus Lupulus (Hops) Extract was predicted to be a slight ocular irritant in HET-CAM and cornea fibroblast assays and a non-irritant in an EpiOcular assay. Another Humulus Lupulus (Hops) Extract was predicted to be non-irritating in an HET-CAM assay and to SIRC fibroblastic cells.

In a 4-week use study of an eye cream that contained Humulus Lupulus (Hops) Extract (.125%), the test material did not demonstrate potential for eliciting ophthalmic irritation.

In a survey of farmers, exposure to *Humulus lupulus* (hops) was reported to cause the greatest number of skin problems; 14 of 73 (19.2%) of the farmers reported work-related skin symptoms, 11% of which were caused by *Humulus lupulus* (hops). There were one, two, three, and four positive SPTs to the following *Humulus lupulus* (hops) allergen preparations, respectively: cone extract in glycerol, cone extract in saline, leaf extract in glycerol, and leaf extract in saline.

In a study of occupational respiratory disease in *Humulus lupulus* (hops) workers, using Workers' Compensation claims filed by *Humulus lupulus* (hops) workers for respiratory disease, the incidence rate of respiratory disease in *Humulus lupulus* (hops) workers was 15 cases per 10,000 full-time workers, which was 30 times greater than the incidence rate for field vegetable crop workers. The authors concluded that occupational exposure to *Humulus lupulus* (hops) dust is associated with respiratory disease; respiratory disease rates were higher in hop workers than in a comparison group of agricultural workers.

In a study of occupational exposure of brewery workers to organic dusts, including *Humulus lupulus* (hops), the potential to affect respiratory function and immunological status was examined. A large number of brewery workers complained of acute symptoms that developed during the work shift. Lung function tests were decreased compared to predicted levels. There was a greater instance of positive SPTs in brewery workers for *Humulus lupulus* (hops) than in controls (15% vs 3%). There were increased serum levels of total IgE in 34 of 97 (45.1%) brewery workers compared to controls, 1 of 76 (2.7%). However, workers with positive SPTs had a prevalence of chronic respiratory symptoms and lung function changes similar to those of workers with negative SPTs. The authors concluded that the data suggests that both smoking and dust exposure in the brewery industry may be responsible for the development of respiratory impairment and immunological reactions.

There were case studies of subjects becoming sensitized to *Humulus lupulus* (hops) plants or the constituent β-myrcene while working with the plant on farms, laboratories, and in breweries.

Discussion

The Panel examined the oral toxicity, genotoxicity, dermal and ocular irritation, and sensitization studies of *Humulus lupulus* (hops)-derived ingredients, as well as studies on occupational exposure. Essential oils, oleoresins, and natural extracts of *Humulus lupulus* (hops) are GRAS for human consumption.

The Panel noted the presence of β-myrcene at up to 25.4% in *Humulus lupulus* (hops) oil. This constituent is a potential irritant, and there is an NTP study showing increased incidences of kidney tumors in male rats and liver tumors in male mice after oral administration of 1.0 g/kg/day β-myrcene for 2 years. The increased incidence of kidney tumors in this study is likely attributable to a mechanism that is not relevant to humans, and the increased incidence of liver tumors is attributable to the high background incidence and susceptibility to the development of liver tumors that is characteristic of the mouse strain used in the study, and is also not predictive of carcinogenicity in humans. Further, the daily dose of β--myrcene administered orally to the rats and mice in the study were much greater than the highest possible exposure to β--myrcene that could occur from *Humulus lupulus* (hops)-derived ingredients in cosmetics. However, concerns about β-myrcene, and possibly other constituents, cannot be addressed fully by the Panel, because the available information is not sufficient to characterize adequately the compositions of *Humulus lupulus* (hops)-derived cosmetic ingredients. The Panel emphasized the importance, generally, of adequately characterizing the compositions of cosmetic ingredients derived from plants, as manufactured and supplied to formulators of cosmetic products.

There were possible estrogenic effects in persons who worked with *Humulus lupulus* (hops) in the field. The studies showed that the purported estrogenic effects were weak and the degree of the exposure to the workers is far greater than any exposure that could occur from the use of cosmetics that contain these ingredients at the reported concentrations of use.

Because final product formulations may contain multiple botanicals, each possibly containing similar constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. For example, in *Humulus lupulus*

(hops)-derived ingredients, the Panel's concerns included the presence of 8-PN, β-myrcene, and quercetin in cosmetics, which could result in estrogenic effects, dermal irritation, and genotoxicity, respectively, as well as other constituents of concern. The Panel noted that IFRA standards to avoid adverse effect have been published for several *Humulus lupulus* (hops) constituents (Table 11). At the reported concentrations of use of these ingredients, the constituents that may cause these effects will be present at levels far below levels of concern, including for sensitization. However, when formulating products with multiple botanically-derived ingredients, manufacturers should avoid reaching levels of plant constituents that may cause sensitization or other adverse health effects.

There were instances of sensitization of persons who worked with *Humulus lupulus* (hops) in the field and in laboratories. The studies showed that the degree of the exposure of these persons is far greater than any exposure that could occur from the use of cosmetics that contain these ingredients at the reported concentrations of use. Additionally, the plant has hairs and bristles that could abrade the skin, thus increasing the chance of dermal penetration of constituents that could cause sensitization. Such abrasion would not occur with exposure to these cosmetic ingredients.

The Panel is reassured by the HRIPT of 10% Humulus Lupulus (Hops) Extract that sensitization should not be a problem under the reported conditions and concentrations of use in cosmetics because the highest reported concentration of use was .2% Humulus Lupulus (Hops) Extract in hair products and .13% in products that come in contact with the skin. Overall, sensitization and constituent profile data show that there should not be any issue with sensitization at the low concentrations of use of these ingredients. The Panel cautions that manufactures use cGMP to formulate products that are non-sensitizing.

There is a substantial data profile on the constituents of the Humulus Lupulus (Hops) Oil; many of these constituents, including potential sensitizers, are similar to those in the Humulus Lupulus (Hops) Extract. Therefore, the safety data on one ingredient informs the other. Concern, however, was expressed about alternative approaches to extraction that might not produce material with the same safety profile described in this safety assessment. There are multiple methods of extraction with multiple solvents (e.g., water/propylene glycol, water/ethanol, water/butylene glycol, and caprylic/capric triglyceride) presented for the ingredient Humulus Lupulus (Hops) Extract and steam distillation for Humulus Lupulus (Hops) Oil. The Panel's safety conclusion is applicable only for Humulus Lupulus (Hops) Extract and Humulus Lupulus (Hops) Oil that are prepared in a manner that produces a similar chemical profile as that described in this report, especially for the constituents of concern. When prepared in a manner resulting in this chemical profile, the Panel's conclusion is that these ingredients do not have significant estrogenic activity, genotoxicity, irritation, or sensitization potential. Ingredients not prepared in a manner that produces a similar chemical profile would be considered safe only if a similar safety test profile was demonstrated.

The Panel expressed concern about pesticide residues, heavy metals, and substances from plants of other species (weeds) that may be present in botanical ingredients. Also, multiple fungi and bacteria have been detected co-localized with *Humulus lupulus* (hops) plants. To address these concerns, the cosmetics industry should continue to use cGMPs to limit impurities.

There were no constituents of concern associated with phototoxicity and there were no indications of phototoxicity in the farmer workers exposed to high amounts of *Humulus lupus* (hops). Accordingly, phototoxicity assays were not deemed to be required.

Also, the Panel noted the limited scope of the in vitro genotoxicity assay in which an aqueous Humulus Lupulus (Hops) Extract increased revertants 2-4 times that of controls. The Panel concluded that these test results were not statistically significant and that there was no significant risk of genotoxicity.

The pulmonary disease associated with working with *Humulus lupus* (hops) plants was reported to be caused by constant inhalation exposure to the plant dust over extended periods (e.g., years). This exposure is far greater than any exposure would be associated with the use of cosmetic products. In this context, the Panel discussed the issue of incidental inhalation exposure from aerosol and pump hair sprays. The limited data on occupational inhalation suggests some potential for respiratory effects at large doses over extended periods of time. Otherwise, there were no inhalation toxicity data available. These ingredients are reportedly used at concentrations up to .0002% in cosmetic products that may be aerosolized and up to .00055% in loose powder products that may become airborne. The Panel noted that most droplets/particles would not be respirable to any appreciable amount. 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. The Panel considered other data available to characterize the potential for *Humulus lupulus* (hops)-derived ingredients to cause toxicity, genotoxicity, irritation, and sensitization. They noted the lack of systemic toxicity, genotoxicity, irritation, and sensitization at relevant doses by *Humulus lupulus* (Hops)-derived ingredients. They also noted that parts of the plants are edible and extractives are GRAS as food additives. 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.

Conclusion

The Expert Panel for Cosmetic Ingredient Safety concluded Humulus Lupulus (Hops) Extract and Humulus Lupulus (Hops) Oil are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-sensitizing.

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