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Final Report of the Cosmetic Ingredient Review Expert Panel Amended Safety Assessment of *Calendula officinalis*–Derived Cosmetic Ingredients

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Abstract

Calendula officinalis extract, *C officinalis* flower, *C officinalis* flower extract, *C officinalis* flower oil, and *C officinalis* seed oil are cosmetic ingredients derived from *C officinalis*. These ingredients may contain minerals, carbohydrates, lipids, phenolic acids, flavonoids, tannins, coumarins, sterols and steroids, monoterpenes, sesquiterpenes, triterpenes, tocopherols, quinones, amino acids, and resins. These ingredients were not significantly toxic in single-dose oral studies using animals. The absence of reproductive/developmental toxicity was inferred from repeat-dose studies of coriander oil, with a similar composition. Overall, these ingredients were not genotoxic. They also were not irritating, sensitizing, or photosensitizing in animal or clinical tests but may be mild ocular irritants. The Cosmetic Ingredient Review (CIR) Expert Panel concluded that these ingredients are safe for use in cosmetics in the practices of use and concentration given in this amended safety assessment.

Keywords

safety, cosmetics, calendula

Introduction

The safety of *Calendula officinalis* extract and *C officinalis* was evaluated by the Cosmetic Ingredient Review (CIR) Expert Panel in an earlier safety assessment.[1] The available data were considered insufficient to support the safety of these ingredients in cosmetics. The data that were needed included (1) current concentration of use; (2) function in cosmetics; (3) UV absorption, and if there is significant UV absorption, photosensitization studies; (4) gross pathology and histopathology in the skin and other major organ systems associated with repeated dermal exposures; (5) dermal reproductive and developmental toxicity; (6) inhalation toxicity, addressing the concentration, amount delivered, and particle size; and (7) genotoxicity testing in a mammalian system and, if positive, a 2-year dermal carcinogenicity assay.

Additional data were provided and the CIR Expert Panel agreed to reopen its safety assessment to consider these new data. This report presents all currently available information.

The naming convention in the *International Cosmetic Ingredient Dictionary and Handbook*[2] has changed for these ingredients. *Calendula officinalis* extract is now termed *C officinalis* flower extract; *C officinalis* is not used as an ingredient name; and a new name, *C officinalis* extract, refers to an extract of the whole plant.

This report will use the current terminology. In addition, cosmetic ingredients derived from the plant, *Calendula officinalis* or *Calendula officinalis* L have been further defined in the *International Cosmetic Ingredient Dictionary and Handbook*[2] to include the ingredients listed in Table 1. All 5 ingredients are included in this amended safety assessment.

In addition to "marigold," the plant *C officinalis* is also known as garden marigold, pot marigold, Marybud, holigold,[3] holligold, and gold-bloom.[4] In Bulgarian, the name is "neven" and in Russian, "nagotki."[5] In French, the name is "souci"; in German, "ringelblume"; in Italian, "calendola"; in Spanish, "maravilla"; and in Dutch, "goudsbloem."[6]

Composition

D'Amelio[7] reported that *C officinalis* contains the following:

- volatile oils,
- saponins,

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Table 1. Current Cosmetic Ingredients Derived from *Calendula officinalis* plants[2]

Ingredient	Definition	Chemical Class	Function	Technical/Other Names
Calendula officinalis extract (no CAS No)	Extract of the whole plant	Biological product	Skin-conditioning agent—miscellaneous	None reported
Calendula officinalis flower (no CAS No.)	The flowers themselves	Biological product	Not reported	None reported
Calendula officinalis flower extract (CAS No 84776-23-8)	Extract of the flower	Biological product	Fragrance ingredient	*Calendula officinalis*, extract
			Skin-conditioning agent—miscellaneous	Marigold extract
Calendula officinalis flower oil (CAS No 70892-20-5)	Volatile oil distilled from the flowers	Essential oil	Fragrance ingredient	Marigold, pot (*Calendula officinalis* L)
			Skin-conditioning agent—miscellaneous	
Calendula officinalis Seed Oil (no CAS No)	Oil expressed from the seeds	Fats and oils	Skin-conditioning agent—occlusive	None reported

- flavonoids,
- calendulin,
- sterols,
- fatty acids,
- calendic and oleanic acids,
- triterpenoids,
- tocopherols, and
- flavonol glycosides of isorhamnetin (and the corresponding quercetin derivatives).

Patri and Silano[6] listed the following constituents of *C officinalis*:

- carotenoids, including carotenes, flavochrome, mutatochrome, aurochrome, flavoxanthin, chyrsantemoxanthin, xanthophyll, and licopenes;
- flavonoids, including isorhamnetin glucoside, quercetin glucoside, and quercetin;
- triterpenic alcohols (mono-ols), including α-amyrin, β-amyrin, taraxasterol, and lupeol;
- triterpenic alcohols (di-ols), including faradiol, arnidiol, brein, erythrodiol, calenduladiol, and ursadiol;
- triterpenic alcohols (tri-ols), including longispinogenine, lupenetriol, ursatriol, heliantriol C, and hiliantriol F;
- mucilages;
- saponins (one specification gives saponins as not less than 2%, calculated as oleanolic acid);
- resins;
- tocopherols; and
- polyprenylquinones.

The European Scientific Cooperative on Phytotherapy (ESCOP) prepared a monograph on the Calendula flower[8] in which triterpene saponins, mainly oleanolic acid glycosides; free and esterified triterpene alcohols, especially faradiol 3-monoesters, carotenoids, flavonoids (not less than 0.4%) based on quercetin and isorhamnetin, polysaccharides, sterols, and sesquiterpenoids; and essential oils were identified.

Kishimoto et al[9] reported the carotenoid composition in petals of *C officinalis* L as a function of orange or yellow petal color. High-performance liquid chromatography (HPLC) fractionation of the carotenoids yielded 19 separate peaks from the orange color petals and 9 peaks in the yellow color petals. Table 2 presents the carotenoids found in the orange cultivar.

The Cosmetic, Toiletry, and Fragrance Association (CTFA) provided a specification for pot marigold from Alban Muller International that included 12% glycosides (roughly 6% mucilages), 5.3% lipids, 10% minerals, and 0.02% essential oil with oxygenated sesquiterpenic derivatives, along with organic acids; phenolic compounds, including phenolic acids (salicylic acid), flavonoids (flavenol heterosides of isorhamnetin and quercetin and isorhamnetin 3-rutinoside), and tannins; terpenoids, including triterpenes (saponins; eg, bidesmosides and monodesmosides of oleanolic acid and phytosterol and taraxasterol), carotenoids (carotenes, lycopenes, violaxanthines, flavoxanthines) and α- and β-amyrins, arnidiol, faradiol, ursadiol, calenduladiol, and heliantriol; lignin).[10]

The CTFA[11] provided the composition for Crodarom's Phytexcell Calendula (proprietary extract of marigold flowers using glycerin, butylene glycol, and water), which included isorhamnetin-3-glucoside; chlorogenic acid; narcissin, rutin; and quercitin (maximum of 450 ppm).

The European Organization of Cosmetics Ingredients Industries and Services (UNITIS) developed a method of evaluating the safety of cosmetic ingredients derived from plant materials that involved the following:

(1) determination of all fractions and compounds identified for the particular plant;
(2) development of a safety profile for each fraction/compound; and
(3) evaluation of skin toxicity studies on those fractions/compounds that may present a risk.[12]

Step (1) was completed with the list of fractions, subfractions, and compounds for *C officinalis* shown in Table 3.

Table 2. Carotenoid Composition From Orange Marigold Cultivar[9]

Carotenoid	% of Total Carotenoids	λ_{max} (nm)
Flavoxanthin	28.5	398, 420, 448
(8'*R*)-luteoxanthin	11.0	398, 422, 448
(All-*E*)-lycopene*	8.7	446, 473, 505
(8*R*,8'*R*)-auroxanthin	7.1	380, 401, 425
(9'*Z*)-lutein-5,6-epoxide	5.0	413, 435, 463
(5'*Z*)-γ-carotene*	4.4	463, 493
(5*Z*,9*Z*)-lycopene*	4.1	442, 467, 497
(5*Z*,9*Z*,5'*Z*,9'*Z*)-lycopene*	4.1	437, 461, 491
(5'*Z*,9'*Z*)-rubixanthin*	4.0	455, 485
(5*Z*,9*Z*,5'*Z*)-lycopene*	3.5	442, 467, 497
β-carotene	3.4	452, 479
(5'*Z*)-rubixanthin*	3.0	461, 491
Lutein	2.0	444, 473
γ-carotene*	2.0	461, 493
Lutein-5,6-epoxide	1.6	416, 438, 469
δ-carotene*	1.4	433, 457, 488
Antheraxantin	1.0	440, 467
α-carotene*	0.8	446, 475
(9*Z*)-lutein	0.6	440, 467

*Found only in the orange cultivar. All other carotenoids found in yellow cultivars as well.

Eyerman[13] evaluated the amount of α-tocopherolquinone in a methanol extraction of *C officinalis* flower heads by HPLC. The standard solution was clearly identified as a peak eluting at around 6.8 minutes, but no detectable signal was found for the extract. The authors concluded that α-tocopherolquinone was not present in the methanol extract at detectable levels.

Eyerman[14] examined the presence of coumarins (esculetin, scopoletin, and umbelliferon) in *C officinalis* flower heads. Dried flowers were frozen, ground into a powder, and methanol extract was prepared. Samples were run on HPLC with 25 mmol/L ammonium acetate/methanol/deionized water (10:40:50) as the mobile phase. Material eluted at 1.8 and 2.3 minutes, but spectroscopic analysis confirmed that these peaks were not coumarins. In the standards and the spiked extracts, coumarins were detected.

Eyerman[15] used both water and methanol extractions to examine the catechol content of dried calendula flowers. High-performance liquid chromatography was used to perform a separation of the extracted material. The sample without the catechol reference standard was free of detectable signal at the elution time for catechol in the reference sample. The author also noted that, since pyrogallol co-elutes with catechol, the absence of detectable levels of pyrogallol was also demonstrated.

Preparation/Extraction

Avramova et al[5] studied the effect of various solvents on the recovery of carotenoids and flavanoids from air-dried marigold flowers. Table 4 presents the recovery in comparison to the initial raw material and separately for carotenoids and flavonoids.

Chemisches Laboratorium Dr Kurt Richter GmbH[16] described a preparation of *C officinalis* flower extract (1%-5%) with soybean oil in which the flowers are gently disintegrated with stabilized soybean oil and the mixture is obtained by filtration.

Grau Aromatic GmbH & Co[17] reported that a mixture of *C officinalis* flower extract was prepared by extracting flowers with 1.2-propylene glycol at a ratio of 1:5.

Ichimaru Pharcos Co Ltd[18] described a similar process except that 1,3-butylene glycol was used as the solvent.

Patri and Silano[6] describe a hydroalcoholic dry extraction in which plant material percolated in a water/alcohol solution is concentrated under vacuum to dryness; a glycolic extraction in which plant material is percolated with propylene glycol and concentrated under vacuum; and an oil tincture in which the plant material is digested with vegetable oil.

The CTFA[10] provided information from Alban Muller International in which propylene glycol, propylene glycol plus water, butylene glycol, butylene glycol plus water, glycerine, glycerine plus water, and vegetable oil were all used as percolation solvents with macerated, dried plant material. The CTFA[11] provided information from Crodarom, stating that they extract marigold flowers with glycerine, butylene glycol, and water.

Natural Product Consulting[12] reported that the currently used extraction methods include lipophilic, water−alcoholic, and supercritical CO_2 extractions.

Lipophilic extractions (eg, with vegetable or mineral oil, octyl palmitate) will include lipophilic hydrocarbons; paraffins; fatty acids and fatty acid esters; steroids; tocopherols; apolar carotenoids; mono-, sesqui-, and triterpenoid esters; and triterpene mono-alcohols and diols. More polar triterpenoid triols, oxygenated carotenoids, and phenolic acids may be found, but extraction likely would not be complete.

Hydroethanolic extracts prepared by maceration and percolation will contain medium-polar and polar classes such as flavonoids, terpenoid glycosides, carotenoids, coumarins, phenolic acids, and tannins.

Extractions with butylene glycol or propylene glycol and water will contain flavonoid and terpenoid glycosides, some polar carotenoids, phenolic acids, tannins, amino acids, and polysaccharides.

Supercritical CO_2 extracts will contain low-polarity compounds, with some medium polarity ones, but the specific compounds will depend on pressure and co-solvents, such as ethanol.[12]

Physical and Chemical Properties

Avramova et al[5] reported that a propylene glycol extraction of marigold flowers, termed Neva, is an orange-brown, viscous liquid. This extract is slightly soluble in water, has a density of 1.021 to 1.060 (at 20°C), an index of refraction of 1.352 to 1.436 (at 20°C), and acid number from 1.2 to 4.9.

According to the Chemisches Laboratorium Dr Kurt Richter GmbH,[16] a mixture of *C officinalis* flower extract (1%-5%), soybean oil (>50%), and tocopherol (<0.1%) is a reddish-yellow, oily liquid with an aromatic herbal odor. This preparation is soluble in fats and oils and has a refractive index (at 20°C) of 1.474 to 1.475, a density of 0.918 to 0.922 g/mL, and an acid value of less than 1.0.

Table 3. Fractions, Subfractions, and Compounds Found in *Calendula officinalis* Plants[12]

Fraction	Proportion	Subfraction/Compound
Mineral	≈6%	Potassium
	≈1.7%	Sodium
	≈0.9%	Magnesium
	≈0.5%	Calcium
Carbohydrates	12%-25%, dry matter	Arabinogalactan PSII 25 kDa (arabinose, galactose)
		Arabinogalactan PSIII 35 kDa (arabinose, galactose)
		Rhamnoarabinogalactan PSI 15 kDa (arabinose, galactose, rhamnose)
	1.5%	Mucilege
Lipids		
Fatty acids	5%, dry matter	9-hydroxy-trans-10,cis-12-octadecadienic acid
		Capric acid
		Caprylic acid
		Dimorphecolic acid
		Lauric acid
		Linoleic acid
		Myristic acid
		Palmitic acid
		Palmitoleic acid
		Pentadecanoic acid
		Stearic acid
		Trans-8,trans-10,cis-12-octadecatrienic acid
Hydrocarbon/ paraffin/waxes	0.15%, fresh petals	$C_{32}H_{62}$
		Dotriacontan
		Hentriacontan
		Heptacosan
		Hexacosan
		Octacosan
		Tetratriacontan
		Triacontan
		Tritriacontan
Phenolic compounds		
Phenolic acids, free and esterified	0.1%, dry matter	Lignin
		Caffeic acid
		Chlorogenic acid
		Ferulic acid
		Gentisic acid
		o-coumaric acid
		p-coumaric acid
		p-hydroxybenzoic acid
		p-hydroxyphenylacetic acid
		Protocatechuic acid
		Salicylic acid (trace only)
		Sinapic acid
		Syringic acid
		Vanillic acid
		Veratric acid
Flavonoids	<1.5%	Astragalin
		Hyperoxide
		Isoquercetin
		Isorhamnetin
		Isorhamnetin-3-neohesperidoside
		Isorhamnetin-3-*o*-(2',6'-dirhamnosyl)-glucoside
		Isorhamnetin-3-*o*-(2'-rhamnosyl)-glucoside
		Isorhamnetin-3-*o*-glucoside
		Isorhamnetin-3-rhamnosyl-(1,2)-rhamnoside
		Isorhamnetin-3β-D-glucopyranosyl-6-1β-l-rhamnofuranoside
		Laempferol
		Manghaslin
		Narcissin
		Neoliesperoside
		Quercetin
		Quercetin-3-neohesperidoside
		Quercetin-3-*o*-(2',6'-dirhamnosyl)-glucoside
		Quercetin-3-*o*-(2'-rhamnosyl)-glucoside
		Quercetin-3-rutinoside
		Rutin
		Syringentin
		Typhaneoside
Tannins	6%-10%	Pyrogallol and catechol types
Coumarins	Not given	Esculetin
		Scopoletin
		Umbelliferon
Steroids and terpenoids		
Sterols and steroids	0.2%	24-methylcholest-5,22-dien-3-β-*ol*
		24-methylcholest-7-en-7-β-*ol*
		24-methylene cholesterol
		24-methylene-lophenol
		28-isofucosterol
		4-α-methyl-24-methylene-cholest -7-en-3-β-*ol*
		4-α-methylstigmasta-7,24(28)-dien-3-β-*ol*
		4-β-methylergosta-7,24(28)-dien-3-β-*ol*
		Campestanol
		Campesterol
		Cholest-7-en-3-β-*ol*
		Cholestanol
		Cholesterol
		Stigmast-7-en-3-β-*ol*
		Stigmastanol (=fucostanol)
		Stamasterol
		α1-sitosterol (=citrostandeniol)
		β-sitosterol
Monoterpenes and sesquiterpenes	0.2%-0.4%	Carvone
		Geranylacetone
		G-terpinene
		Linalool

(continued)

Table 3 (continued)

Fraction	Proportion	Subfraction/Compound
		Menthone
		p-cymen
		Sabinen
		Terpinen-4-*ol*
		α-pinene
		α-terpineol
		α-thujen
		Isomenthone
		Aloaromadendrol
		Calamenen
		Caryophyllene
		Cubenol
		D-cadinene
		D-cadenol (= torreyol)
		Epicubebol
		G-cadinene
		Germacren D
		Guiaiol
		Oplopanone
		Palustrol
		t-cadinol
		t-muurolol
		Transcaryophyllene oxide
		α-cadinol (0.05-0.15%)
		α-humulen
		α-muurolene
		β-eudesmol
		Officinosides C and D (sesquiterpine oligoglycosides)
Free and esterified triterpenic alcohols	<5%	Arnidiol
		Brein (mainly as fatty acid esters)
		Calenduladiol (mainly as fatty acid esters)
		Coflotriol
		Erythrodiol
		Faradiol (mainly as fatty acid esters)*
		Heliantriol A0
		Heliantriol A1
		Heliantriol B0
		Heliantriol B1
		Heliantriol B2
		Heliantriol C
		Heliantriol F
		Longispinogenine
		Lupentriol
		Lupeol (and its esters)
		Maniladiol (and its esters)
		Olean-12-ene-3β,16β,28-triol
		Pseudotaraxasterol
		psi-taraxasterol
		Taraxasterol
		Ursadiol
		Ursatriol
		α-amyrin
		β-amyrin
Triterpenic glycosides	2%-10%, dry matter	Calendulosides A, B, C, D, E, F, G, H (oleanolic acid glycosides)
		Calendasaponins A, B, C, and D (oleanolic acid glycosides)
Tocopherols	Not given	7-methyltocol
		5,7-dimethyltocol
		5-methyltocol
		8-methyltocol
		α-tocopherol
		β-tocopherol
		γ-tocopherol
		δ-tocopherol
Quinones (mainly as polyprenylquinones)	Not given	5-phytyltoluquinone
		6-phytyltoluquinone
		α-tocopherolquinone
Others	3.4%-4.5%, dry matter	Free ascorbic acid and malic acid
		Amino acids
		Resins

*Found only in the orange cultivar. All other carotenoids found in yellow cultivars as well.

Grau Aromatic GmbH & Co[17] reported that a mixture of *C officinalis* flower extract (10%-15%) and propylene glycol (>75%) is a clear, brown liquid with a faint herbal odor. This preparation is soluble in water and has a refractive index (at 20°C) of 1.425 to 1.445, a density of 1.035 to 1.055 (at 20°C), and a pH range of 5.5 to 6.5.

Ichimaru Pharcos Co Ltd[18] described a mixture of *C officinalis* flower extract, butylene glycol, and water (proportions not given) as a yellowish-brown, transparent liquid with a bitter taste. This preparation had a specific gravity of 1.01 to 1.05 (at 20°C) and a pH range of 5.0 to 6.0.

The CTFA[11] described Crodarom's Phytexcell Calendula (proprietary extract of marigold flowers using glycerin, butylene glycol, and water) as a brown to red-brown, clear liquid with a slightly characteristic odor. This preparation had a density of 1.140 to 1.170 kg/L (at 20°C), a refraction index of 1.425 to 1.455 (at 20°C), and a pH range of 4.5 to 6.5 (10% in distilled water at 20°C).

Analytical Methods

High-performance liquid chromatography, reverse-phase HPLC, and micellular electrokinetic capillary chromatography have been used to analyze *C officinalis*.[19,20] Fractionation by column chromatography and thin layer chromatography have been used to determine terpene derivatives in *C officinalis*.[21]

Impurities

Ichimaru Pharcos Co Ltd[18] reported that a mixture of *C officinalis* flower extract, butylene glycol, and water contained ≤10 ppm heavy metals, including ≤1 ppm arsenic.

Table 4. Extraction of Marigold Flowers as a Function of Solvent[5]

Solvent	% of Raw Material Recovered	% Carotenoid	% Flavonoid	Color
Chloroform	8.61	1.65	Not reported	Dark orange
Petroleum ether	7.27	1.43	Not reported	Yellow-orange
Dichloroethane	8.26	1.58	Not reported	Dark brown-orange
Ethyl alcohol	5.08	1.12	0.54	Light yellow
Propylene glycol	Not reported	Not reported	0.22	Brown-orange

Table 5. Absorption Peaks for *Calendula officinalis* Flower Oil, *C officinalis* Flower Extracts, and a Trade-Name Mixture With *C officinalis* Flower Extract[22]

Ingredient	UVC Absorption Peaks (nm)	UVB/UVA Absorption Peaks (nm)	Visible Light Absorption Peaks (nm)
Calendula officinalis flower oil	269, 280	None	656
Calendula officinalis flower extract	262, 272	283[a]	610, 669
Calendula officinalis flower extract	272	378[b]	423, 449, 610, 670
Trade-name mixture containing *C officinalis* flower extract	270	None	488, 583, 660

[a] 0.4 AU at 272 nm and 0.315 AU at 283 nm.
[b] 0.4895 AU at 272 nm and .08637 AU at 378 nm.

UV Absorption

The CTFA[22] provided absorption spectra over the range of 250 to 800 nm for several preparations. *Calendula officinalis* flower oil (neat) was diluted to 1% with USP ethanol. *Calendula officinalis* flower extract (neat) diluted to 1% with spectral grade cyclohexane and then further diluted 1:50 and then 3:25. *Calendula officinalis* flower extract (neat) from another source was diluted to 1% with spectral grade cyclohexane and then further diluted 1:50. A trade-name mixture with *Calendula officinalis* flower extract (51.5%) was diluted to 1% with deionized water and then further diluted 1:50.

Table 5 presents the UV absorption results for these 4 materials. For reference, generally speaking, red wavelengths are 625 to 740 nm; orange, 590 to 625 nm; yellow, 565 to 590 nm; green, 520 to 565 nm; cyan, 500 to 520 nm; blue, 435 to 500 nm; violet, 380 to 435; UVA, 315 to 400 nm; UVB, 280 to 315 nm (solar UVB below 290 nm is mostly blocked by the earth's atmosphere); and UVC, less than 280 nm.

Use

Cosmetic

As given in the *International Cosmetic Ingredient Dictionary and Handbook*, cosmetic ingredients derived from *C officinalis* have the definitions and functions as shown in Table 1. Under the Food and Drug Administration (FDA) voluntary cosmetic registration program (VCRP), manufacturers provide information on their use of individual cosmetic ingredients as a function of product type. Those data are given in Table 6.

The CTFA conducted a survey of current use concentrations for cosmetic ingredients derived from *C officinalis* and the results of that survey also are given in Table 6.[24] *Calendula officinalis* seed oil is not reported to be used.

Uses were reported in the VCRP of Calendula wax and Calendula fluid extract, but it is not known to which of the *C officinalis* derivatives listed as cosmetic ingredients these correspond, so they are listed separately in Table 6. One use of marigold oil reported in the VCRP was assumed to be *Calendula officinalis* flower oil and 5 uses of marigold flower were assumed to be *Calendula officinalis* flower and placed in Table 6 accordingly.

The only ingredients for which current use concentration data are available are *C officinalis* flower extract and *C officinalis* flower oil. Although the CTFA survey yielded information on the use concentrations for many of the product types in which these ingredients are used, use concentrations were not provided for others such as *C officinalis* flower extract in 7 products in the baby lotions category. In other cases, such as the use of *C officinalis* flower oil in makeup foundations at 0.02%, there was no corresponding report of uses to FDA.

In the original safety assessment by the CIR Expert Panel,[1] only uses of the flower extract were reported in 178 products, compared with 295 current uses in Table 6. In the earlier safety assessment, limited use concentration data were available.

In Europe,[25] *C officinalis* is defined as a plant material derived from the flowers of the calendula, *C officinalis*, Compositae, that functions as an emollient. *Calendula officinalis* extract is defined as an extract of the flowers of the calendula, *C officinalis*, Compositae, that functions as an emollient. And *C officinalis* oil is defined as the oil derived from the flowers of *C officinalis*, Compositae, that functions as an emollient. No restrictions on the use of these ingredients in Europe were given.

Table 6. Current Uses and Use Concentrations for Cosmetic Ingredients Derived From *Calendula officinalis*

Product Category (Total number of products in each category [FDA 2006[23]])	Frequency of Use[23]	Concentration of Use (%)[24]
Calendula officinalis flower extract		
Baby products		
Shampoos	2	—
Lotions, oils, powders, and creams	7	—
Other	9	—
Bath products		
Oils, tablets, and salts	1	—
Bubble baths	3	—
Other	2	0.1[a]
Eye makeup		
Eyeliners	2	—
Eye shadow	5	—
Eye lotions	2	—
Other	2	0.0001-1.0
Fragrance products		
Other	1	—
Noncoloring hair-care products		
Conditioners	23	0.0004-0.01
Sprays/aerosol fixatives	3	—
Rinses	3	—
Shampoos	29	0.001-0.01
Tonics, dressings, etc	12	0.004-0.01
Other	10	—
Makeup		
Blushers	2	0.01
Face powders	3	—
Foundations	1	—
Lipsticks	4	0.0002-0.5
Other	2	—
Oral hygiene products		
Dentifrices	1	—
Mouthwashes and breath fresheners	2	—
Personal hygiene products		
Soaps and detergents	13	0.002-0.05
Underarm deodorants	2	—
Other	3	—
Shaving products		
Aftershave lotions	3	—
Shaving cream	2	0.01
Other	1	—
Skin-care products		
Skin-cleansing creams, lotions, liquids, and pads	18	0.0002-0.1
Depilatories	1	—
Face and neck creams, lotions, powder and sprays	12	0.001-0.2
Body and hand creams, lotions, powder and sprays	25	0.002-0.4
Moisturizers	28	0.001-0.8
Night creams, lotions, powder and sprays	8	—
Paste masks/mud packs	7	0.01
Skin fresheners	9	0.2-0.5
Other	22	0.5-6.0[b]
Suntan products		
Suntan gels, creams, liquids and sprays	2	0.02
Indoor tanning preparations	8	—
Total uses/ranges for *Calendula officinalis* Flower Extract	295	0.0001-6
Calendula officinalis flower oil		
Baby products		
Lotions, oils, powders, and creams	2	—
Other	1	—
Bath products		
Oils, tablets, and salts	—	0.1
Other	—	0.1
Eye makeup		
Eye makeup remover	1	—
Noncoloring hair-care products		
Conditioners	1	—
Makeup		
Foundations	—	0.02
Personal hygiene products		
Soaps and detergents	1	—
Shaving products		
Aftershave lotions	2	—
Other	1	—
Skin care products		
Skin-cleansing creams, lotions, liquids, and pads	5	—
Face and neck creams, lotions, powder and sprays	3	0.1
Body and hand creams, lotions, powder and sprays	6	0.05
Moisturizers	9	0.1
Other	5	—
Suntan products		
Suntan gels, creams, liquids and sprays	1	—
Total uses/ranges for *C officinalis* flower oil	39	0.02-0.1
Calendula officinalis extract		
Noncoloring hair-care products		
Conditioners	2	—
Tonics, dressings, etc	1	—
Makeup		
Lipsticks	1	—
Skin-care products		
Face and neck creams, lotions, powder and sprays	1	—
Body and hand creams, lotions, powder and sprays	2	—
Moisturizers	1	—
Night creams, lotions, powder and sprays	1	—
Paste masks/mud packs	2	—
Total uses/ranges for *Calendula officinalis* extract	11	—
Calendula officinalis flower		
Baby products		
Shampoos	1	—

(continued)

Table 6 (continued)

Product Category (Total number of products in each category [FDA 2006[23]])	Frequency of Use[23]	Concentration of Use (%)[24]
Lotions, oils, powders, and creams	1	—
Fragrance products		
Other	1	—
Noncoloring hair-care products		
Other	1	—
Makeup		
Lipsticks	1	—
Personal hygiene products		
Other	2	—
Skin-care products		
Moisturizers	1	—
Night creams, lotions, powder and sprays	2	—
Other	3	—
Total uses/ranges for *C officinalis* flower	9	—
Calendula wax		
Skin-care products		
Skin-cleansing creams, lotions, liquids, and pads	1	—
Moisturizers	5	—
Night creams, lotions, powder and sprays	2	—
Other	1	—
Total uses/ranges for Calendula wax	9	—
Calendula fluid extract		
Skin-care products		
Paste masks/mud packs	1	—
Total uses/ranges for Calendula fluid extract	1	—

[a] Body scrub.
[b] 6% in a Calendula balm.

In Japan, the Ministry of Health, Labor, and Welfare (MHLW) has not listed *C officinalis* extract, *C officinalis* flower, *C officinalis* flower extract, *C officinalis* flower oil, or *C officinalis* seed oil as prohibited or restricted cosmetic ingredients, or as quasi-drugs.[26,27]

Cosmetic Aerosols

Cosmetic ingredients derived from *C Officinalis* are used in hair sprays and effects on the lungs that may be induced by aerosolized products containing these ingredients are of concern. The potential adverse effects of inhaled aerosols depend on the specific chemical species, the concentration, the duration of the exposure, and the site of deposition within the respiratory system.[28] In general, the smaller the particle, the farther into the respiratory tree the particle will deposit and the greater the impact on the respiratory system.[29]

Anhydrous hair spray particle diameters of 60 to 80 μm have been reported, and pump hair sprays have particle diameters of ≥80 μm.[30] The mean particle diameter is around 38 μm in a typical aerosol spray.[31] In practice, aerosols should have at least 99% of particle diameters in the 10 to 110 μm range. This means that most aerosol particles are deposited in the nasopharyngeal region and are not respirable.

Noncosmetic

Calendula officinalis L is generally recognized as safe (GRAS) by the FDA in spices and other natural seasonings and flavorings.[32]

Calendula officinalis has been used in traditional herbal medicine.

General Biology

Absorption, Distribution, Metabolism, and Excretion

Published data on the absorption, distribution, metabolism, and excretion of ingredients derived from *C officinalis* were not found, nor were unpublished data provided.

Cytotoxicity

Boucaud-Maitre tested the cytotoxicity of 5 *C officinalis* extracts using MRC5, Hep2, and Ehrlich cell lines. Saline was used as the control. All the extracts were cytotoxic in the range of 0.02 to 0.2 g/L. For MRC5 cells, cell death was seen in 30% and 99% at the 2 doses, respectively, with control cell death between 16% and 20%. For Hep2 cells, cell death was 2% and 99%, respectively, with control cell death between 2% and 4%. For Ehrlich cells, cell death was 10% and 100%, respectively, with control cell death less than 2%.

Immunologic Effect

Wagner et al[33] studied the immunostimulating properties of polysaccharides isolated from higher plants. One preparation studied was a polysaccharide from an aqueous/NaOH extract of *C officinalis* L in the 25 000 to larger than 500 000 Da size range. According to the authors, this material exhibited significant immunostimulating activity in both the granulocyte and carbon clearance test used for screening in this laboratory.

Animal Toxicology

Acute Oral Toxicity

According to CTFA,[34] the median lethal dose (LD_{50}) of *C officinalis* flower extract for rats was greater than 4.64 g/kg.

Chemisches Laboratorium Dr Kurt Richter GmbH[16] reported that the LD_{50} of a mixture containing *C officinalis* flower extract (1%-5%), soybean (Glycine Soja) oil (>50%), and tocopherol (<0.1%) was greater than 20 mL/kg.

Silva et al[35] conducted a study of the acute effects of a hydroalcohol extract of *C officinalis* L in rats and mice. The extract was prepared from dried flowers extracted by hydroalcohol percolation. The material was evaporated to dryness and suspended in distilled water at 350 to 450 mg/mL. Adult male and female Wistar rats and albino Swiss mice were used in this study (10 animals per group). Animals were fasted 12 hours

prior to treatment, then administered the extract at 0 (distilled water only), 0.625, 1.25, 2.5, and 5.0 g/kg. Animals were observed daily for general behavioral changes, morbidity, and mortality. No deaths or morbidity were found in the control group or the treatment groups.

Harikumar et al[36] reported an acute toxicity study of lutein and lutein ester isolated from marigold flowers using female Wistar rats. Animals (10 per group) were given sunflower oil alone (control—no detectable lutein), or 1, 2, or 4 g/kg lutein or 1, 2, or 4 g/kg lutein ester via oral gavage. For lutein, doses were delivered as 4 equal 2 mL doses at 2-hour intervals. For lutein esters at 1 and 2 g/kg, the same dosing was done. For lutein esters at 4 g/kg, doses were delivered as 6 equal 2 mL doses at 2-hour intervals. Control animals received 6 equal 2 mL sunflower oil doses at 2-hour intervals. Animals were monitored for 12 days for mortality and morbidity; body weights were measured, and feed consumption was estimated.

No mortality was seen at any dose level. Feed consumption in all animals initially was low but increased to control levels at day 3 postexposure. Diarrhea was observed in all animals for the first 2 days postexposure, which lessened from day 3 on. Both signs were attributed to the sunflower oil in the diet. The authors concluded that lutein and lutein ester did not produce any mortality at doses up to 4 g/kg.[36]

Acute Parenteral Toxicity

Dhar et al[37] determined the intraperitoneal LD_{50} of *C officinalis* flower extract, using 2 to 3 albino mice per dose group (doses not given), to be 300 mg/kg.

Short-Term Oral Toxicity

Ichimaru Pharcos Co Ltd[18] reported a study in which groups of dd-mice (number not stated) were given a mixture of *C officinalis* flower extract, butylene glycol, and water (neither concentrations nor relative proportions given) daily for 14 days at 5, 10, or 20 mL/kg. No animals died and the LD_{50} was reported to be greater than 20 mL/kg.

Hindle et al[38] conducted a study of the use of calendula meal in the diet of 8- to 13-week-old crossbred pigs at levels of 2%, 6%, 10%, or 20% for 37 days. A control group received diet with no calendula meal. There were 8 animals per group and duplicate groups were tested for a total of 18 animals at each exposure/control level. Only castrated or female pigs were used. Calendula meal was prepared from the kernel fraction of seeds from which the hulls had been removed. The daily weight gain was highest for the 2% group, significantly higher than the control. As the percentage of calendula meal in the diet increased, weight gain decreased and, at the 20% level, was less than the control. Blood levels of hematocrit, hemoglobin, oxygenated hemoglobin, creatinine, and zinc were unchanged by calendula meal in the diet at any level and aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase activities were within the normal range as well.

Silva et al[39] reported an oral study in which a hydroalcoholic extract of *C officinalis* was given to female Wistar rats (10 per group). The hydroalcoholic extract of the dried flowers of *C officinalis* was supplied by Simões Laboratory, RJ, Brazil (lot No 02.001) and concentrated by evaporating the solvent. The material was resuspended in distilled water and stored in aliquots at $-20^{\circ}C$.

Daily doses of 0.25, 0.5, and 1.0 g/kg of the Calendula extract were given for 30 days. The control group received distilled water only. The animals were observed for signs of toxicity, body weights were recorded weekly, and water and feed consumption were monitored. Animals were fasted for 12 hours after the last treatment, anesthetized, and blood collected for evaluation. Biochemical analysis included glucose, blood urea nitrogen (BUN), creatinine, AST, ALT, total cholesterol, triglycerides, alkaline phosphatase (ALP), total and direct bilirubin, total protein, albumin, and globulin. Hematological parameters included erythrocytes, leukocytes, platelets, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and differential leucocyte counts. After blood collection, animals were killed, and the brain, heart, liver, kidneys, adrenal glands, spleen, uterus, and ovaries were removed, weighed, and evaluated for external signs of toxicity.

Treatment did not have a statistically significant effect on body weight gain, or consumption of water or feed. No clinical signs of toxicity were observed in any treated animals and all survived. Biochemical parameters were not significantly different from controls, except that the BUN level was elevated in the high-dose group (51.2 ± 1.6 mg/dL) compared with controls (41.4 ± 2.3 mg/dL), and ALT activity was elevated at all dose levels. Several statistically significant differences were noted in hematological parameters. The number of neutrophils was reduced in the low- and high-dose groups compared with controls, but not in the middle-dose group. The lymphocyte count was elevated in the high-dose group compared with controls. The number of monocytes was reduced in the middle- and high-dose groups. No external signs of toxicity were observed in any organ, and there were no organ weight differences between treated and control animals. The authors concluded that the data supported an absence of toxicity for this Calendula extract; however, they also suggested that the elevated BUN and ALT could be signs of possible renal and hepatic overload, which should be further investigated.[39]

This same laboratory[35] repeated the previously described study in male Wistar rats using oral doses of 0 (distilled water only), 0.025, 0.25, 0.5, and 1.0 g/kg for 30 days. No deaths were found in any group and there were no signs of morbidity in any animal. Body weights and food and water consumption were not different between control and treatment groups. The authors concluded that the extract as given did not produce significant alterations in most parameters, but that the elevated hematological values and the liver histology could suggest renal and hepatic overload. Statistically significant findings (compared with controls) included a slight increase in erythrocyte count at all doses, decrease in MCV and MCH at all doses,

an increase in eosinophils at the high dose, and a decrease in monocytes at 0.25 and 0.5 g/kg, but not at 0.025 or 1.0 g/kg. There was a statistically significant dose-dependent increase in BUN levels and an increase in ALT activity in all test groups, except the 0.025 g/kg group.

Harikumar et al[36] reported a 4-week study of lutein and lutein ester isolated from marigold flowers using male and female Wistar rats. Animals (5 male and 5 female per group) were given sunflower oil alone (control—no detectable lutein), or 4, 40, or 400 mg/kg lutein or 4, 40, or 400 mg/kg lutein ester via oral gavage. Animals were monitored for mortality and morbidity, body weights were measured, and feed consumption was estimated. After the exposures were completed, the animals were killed, blood was collected, and a necropsy was performed, including retrieval and weighing of organs (liver, lungs, thymus, spleen, kidney, brain, and eyes) and histopathological examination.

Neither morbidity nor mortality was observed. No body weight changes or changes in feed consumption were noted in treated animals compared with controls. No differences in organ weights between treated and control animals were reported, and there were no pathological lesions in any organ. The only statistically significant changes were increased high-density cholesterol in some, but not all, lutein-treated groups (dose levels not given). The authors concluded that 4-week treatment of Wistar rats with lutein or lutein ester was not toxic.

Subchronic Toxicity

Harikumar et al[36] reported a 13-week study of lutein and lutein ester isolated from marigold flowers using male and female Wistar rats. Animals (5 male and 5 female per group) received sunflower oil alone (control—no detectable lutein), or 4, 40, or 400 mg/kg lutein or 4, 40, or 400 mg/kg lutein ester via oral gavage. Animals were monitored for mortality and morbidity, body weights were measured, and feed consumption was estimated. At the conclusion of the study, animals were killed, and blood was collected via heart puncture for hematological parameters. A necropsy was performed, and organs (liver, lungs, thymus, spleen, kidneys, brain, and eyes) were weighed and analyzed histologically.

Neither morbidity nor mortality was reported. Body weight gains and feed consumption were not different between treatment and control animals.

Isolated statistically significant differences were noted in hematological parameters, but there was no dose response.

No differences in organ weights were found, except for a reduction in liver weight in the low-dose lutein ester group (but not in the 10× and 100× higher dose groups). No pathology was observed in the histopathological analyses of organ tissues. The authors concluded that lutein and lutein esters are not toxic to rats.[36]

Chronic Toxicity

Available studies are discussed in the section on Carcinogenicity.

Dermal Irritation

The CTFA[40] reported the dermal irritation potential of a 10% aqueous *C officinalis* flower extract using 9 rabbits in a single-insult occlusive patch test (SIOPT). As tested, the material produced no irritation, had a primary irritation index of 0.0, and was not considered an irritant.

The CTFA[41] reported that an SIOPT to determine the dermal irritation potential of an eye cream containing 1.0% *C officinalis* flower extract resulted in minimal irritation (no primary irritation index provided).

Chemisches Laboratorium Dr Kurt Richter GmbH[16] stated that a mixture of *C officinalis* flower extract (1%-5%), soybean (Glycine Soja) oil (>50%), and tocopherol (<0.1%) all diluted to 10% in liquid paraffin was not irritating to rabbit skin.

Ichimaru Pharcos Co Ltd[18] determined the irritation potential of a mixture of *C officinalis* flower extract, butylene glycol, and water (neither concentrations nor relative proportions given). The test material was applied (0.5 mL) to intact and abraded skin of 6 albino rabbits. Test sites were scored at 4, 24, and 48 hours after application. The authors reported very slight erythema after 4 hours. In another test of the same material, 0.5 mL was applied to the skin of 5 guinea pigs over a 4-week period. Neither erythema nor edema was observed.

Natural Product Consulting[12] summarized results from skin irritation tests of 3 hydroalcoholic extracts and 5 lipophilic extracts of *C officinalis*. These extracts were intended to test the full range of extract compositions. Water/alcohol extracts are considered rich in carbohydrates, flavonoids, tannins, coumarins, triterpenic saponins, and organic acids. Lipid extracts are rich in fatty acids, hydrocarbons, monoterpenes, sesquiterpenes, sterols and steroids, carotenoids, triterpenic alcohols, tocopherols, and quinones.

One extract was prepared when plant flowers were macerated for a prolonged time in a water−propylene glycol mixture. The extract was diluted to 15% in sterile water and placed on intact skin of 6 albino male rabbits (0.5 mL per animal) for 4 hours and covered with a patch.[42] After patch removal, the skin was examined immediately and at 24, 48, and 72 hours postexposure. No erythema or edema was observed, and the water/propylene glycol extract was classified as a nonirritant in this skin test. The same extract was tested neat and evaluated after 72 hours. The pure extract also was classified as a nonirritant.

Another material tested was a hydroglycolic extract from dried *C officinalis* flowers. The treatment was performed as previously described using 6 albino male rabbits.[43] After patch removal, the skin was examined immediately and at 24, 48, and 72 hours postexposure. No erythema or edema was observed, and the hydroglycolic extract was classified as a nonirritant in this skin test.

A hydroalcoholic extract of *C officinalis* was tested in 6 albino male rabbits.[42] The extract was placed on the skin (volume not stated). No erythema or edema was observed. The hydroalcoholic extract was classified as a nonirritant in this skin test.

An extract of *C officinalis* was prepared by prolonged maceration in a vegetable oil and mineral oil mixture

(proportions not given). The extract was diluted to 20% in olive oil and placed on the skin (volume not given) of 6 rabbits.[42] No further details were given. Evaluations were made at 24 and 72 hours. The vegetable/mineral oil extract was classified as a nonirritant in this skin test.

A glycolic extract of *C officinalis* was prepared by prolonged maceration with an esterified oil (oil not identified). The extract was diluted to 20% in water and placed on the skin (volume not given) of 6 rabbits.[42] Evaluations were made at 24 and 72 hours. The glycolic extract was classified as a nonirritant in this skin test.

A liposoluble extract was tested using 3 rabbits.[42] The extract (0.5 mL) was placed on shaved dorsal skin for 4 hours. Evaluations were made at 1, 24, 48, and 72 hours. Well-defined irritation was observed following exposure, which resolved after 72 hours. No systemic changes were noted in the 3 animals, and body weight changes were not remarkable. The liposoluble extract caused little or no irritation in this skin test.

Biolab SGS[42] tested another liposoluble extract using rabbits (number not given). The extract was diluted to 10% in paraffin oil and applied to shaved dorsal skin. The primary irritation index was given as 0.2, and this liposoluble extract was classified as a nonirritant in this skin test.

Dermal Sensitization

The CTFA[44] reported that the sensitization potential of *C officinalis* flower extract was determined in a modified Magnusson-Kligman maximization test using 10 female Dunkin Hartley guinea pigs. During induction, intradermal injections of 0.05 mL of a mixture of 50% aqueous Freund complete adjuvant, 5% *C officinalis* flower extract in propylene glycol, and 5% flower extract in 50% Freund complete adjuvant were made to sites on the upper back of each animal. A control group of 10 animals received the injections without flower extract.

At 1 week postinjection, a booster dermal exposure of 20% *C officinalis* flower extract (a dose determined to be slightly irritating) was made to the site for 48 hours under an occlusive patch. Two weeks after the booster application, the animals were challenged with 5% and 10% *C officinalis* flower extract for 24 hours under an occlusive patch. The challenge sites were graded 24 and 48 hours after patch removal. No sensitization reactions were reported.[44]

Chemisches Laboratorium Dr Kurt Richter GmbH[16] stated that a mixture of *C officinalis* flower extract (1%-5%), soybean (Glycine Soja) oil (>50%), and tocopherol (<0.1%) was not sensitizing to guinea pigs.

Ichimaru Pharcos Co Ltd[18] determined the sensitization potential of a 50% mixture of *C officinalis* flower extract, butylene glycol, and water (neither concentrations nor relative proportions given) in a guinea pig maximization test (further details not given). The authors reported that neither erythema nor edema was observed.

Natural Product Consulting[12] summarized results from a local lymph node assay of a hydroalcoholic extract and a lipophilic extract of *C officinalis*. These extracts were intended to test a range of extract compositions. The report stated that a liposoluble extract of *C officinalis* was tested using a local lymph node assay, but no details were provided. The liposoluble extract was described as not sensitizing.

A hydroalcoholic extract of *C officinalis* was tested using guinea pigs (20 animals). Biolab SGS[42] performed a maximization induction with 3 double injections (0.1 mL) of the following: (1) 0.5 g of the extract, (2) 0.5 g of an extract/Freund complete adjuvant (1:1), and (3) 0.5 g of Freund complete adjuvant. On day 7, the 20 pretreated animals were exposed to the extract (0.5 g) and 10 controls received a topical application of distilled water. On day 21, 0.5 g of the extract was applied to the skin of the 20 induced and 10 control animals. In 14 of the 20 treated animals and 10 of 10 control animals, slight erythema was observed, which was attributed to an irritant reaction (no irritation was reported during the induction phase). The hydroalcoholic extract was classified as nonsensitizing.

Photosensitization

Ichimaru Pharcos Co Ltd[18] determined the phototoxicity of a 50% mixture of *C officinalis* flower extract, butylene glycol, and water (neither concentrations nor relative proportions given) using 6 guinea pigs. The test material was applied (0.1 mL) to a site on the back and then exposed to UVB radiation (spectrum not given) for 15 minutes to give a minimal erythemal dose. A control site received UVB radiation only. The *C officinalis* flower extract mixture did not produce phototoxicity.

Ocular Irritation

The CTFA[40] reported the results of a study using 6 rabbits in which a 10% aqueous mixture of *C officinalis* flower extract (volume not stated) was instilled into the conjunctival sac of each animal, without rinsing. The test material was considered a minimal ocular irritant.

The CTFA[41] reported that a study to determine the ocular irritation potential of an eye cream containing 1.0% *C officinalis* flower extract resulted in no-to-minimal irritation in the animals tested (number and species not given).

Chemisches Laboratorium Dr Kurt Richter GmbH[16] stated that a mixture of *C officinalis* flower extract (1%-5%), soybean (Glycine Soja) oil (>50%), and tocopherol (<0.1%) all diluted to 10% in liquid paraffin was not irritating to rabbit eyes in a Draize test.

Ichimaru Pharcos Co Ltd[18] determined the ocular toxicity of a 50% mixture of *C officinalis* flower extract, butylene glycol, and water (neither concentrations nor relative proportions given) using 6 albino rabbits. The test material (0.1 mL) was instilled into the conjunctival sac (not stated if the material was subsequently rinsed). A conjunctival irritation reaction was observed in 1 rabbit.

Natural Product Consulting[12] summarized results from an ocular irritation test of 3 hydroalcoholic extracts and 5

lipophilic extracts of *C officinalis*. These extracts were intended to test a range of extract compositions.

One extract was prepared when plant flowers were macerated for a prolonged time in a water−propylene glycol mixture. According to Biolab SGS,[42] the extract was diluted to 15% in sterile water and instilled into the conjunctival sac of 6 albino male rabbits (0.1 mL per animal). The eyes were examined at 24 hours. No abnormalities were reported, and the water/propylene glycol extract was classified as a nonirritant in this ocular test.

Another material tested was a hydroglycolic extract from dried *C officinalis* flowers. The treatment was performed as described previously using 6 albino male rabbits.[43] Eyes were examined at 1, 24, 48, and 72 hours. In all animals, this extract produced a slight conjunctival congestion at 1 hour, but this disappeared at 24 hours in 4 animals and at 48 hours for the other 2 animals. The hydroglycolic extract was classified as a nonirritant in this ocular test.

Biolab SGS[42] tested a hydroalcoholic extract of *C officinalis* in albino male rabbits (number not stated). The extract was placed in the conjunctival sac (volume not stated). At 1 hour posttreatment, a deep crimson red color and slight chemosis were noted in the conjunctivae. The chemosis disappeared after 48 hours, and the crimson red color disappeared after 7 days. The hydroalcoholic extract was classified as a nonirritant in this ocular test.

An extract of *C officinalis* was prepared by prolonged maceration in a vegetable oil and mineral oil mixture (proportions not given). The extract was diluted to 20% in olive oil and instilled (volume not given) into the eyes of 6 rabbits.[42] Evaluations were made at 1, 24, and 48 hours. The vegetable/mineral oil extract was classified as a nonirritant in this ocular test.

A glycolic extract of *C officinalis* was prepared by prolonged maceration with an esterified oil (oil not identified). The extract was diluted to 20% in water and instilled (volume not given) into the eyes of 6 rabbits.[42] Evaluations were made at 1, 24, 48, and 72 hours, and at 4 and 7 days. The glycolic extract was classified as a nonirritant in this ocular test.

A liposoluble extract of *C officinalis* was tested using 3 rabbits.[42] The extract (0.1 mL) was instilled into the right eye. Evaluations were made at 1, 24, 48, and 72 hours. Slight to well-defined conjunctival irritation was observed following exposure, which resolved after 24 hours. No systemic changes were noted in the 3 animals, and there were no changes in body weights. The liposoluble extract was classified as a minor ocular irritant in this ocular test.

Biolab SGS[42] tested another liposoluble extract using rabbits (number not given). The extract was diluted to 10% in paraffin oil and instilled (volume not given) into the eye. The ocular irritation index was maximum at 24 hours, but there was no irritation at 72 hours. This liposoluble extract was classified as a slight irritant in this ocular test.

The final extract was obtained by prolonged maceration of *C officinalis* flowers in a vegetable oil and tested in an in vitro test using hen's egg chorion−allantoic membrane to evaluate the potential for ocular toxicity. Details of the test were not given. The vegetable oil extract was classified as having no potential for ocular irritation in this in vitro test.

Reproductive and Developmental Toxicity

Published reproductive and developmental toxicity studies on ingredients derived from *C officinalis* were not found, nor were unpublished data provided. Data, however, were available for selected components found in these ingredients.

Bickers et al[45] reviewed data from a reproductive and developmental toxicity study of coriander oil, containing almost 80% linalool and approximately 20% of other terpenes, including camphor, *p*-cymene, α-pinene, γ-terpinene, limonene, geranyl acetate, myrceen, α-terpinol, and camphene. Coriander oil was given to female rats by gavage at 250, 500, or 1000 mg/kg per d from 7 days prior to mating through gestation, delivery, and 4 days postpartum.

Excess salivation was observed in all groups but was statistically significant in the 500 and 1000 mg/kg per d groups, and maternal weight changes and feed consumption changes were noted. Decreases in litter size and pup mortality on day 1 were statistically significant at the high-dose level only.

This review further presented data from a 28-day subchronic toxicity study using rats. Coriander was given by gavage at 160, 400, and 1000 mg/kg per d. There were reported increases in liver weights, degenerative lesions in the renal cortex, and a high incidence of slight periportal hepatocellular cytoplasmic vacuolization. The no observed effect level (NOEL) was determined to be 160 mg/kg per d. Macro and microscopic examination of the reproductive organs uncovered no adverse effects at the high dose of 1000 mg/kg per d.

The review concluded that the adverse effects on reproduction and development occurred at maternally toxic levels and not at levels that were not maternally toxic.[45]

Genotoxicity

Elias et al[46] performed an Ames test of 6 saponins isolated from the dried flowers of *C officinalis* using *Salmonella typhimurium* TA98, with and without metabolic activation. No increase in mutation frequency of the test substances was found compared with controls. In a preliminary spot test using *S typhimurium* TA97, TA98, TA100, and TA102, no evidence of toxicity was found.

Graf et al[47] performed somatic mutation and recombination assays using *Drosophila melanogaster* exposed to *C officinalis* herbal tea extract at 20% and 40% in their drinking water. No increase in mutation frequency was found in either assay. A test of 2 flavonols, quercetin and rutin, produced weak genotoxic activity in these assays.

Ramos et al[48] studied the genotoxicity of an extract of *C officinalis* L using 3 short-term assays: an Ames test in *S typhimurium* TA1535, TA1537, TA98, and TA100, with and without metabolic activation; a mitotic segregation assay in a diploid strain (D-30) of *Aspergilis nidulans*; and a mouse micronucleus test. Dried marigold flowers were extracted using

4 rounds of hydroalcoholic percolation. The extract contained 101 mg/mL plant solids and 60% ethanol (vol/vol).

For the Ames test, the ethanol was evaporated and the solid material redissolved in dimethyl sulfoxide (DMSO) to a final concentration of 50 mg/mL and added to culture plates at 50 to 5000 μg/plate. Positive controls appropriate for each *S typhimurium* strain tested, with and without metabolic activation, were used. For example, for strain TA1535, sodium azide was the positive control without metabolic activation, and cyclophosphamide was the positive control with metabolic activation.

For the mitotic segregation assay, the extract in ethanol was added to complete media at 0.1, 0.2, 0.4, 0.8, and 1.0 mg/mL. An untreated control was performed and ethanol served as the vehicle control. Methylmethane sulfonate was the positive control. Conidia were inoculated to each plate by a single puncture at the center, and incubation was done at 37°C for 72 hours for toxicity evaluation and for 6 to 10 days for mitotic segregation.

For the mouse micronucleus test, Swiss mice (5 weeks old, 20-25 g) were fed a rodent diet and given water ad libitum. The extract in ethanol was given orally at 10 mL/kg at dilutions that yielded doses of 0.25, 0.50, and 1.0 g/kg of plant solids. An untreated control was performed, and ethanol served as the vehicle control. Cyclophosphamide was the positive control. Five animals of each gender were used in each exposure group. Two administrations were given, and the animals were killed at 24-hour intervals. Bone marrow smears were prepared, and the number of micronuclei in polychromatic erythrocytes were recorded (1000 counted per animal). The ratio of polychromatic-to-normochromatic erythrocytes was determined by counting the normochromatic erythrocytes present for each 250 polychromatic cells in each slide.

The authors reported that there was no increase in mutation frequency in any of the Ames tests, with or without metabolic activation, for any concentration of the extract. In each case, the positive controls yielded the expected results. A statistically significant and concentration-dependent increase in mitotic segregation (sectors per colony) was reported. The authors also reported a dose-dependent increase in the number of adjacent-color sectors that they considered indicative of somatic crossing over. The authors reported no increase in micronuclei in polychromatic cells and no change in the ratio of polychromatic-to-normochromatic cells.[48]

Carcinogenicity

Avramova et al[5] reported that *C officinalis* flower extract (propylene glycol extraction solvent) was tested for carcinogenesis in a study done in 1985 (the original study was not available). Few details were given. One study used B_6 rats (50 of each gender tested, 50 controls) given the extract 0.15 g/kg orally with food for 21 months. Another study used golden hamsters (50 of each gender tested, 50 controls) given the extract 0.15 g/kg orally with food for 18 months. No carcinogenic effect was found.

The International Agency for Research on Cancer (IARC) evaluated the human carcinogenicity potential of coumarin, concluding that this chemical is not classifiable as to its carcinogenicity to humans.[49] The evaluation did state that there is limited evidence in experimental animals for the carcinogenicity of coumarin. In particular, in mice of 1 strain, it produced increases in lung adenomas and carcinomas in both males and females and in hepatocellular adenomas in females. There was no increase in tumor incidences in another strain of mouse. In 1 study in rats, coumarin produced a low incidence of renal tubule adenomas in males, seen only after step sectioning of the kidney.

Hard et al[50] reevaluated kidney tumors and renal histopathology in a 2-year rat carcinogenicity study (National Toxicology Program [NTP]) of quercetin in male and female rats given 0, 1000, 10 000, and 40 000 ppm daily. The authors undertook the work in an attempt to understand the mechanism that may have been responsible for the slight increase in renal tubule tumors and an increase in the severity of chronic nephropathy. They noted that the original finding of adenomas (and 1 adenocarcinoma in the high-dose group) was predicated on an extended evaluation of the kidney by step sectioning to produce multiple samples for each kidney. The authors confirmed the original findings and suggested that the 2 findings were linked. Quercetin, in this model, exacerbates chronic nephropathy, leading to renal tumors and was considered a secondary mechanism for renal tumor development. The authors also suggested that the single adenocarcinoma, along with a group of 4 other lesions (ranging from hyperplasia to adenomas) had a unique phenotype associated with neoplasms of spontaneous and familial origin.

Antitumor Activity

In their study of cytotoxicity, Boucaud-Maitre et al (1988) also examined the effect of 5 extracts of *C officinalis* on mouse Ehrlich ascites carcinoma. They reported that 1 extract had no effect on tumor growth and 3 extracts were minimally effective in limiting tumor growth. The fifth extract (the most saponin-rich extract) was effective in curtailing tumor growth.

Clinical Assessment of Safety

Dermal Irritation

The CTFA[41] reported a study of the dermal irritation potential of a cosmetic formulation containing 1.0% *C officinalis* flower extract using 14 participants. No adverse reactions were observed, and the material was given a primary irritation index of 0.0.

Hilltop Research[51] tested the cumulative irritation potential of a cosmetic formulation containing 1.0% *C officinalis* flower extract using 13 participants (3 male, 10 female). The test material (0.2 mL) was applied to a site on the back of each participant for 23 hours under an occlusive patch. This was repeated daily for 20 days. The test sites were scored 1 hour after patch removal. The report stated that the cosmetic

formulation containing 1.0% *C officinalis* flower extract was a mild material.

The CTFA[52] stated that a cosmetic eye product containing 1.0% *C officinalis* flower extract tested in a 4-day mini-cumulative irritation test (under occlusion) had a primary irritation index of 0.24.

Derma Consult GmbH[53] tested an extract obtained by prolonged maceration of *C officinalis* flowers in a vegetable oil in 50 healthy, adult human volunteers. A single application of the test material (100% and 10% concentrations) was made to the skin of the back under occlusive conditions for 48 hours. Water was the negative control and 1% sodium dodecyl sulfate (SDS) in water was the positive control. No reactions were reported to the test material or water. Sodium dodecyl sulfate exposures produced the expected erythemal reactions. The vegetable oil extract was classified as very well tolerated.

Sensitization

Rodriquez and Mitchell[54] applied commercial grade absolute of calendula, 1.0% in petrolatum, to 3 participants who were sensitive to numerous Compositae species and sesquiterpene lactones and to 6 patients with eczema. The authors described an absolute as a highly concentrated refined perfume material that has undergone at least 2 alcohol extractions. No positive reactions to the calendula absolute were observed.

The CTFA[55] reported a study in which an eye cream containing 1.0% *C officinalis* flower extract was tested in a repeated insult patch test (RIPT) using 109 participants (11 males, 98 females). The test material was applied (0.1 mL) to a test site on the back under an occlusive patch for 24 hours. This was repeated 3 times per week for 3 weeks. Following a 2-week nontreatment period, a challenge patch was applied to a previously unpatched site for 24 hours. The site was scored 24 and 48 hours after removal of the challenge patch. No erythematous reactions were observed during induction or challenge, and the eye cream was not considered a sensitizer.

TKL Research[56] conducted an RIPT of a cosmetic formulation containing 1.0% *C officinalis* flower extract using 102 participants. Induction and challenge were done as described earlier. One participant had a reaction at challenge indicative of a possible sensitization response. On re-challenge using occlusive and semiocclusive patches, the reaction was considered irritation and not sensitization and the material was not considered a sensitizer.

de Groot et al[57] reported the results of a multicenter sensitization study of 119 participants with allergic contact dermatitis using the European standard series of materials and 1 10% Calendula extract in alcohol. The extract caused a positive reaction in 1 participant.

Bruynzeel et al[58] reported the results of a sensitization study of 1032 participants from 6 patch test centers. The European standard series of materials and several ointment bases were tested, and 1 of the ointment bases contained calendula tincture (10%). Two participants had positive reactions to the calendula tincture and 1 of these also had a positive reaction to wool fat. The authors expressed concern about the relevance of the findings because participants often do not know whether they have previously used an ointment and because the ointments may not be a suitable vehicle for such testing.

Wrangsjö et al[59] performed a patch test using 15 dermatitis patients with the European standard series and a 10% calendula extract in petrolatum, calendula pollen, and calendula flowers (fresh and frozen for 6 months). Test material was applied for 24 hours, and the test sites were scored at 20 and 60 minutes, and at 48 and 96 hours. The calendula extract, the calendula pollen, and both the fresh and frozen flowers produced a positive reaction in 1 participant.

Paulsen et al[60] included a sesquiterpene lactone mix (0.1% in petrolatum) in a standard patch test series used on 686 clinical patients. Of 79 patients with a reaction to the sesquiterpene mix or who were suspected of having Compositae dermatitis were tested with a Compositae mix (6% in petrolatum). Of these 79 patients, 31 had positive reactions to one or both mixes. One patient with a demonstrated Compositae allergy was patch tested with 10% *C officinalis* L and had no response.

Component Safety Assessments

Table 7 briefly presents the *C officinalis* components for which safety assessments have been completed.

The CIR Expert Panel has evaluated the safety of fatty acids (oleic, lauric, palmitic, myristic, and stearic) used in cosmetics and found them to be safe as used[62] and reaffirmed that conclusion.[69]

The CIR Expert Panel has evaluated the safety of paraffin and other waxes and found them to be safe as used and reaffirmed that conclusion.[64,71]

Data on *p*-hydroxybenzoic acid are available in the CIR Expert Panel safety assessment of parabens (2006), which were found safe for use in cosmetics as preservatives at concentrations higher than would be present in cosmetics from the use of *C officinalis* derivatives.

Data on salicylic acid are available in the CIR Expert Panel safety assessment of this ingredient, which found salicylic acid and its salts and simple esters to be safe when formulated to avoid irritation and increased sun sensitivity.[45]

The CIR Expert Panel reviewed the safety of pyrogallol and found this ingredient safe in the practices of use (oxidative hair dye) and concentration (up to 5%).[72] The CIR is awaiting completion of an NTP report on further testing. The CIR Expert Panel reviewed the safety of pyrocatechol (aka catechol) and found this ingredient to be unsafe for use in leave-on products (carcinogenicity and co-carcinogenicity concerns) and concluded that the available data were insufficient to support its safety in hair dyes.[61]

The CIR Expert Panel reviewed the safety of t-butylhydroquinone and found that it may be used safely as a cosmetic ingredient up to 0.1% and this conclusion was reaffirmed.[65]

In the safety assessment of polyethelyne glycol (PEG) soy sterols, data on the safety of plant sterols were considered,

Table 7. Components of *C officinalis* for Which Safety Assessments Have Been Performed

Component	Finding	Reference
t-butylhydroquinone	Safe as a cosmetic ingredient at concentrations up to 0.1%	CIR 2007
Catechol (aka pyrocatehol)	Unsafe for use in leave-on products (carcinogenicity and co-carcinogenicity concerns) and concluded that the available data were insufficient to support its safety in hair dyes	61
Coumarin	Not classifiable as to its carcinogenicity to humans	49
Fatty acids (oleic, lauric, palmitic, myristic, and stearic)	Safe in the present practices of use (up to 25% for lauric and palmitic acids; up to 50% for oleic and myristic acids; >50% for stearic acid	62,63
p-hydroxybenzoic acid	Breakdown product of parabens, which were found safe for use in cosmetics (0.4% when used singly or 0.8% when used in combination)	CIR 2006
Linalool	Safe at up to 4.3% (20% in a consumer fragrance)	Bickers et al[45]
Paraffin	Safe as used (up to 99%)	Elder[64]
Pyrogallol	Safe as an oxidative hair dye (concentrations up to 5%)	Elder[65]
Salicylic acid	Safe when formulated to avoid irritation and increased sun sensitivity (uses up to 3%)	66
Tocopherol	Safe as used (up to 5%)	67
Sterols (PEGs soy sterols and cholesterol)	PEGs Soy Sterols are safe as used (up to 2%) in cosmetics Cholesterol is safe as used (up to 3%) in cosmetics	68 69,70

which demonstrated an absence of estrogenic activity and suggested that compounds such as β-sitosterol were not present in biologically relevant levels.[68] Cholesterol in cosmetics was found safe as used in cosmetics,[70] as was tocopherol.[67]

The Research Institute for Fragrance Materials (RIFM) expert panel reviewed the available safety data (acute oral and dermal toxicity, subchronic oral and dermal toxicity, genotoxicity, reproductive and developmental toxicity, and dermal irritation, sensitization, photoirritation, and photosensitization) for linalool and its related esters when used as fragrance ingredients and concluded that there were no safety concerns at the current levels of use and resulting exposure.[45]

Safety Assessment Approach for Botanicals

Quercetin

The Council of Europe[73] Committee of Experts on Cosmetic Products published a monograph on quercetin, noting that this chemical may be found in many plants, including *C officinalis*. Based on the information the committee reviewed, quercetin was genotoxic in vitro but not in vivo. They suggested that rapid metabolic inactivation may explain the different genotoxicity findings. Some evidence for carcinogenicity (renal tumors) was found in one of several studies, in one species (rat), in one gender (male). The antioxidant properties of quercetin were noted, as were its estrogenic properties, consistent with that of other flavonoids. Overall, the committee concluded that quercetin did not present potential risks for human health, but that skin effects and dermal penetration data are needed to complete the toxicological profile.

Harwood et al[74] reviewed the available quercetin genotoxicity data, with a focus on reconciling the results of in vitro studies, which consistently demonstrate quercetin mutagenicity, and in vivo studies, which demonstrate that quercetin is not carcinogenic. As in the above evaluation, these authors identified the mechanisms, including glutathione conjugation, protein complexed metal ion such as copper and iron, and biodegradation, which limit the antioxidant properties of ingested quercetin. The authors acknowledged the findings of Kitamura et al[75] in which 1% quercetin in the diet of rats prone to accumulate copper exacerbated renal tubular necrosis. After reviewing available metabolic and other data, including the Hard et al[50] review of an NTP 2-year carcinogenicity study presented earlier in this document, the authors concluded that the weight of evidence supports a finding that quercetin, at estimated dietary levels as a dietary supplement (200-1200 mg/d) would not produce adverse health effects.

Calendula

The European Organization of Cosmetics Ingredients Industries and Services (UNITIS) developed a method of evaluating the safety of cosmetic ingredients derived from plant materials that involves the following: (1) determining all fractions and compounds identified for the particular plant; (2) developing a safety profile for each fraction/compound; and (3) evaluating skin toxicity studies on those fractions/compounds that may present a risk.[12]

In the UNITIS report, this approach was used for the plant *C officinalis*. Step (1) was completed with the list of fractions, subfractions, and compounds listed in Table 2 earlier in this report. Although not a part of the approach described, the report went on to provide information on general safety considerations for *C officinalis.* This information was not different from that presented earlier in this report.

Step (2) in the process was to develop a safety profile for each fraction. Information provided by the UNITIS[12] was combined with information from the Research Institute for Fragrance Materials and from previous safety assessments conducted by the CIR.

Mineral Matter—based on a low dermal absorption for minerals and their salts, these were not considered to present any danger. Neither toxic minerals nor heavy metals were considered present at a significant rate, unless by contamination, according to the UNITIS report.

Carbohydrates—arabinogalactan was nontoxic in rats and mice and human consumption produced no adverse effects, only a positive effect on fecal chemistry, and some bloating and flatulence at high intakes. Arabinogalactan was not mutagenic in an Ames test. The UNITIS considered that the carbohydrates that may be present in *C officinalis* were safe.

Fatty Acids—the fatty acids that may be present were among those that are essentially nontoxic and were considered by the UNITIS to be safe for cosmetic preparations.

Hydrocarbons/paraffins/waxes—the UNITIS report acknowledged that data are lacking on which to base an evaluation, but notes that the level was sufficiently low as to present no risk. Having said data were lacking, the UNITIS report went on to cite CIR Expert Panel safety assessments of paraffin and various waxes in which CIR concluded that these ingredients were safe as used in cosmetics.

Phenolic acids—the UNITIS report focused on caffeic acid, ferulic acid, chlorogenic acid, gentisic acid, benzyl derivatives, including *p*-hydroxybenzoic acids, salicylic acid, and courmaric acid. The principal argument for safety of these components rested on their low concentration, in the 0.01% range, in the dry plant material itself. The UNITIS report noted that caffeic acid and ferulic acid (cinnamic acid derivatives) did penetrate skin, had UV protoprotective activity, and that the 1993 IARC report stated that there was evidence for carcinogenicity for caffeic acid in animals and the effect in humans is not conclusive.[76]

Chlorogenic acid is an antioxidant that inhibited tumor promotion by phorbol esters in mice. Although some controversy existed over allergic reactions to chlorogenic acid in green coffee beans, it was accepted that chlorogenic acid is not the allergen. Gentisic acid is a metabolite of aspirin in humans and there were no known adverse effects.

The biological activity of substituted benzyl derivatives, including *p*-hydroxybenzoic acid, was only briefly described.

Salicylic acid biological activity was also briefly described. The UNITIS report noted that salicylic acid was approved for use in Europe at levels up to 0.5%.

Courmaric acid and the rest of the phenolic acids were negative in bacterial genotoxicity tests. Evidence was found for genotoxicity (chromosome aberrations) in mammalian cells in vitro, but no evidence was found in vivo.

Flavonoids—epidemiological studies implicated high dietary intake levels of flavonoids in heart disease, but a study of cancer risk failed to find a link.

Based on some evidence of genotoxicity in bacterial assays, further studies were described. Among the flavonoids found in *C officinalis*, quercetin and kaempferol were positive in Ames testing. Genotoxicity for these chemicals also was reported in other bacterial assays, in yeast, and in fruit flies. Quercetin and kaempferol were genotoxic in mammalian cell assays. The UNITIS report stated that flavonoids do not appear to be genotoxic to mammals in vivo.

The UNITIS report acknowledged that an NTP study suggested some evidence of carcinogenic activity (renal tubular cell adenomas) in rats that received 4% quercetin in the diet, but that most other studies failed to find any evidence of carcinogenesis. A recent reevaluation of the NTP study suggested that some adenomas and the 1 adenocarcinoma were phenotypically similar to tumors of spontaneous or familial origin.

The flavonoids are not considered significant allergens. Quercetin was demonstrated to reduce histamine release from antigen-induced human basophil cells.

The UNITIS report stated that the toxicity data on flavonoids, particularly quercetin, were lacking, but they argued that the low dermal penetration suggests that any risks would be low.

Tannins—based on an absence of data in humans and limited evidence of carcinogenicity in animals, the UNITIS report acknowledged that IARC concluded that tannins are not classifiable as to their carcinogenicity. The UNITIS report suggested that tannins applied to the skin should be considered safe.

Coumarins—the UNITIS report noted the paucity of toxicology data relevant to the topical use of these compounds. The UNITIS report stated that coumarins are uncommon in the genus *Calendula* and that those that were found were also widespread in other plants. The risk of adverse effects was considered low. The IARC's evaluation of the human carcinogenicity potential of coumarin, discussed earlier, concluded that this chemical was not classifiable as to its carcinogenicity to humans.[49]

Sterols and steroids—the UNITIS report relied on the FDA GRAS determination for plant sterol/stanol esters and the conclusion of the European Commission Scientific Committee on Foods that phytosterol esters in margarine were safe for human use. The UNITIS report cited many of the same studies that were considered by the CIR Expert Panel in their evaluation of PEGs soy sterols, including the concern about the potential activity of β-sitosterol[68] and the evaluation of cholesterol (Andersen 2006).[70]

Monoterpenes—the UNITIS report stated that monoterpenes are key components of essential oils. The report acknowledges that these chemicals may be skin irritants but suggests that the low concentration of essential oils in *C officinalis* (0.2%-0.4%) means that they may be considered safe. One of the monoterpenes, linalool, has been listed as a fragrance allergen by the European Commission. The UNITIS report presented the information that linalool makes up only 0.21% of the *C officinalis* essential oil, resulting in less than 0.001% in dry flowers.

Based on the low concentration, linalool was not considered to present a risk of allergenicity.

Sesquiterpenes—some evidence of cytotoxicity in human cell cultures was reported, but no other evidence of toxicity was found. β-caryophyllene was reported to have a gastric cytoprotective effect against necrotizing agents. The UNITIS report suggested that the chemicals of concern in this group would be the sesquiterpene lactones but noted that these chemicals are not found in *C officinalis* essential oils.

Carotenoids—these compounds were not included in Table 5, but the UNITIS report noted that these pigments are responsible for the bright colors of orange and yellow marigolds and may be present at levels from 0.2% to 4.7%. Although the concentration may be significant, the report states that there is no evidence of adverse biological activity associated with carotenoids. Acute, short-term, and subchronic toxicity studies of lutein and lutein esters using rats described earlier found no evidence of toxicity.[36]

Triterpenic Alcohols—the UNITIS report noted that studies suggest hepatoprotective and anticarcinogenic activity for lupeol, but that otherwise there is a lack of toxicity studies and data, making any conclusion about the safety of these compounds (present at concentrations <5% in *C officinalis*) difficult.

Triterpenic glycosides and saponins—limited data from a study of the antiulcerous activity of calenduloside B found no adverse biological activity. No data were available for the other glycosides or saponins found in *C officinalis*.

Tocopherols—the UNITIS report cited many of the same data that were used by the CIR Expert Panel in its safety assessment of tocopherol and its esters and ethers[67] and concluded that this *C officinalis* component can be considered safe.

Quinones—the UNITIS report stated that there are limited safety data on the 2 phytyltoluquinone isomers, but that tocopherolquinone is a metabolic by-product of vitamin E and should be considered safe.

Organic Acids—the UNITIS report cited the CIR Expert Panel safety assessment of malic acid and of ascorbic acid, noting that accepted levels in those assessments are well above 6 and 3 ppm, respectively, and that these acids are present in *C officinalis*. The UNITIS report did not comment on amino acids, which were stated to be 4.5% of dry matter, or resins, which were stated to be 3.4% of dry matter.

The UNITIS report concluded that most of the fractions and subfractions may be considered safe. Several were considered of low risk, including carbohydrates and lipids, flavonoids, and coumarins. Others were considered of intermediate risk, including triterpene alcohols and saponins and quinones.

To address these areas of concern, several safety studies were undertaken and reported earlier in this report. Those studies include an in vitro ocular tolerance test; animal ocular irritation, skin irritation, and skin sensitization; and a human skin tolerance test on both hydroalcoholic and lipophilic extracts.

Water/alcohol extracts are considered rich in carbohydrates, flavonoids, tannins, coumarins, triterpenic saponins, and organic acids. Lipid extracts are rich in fatty acids, hydrocarbons, monoterpenes, sesquiterpenes, sterols and steroids, carotenoids, triterpenic alcohols, tocopherols, and quinones.

The authors of the UNITIS report also stated that, considering those fractions and subfractions known to be safe and those for which test data were provided, the use of extracts of *C officinalis* in cosmetics does not present a risk to consumers.

Threshold of Toxicologic Concern Analysis

Re et al[77] applied a threshold of toxicologic concern approach to evaluating the safety of Calendula flowers and *C officinalis* extracts. As a starting point, these authors considered the list of chemical constituents given in Table 3 of this report.

In recognition that Calendula flowers are rich in carotenoids, but that specific carotenoids are not listed in Table 3, these authors created a list of carotenoids present in Calendula flowers from Duke (1996) and Kishimoto et al.[9] Based on the original proposal by Cramer et al (1978) that there are different thresholds for toxicity for different compounds, these authors described 3 classes of compounds: class I—1800 μg per person per day; class II—540 μg per person per day; and class III—90 μg per person per day.

For those chemicals identified in the National Library of Medicine's ChemID database, a SMILES (simplified molecular input line entry specification) notation was used as input to the ToxTree[78] open-source application software. Molecular weight and octanol:water partition coefficient data were used to predict dermal penetration. To be conservative in approach, it was assumed that all of a component extracted into a vehicle would be available for absorption. Exposure was predicated on a use concentration of 0.1% in formulation and a formulation usage of 18 g/d.

The authors noted several exceptions to the software prediction of Cramer class, including longispinogen and ursodiol (predicted as Cramer class III, but more properly in class I) and tocopheols (predicted as Cramer class II, but more properly in class I).

The authors assumed that several categories of chemicals that could be isolated from Calendula flowers would not pose any safety concern. These include mineral matter (~9%); high molecular weight carbohydrates, for example, mucilage (12 25%); fatty acid esters (5%); amino acids (4.5%); resins (3.4%); components with molecular weights greater than 1000 (because skin absorption is negligible); inert plant material, for example, cellulose; and components present at less than 0.5%.

Table 8 compares the calculated systemic exposure of Calendula constituents with the Cramer toxicity class of that constituent. The systemic exposures for quercetin, kaempferol, and isorhamnetin were higher than the 90 μg/d established for Cramer class III.

Systemic exposures for all other components in Table 8 were expected to produce systemic exposures below the threshold of toxicologic concern. The authors suggested that little

Table 8. Threshold of Toxicologic Concern Analysis of Calendula Constituents[77]

Constituent	CAS No	Molecular Weight	Calculated logP	Estimated J_{max}	Default % Absorption	Cramer Toxicity Class	Systemic Exposure (μg/d)
Flavonoids < 1.5%							
Astragalin	480-10-4	448.378	0.49 ± 1.11	<0.1	10	III	<27
Hyperoside	482-38-0	484.377	0.30 ± 1.34	<0.1	10	III	<27
Isoquercitrin	21637-25-2	484	–0.1	<0.1	10	III	<27
Isorhamnetin	480-19-3	316.264	2.39 ± 0.54	>0.1 - <1.0	40	III	<108
Neohesperidin (surrogate forneoliesperoside)	13241-33-3	610.518	0.05 ± 1.60	<0.1	10	III	<27
Narcissin	604-80-8	624.546	0.45 ± 1.35	<0.1	10	III	<27
Quercetin	117-39-5	302.237	1.48	>0.1 - <1.0	40	III	<108
Rutin	153-18-4	610.518	–2.02	<0.1	10	III	<27
Syringenin (surrogate for syringentin)	118-34-3	372.368	–0.45 ± 1.35	<0.1	10	III	<27
Typhaneoside	104472-68-6	770.686	7.57 ± 1.23	<0.1	10	III	<27
Tannins 6%-10%							
Pyrogallol and catechol types	N/A	>500	Various	<0.1	10	III	180
Free and esterified triterpenic alcohols < 5%							
Arnidiol	6750-30-7	442.723	7.57 ± 1.23	<0.1	10	I	<90
Calenduladiol	10070-48-1	442.723	7.65 ± 1.17	<0.1	10	I	<90
Erythrodiol	545-48-2	442.723	7.71 ± 0.76	<0.1	10	I	<90
Faradiol	2054-95-4	442.723	7.53 ± 0.87	<0.1	10	I	<90
Longispinogenin	465-94-1	472.7486	8.91 ± 0.76	<0.1	10	I	<90
Lupeol	545-47-1	426.724	9.23	<0.1	10	I	<90
Maniladiol	595-17-5	442.723	7.65 ± 0.62	<0.1	10	I	<90
Pseudotaraxasterol or psi-taraxasterol	464-98-2	426.724	8.91 ± 1.31	<0.1	10	I	<90
Taraxasterol	1059-14-9	426.724	8.69 ± 1.46	<0.1	10	I	<90
Ursasiol	128-13-2	382.576	3.00	>0.1 - <1.0	40	I	<350
α-Amyrin	636-95-9	426.724	9.16	<0.1	10	I	<90
β-Amyrin	559-70-6	426.73	9.19	<0.1	10	I	<90
Triterpenic Glycosides 2%-10%							
Calenduloside	34381-96-1	943.125	2.49 ± 2.46	<0.1	10	II	180
Carotenes							
β-carotene	7236-40-7	536.87	17.62	<0.1	10	II	<90
ζ-carotene	N/A	540.92	15.7	<0.1	10	I	<90
Lycopene	502-55-8	536.87	17.64	<0.1	10	I	<90
Lutein	127-40-2	568.87	14.82	<0.1	10	II	<90
Citroxanthin	515-06-0	552.87	9.2	<0.1	10	III	<90
Flavoxanthin	512-29-8	584.87	13.22	<0.1	10	III	<90
Violaxanthin	126-29-4	600.87	11.98	<0.1	10	III	<90
Antheraxanthin	640-03-8	584.87	9.46	<0.1	10	III	<90
Rubixanthin	3763-55-1	552.87	11.45	<0.1	10	III	<90

concern should exist for the 3 chemicals that exceeded that threshold because they (1) did not exceed the level for their Cramer class by much, (2) the assumptions in estimating systemic exposure were conservative, especially skin penetration, and (3) the no observable adverse effect levels (NOAELs) for quercetin and kaempferol were around 400 mg/kg per d.

For a number of other chemicals in Table 3, published NOAELs are available that suggest the absence of toxicity at levels well above the threshold of toxicologic concern. These include coumarin at 10 mg/kg per d; lutein at 400 mg/kg per d; and tannic acid at around 125 mg/kg per d. The authors also noted the established acceptable or tolerable food intake values for coumarin (1.2 mg/d), α-tocopherol (279 mg/d), and β-carotene (5 mg/kg/d).

There are components that may be present in Calendula flowers for which the percentage of total material is not known (see Table 3), including coumarins, tocopherols, and quinones. Predicated on a systemic exposure that would not exceed the threshold of toxicologic concern for each of these chemical categories, the authors back calculated to determine the maximum allowable concentration in the dried flowers. For coumarins, the maximum allowable concentration in the dried flowers was 0.6%; for tocopherols, 100%; and quinones, 5%, to not exceed the threshold of toxicologic concern.

To further illustrate the application of the threshold of toxicologic concern for cosmetic products, the authors examined faradiol monoesters. As reported by European Medicines Agency (EMEA),[79] a supercritical CO_2 extract of Calendula flowers contains 21% of faradiol monoesters, suggesting a faradiol concentration of 11.9%. The use of this extract at 1% in a facial toner applied at a rate of 1.6 g/d would produce a systemic exposure of 190 μg/d. The use at 1% in a leave-on body lotion applied at 9.6 g/d, would produce a systemic exposure of 1142 μg/d. The use at 1% in a leave-on applied full body at 18 g/d would result in a systemic exposure of 2142 μg/d. Only in this latter case, would the threshold of toxicologic concern of 1800 μg/d be exceeded.

The authors acknowledged that chemicals present in Calendula flower extracts at levels below 0.5% were not further evaluated because they would result in exposures less than 90 μg/d, by definition below the lowest established threshold of toxicologic concern. Were an extract to have concentrated levels of such minor chemicals, however, an evaluation using the approaches outlined would be warranted.[77]

Summary

The cosmetic ingredients derived from *C officinalis* include *C officinalis* extract, *C officinalis* flower, *C officinalis* flower extract, *C officinalis* flower oil, and *C officinalis* seed oil. Many of these materials retain the carotenoid-based orange or yellow color of the flowers.

In 1 complete analysis of the components that may be found in *C officinalis*, more than 150 separate chemicals were identified in different fractions and subfractions that included minerals, carbohydrates, lipids, phenolic acids, flavonoids, tannins, coumarins, sterols and steroids, monoterpenes, sesquiterpenes, triterpenes, tocopherols, quinones, amino acids, and resins. Which of those fractions will be present in a given extract depends on the method of extraction. Currently used extraction methods include lipophilic, water-alcoholic, and supercritical CO_2 extractions.

Lipophilic extractions (eg, with vegetable or mineral oil, octyl palmitate) will include lipophilic hydrocarbons; paraffins; fatty acids and fatty acid esters; steroids; tocopherols; apolar carotenoids; mono-, sesqui-, and triterpenoid esters; and triterpene mono-alcohols and diols. More polar triterpenoid triols, oxygenated carotenoids, and phenolic acids may be found, but extraction likely would not be complete. Hydroethanolic extracts prepared by maceration and percolation will contain medium-polar and polar classes such as flavonoids, terpenoid glycosides, carotenoids, coumarins, phenolic acids, and tannins. Extractions with butylene glycol or propylene glycol and water will contain flavonoid and terpenoid glycosides, some polar carotenoids, phenolic acids, tannins, amino acids, and polysaccharides. Supercritical CO_2 extracts will contain low-polarity compounds, with some medium polarity ones, but the specific compounds will depend on pressure and co-solvents, such as ethanol.

Catechol and pyrogallol, coumarins (esculetin, scopoletin, and umbelliferon), and α-tocopherolquinone, although listed as chemicals found in *C officinalis*, were not found in assays of extracted Calendula flowers.

Extracts and oils absorb in the UVC region of the spectrum and in the visible spectrum as dictated by the color of the preparation. One extract had an absorption peak at 283 nm in the UVB region, but below the wavelength cut-off of 290 nm imposed on sunlight by the earth's atmosphere. Another extract had an absorption peak at 378 nm, but the absorbance was 5 times lower than the expected peak at 272 nm. Extracts may contain heavy metals at low levels, that is, 10 ppm or less.

The most frequently used *C officinalis* derivative is *C officinalis* flower extract, used in 295 cosmetic formulations, generally at concentrations from 0.0001% to 0.8%, although a 6% concentration was reported in 1 calendula balm product. *Calendula officinalis* flower oil is used in 39 products at concentrations from 0.02% to 0.1%. *Calendula officinalis* extract is used in 11 products and *C officinalis* flower is used in 9 products, but use concentrations are not available. Calendula wax and Calendula fluid extract are reported to be used but do not correspond to any of the listed ingredients. *Calendula officinalis* seed oil is not reported to be used.

Calendula officinalis L is a GRAS food seasoning. *Calendula officinalis* is used in herbal medicine for a wide range of reported effects. One study of wound-healing effects concluded that the unguentum vehicle performed better than the *C officinalis* derivative in unguentum.

No data are available on the absorption, distribution, metabolism, or excretion of *C officinalis* derivatives.

Calendula officinalis extracts were cytotoxic in 1 study and had immunostimulating properties in another. The oral LD_{50} for *C officinalis* extract was greater than 4650 mg/kg in rats. The oral LD_{50} was greater than 5000 g/kg for a hydroalcohol extract of *C officinalis* L in rats and mice. The intraperitoneal (ip) LD_{50} for *C officinalis* extract was 300 mg/kg.

No deaths were found in any dose group, and there were no signs of morbidity in a study of male Wistar rats given doses of 0.025, 0.25, 0.5, and 1.0 g/kg of a hydroalcoholic extract of *C officinalis* orally for 30 days. Some hematology results were significantly different from controls at certain dose levels, but the results were not dose dependent and were within the normal range for these parameters. Blood urea nitrogen levels were significantly increased in a dose-dependent manner at 0.25 g/kg and above, and centrilobular liver cells with acidophilic cytoplasm and heterochromatic nuclei, and periportal inflammation (leucocytes, plasmacytes, and some mononuclear cells) were reported at the 1.0 g/kg dose only. In an acute toxicity study using rats, lutein and lutein ester did not produce any mortality at doses up to 4 g/kg.

A reproductive and developmental toxicity study of coriander oil, containing almost 80% linalool and approximately 20% of other terpenes reported developmental toxicity only at maternally toxic levels. The NOEL was determined to be 160 mg/kg per d. Gross and microscopic examination of the reproductive organs uncovered no adverse effects at the high dose of 1000 mg/kg per d.

Ames tests of material isolated from the dried flowers of *C officinalis* were negative. A somatic mutation and recombination assay using *Drosophila melanogaster* exposed to *C officinalis* herbal tea extract was negative, but quercetin and rutin produced weak genotoxic activity. An extract of *C officinalis* was negative in a mouse micronucleus test but was positive in a mitotic segregation assay using *Aspergilis nidulans*.

Calendula officinalis flower extract (propylene glycol extraction solvent) reportedly was not carcinogenic in rats in a 21-month or in hamsters in an 18-month feeding study. Some extracts of *C officinalis* inhibited growth of mouse Ehrlich ascites carcinoma. Coumarin was not classifiable as to its carcinogenicity to humans according to the International Agency for Research on Cancer.

Calendula officinalis extract at concentrations up to 10% aqueous, cosmetic products containing 1.0% *C officinalis* extract and mixture of *C officinalis* extract (up to 5%) were neither irritating nor sensitizing in animal tests. One series of studies examined the irritation and sensitization of *C officinalis* extracts performed with hydroalcoholic and lipophilic extractions to generate different distributions of fractions and subfractions. In one or the other extraction, a high level of each fraction or subfraction was present, yet none of the extracts were irritants or sensitizers.

Lipophilic extracts prepared in this series of studies were mild ocular irritants, but no ocular irritancy was observed for the hydroalcoholic extracts. Ocular tests of 10% aqueous *C officinal* extracts, a cosmetic product with 1.0% *C officinalis* extract, and mixtures containing up to 5% *C officinalis* extract were all negative.

A 50% mixture of *C officinalis* extract was not phototoxic in guinea pigs exposed to a minimal erythemal dose of UVB radiation.

In clinical testing, cosmetic formulations with up to 1.0% *C officinalis* extract were not irritating in short-term tests, not irritating in cumulative irritation tests, and not sensitizing in RIPT tests. Predictive testing for allergic reactions among dermatitis patients uncovered 1 of 109 positives in one study, 2 of 1032 in another, and 1 of 15 participants in a third. Sesquiterpene lactones elicited around 10% positive reactions in 686 patients, many of whom were known to be sensitive to Compositae.

The CIR safety assessments have been completed separately on many components of Calendula extracts, including fatty acids (oleic, lauric, palmitic, myristic, and stearic), sterols (PEGs Soy Sterols and Cholesterol), paraffin, *p*-hydroxybenzoic acid, salicylic acid, pyrogallol, catechol (aka pyrocatehol), tocopherol, and quinone (t-butylhydroquinone). Other safety assessments have addressed linalool and coumarin.

A method of evaluating the safety of cosmetic ingredients derived from plant materials was described that involves (1) determining all fractions and compounds identified for the particular plant; (2) developing a safety profile for each fraction/compound; and (3) evaluating skin toxicity studies on those fractions/compounds that may present a risk. Application of this approach to *C officinalis* identified many fractions for which the available data were considered to support safety. For those fractions where safety was not fully demonstrated, dermal irritation and sensitization studies were performed to support their safety. Given the claimed perpetual uncertainty in the exact composition of specific plant extracts, this approach was described as the only practical way to consider the safety of plant extracts.

A threshold of toxicologic concern analysis, using conservative assumptions about dermal absorption, applied to a number of chemicals found in *C officinalis* flowers resulted in systemic exposure values below the threshold of toxicologic concern values established for the relevant Cramer class, except for quercetin, kaempferol, and isorhamnetin. Available NOAELs suggested that these chemicals would not present any toxicity at concentrations used in cosmetics.

Discussion

The Panel noted that animal safety test data on Calendula extracts were available addressing acute, short-term, and subchronic toxicity; dermal irritation, sensitization, and photosensitization; ocular irritation; and genotoxicity. These data demonstrated an absence of adverse effects. Although limited carcinogenicity data were available in animal tests, one study suggested antitumor activity in vitro. Clinical testing demonstrated an absence of dermal irritation and infrequent sensitization reactions. Other safety test data of individual chemical components of Calendula (eg, lutein), likewise, did not suggest any adverse effects. There are no dermal reproductive or developmental toxicity data on Calendula extracts, but data on coriander oil, high in linalool and other terpenes, demonstrated that adverse effects occurred only at maternally toxic levels and did not occur at levels that were not maternally toxic.

Previous CIR safety assessments of fatty acids, plant sterols, paraffin, *p*-hydroxybenzoic acid, salicylic acid, and tocopherol, all of which are chemical components of Calendula extracts, supported that these chemical components of Calendula extracts would be safe at the levels found in the extracts and at the use concentration of the extracts. In previous CIR safety assessments of other listed chemical components of Calendula extracts, including pyrogallol, pyrocatechol, and t-butylhydroquinone, adverse effects were identified. These concerns were considered relevant to this safety assessment because, for example, tannins comprise 6% to 10% of material derived from Calendula and catechol is a subset of tannins. Analysis of actual Calendula extracts, however, demonstrated that catechol and pyrogallol, coumarins (esculetin, scopoletin, and umbelliferon), and α-tocopherolquinone were not present at detectable levels. Given the low use concentrations of the extract, and the concentration of components that are only a small percentage of the total ingredient (below the level of detection in some cases), the Panel concluded that these extracts, as described, did not present a concern as used in cosmetics.

The Panel recognized that every extract would likely be somewhat different and that the characterization of the composition of these plant-derived ingredients presented in this safety assessment is broad. Nonetheless the composition does represent what commonly would be found in these ingredients prepared in the manner described. The conclusion regarding safety, therefore, is valid only for ingredients prepared in a manner that produces a similar chemical profile as that described in this report. Extracts not prepared in a manner that produces a similar chemical profile, could be considered safe only if they have a similar safety test profile.

Additional considerations included the existing determination by the FDA that *C officinalis* L is generally recognized as safe as a spice; and that *Calendula* extracts have a long history of use as a food.

The Panel noted that these ingredients may be used in products that may be aerosolized, but that inhalation toxicity data are not available. In the absence of inhalation toxicity data, the Panel determined that these ingredients can be used safely in such products, because the ingredient particle size is not respirable. The Panel reasoned that the particle size of aerosol hair sprays ($\sim$38 μm) and pump hair sprays (>80 μm) is large compared with respirable particulate sizes ($\leq$10 μm).

The Expert Panel expressed concern regarding pesticide residues and heavy metals that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use the necessary procedures to limit these impurities in the ingredient before blending into cosmetic formulations.

Amended Conclusion

The CIR Expert Panel concluded that *C officinalis* extract, *C officinalis* flower, *C officinali*s flower extract, *C officinalis* flower oil, and *C officinalis* seed oil are safe for use in cosmetics in the practices of use and concentration given in this amended safety assessment. The Panel recognized that *C officinalis* seed oil is not currently used. Were it to be used as a cosmetic ingredient in the future, the expectation is that it would be used in products and at concentrations comparable with the other ingredients in this safety assessment.

Author's Note

The 2010 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, MD, FACP; Donald V. Belsito, MD; Ronald A. Hill, PhD; Curtis D. Klaassen, PhD; Daniel C. Liebler, PhD; James G. Marks Jr, MD, Ronald C. Shank, PhD; Thomas J. Slaga, PhD; and Paul W. Snyder, DVM, PhD.

The CIR Director is F. Alan Andersen, PhD. This report was prepared by Valerie C. Robinson, former Scientific Analyst/Writer.

Unpublished sources cited in this report are available from the Director, Cosmetic Ingredient Review, 1101 17th St., Suite 412, Washington, DC 20036, USA.

Conflict of Interest

The author's declared no potential conflict of interest relevant to this article was reported. F. Alan Andersen is employed by the Cosmetic Ingredient Review.

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