Safety Assessment of *Centella asiatica*-derived Ingredients as Used in Cosmetics

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All interested persons are provided 60 days from the above release date to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. Lillian Gill.

The 2015 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst.

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Abstract: The Cosmetic Ingredient Review Expert Panel (the Panel) reviewed the safety of 9 *Centella asiatica*-derived ingredients, which function primarily as skin conditioning agents in cosmetic products. The Panel reviewed relevant animal and human data on these ingredients. The Panel concluded that centella asiatica leaf extract and centella asiatica meristem cell culture are safe in the present practices of use and concentration in cosmetics, as described in this safety assessment, but that the available data are insufficient to make a determination that the following ingredients are safe under the intended conditions of use in cosmetics: centella asiatica extract, centella asiatica callus culture, centella asiatica flower/leaf/stem extract, centella asiatica leaf cell culture extract, centella asiatica leaf water, centella asiatica meristem culture extract, and centella asiatica root extract.

INTRODUCTION

The safety of the following 9 ingredients in cosmetics is reviewed in this safety assessment:

- centella asiatica extract
- centella asiatica callus culture
- centella asiatica flower/leaf/stem extract
- centella asiatica leaf cell culture extract
- centella asiatica leaf extract
- centella asiatica leaf water
- centella asiatica meristem cell culture
- centella asiatica meristem cell culture extract
- centella asiatica root extract

These ingredients function primarily as skin conditioning agents in cosmetic products.[1] A detailed list of *Centella asiatica*-derived ingredient functions is included in Table 1. In addition to data on *Centella asiatica*-derived ingredients, data on the composition and biological activity/toxicity of *Centella asiatica* are included.

CHEMISTRY

Definition and Characterization

Centella asiatica (hydrocotyle; Indian pennywort) is a herbaceous plant of the *Umbelliferae* family.[2] The definitions and functions, in cosmetics, of the *Centella asiatica*-derived ingredients reviewed in this safety assessment are presented in Table 1.[1]

Method of Manufacture

Centella Asiatica Extract

In the production of centella asiatica extract, the stalks and leaves of *Centella asiatica* are macerated in propylene glycol and water for several days. The material is then drained and pressed, followed by a sterilizing filtration.[3]

According to another source, the dried raw material (*Centella asiatica*) is extracted with an 80% propylene glycol solution or with ethanol.[4] For the propylene glycol extract, extraction is followed by filtration, sedimentation, filtration, and packaging. For the ethanol extract, extraction is followed by filtration, concentration, sedimentation, filtration, and packaging.

The methanolic extract of *Centella asiatica* has been prepared as follows:[5] The whole plant was washed, dried, and powdered. The dry powder (5 g) was extracted with 50 ml of 80% methanol, the extract was filtered, and the filtrate was evaporated to dryness in a vacuum. The yield of the solvent free extract was 20% (i.e. 1 g).

Centella Asiatica Leaf Extract

Centella asiatica leaf extract has been prepared as follows:[6] The *Centella asiatica* plant was cleaned with triple-distilled water and the leaves were separated and freeze-dried. The leaves were boiled in triple-distilled water, and the extract was then lyophilized and stored at -80°C.

According to another method, the fresh leaves of the *Centella asiatica* plant are air-dried at 40˚C and ground to powder, which is then subjected to exhaustive extraction using ethanol in a Soxhlet apparatus.[7] The dark-green liquid extract is concentrated under vacuum, and the resulting dried extract is lyophilized and preserved in a refrigerator at 4˚C.

Centella Asiatica Meristem Cell Culture

Centella asiatica meristem culture is obtained from a cell culture of *Centella asiatica* consisting of a population of undifferentiated stem cells originating from leaves.[8] The cells are then filtered in order to remove the culture medium. Glycerin is added to the cells, which results in extraction of the internal soluble substances and the external cell walls (largely insoluble in water and solvents).

Composition/Impurities

Centella Asiatica Extract

Centella asiatica plant extract consists of the following:[9]

- plant sterols
- flavonoids
- tannins (20 to 25%)
- essential acid (0.1% with β-chariophylen, trans-β-pharnesen, and germachrene D)
- phytosterols (campesterol, sitosterol, stigmasterol)
- mucilages
- resins
- free amino acids (alanine, serine, aminobutyrate, aspartate, glutamate, lysine, and threonine)
- flavonoids (derivatives of chercetin and kempferol)
- an alkaloid (hydrochotine)
- vallerine
- fatty acids (linoleic, linolenic, oleic, palmitic, and stearic acids)

According to another source, both the ethanol and propylene glycol extracts of *Centella asiatica* contain tannins and saponins.[4]

Centella asiatica

The composition of *Centella asiatica* has been described to include:[10,11]

- asiaticoside*
- centelloside*
- madecassoside*
- asiatic acid*
- volatile oils
- flavonoids
- tannins
- phytosterols
- amino acids
- sugars
- centellin (6-acetoxy-trideca-1,7-dien-4-yn-3-ol)
- asiaticin (p-benzoyloxy methyl-butyl benzoate)
- centellicin (1-(2',3'-dihydroxypropyl)-2-en-3-methyl-6-hydroxy-9-yn-undecanoate)

The most important constituents isolated from *Centella asiatica* were triterpenoid saponins known as centelloids (identified by an asterisk above). Chemical structures of triterpenoid saponins are presented in Figures 1, 2, and 3.[12,13]

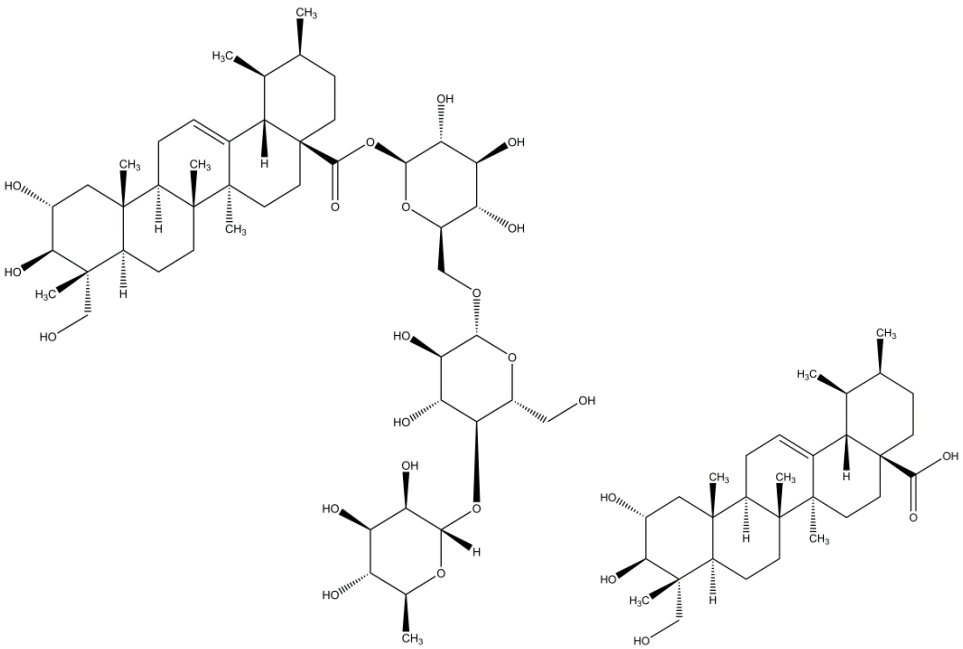

Figure 1. Asiaticoside and Asiatic Acid

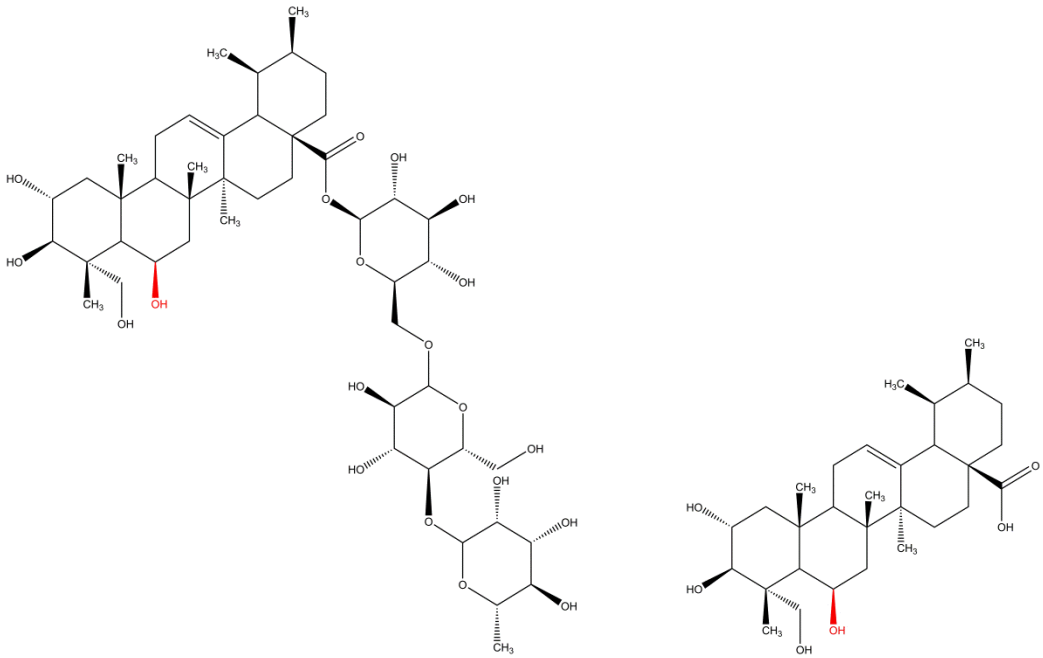

Figure 2. Madecassoside and Madecassic Acid (difference from Asiaticoside and Asiatic Acid in red)

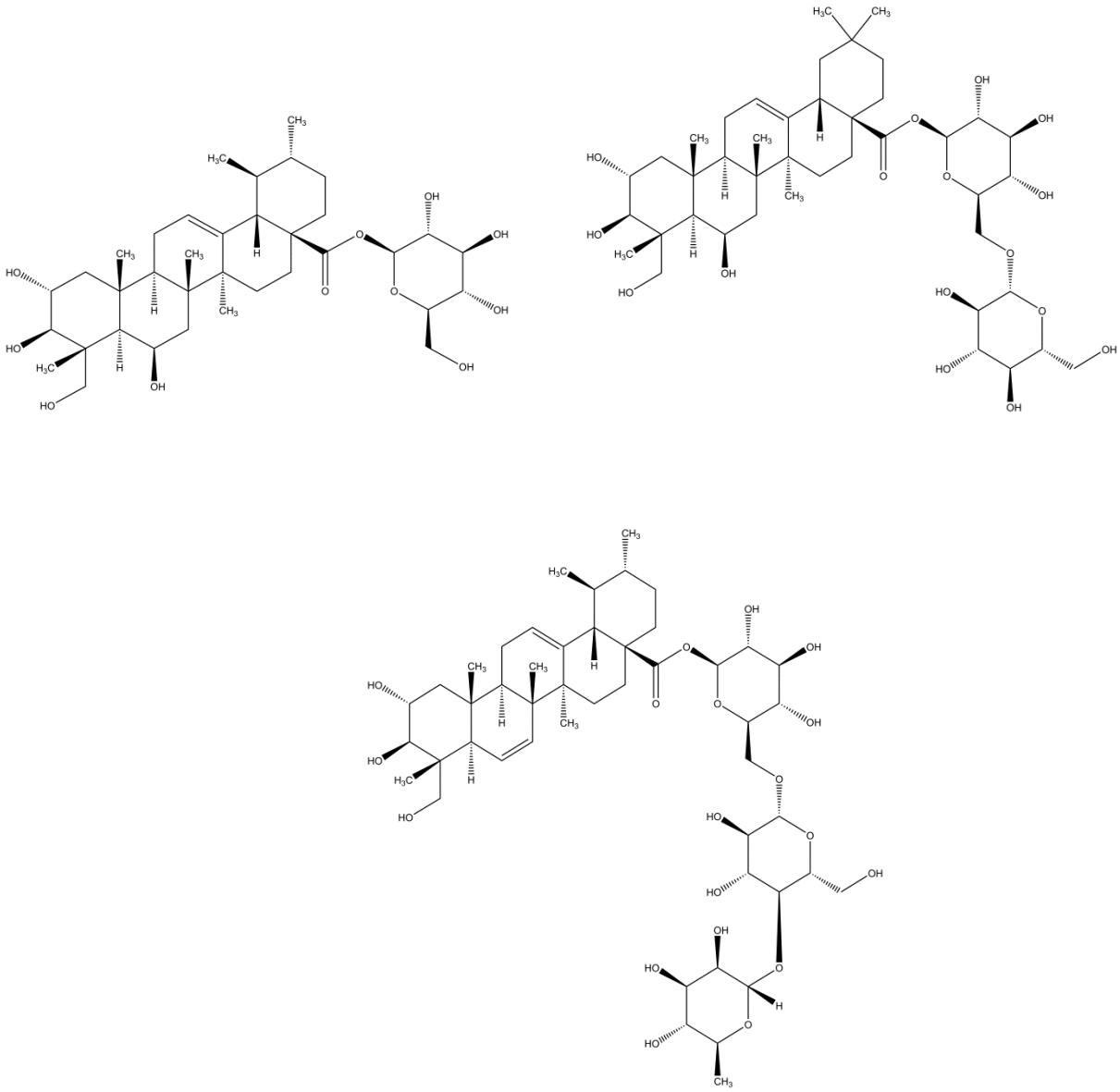

Figure 3. Centellosides

Saponins may account for 1% to 8% of all *Centella asiatica* constituents.[14] The variable quantity of saponins depends mainly on the origin of the plant and can be established by high-performance liquid chromatography with an ultraviolet detector (HPLC-UV). Other constituents of *Centella asiatica*, identified as centellosides, are primarily ursane- and oleanane-type pentacyclic triterpenoid saponins. The pharmacological activity (e.g., treatment of venous hypertension)[9] of the centellosides is attributed to the compounds asiaticoside, madecassoside, asiatic acid, and madecassic acid. Asiaticoside also induces type I collagen synthesis and stimulates angiogenesis. Other centellosides occurring in *C. asiatica* include triterpenic acids (e.g., brahmic acid, madasiatic acid, terminolic acid, centellic acid) as well as their glycosides, namely, brahminoside, madasiaticoside and centelloside. *Centella asiatica* also contains volatile oils (0.1%).

In the *Centella asiatica* plant, grown in Peninsular Malaysia, barium concentrations (μg/g dry weight) ranged from 5.05 to 21.88 μg/g in roots, 3.31 to 11.22 μg/g in leaves, and 2.37 to 6.14 μg/g in stems.[15] This study was performed because, at the time, there was no established background level of barium in soils and in edible *Centella asiatica* for Malaysia.

Centella Asiatica Meristem Cell Culture

Centella asiatica meristem culture is composed mainly of primary metabolites (lipids, glucides (carbohydrates), and amino acids).[8] Only traces of secondary metabolites are detected. Saponin derivatives from asiatic and madecassic acids have never been detected in this product. The total control of culture conditions guarantees the absence of environmental contaminants such as pesticides, heavy metals, and biological pollutants.

USE

Cosmetic

The safety of *Centella asiatica*-derived ingredients is evaluated based on the expected use of these ingredients in cosmetics. The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) uses data received from the Food and Drug Administration (FDA) and the cosmetics industry to determine expected cosmetic use. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by Industry in response to surveys of maximum reported use concentrations, by product category, that are conducted by the Personal Care Products Council (Council). Collectively, the use frequency and use concentration data indicate that 4 of the 9 *Centella asiatica*-derived ingredients are used in cosmetic products.[16,17] According to these data, the following 5 ingredients are not being used in cosmetics:

centella asiatica callus culture
centella asiatica leaf cell culture extract
centella asiatica leaf water
centella asiatica meristem culture extract
centella asiatica root extract

According to the 2015 VCRP survey, the greatest reported use frequency is for centella asiatica extract (454 formulations, mostly leave-on), followed by silk powder (66 formulations, mostly leave-on) (Table 2).[16] Lower use frequencies are being reported for the remaining *Centella asiatica*-derived ingredients. The results of a concentration of use survey conducted by the Personal Care Products Council (Council) and provided in 2015 indicate that centella asiatica extract has the highest maximum concentration of use; it is used at concentrations up to 0.5% in leave-on products (face and neck products [not spray]) (Table 2).[17] In some cases, reported uses appear in the VCRP database, but concentration of use data were not provided, and vice versa.

Cosmetic products containing *Centella asiatica*-derived ingredients may be applied to the skin and hair or, incidentally, may come in contact with the eyes and mucous membranes. Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

Centella asiatica extract is reported as being used in face powders that may be loose powder products (but not spray products), and could possibly be inhaled. Industry can minimize airborne particles from cosmetic powder products by controlling the milling of the ingredients and adding binding materials, such as oils, waxes or hygroscopic ingredients, in the formulations.[18] The binding materials foster the agglomeration of the ingredients and substantially increase their cohesivity. These measures increase the size of the particles in the product, and can ensure that airborne particles produced during the use of such products are not respirable to any appreciable amount.

Noncosmetic

Centella asiatica

The herb *Centella asiatica* (also known as gotu kola) has been used in traditional Asian medicine for many years, especially to treat dermatological conditions, including small wounds, scratches, and burns, and as a hypertrophic wound healing agent and an anti-inflammatory agent, particularly in eczema.[14] However, gotu kola, centella asiatica extract, and an ointment that contains centella asiatica extract (Madecassol ointment) are not included in FDA's database of FDA-approved drug products.[19]

Centella Asiatica Extract

The extract from the fresh and dried leaves and stems of *Centella asiatica* contains triterpenic derivatives (madecassic acid, asiatic acid, and asiaticoside), which have been shown to promote epithelialization and have anticellulitic and vasotonic activity.[20] According to another source, the active principle of *Centella asiatica* is a triterpenic derivative, from which a glycolic extract is obtained.[2] Reportedly, this glycolic extract is used widely, topically in dermatology, to promote the epithelialization of wounds and ulcers, and as an anticellulitic and vasotonic. Information relating to the underlying mechanism(s) for centella asiatica extract in keloid management/wound healing is included in the section on Other Effects.

Centella asiatica preparations are used as drugs in Europe. The European Medicines Agency reports that, for cutaneous use in the treatment of leg ulcers, wounds, and burns, etc., ointments contain 1% titrated extract of *Centella asiatica* (TECA). A cutaneous powder containing 2% TECA is used for the treatment of scars, keloid scars, and burns.[21]

TOXICOKINETICS

Non-Human

Centella asiatica

The disposition and metabolism of madecassoside (see Figure 2), triterpenoiod saponin in *Centella asiatica*, was evaluated using groups of 6 Sprague-Dawley rats.[22] The test substance was administered orally at a single dose of 100 mg/kg. Plasma, heart, liver, spleen, lung, kidney, and brain tissues, and bile, urine and feces were collected from 0 h to 72 h post-dosing. Madecassoside concentrations in biological samples were determined using liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). After oral dosing, madecassoside was widely distributed to the heart, liver, spleen, lung and kidney of rats, and the concentrations of madecassoside in liver and kidney were relatively higher than in other organs. Values for the excretion of madecassoside in bile, urine, and feces were 7.16% (0-12h), 0.25% (0-72h) and 24.68% (0-72h) of the administered dose, respectively. Madecassoside was metabolized by hydrolase isozymes produced by intestinal bacteria, and the following 3 deglycosylated metabolites identified in rat feces were consistent with the sequential cleavage of C-28 glycoside bonds: O-glucopyranosyl(1,6)-glucopyranosyl-2,3,6,23-tetrahydroxyurs-12-en-28-oate; O-glucopyranosyl-2,3,6,23-tetrahydroxyurs-12-en-28-oate, and madecassic acid.

Human

Centella Asiatica Extract

Following a single 30 mg and 60 mg oral dose of centella asiatica extract administered to 12 human subjects, maximum plasma levels of asiatic acid were attained in 4.5 h and 4.2 h, respectively.[10,23] Plasma half-lives were 2.2 h (30 mg dose) and 3.4 h (60 mg dose), with no detectable levels of the saponin in plasma 24 h post-dosing. The same doses of centella asiatica extract administered orally for 7 days resulted in higher peak plasma concentrations, longer half-lives, and greater area-under-the-curve values. The authors noted that the 3 principal components of the triterpenoid fraction (TTF) of *Centella asiatica* are asiatic acid, madecassic acid, and asiaticoside. Furthermore, asiatic and medecassic acids together account for approximately 60%, and asiaticoside accounts for 40% of the composition of TTF.

TOXICOLOGY

Acute Toxicity

Oral

Non-Human

Centella Asiatica Leaf Extract

The acute oral toxicity of centella asiatica leaf extract was evaluated using groups of 8 adult Wistar albino male rats.[7] The test substance was administered orally (intubation) at a single dose of 100, 500, 1000, or 2000 mg/kg. The LD_{50} was 200 mg/kg (calculated value). Additional study details were not included.

Repeated Dose Toxicity

Oral

Human

Centella Asiatica Extract

A study was performed to evaluate the clinical efficacy of centella asiatica extract (plant part and extraction method not specified) oral administration and identify any side effects.[24] The study involved 84 diabetic wound patients receiving oral doses of the extract and a placebo group consisting of 86 patients (mean age = 59 years, all patients). Two centella asiatica extract capsules (50 mg of extracted asiaticoside/capsule) were taken after a meal 3 times per day for 21 days. *Centella asiatica* extract capsules promoted the wound healing process (rapid wound contraction), when compared to the placebo group. No systemic side effects or complications were reported.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Centella Asiatica Leaf Extract

The reproductive toxicity of centella asiatica leaf extract (in distilled water) was evaluated using 5 groups of 8 Wistar adult male rats.[7] Four groups received oral doses (gavage; dose volume = 1 ml) of 10, 50, 80, and 100 mg/kg/day, respectively, for 8 weeks. The fifth group was given distilled water and served as the control. Animals were killed on the last day of dosing (day 60). When compared to the control group, statistically significant ($p < 0.01$ or $p < 0.001$) reductions in sperm viability and motility were noted in each group dosed with centella asiatica leaf extract. In each experimental group, histopathological examination of the testis revealed a significant (p value not stated) decrease in the number of spermatogenic cells (spermatogonia, spermatocyte, spermatid, and sperm) in the seminiferous tubules. Also, when compared to the control group, intertubular spaces and venous congestion were increased in experimental groups. The authors noted that the reported loss in testicular weight likely corresponded to a dose-dependent decrease in mean spermatogenic cells in seminiferous tubules. At the 100 mg/kg/day dose, the mean number of sperms from the cauda epididymis ($\times 10^6$) was 36.7 ± 4.8, compared to a mean value (control) of 61.60 ± 2.34; this difference was statistically significant ($p < 0.001$). Additionally, degeneration of seminiferous tubules was reported. It was concluded that centella asiatica leaf extract was toxic to the reproductive system of male rats.

Centella Asiatica Extract

A study was performed to evaluate the effects of centella asiatica extract (ethanol extract) on the rat testis.[25] The following groups of 8 male Sprague-Dawley rats (dosed orally) were used in the study: low-dose group (100 mg/kg body weight), mid-dose group (200 mg/kg body weight), high-dose group (300 mg/kg body weight), and control group (distilled water). The groups were force fed (using force feeding needle) for 42 consecutive days, after which the animals were killed and the testis removed for histological examination. Animals of all dose groups had some degeneration of spermatogenic cells and reduction of spermatozoa in the lumen of the seminiferous tubules. When compared to the control group, the serum testosterone level decreased in a dose-dependent manner and there was a significant decrease in cauda epididymal sperm

count. A statistically significant reduction ($p < 0.05$) in sperm count was observed in the 200 mg/kg and 300 mg/kg dose groups, but not in the 100 mg/kg dose group. Differences in sperm motility were also observed. Slow or sluggish progressive sperm motility was reported for the control and 100 mg/kg dose group. Non-progressive motility (< 5 µm/second) was reported for both the 200 mg/kg and 300 mg/kg dose groups. In control animals, the testis had normal features, with successive stages of transformation of the seminiferous epithelium into spermatozoa. However, abnormalities in seminiferous tubules were observed in all dose groups. Complete arrest of the seminiferous tubules was observed only in the 300 mg/kg dose group. It was concluded that centella asiatica extract (ethanol extract) was a reproductive toxicant in male rats.

GENOTOXICITY

In Vitro Assays

Centella Asiatica Leaf Extract

The genotoxicity of centella asiatica leaf extract (acetone extract) was evaluated in a chromosomal aberration assay using human peripheral blood lymphocytes.[26] Results were negative over the range of test concentrations (1.075×10^{-4} to 4.17×10^{-4} g/ml). Results for the dimethylsulfoxide (DMSO, 5 µl/ml) control were negative. A sister chromatid exchange assay was also used to evaluate the genotoxicity of the same test substance using human peripheral blood lymphocytes. Results were negative over the same concentration range tested in the chromosomal aberration assay. Again, results for the DMSO control were negative.

Centella Asiatica Extract

The genotoxicity of centella asiatica extract (aqueous extract of edible plant parts) was evaluated in the Ames test using *Salmonella typhimurium* strains TA98 and TA100.[27] The extract was tested, with and without metabolic activation, at concentrations of 2 and 5 mg/plate. Results were uniformly negative.

Centella Asiatica Meristem Cell Culture

The genotoxicity of centella asiatica meristem cell culture was evaluated in the Ames test at doses up to 100 mg/plate using the following bacterial strains, with and without metabolic activation:[28] *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and TA102. Negative control cultures with and without solvent (water) were used. The positive controls without metabolic activation were as follows: sodium azide, 9-aminoacriine, 2-nitrofluorene, and mitomycin C. 2-Aminoanthraceneand cyclophosphamide served as positive controls with metabolic activation. The test material was not genotoxic in any of the strains tested, with or without metabolic activation. The positive controls were genotoxic.

CARCINOGENICITY

Data on the carcinogenicity of *Centella asiatica*-derived ingredients were not found in the published literature and unpublished data were not submitted.

Asiaticoside

The dermal carcinogenicity of asiaticoside was evaluated using hairless mice (number of animals not stated). The test substance was painted on dorsal skin twice weekly for up to approximately 20 months.[29,21] It was noted that some of the mice had previously been initiated with a small dose of 20-methylcholanthrene (MCA). A control group that had received benzene (solvent) only after MCA-initiation was also included. These carcinomas did not appear before approximately 16 months of observation. Prior to this, the painted, MCA-initiated animals had a significantly lower number of skin papillomas, when compared to the corresponding control group treated with benzene only. At necropsy, it was found that approximately 30% of the corresponding MCA-initiated, benzene or asiaticoside-treated animals had such neoplasms. Asiaticoside was used at a concentration of 0.10% in benzene. Asiaticoside, dissolved in benzene, caused an increased yield of papillomas and, also, a 2.5% incidence of skin sarcomas, indicating an effect on the dermis as well. It was also noted that asiaticoside did not cause necrosis or acantholysis of the skin and did not appear to be toxic.

IRRITATION AND SENSITIZATION

Irritation and Skin Sensitization

Dermal

Non-Human

Centella Asiatica Extract

The skin irritation threshold of centella asiatica extract, in emulsion prepared from Freund's complete adjuvant (FCA) and physiological saline, was determined using 10 guinea pigs (test protocol not included).[30] Unprocessed dry leaves of *Centella asiatica* were extracted with diethyl ether and ethanol. The irritancy threshold of the extract was determined to be greater than 30%.

The skin sensitization potential of 30% centella asiatica extract (extracted with diethyl ether and ethanol) was evaluated in the guinea pig maximization test using 10 female guinea pigs.[30] The extract (30 mg in FCA and saline) was injected intradermally into the shoulder area. Following an 11-day non-treatment period, the animals were challenged on day 20. During the challenge phase, centella asiatica extract (30%), dissolved in a mixture of acetone/ethanol (1:1), was applied epicutaneously (open) to skin of the right flank. The following reactions, scored in accordance with International Contact Dermatitis Research Group criteria, were reported: seven + reactions (at 24 h reading), three + reactions (at 48 h reading), and two + reactions (at 72 h reading). Centella asiatica extract was classified as a weak sensitizer in this study.

In another study, the skin sensitization potential of TECA (water extract) was evaluated using 10 guinea pigs (ages and strain not stated), according to OECD protocol 406.[31] A negative control group was included; however the number of animals was not stated. The induction phase consisted of topical applications of undiluted TECA. Following a 17-day non-treatment period, the animals were challenged with undiluted TECA and 50% TECA in paraffin oil (each under an occlusive dressing for 24 h). The test substance (on 2 x 4 cm filter paper) was applied to the flank. No macroscopic cutaneous reactions attributable to allergy were observed during the challenge phase. Similarly, no cutaneous intolerance reactions were observed in the negative control group.

Human

Centella Asiatica Extract

Negative patch test results were reported for 20 subjects patch tested with centella asiatica extract at concentrations of 1% and 5% in petrolatum.[32] These 20 subjects comprised the control group in a case report (42-year old patient) on centella asiatica extract that is summarized later in the report text.

Centella Asiatica Leaf Extract

The skin irritation and sensitization potential of an eye lotion containing 0.2% centella asiatica leaf extract was evaluated using 54 subjects (men and women).[33] An occlusive patch containing approximately 0.1 g to 0.15 g of the lotion (≈ 25 to 38 mg/cm^2) was applied to the back (between the scapulae and waist, adjacent to the spinal midline) of each subject. This procedure was repeated for a total of 9, 24-h induction applications. Following a 2-week non-treatment period, a challenge patch was applied to a new site. Reactions were scored at 24 h and 72 h post-application. The lotion did not cause skin irritation or allergic contact dermatitis in any of the subjects tested.

Centella Asiatica Root Extract

A material with the following composition was evaluated for skin irritation and sensitization potential in 47 subjects:[34] centella asiatica root extract, as % solid content in overall extract composition (0.1%), water (98.3%), potassium sorbate and phenoxyethanol preservative blend (0.6%), and Saccharomyces lysate extract (0.1%). The preceding repeated insult patch test procedure was used. The test material did not cause dermal irritation or allergic contact sensitization in any of the subjects tested.

Ocular

Centella Asiatica Leaf Extract

The ocular irritation potential of an eye lotion containing 0.2% centella asiatica leaf extract was also evaluated using a three-dimensional human corneal epithelial (HCE) model.[35] The model consisted of human corneal cells cultured on an inert polycarbonate filter at the air-liquid interface. The objective of this assay was to assess, quantitatively, the effects of the test material on cell survival through the 4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Determination of cell viability was based on cellular dehydrogenase activity, which was measured by MTT reduction and conversion into blue formazan salt (quantified after extraction from tissues). The reduction of cell viability was compared to the negative control (phosphate-buffered saline) and expressed as a percentage. The percentage reduction in cell viability was used to predict ocular irritation potential. The time to toxicity (ET_{50}; MTT activity reduced to 50% of the control condition) was determined.

The eye lotion directly reduced MTT in this study, and, therefore, a killed-control experiment (functional check using freeze-killed control tissue) was performed to evaluate whether residual test article was binding to the tissue and causing a false MTT reduction signal. The results of the killed control experiment indicated that there was little or no direct MTT reduction in the test material-treated killed control, compared to the negative control-killed controls, and the MTT reduction in the test material-treated viable tissue was ascribed to the viable cells. An ET_{50} of 21.5 h was reported for the eye lotion, and an ET_{50} of 28.2 minutes was reported for the 0.3% Triton-X-100 positive control. The meaning of these reported ET_{50} values in terms of irritation potential was not stated.[35]

Centella Asiatica Meristem Cell Culture

A water-soluble raw material for use in cosmetic products (liquid containing glycerol [80%], centella asiatica (Aplaceae) cells [20%], xanthan gum [0.3%]) was evaluated for ocular irritation potential using the HCE model and MTT assay that are described in the preceding study.[36] The test material was considered a non-irritant.

Centella Asiatica Root Extract

The following material (diluted with distilled water to 1%; data extrapolated to 5%) was evaluated for ocular irritation potential using the HCE model and MTT assay:[37] centella asiatica root extract, as % solid content in overall extract composition (0.8% to 1.2%), water (97% to 98%), phenoxyethanol (1%), and sodium benzoate (0.3%). The ET_{50} was greater than 256 minutes. Therefore, at 5%, the estimated Draize ocular irritation score for the test material was 0 (non-irritating).

Case Reports

Centella Asiatica Extract (from leaf and stem)

Erythema and mild eczematous lesions were observed on keloid skin of a 33-year-old woman after application of an ointment that contained *Centella asiatica* (composition described below).[38] Patch test results were positive (++) for this ointment. The area of the application site and patch test protocol were not stated.

- TECA (1g)*
- Asiaticoside (0.4 g in TECA)*
- Asiatic acid (0.3 g in TECA)*
- Madecassic acid (0.3 g in TECA)*
- Glycol stearate (15 g)
- Propylene glycol (30 g)
- White Vaseline (5 g)
- Lavender oil (0.143 ml)
- Geranium oil (0.143 ml)

*TECA (1 g) contains Asiaticoside (0.4 g) + Asiatic Acid (0.3 g) + Madecassic Acid (0.3 g).

Negative patch test results were reported for TECA at concentrations of 1% and 10% in petrolatum. In a second case report, a 23-year-old woman applied the same ointment on the donor site of her skin graft and itchy, oozing erythematous lesions were observed. Patch test results were positive for the following materials: 10% TECA, the ointment, propylene glycol (20%, 30%, 40%, and 50%), and geranium oil (20%).[38] According to comments on these test results that were received from the cosmetics industry, the ointment tested contains excipients such as lavender oil and geranium oil, which are known to be allergenic.[39]

A case of allergic contact dermatitis in a 54-year-old woman, with no history of atopic or allergic contact dermatitis, after application of an ointment containing centella asiatica extract was reported.[20] The patient was patch tested with centella asiatica extract (1% and 10% in petrolatum; and 2% in ethanol 70˚ [70˚ = alcohol proof]). Patch testing with the 1% centella asiatica extract resulted in a + reaction at 48 h and a ++ reaction at 72 h and 96 h. A +++ reaction to 2% and 10% centella asiatica extract was observed at 48 h, 72 h, and 96 h.

Centella Asiatica Extract

A 42-year-old woman, with no history of atopy, developed severe dermatitis of the legs after application of a vasotonic cream containing centella asiatica extract (2% in alcohol 70˚).[2] The extraction method was not specified. The patient was patch tested using the Finn Chamber® and thin-layer rapid-use (TRUE) test® methods. Reactions were scored at 2 and 4 days. A +++ reaction to centella asiatica extract was reported.

A red vesicular reaction, with exudation and intense itching, was observed in a 39-year-old woman after applying a cream containing centella asiatica extract (concentration in cream not stated).[40] The extraction method was not specified. Patch testing with the cream yielded a +++ reaction. Patch testing with centella asiatica powder (1% in petrolatum) yielded a +++ reaction on days 2 and 3. However, negative reactions were observed in 50 control subjects patch tested with 1% centella asiatica powder in petrolatum.

An eczematous reaction on both knees of a 38-year-old man with joint pain was observed after topical application of a cream that contained centella asiatica extract (concentration not stated).[41] The extraction method was not specified. Patch test results for centella asiatica extract were positive (+++ reaction).

Localized, severe eczema on the neck and upper chest was observed in a 42-year-old non-atopic woman after treatment of a scar with an ointment that contained *Centella asiatica* (composition described at beginning of section).[32] Patch test results were positive for the ointment and centella asiatica extract (extraction method not specified) at concentrations of 1% and 5% in petrolatum.

Centella asiatica

Three women (61, 52, and 49 years old) developed jaundice after taking *Centella asiatica* tablets (for weight loss) during 30, 20, and 60 days, respectively.[42] Neither the dose of *Centella asiatica* per tablet ingested nor the number of tablets ingested per day was stated. The respective diagnoses were: granulomatous hepatitis with marked necrosis and apoptosis; chronic hepatitis with cirrhotic transformation and intense necroinflammatory activity; and granulomatous hepatitis. All three cases recovered after discontinuing use of these tablets.

OTHER EFFECTS

Wound Healing

Centella Asiatica Extract

The active ingredients of centella asiatica extract in the process of wound healing are the triterpenoid compounds asiatic acid, madecassic acid, asiaticoside, and madecassoside.[24]

Centella asiatica extract has a history of use in keloid management (i.e., anti-scar activity), and asiatic acid is one of the extract's principle bioactive components.[43] Keloids are fibroproliferative disorders characterized by exuberant extracellular matrix deposition. Furthermore, keloid formation, a result of abnormal wound healing, is characterized as exuberant collagen deposition and invasive growth beyond original wound margins. The transforming growth factor (TGF)-β/Smad pathway plays a pivotal role in keloid pathogenesis. In an *in vitro* assay, asiatic acid inhibited TGF-β1-induced collagen and plasminogen activator inhibitor-1 (PAI-1) expression in keloid fibroblasts through peroxisome proliferator-activated receptor- γ (PPAR-γ) activation. Thus, asiatic acid inhibited collagen type I expression in keloid fibroblasts. The authors noted that this finding suggests that asiatic acid was one of the active constituents of *Centella asiatica* responsible for success in keloid management.

Human foreskin fibroblast cells were incubated with centella asiatica extract (100 µg/ml) for 48 h.[44] The extraction method was not specified. Centella asiatica extract stimulated collagen and fibronectin synthesis in fibroblasts. When

compared to control cultures, collagen synthesis was statistically significantly increased ($p < 0.05$) and fibronectin synthesis was elevated by approximately 1.5-fold.

Centella asiatica

Following oral and topical administration of *Centella asiatica* in rats, increased cellular hyperplasia and collagen production were observed at the site of injury in a wound healing assay. The following served as measurements of increased cellular hyperplasia and collagen production: increased levels of DNA, protein, total collagen, and hexosamine in granulation tissue. More rapid maturation and cross-linking of collagen were observed in animals treated with centella asiatica extract, as determined by elevated stability of acid-soluble collagen and increases in aldehyde content and tensile strength. When compared to control wounds, rats treated with *Centella asiatica* had a higher degree of epithelialization and a higher rate of wound contraction.[10,45,46]

Effect on Mucopolysaccharide Metabolism

Centella asiatica

Individuals with varicosities receiving (mode of administration not stated) 30 mg total triterpenoid fraction of *Centella asiatica* twice daily for 3 months had significantly reduced levels of serum enzymes involved in mucopolysaccharide metabolism (beta-glucuronidase, beta-*N*-acetylglucosaminidase, and arylsulfatase), compared to baseline values ($p < 0.01$).[10,47]

Effect on Nerve Regeneration

Centella Asiatica Extract

Following a sciatic nerve crush injury, male Sprague-Dawley rats given centella asiatica extract (ethanol extract) in drinking water (300 to 350 mg/kg daily) for 18 days recovered more quickly from this nerve damage, compared to controls. Increased axonal regeneration and more rapid functional recovery were observed.[10,48] It should be noted that dried centella asiatica extract (ethanol extract) was dissolved in drinking water at a concentration of 2 mg/ml. Based on the amount of water that was consumed, the average dose for each rat was calculated to be 300 to 330 mg/kg daily over the 18-day study. The authors noted that the capacity to regenerate axons is an important component of healing after nerve damage.

Cytotoxicity

Centella Asiatica Leaf Extract

The cytotoxic activity of centella asiatica leaf extract (aqueous extract) against four cancer cell lines and one normal cell line was studied using the MTT assay, a colorimetric assay for assessing cell viability.[49] Cultures were incubated with centella asiatica leaf extract at concentrations ranging from 0.1 to 1000 µg/ml. The 50% inhibitory concentrations (IC_{50}) were calculated by linear regression over the range of test concentrations. Centella asiatica leaf extract was cytotoxic to the following cancer cell lines: human breast cancer MDA-MB 231 (IC_{50} = 648 µg/ml), mouse melanoma $B16F_1$ (IC_{50} = 698 µg/ml), and rat glioma C6 (IC_{50} = 1,000 µg/ml). The leaf extract was not cytotoxic at concentrations up to 1,000 µg/ml to the human lung carcinoma (A549) and normal hamster kidney (BHK-21) cell lines.

Centella Asiatica Extract

The potential for centella asiatica extract (methanolic extract of whole plant) to induce apoptosis was evaluated in the following cancer cell lines: MCF-7, HeLa, HepG2, and SW 480.[5] In the manufacturing process, the yield of the solvent free extract was 20% (i.e. 1 g). In cell viability assays, cells grown in 96-well microtitre plates (7000 cells/well) were incubated for 48 h with and without centella asiatica extract (10.5 to 82 µg/100 µL). The MCF-7 cell line was found to be most sensitive to *in vitro* growth inhibitory activity. Centella asiatica extract inhibited proliferation of the MCF-7 cell line in a concentration-dependent manner (LD_{50} = 66 µg [calculated value]). The highest test concentration of the extract (82 µg/100 µL) inhibited MCF-7 cell growth to an extent that was almost equivalent to tamoxifen (10 mM)-induced inhibition. Centella asiatica extract induced apoptosis in MCF-7 cells, which was consistent with the observed nuclear condensation, increased annexin staining, loss of mitochondrial membrane potential, and DNA breaks.

Photocytotoxicity

Centella Asiatica Meristem Cell Culture

The cytotoxicity of centella asiatica meristem cell culture, in the presence and absence of exposure to a non-cytotoxic dose of simulated solar light, was evaluated using the 3T3 neutral red uptake (NRU) photocytotoxicity test.[50] The test material, in phosphate-buffered saline, was tested at dilutions ranging from 0.15 g/l to 30 g/l. Each dilution was applied to Balbc 3T3 fibroblasts for 1 h prior to UV exposure (5 J/cm^2) for 50 minutes. Non-exposed cultures remained in the dark. Cell viability was determined by vital dye (neutral red) uptake. The assessment parameter obtained was the IC_{50} (concentration of test material inhibiting 50% survival and cell growth). Centella asiatica meristem cell culture was classified as non-phototoxic over the range of dilutions tested.

Effect on Neurotoxicity

Centella Asiatica Extract

Centella asiatica extract (aqueous extract) (100 µg/mL) mitigated amyloid-β-induced cell death in the MC65 and SH-SY5Y neuroblastoma cell lines.[51] The attenuation of amyloid-β-induced alterations in tau expression and phosphorylation in both cell lines was also noted. The authors noted that the accumulation of amyloid-β is a hall mark of Alzheimer's disease, and is known to result in neurotoxicity both *in vivo* and *in vitro*.

Immunomodulatory Activity

Centella Asiatica Extract

The effects of centella asiatica extract (aqueous and ethanol extracts, whole plant) on cell-mediated and humoral immune responses was evaluated.[52] In human peripheral blood mononuclear cells (PBMCs**),** the aqueous extract of centella asiatica significantly increased ($p < 0.05$) proliferation and the production of IL-2 and TNF-α. In contrast, the ethanol extract of centella asiatica inhibited human PBMC mitogenesis and the production of IL-2 and TNF-α (i.e., exhibited immunosuppressive activity).

In another experiment, 6 male BALB/c mice were fed centella asiatica extract (aqueous extract, 100 mg/kg body weight) and immunized with bovine serum albumin (BSA). The control group (6 mice) received distilled water. The experimental group had greater responses to both primary and secondary antibodies against BSA when compared to the non-treated group. It was concluded that centella asiatica extracts (aqueous and ethanol) had immunomodulating activity with respect to both non-specific cellular and humoral immune responses.[52]

SUMMARY

Centella asiatica, the plant source of ingredients reviewed in this safety assessment, is an herbaceous plant of the *Umbelliferae* family.

Collectively, information supplied to FDA by industry as part of the VCRP and a survey of ingredient use concentrations conducted by the Council indicate that the following *Centella asiatica*-derived ingredients are being used in cosmetic products: centella asiatica extract, centella asiatica flower/leaf/stem extract, centella asiatica leaf extract, and centella asiatica meristem cell culture. The Council survey data also indicate that *Centella asiatica*-derived ingredients are being used in cosmetics at maximum ingredient use concentrations up to 0.5% (i.e., for centella asiatica extract in face and neck products).

The European Medicines Agency reports that, for cutaneous use in the treatment of leg ulcers, wounds, and burns, etc., ointments contain 1% titrated extract of *Centella asiatica* (TECA). A cutaneous powder containing 2% TECA is used for the treatment of scars, keloid scars, and burns.

Oral dosing with centella asiatica extract in human subjects yielded plasma half-lives of 2.2 h (30-mg dose) and 3.4 h (60-mg dose) for asiatic acid, with no detectable levels of the saponin remaining at 24 h post-dosing. After oral dosing of rats with madecassoside, this saponin component of *Centella asiatica* was widely distributed to the heart, liver, spleen, lung and kidney, and the levels of madecassoside in the liver and kidneys were relatively higher than in other organs. Values for the excretion of madecassoside in bile, urine, and feces were 7.16% (0-12h), 0.25% (0-72h) and 24.68% (0-72h) of the administered dose, respectively.

In an acute oral toxicity study of centella asiastica leaf extract involving rats, an LD_{50} of 200 mg/kg was reported. No systemic toxicity or complications were reported in a study in which 84 diabetic wound patients received oral doses of centella asiatica extract (50 mg extracted asiaticoside/capsule) 3 times per day for 21 days.

The skin irritancy threshold of centella asiatica extract was determined to be > 30% in a test involving 10 guinea pigs. In the skin sensitization phase of this study, 30% centella asiatica extract was classified as a weak sensitizer. In a sensitization study on TECA (undiluted and at 50% in paraffin oil) involving guinea pigs, neither test material induced skin sensitization.

Negative patch test results were reported for 20 subjects patch tested with centella asiatica extract at concentrations of 1% and 5% in petrolatum. An eye lotion containing 0.2% centella asiatica leaf extract did not cause skin irritation or allergic contact dermatitis in any of the 54 subjects tested in a human repeated insult patch test (HRIPT). A material with the following composition was evaluated for skin irritation and sensitization potential in an HRIPT involving 47 subjects: centella asiatica root extract, as % solid content in overall extract composition (0.1%), water (98.3%), potassium sorbate and phenoxyethanol preservative blend (0.6%), and Saccharomyces lysate extract (0.1%). The results of this study were also negative.

In case reports, patch test results for centella asiatica extract were positive at concentrations as low as 1%. Centella asiatica extract (10%, from leaf and stem) yielded a positive and negative reaction in separate case reports.

Results for the following ingredients were negative in the *in vitro* MTT assay for evaluating ocular irritation potential: 0.2% centella asiatica leaf extract, 20% centella asiatica meristem cell culture, and 1% centella asiatica root extract.

Degeneration of seminiferous tubules and a significant dose-dependent reduction in sperm density were reported in male rats dosed orally with centella asiatica leaf extract (up to 100 mg/kg/day). Centella asiatica extract caused antispermatogenic and antifertility effects on the reproductive system of male rats. A significant reduction ($p < 0.05$) in sperm count was observed in the 200 mg/kg/day and 300 mg/kg/day dose groups, but not in the 100 mg/kg/day dose group.

Centella asiatica leaf extract was not genotoxic in a chromosomal aberration assay involving human peripheral blood lymphocytes. Negative results were also reported for centella asiatica extract and centella asiatica meristem cell culture in the Ames test with and without metabolic activation.

Asiaticoside, dissolved in benzene, caused an increased yield of papillomas and, also, a 2.5% incidence of skin sarcomas in hairless mice, indicating an effect on the dermis as well.

In vitro studies on centella asiatica extract and centella asiatica leaf extract showed that these botanicals are cytotoxic to various cancer cell lines.

The cytotoxicity of centella asiatica meristem cell culture, in the presence and absence of exposure to a non-cytotoxic dose of simulated solar light, was evaluated using the 3T3 neutral red uptake (NRU) photocytotoxicity test. The test material (in phosphate-buffered saline) was evaluated at dilutions ranging from 0.15 g/l to 30 g/l, and was classified as non-phototoxic.

DISCUSSION

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

Because final product formulations may contain multiple botanicals, each possibly containing similar constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. For *Centella asiatica*-derived ingredients, the Panel was concerned about the presence of asiaticoside, centelloside, madecassoside, and asiatic acid in cosmetics, which could result in sensitization. Therefore, when formulating products, manufacturers should avoid reaching levels of plant constituents that may cause sensitization or other adverse health effects.

Centella asiatica extract (30%), extracted with diethyl ether and ethanol, was classified as a weak sensitizer in the guinea pig maximization test. After considering these data, the Panel noted that there is little concern over sensitization

potential in humans, given the low reported maximum use concentration of 0.5% in cosmetic products. The Panel also noted different effects of centella asiatica extract relating to the cell-mediated immune response, depending on the method of ingredient extraction. Centella asiatica extract (aqueous extract) stimulated cytokine production, whereas centella asiatica extract (ethanol extract) inhibited cytokine production.

Centella asiatica extract was a reproductive toxicant in male rats at daily oral doses ranging from 100 to 300 mg/kg/day, and the same was true for centella asiatica leaf extract in male rats at a daily oral dose of 100 mg/kg/day. The Panel noted that the male reproductive toxicity induced by both extracts was observed at high doses, but that this level of exposure would not be associated with daily use of cosmetic products at maximum reported ingredient use concentrations up to 0.5% (centella asiatica extract) and 0.2% (centella asiatica leaf extract). Thus, the Panel agreed that the level of use of centella asiatica leaf extract and centella asiatica meristem cell culture in cosmetics should be below the threshold of toxicologic concern for that endpoint. Centella asiatica leaf extract and centella asiatica meristem cell culture are the only ingredients in this safety assessment for which the available data are sufficient for determining safety.

The following data are needed for completion of the safety assessment on centella asiatica extract, centella asiatica callus culture, centella asiatica flower/leaf/stem extract, centella asiatica leaf cell culture extract, centella asiatica leaf water, centella asiatica meristem cell culture extract, and centella asiatica root extract:

1) Method of manufacture, composition, and impurities data on the ingredients stated above
2) Irritation and sensitization data for all ingredients
3) 28-day dermal toxicity data on centella asiatica extract or centella asiatica root extract. If it is determined that centella asiatica root extract is a component of centella asiatica extract, only data on centella asiatica extract are needed.

CONCLUSION

The CIR Expert Panel concluded that centella asiatica leaf extract and centella asiatica meristem cell culture are safe in the present practices of use and concentration in cosmetics, as described in this safety assessment, when formulated to be non-sensitizing. The Panel also concluded that the available data are insufficient to make a determination that the following ingredients are safe under the intended conditions of use in cosmetics:

centella asiatica extract
centella asiatica callus culture*
centella asiatica flower/leaf/stem extract
centella asiatica leaf cell culture extract*
centella asiatica leaf water*
centella asiatica meristem cell culture extract*
centella asiatica root extract*

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

Table 1. Definitions and functions of the ingredients in this safety assessment.[1]

Ingredient/CAS No.	Definition	Function
Centella asiatica extract 84696-21-9 84776-24-9	Centella asiatica extract, also known as gotu kola extract, is the extract of the whole plant, *Centella asiatica.*	Skin-Conditioning Agents - Miscellaneous
Centella asiatica callus culture	Centella asiatica callus culture is a suspension of the cultured callus cells of *Centella asiatica*.	Antioxidants; Skin-Conditioning Agents - Miscellaneous
Centella asiatica flower/leaf/stem extract	Centella asiatica flower/leaf/stem extract is the extract of the flowers, leaves and stems of *Centella asiatica.*	Skin-Conditioning Agents - Miscellaneous
Centella asiatica leaf cell culture extract	Centella asiatica leaf cell culture extract is the extract of a culture of the leaf cells of *Centella asiatica.*	Antioxidants; Skin Protectants
Centella asiatica leaf extract	Centella asiatica leaf extract is the extract of the leaves of *Centella asiatica.*	Skin-Conditioning Agents - Miscellaneous
Centella asiatica leaf water	Centella asiatica leaf water is an aqueous solution of the steam distillate obtained from the leaves of *Centella asiatica.*	Skin-Conditioning Agents - Miscellaneous
Centella asiatica meristem cell culture	Centella asiatica meristem cell culture is a suspension of the cultured meristem cells of *Centella asiatica.*	Antioxidants; Skin Protectants
Centella asiatica meristem cell culture extract	Centella asiatica meristem cell culture extract is the extract of centella asiatica meristem cell culture.	Skin-Conditioning Agents - Emollient
Centella asiatica root extract	Centella asiatica Root Extract is the extract of the roots of *Centella asiatica.*	Skin-Conditioning Agents - Miscellaneous

Table 2. Current Frequency and Concentration of Use According to Duration and Type of Exposure.[16,17]

	Centella Asiatica Extract		**Centella Asiatica Flower/Leaf/Stem Extract**		**Centella Asiatica Leaf Extract**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	454	0.00002-0.5	1	0.001	66	0.0017-0.2
Duration of Use						
Leave-On	262	0.00002-0.5	NR	NR	63	0.0017-0.2
Rinse off	65	0.00002-0.082	1	0.001	3	0.02
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Exposure Type						
Eye Area	42	0.1-0.3	NR	NR	7	0.0017-0.2
Incidental Ingestion	6	0.0003-0.01	NR	NR	13	NR
Incidental Inhalation- Sprays	129*	0.0033*	NR	NR	38*	NR
Incidental Inhalation- Powders	111**	0.0032-0.5**	NR	NR	35**	0.02-0.1**
Dermal Contact	368	0.00002-0.5	1	0.001	51	0.0017-0.2
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	38	0.0005-0.003	NR	NR	2	NR
Hair-Coloring	3	0.028	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	17	0.0001-0.01	NR	NR	14	NR
Baby Products	3	NR	NR	NR	NR	NR
	Centella Asiatica Meristem Cell Culture					
	# of Uses	Conc. (%)				
Totals/Conc. Range	4	0.05-0.1				
Duration of Use						
Leave-On	4	0.05-0.1				
Rinse off	NR	NR				
Diluted for (bath) Use	NR	NR				
Exposure Type						
Eye Area	1	0.05				
Incidental Ingestion	NR	NR				
Incidental Inhalation- Sprays	3*	NR				
Incidental Inhalation- Powders	3**	0.1				
Dermal Contact	4	0.05-0.1				
Deodorant (underarm)	NR	NR				
Hair - Non-Coloring	NR	NR				
Hair-Coloring	NR	NR				
Nail	NR	NR				
Mucous Membrane	NR	NR				
Baby Products	NR	NR				

NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for (Bath) Use Product Uses.
*It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.
**It is possible that these products may be powders, but it is not specified whether the reported uses are powders.
Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

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