Safety Assessment of *Equisetum arvense*-Derived Ingredients as Used in Cosmetics

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ABSTRACT: The Expert Panel for Cosmetic Ingredient Safety (Panel) reviewed the safety of 5 *Equisetum arvense*-derived ingredients as used in cosmetic products; all of these ingredients are reported to function as skin-conditioning agents in cosmetics. Industry should use current good manufacturing practices to minimize impurities that could be present in botanical ingredients. The Panel reviewed data relevant to the safety of these ingredients in cosmetic formulations, and concluded that the 5 *Equisetum arvense*-derived ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

The safety of the following 5 *Equisetum arvense*-derived ingredients as used in cosmetics is reviewed in this assessment.

Equisetum Arvense Extract
Equisetum Arvense Juice
Equisetum Arvense Leaf Extract
Equisetum Arvense Leaf Powder
Equisetum Arvense Powder

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), all of the *Equisetum arvense*-derived ingredients are reported to function as skin conditioning agents in cosmetic products (Table 1).[1] Common names for the herb *Equisetum arvense* include horsetail and field horsetail.[2]

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated.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 Unpublished data may be provided by the cosmetics industry, as well as by other interested parties.

Botanicals, such as *Equisetum arvense* -derived ingredients, may contain hundreds of constituents. In this assessment, the Panel is evaluating the potential toxicity of each of the *Equisetum arvense* - derived ingredients as a whole, complex substance; toxicity from single components may not predict the potential toxicity of botanical ingredients.

Also, with botanicals, it is often not known how the substance being tested in a study compares to the cosmetic ingredient. In the report text, if it is known that the material being tested is a cosmetic ingredient, the INCI naming convention is used (i.e., the names of cosmetic ingredients are capitalized, without italics (e.g., Equisetum Arvense Extract)). If it is not known that the test substance is the same as the cosmetic ingredient, the taxonomic naming conventions (i.e., with genus and species name italicized (e.g., an *Equisetum arvense extract*)) is used.

CHEMISTRY

Definition and Plant Identification

The ingredients in this report are related as derivatives from the same species, *Equisetum arvense.* The definitions of these *Equisetum arvense*-derived ingredients are presented in Table 1; the generic CAS number for 3 of these ingredients is 71011-23-9.[1]

Equisetum arvense (horsetail) has been described as a non-flowering weed (a perennial with hollow stems and shoots) that is found throughout parts of Europe, Asia, the Middle East, and North America.[3] *Equisetum arvense* is distributed throughout temperate and arctic areas of the northern hemisphere, growing typically in moist soils.[4] It has also been described as an herbaceous perennial relative of ferns consisting of 2 types of stems, namely, sterile non-reproductive and photosynthetic, and reproductive and non- photosynthetic. The latter, which is 10 to 25 cm long with brown scale leaves and a 10 to 40 mm long spore cone, emerges in spring and then withers, giving rise to the sterile, photosynthetic stems. These stems persist from summer until the first frost. According to another source, *Equisetum arvense* has aerial stems, branched with regular verticilies (2 - 23 mm in diameter) and terminal strobile in the branches and in the main stem (10 mm long and 4 mm in diameter).[5]

Physical and Chemicals Properties

Equisetum Arvense Extract

Equisetum arvense is available as a dried extract in powdered form or as a liquid extract.[5] As the plant dries, silica crystals form in the stems and branches.[3]

Method of Manufacture

Equisetum Arvense Extract

Equisetum Arvense Extract is an extraction directly into the solvent mixture (water/glycol) by maceration.[6] The method of production for an Equisetum Arvense Extract in ethanol and water (0.7% solids w/v) tradename material is described as follows: dried raw material ⇒ extract with ethanol ⇒ filtrate ⇒ concentration ⇒ adjustment ⇒ sedimentation ⇒ filtrate ⇒ adjustment ⇒ packaging.[7]

An Equisetum Arvense Extract in butylene glycol (0.54% solids w/v) tradename material is produced according to the following process: dried raw material ⇒ extract with 50 vol% 1,3-butylene glycolic solution ⇒ filtrate ⇒ sedimentation ⇒ filtrate ⇒ packaging.[7] The method of production of an Equisetum Arvense Extract in ethanol and water tradename material is described as follows: dried raw material ⇒ extract with 30 vol% ethanolic solution ⇒ filtrate ⇒ sedimentation ⇒ filtrate ⇒ packaging.

A method of manufacture relating to the preparation of three different extracts of *Equisetum arvense* (sterile stems) is also available in the published literature.[8] Air-dried and powdered plant material (100 g) was macerated with petroleum ether overnight, and afterwards with 70% methanol (24 h). After filtration, the methanolic extract was concentrated to dryness. The dry residue was dissolved in hot water and then separated by liquid-liquid extraction into the chloroform, ethyl acetate, and *n*-butanol extracts.

Composition/Impurities

The following composition data could be characterized as general information relating to *Equisetum arvense* or *Equisetum arvense* extracts, and it is unknown if it applies to the cosmetic ingredients that are being reviewed in this safety assessment.

Data on flavonoid composition reveal the existence of 2 chemotypes of *Equisetum arvense*, one in Asia and North America, and the other in Europe.[8] *Equisetum arvense* from Asia and North America contains luteolin-5-*O*-glucoside (quantitative information absent) and its malonyl ester (quantitative information absent), but these compounds are not found in *Equisetum arvense* from Europe. The dominant compounds in *Equisetum arvense* from Europe are quercetin 3-*O*-glucoside, apigenin 5-*O*-glucoside, and dicaffeoyl-*meso*-tartaric acid (quantitative information absent). Di-*E*-caffeoyl-*meso*-tartaric acid (quantitative information absent) is a marker for both chemotypes. According to another source, quercetin 3-*O*-(6"-*O*-malonyl-β-D-glucopyranoside) has been found to be the major flavonoid in European plants (*Equisetum arvense*), comprising between 28% and 50% of the total flavonoid content.[9] In plants from Taiwan and China, luteolin 5-*O*-β-D-glucopyranoside has been reported as the major flavonoid, comprising 50% to 60% of the total flavonoid content.

Additional data on the composition of *Equisetum arvense* indicate that it contains more than 10% inorganic substances (two-thirds of which are silicic acid and potassium salts). Specifically, the aerial parts of *Equisetum arvense* contain flavonoids, saponins, caffeic acid and other phenolic compounds, alkaloids, sterols (β-sitosterol, campesterol, and isofucosterol), and minerals (primarily silicon and potassium salts).[2,8] According to other sources, acids that have been isolated from *Equisetum arvense* include aconitic acid (tricarboxylic acid), ascorbic acid (ketolactone), malic acid (dicarboxylic acid), oxalic acid (dicarboxylic acid), and the following phenolic acids: caffeic acid, cinnamic acids, *p*-coumaric acid, gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, and vanillic acid.[8,10] Other components include polyenic acids, rare dicarboxylic acids (equisetolic acid), flavonoids, and styrylpyrones.[8]

The concentration ranges for some essential elements in *Equisetum arvense* have been determined to be: iron (193.4 - 1757.9 µg/g), manganese (23.6 - 143.7 µg/g), zinc (15.4 - 32.7 µg/g), selenium (0.13 - 0.92 µg/g), and copper (11.3 - 21.8 µg/g).[11] Among the components (oligo-β-glucans) of the *Equisetum arvense* cell wall are the tetrasaccharide, β-glucosyl-(1→4)-β-glucosyl-(1→4)-β-glucosyl(1→3)-glucose and the trisaccharide, mixed-linkage (1→3, 1→4)-β-D-glucan.[12] The enzyme thiaminase (which breaks down vitamin B_1) also occurs in *Equisetum arvense*.[13]

Equisetum Arvense Extract

According to a cosmetics industry source, a tradename mixture of Equisetum Arvense Extract has the following composition: Equisetum Arvense Extract (~ 2% dry extract), propylene glycol (~ 66%), phenoxyethanol (~ 0.36%), methylparaben (~ 0.08%), ethylparaben (~ 0.02%), propylparaben (~ 0.04%), and water (qsp 100%).[14]

An Equisetum Arvense Extract in ethanol and water (approximately 0.7% solids w/v) tradename mixture contains tannin and saponin.[7] Tannin and flavonoid are present in an Equisetum Arvense Extract in butylene glycol (approximately 0.54% solids w/v) tradename mixture and in an Equisetum Arvense Extract in ethanol and water tradename mixture.

Whether or not flavonoids or phenolic acids are the predominant compounds in *Equisetum arvense* extracts is dependent upon the extractant that is used. In one publication, flavonoids were the main compounds in ethyl acetate and butanol *Equisetum arvense* extracts, and phenolic acids were the major constituents in the aqueous *Equisetum arvense* extracts.[8] These data are summarized in Table 2. According to another source, the following water-soluble acids have been detected in an aqueous extract of *Equisetum arvense* (whole, air-dried plant): aconitic acid, arabinonic acid, citric acid, ferulic acid, fumaric acid, gluconic acid, glyceric acid, malic acid, malonic acid, phosphoric acid, quinic acid, and threonic acid.[10]

Composition data on a methanol extract of *Equisetum arvense* (aerial parts) indicate the presence of 2 phenolic petrosins, namely onitin and onitin-9-*O*-glucoside, and the following 4 flavonoids: apigenin, luteolin, kaempferol-3-*O*-glucoside, and quercetin-3-*O*-glucoside.[15] The following % composition values for flavonoid and caffeic acid derivatives of the hydro-alcoholic (20:80, v/v) extract of *Equisetum arvense* stems have been calculated (computed from the high performance liquid chromatography peak areas): quercetin (21.1%), quercetin 3-*O*-glucoside (49.6%), quercetin 3-*O*-(6"-*O*-malonylglucoside) (8.8%), 5-*O*-caffeoyl shikimic acid (4.4%), monocaffeoyl *meso*-tartaric acid (3%), and dicaffeoyl *meso*-tartaric acid (1.6%).[16]

Composition data on *Equisetum arvense* (water and methanol extract) grown in Asia versus *Equisetum arvense* grown in Europe are presented in Table 3.[17] In addition to these data, the researchers presented an accumulation profile (graph) of quercetin glucosides (absolute content, % dry weight) in *Equisetum arvense* during 2 growing seasons which indicated that development of

the total amount (% dry weight) of the main flavonoids quercetin 3-*O*-glucoside and quercetin 3-*O*-(6"-*O*-malonylglucoside) was different over several years of observation. An accumulation profile (graph) of quercetin glucosides (proportional content, % total flavonoids) in *Equisetum arvense* during 2 growing seasons indicated that few differences were found in the proportional content (% of flavonoid content) of the 2 main flavonoids in several years of observation. Also, it was found that there was a decrease in quercetin 3-*O*-(6"-*O*-malonylglucoside) and a simultaneous increase in quercetin 3-*O*-glucoside toward the end of the growing period.

One source indicates the following mean values for 3 toxic metals in *Equisetum arvense*: lead (14.07 mg/kg), cadmium (0.139 mg/kg), and mercury (0.014 mg/kg).[18] In this analysis, *Equisetum arvense* herb (above ground plant parts, dried raw material) was studied.

Nicotine has been detected in British species of *Equisetum arvense*.[19] The amount of nicotine obtained from 5 g of dried plant material (British *Equisetum arvense*) has been estimated, using ultraviolet (UV) spectrophotometry, to be not more than 2 mg.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database.[20] Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.[21]

According to 2021 FDA VCRP data, Equisetum Arvense Extract is reported to be used in 186 cosmetic products (125 leave-on products, 59 rinse-off products, and 2 products that are diluted for (bath) use; Table 4).[20] Of the *Equisetum arvense*-derived ingredients that are being reviewed in this safety assessment, this is the greatest reported use frequency. The results of a concentration of use survey completed in 2018 and provided by the Council in 2019 indicate that Equisetum Arvense Extract is being used at maximum use concentrations up to 0.4% in leave-on products (body and hand products [not spray]), and at maximum use concentrations up to 0.00078% in rinse-off products (skin cleansing products).[21] Equisetum Arvense Extract is the only *Equisetum arvense*-derived ingredient in this safety assessment for which use concentration data were provided in response to the Council survey. Additionally, according to both VCRP and Council survey data, Equisetum Arvense Juice and Equisetum Arvense Leaf Powder are not reported to be used in cosmetic products.

It should be noted that frequency of use data on *Equisetum arvense* (horsetail), from 2021 FDA VCRP, are also included in Table 4. Because neither the plant part(s) associated with the name *Equisetum arvense* nor whether the name corresponds to a plant part extract is stated, it is not possible to specifically associate the frequency of use data on *Equisetum arvense* with any of the 5 *Equisetum arvense*-derived ingredients that are reviewed in this safety assessment.

Cosmetic products containing *Equisetum arvense*-derived ingredients may be applied to the skin/hair, or incidentally, may come in contact with the eyes (e.g., Equisetum Arvense Extract and Equisetum Arvense Leaf Extract). Equisetum Arvense Extract is used in products that come in contact with mucous membranes during product use (e.g., mouthwashes and breath fresheners at up to 0.0002%); thus, Equisetum Arvense Extract may be incidentally ingested. Products containing *Equisetum arvense*-derived ingredients may be applied as frequently as several times per day and may come in contact with the skin for variable periods following application. Daily or occasional use may extend over many years.

Equisetum Arvense Extract is reported to be used in cologne and toilet waters, and in other fragrance preparations (concentrations unknown).[20] Equisetum Arvense Leaf Extract is reported to be used in hair spray (aerosol fixatives) (concentrations unknown). In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm, compared with pump sprays.[22-25] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[22,23] Equisetum Arvense Extract is reported to be used in face powders (concentrations unknown).[20] Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[26-28]

The *Equisetum arvense*-derived ingredients are not restricted from use in any way under the rules governing cosmetic products in the European Union.[29]

Non-Cosmetic

Equisetum arvense (horsetail) is an herbal remedy that dates back to ancient Rome and Greece.[3] Traditionally, it was used to stop bleeding, heal ulcers and wounds, and for the treatment of tuberculosis and kidney problems. The aboveground parts of this plant are used for medicinal purposes. Because *Equisetum arvense* contains silicon, which strengthens bone, some practitioners recommend it as a treatment for osteoporosis (not a US FDA-approved use). It is also used as a diuretic, and the diuretic effects of

Equisetum arvense may enhance the toxic effects of certain medications, such as digoxin (used to treat congestive heart failure), phenytoin (for seizures), anticoagulants, and others.[30] Thus, individuals taking prescription medications should not take *Equisetum arvense* without first consulting a health care provider.

In Japan, *Equisetum arvense* (field horsetail) sporophyte (*tsukushi*) is consumed as food in sweetened vinegar, cooked food, and chopped fish.[16] Furthermore, in Asian traditional medicine, the aerial parts of *Equisetum arvense* have been used to treat hemorrhage, urethritis, jaundice, and hepatitis.[31,32] Sterile stems of *Equisetum arvense* are used in herbal medicine in various countries, constituting the "Equiseti herba" of European Pharmacopeias.[8] According to another source, *Equisetum arvense* is used mainly for its diuretic properties, and also has the following uses: analgesic, hemostatic, astringent, and treatment for digestive disorders and kidney/bladder stones.[33]

According to the US FDA, *Equisetum arvense* is among the ingredients that have been present in over-the-counter (OTC) drug products for use as a digestive aid (21 CFR 310.545). However, based on evidence currently available, there are inadequate data to establish general recognition of safety and effectiveness of this ingredient for this specified use.

TOXICOKINETIC STUDIES

No relevant toxicokinetic studies on *Equisetum arvense*-derived ingredients were found in the published literature. In general, toxicokinetics data are not expected to be found on botanical ingredients because each botanical ingredient is a complex mixture of constituents.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

Equisetum Arvense

A single-dose, oral toxicity study on *Equisetum arvense* was performed using groups of male and female rats (strain and number per group not stated).[34] Doses of 800 mg/kg, 2000 mg/kg, and 5000 mg/kg were administered orally (method not stated). No deaths or abnormal changes in body weight occurred, and no toxicity signs were observed at necropsy. The LD_{50} was > 5000 mg/kg.

Equisetum Arvense Extract

In an acute oral toxicity study involving 10 mice (strain not stated), an LD_0 of ≥ 20 ml/kg was reported for Equisetum Arvense Extract (hydroglycolic extract containing ~2% dry extract).[6] Data on the composition of this extract are included in the section on Composition/Impurities. Details relating to the test protocol are not included.

Intraperitoneal

The acute toxicity of an *Equisetum arvense* extract (hydroalcoholic extract) was evaluated using groups of 8 male Wistar rats.[35] The groups received intraperitoneal (i.p.) doses of 1000 mg/kg, 2000 mg/kg, and 5000 mg/kg. Control animals were dosed with saline. The number of survivors was recorded on the following day. Mortalities were observed in the 2000 mg/kg group (12.5% of the animals) and in the 5000 mg/kg group (37.5% of the animals). In all 3 dose groups, transitory respiratory depression and elevated sedation were observed. Both signs persisted to the end of the 240 min observation period, and were dose-dependent.

Short-Term Toxicity Studies

Oral

Equisetum Arvense Powder

In a short-term study, male Sprague-Dawley rats (groups of 6) were fed an *Equisetum arvense* powder (0.4% or 4%) in a 20% casein diet, with and without cholesterol (0.5% cholesterol and 0.15% sodium cholate), for 14 d.[36] At a concentration of 0.4% or 4% in either diet, the test material did not influence food intake or growth. There also were no apparent effects on serum or liver lipids after feeding with either concentration in both diets. However, on days 9 to 12 of feeding with 4% of a *Equisetum arvense* powder in the cholesterol diet, 4 of 6 rats lost their hair, and non-specific ulcerative dermatitis was observed on the neck, head, nose, and back. At microscopic examination, dense infiltration of neutrophils and lymphocytes was observed in the dermis and subcutaneous tissue. At the center of the eruption, the dermis was ulcerated. The number of mast cells was also increased. These changes at microscopic examination were diagnosed as nonspecific inflammatory lesion of the skin. Reversal of the dermatitis was noted when the diet was changed to commercial pellets. Serum immunoglobulin E (IgE) levels, measured by enzyme-linked immunoassay, indicated that the induction of IgE may not necessarily be involved in the dermatitis caused by *Equisetum arvense* intake. In 2 additional experiments (21 rats total), rats were fed a *Equisetum arvense* powder (concentration not stated) in a cholesterol diet (composition not stated) for 4 wk and 6 wk. Six of 21 rats from the 2 experiments had dermatitis on the neck and back. The incidence of dermatitis after feeding for 4 wk and 6 wk was approximately 20% and 30%, respectively.

Intraperitoneal

Equisetum Arvense Extract

An *Equisetum arvense* extract (dried stem, ethanol and water extract) was administered to 10 male Wistar rats, at a daily i.p. dose of 50 mg/kg for 8 wk.[37] Signs of toxicity were not observed during the treatment period.

Subchronic Toxicity Studies

Oral

Equisetum arvense

The subchronic oral toxicity of *Equisetum arvense* (powder extracted with hot water; plant part not stated) was evaluated in a study involving groups of 10 male and 10 female F344 rats.[5] The groups were fed *Equisetum arvense* at a concentration of 0.3%, 1%, or 3% in the diet (powdered basal diet) for 13 wk. Animals of the control group received diet only. Test and control animals were killed at the end of the study, and histopathological examinations of internal organs were performed. If lesions were frequently found in the 3% dietary group, then histopathological examination was extended to all tissues of the 0.3% and 1% dietary groups. None of the animals died and no obvious clinical signs were observed in any of the animals during the study. Body weights and cumulative body weight gains in all dietary groups were similar to control values. Additionally, there were no differences in food consumption between the groups. Urinalyses revealed no significant differences in any of the parameters evaluated among the groups. However, the protein levels in males of the 1% and 3% dietary groups were decreased. Additional results are summarized below.

Statistically significant alterations in hematological parameters (e.g., mean corpuscular hemoglobin and platelet count) were observed, but no dose-dependence was apparent. However, a trend toward a dose-dependent decrease in the white blood cell count was noted in females. There were no statistically significant differences in organ weights. However, a tendency toward a decrease in absolute adrenal weights was observed in males. No treatment-related macroscopic changes were observed at necropsy. However, the following histopathological changes (minimal grade changes) were observed in treated animals: spontaneous inflammatory/proliferative lesions in the liver (3% dietary group, 2 males), spontaneous inflammatory and proliferative lesions in the pancreas (1 female, 3% dietary group), liver microgranulomas (3% dietary group, 1 male and 2 females), kidney atrophy (3 males [0.3% group], 2 males [1% group], and 1 male [3% group]; 1 female [3% group]), and ovarian cysts (3% dietary group, 1 female). Eosinophilic bodies and alpha 2u-globulin expression in the proximal tubules of the kidney were observed in all male rats, including the control group. No treatment-related findings were observed in other tissues and organs of male or female rats. The no-observed-adverse-effect level (NOAEL) for *Equisetum arvense* was determined to be more than 3% in male and female rats (> 1.79 g/kg bw/d males; > 1.85 g/kg bw/d females).

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

In Vitro

Equisetum arvense

A study was performed to determine the effect of *Equisetum arvense* and thymol during early development of the zebrafish.[38] Embryos resulting from the natural spawning of adult wild-type zebrafish (*Danio rerio*, AB strain) were used. The source of *Equisetum arvense* (natural extract) was a commercial formulation containing horsetail extract (*Equisetum arvense*) decoction (95.2% decoction of horsetail (*Equisetum arvense* 7%). Stock solutions of *Equisetum arvense* (500 mg/l, 6250 mg/l, and 80,000 mg/l) were prepared. Exposure solutions were freshly prepared in embryo water (200 mg/l instant ocean salt and 100 mg/l sodium bicarbonate, UV sterilized) prepared from filtered tap water. The lethal concentration that causes 50% mortality (LC_{50}) was determined using a modification of Organization for Economic Co-operation and Development (OECD) Test Guideline 236. The 96-h LC_{50} and 95% confidence limits for *Equisetum arvense* were 1.98 mg/l (0.50 - 4.13). Based on this LC_{50} value, 3 sublethal concentrations (0.00625 mg/l, 0.0625 mg/l, and 0.625 mg/l) were selected for the assay. The incubation period for tested embryo cultures was 96 h. Embryo cultures incubated in embryo water served as the blank control. The experiment was repeated independently 5 times. Lethal parameters such as failure of somites, eye and otolith development, missing heartbeat, and non-detached tail and head were recorded at 24, 48, 72, and 98 h post fertilization (hpf). The spontaneous movements at 24 hpf, pigmentation formation and heart rate at 48 hpf, and hatching rate at 72 hpf were evaluated as sublethal endpoints. Morphological abnormalities (body length, area of egg yolk, area of heart and eye, and head to body angle) were screened at 98 hpf in 10 randomly 3% methylcellulose-immobilized eleutheroembryo. The authors noted that the results of this study demonstrated no teratogenic potential of *Equisetum arvense* at sublethal concentrations during the early development of zebrafish. At 98 hpf, embryo development in control cultures was, as expected, around 80% with normal development. Thymol was tested at concentrations of 0.008 mg/l, 0.08 mg/l, and 0.8 mg/l in this assay, based on 96-h LC_{50} and 95% confidence limits of 2.35 mg/l (0.78 - 5.55). At 98 hpf, malformations were observed at the highest concentration of thymol (0.8 mg/l), namely, pericardial edema, yolk and eye deformations, and decreased body length. Increased lethality was also noted.

GENOTOXICITY STUDIES

In Vitro

Equisetum arvense

The genotoxicity potential of *Equisetum arvense* (plant part, method of preparation, and doses not stated) was evaluated in a reverse mutation test using the following bacterial strains: *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2uvrA.[34] Details relating to the test protocol were not included. The number of revertant colonies per plate was not increased in any bacterial strain, and *Equisetum arvense* was non-genotoxic in this assay.

A chromosomal aberration test on *Equisetum arvense* (plant part, method of preparation, and doses not stated) was performed using Chinese hamster lung cells.[34] Details relating to the test protocol were not included. However, it was stated that the short treatment method and the continuous treatment method were used. Using both methods, the incidence of cells with chromosomal aberrations was less than 5%. It was concluded that *Equisetum arvense* did not have any potential for inducing chromosomal aberrations.

Equisetum Arvense Extract

The cytogenetic effects of *Equisetum arvense* extract (ethanol extract of whole, or cut, dried sterile aerial parts; 0.025, 0.05, 0.1, and 0.2 mg/ml) on irradiated human blood lymphocytes was studied. [39] The acquired micronucleus formation in unirradiated and irradiated samples of cultured blood lymphocytes using the cytochalasin block micronucleus test. All lymphocyte cultures were done in parallel in a single experiment with 2 concurrent controls; inherent (native) control of lymphocytes and lymphocytes plus adjuvant (lactose monohydrate). Irradiation of blood samples (in 15 x 15 cm Plexiglass container) with *Equisetum arvense* extract was performed using a Co γ-ray source. The radiation dose was 2 Gy, given at a dose rate of 0.45 Gy/min and delivered to a field of 20 x 20 cm at a distance of 74 cm from the source. Centromere-positive micronuclei were identified by fluorescence in situ hybridization, using a DNA probe labeled with alpha-satellite digoxigenin. *Equisetum arvense* extract had weak clastogenic properties. The yield of micronuclei in unirradiated samples was increased and the level of radiation-induced micronuclei was reduced in a concentration-dependent manner. In the control, unirradiated samples, 36.8% of micronuclei were centromere positive (MNC+). In the control irradiated samples, the percentage of MNC+ ranged from the percentage of MNC+ ranged from 10.8% to 15.3%. These results for controls were indicative of a clastogenic mechanism for micronuclei formation. The authors noted that the results of this study indicate that *Equisetum arvense* extract weakly induced micronuclei in a concentration-dependent manner.

The genotoxicity of an *Equisetum arvense* extract (stem hydro-alcoholic (20:80, v/v) extract) was evaluated in the micronucleus test.[16] Human blood samples were cultured with the extract (62.5 µg/ml) for a total of 67 h. Cell cultures without the extract (also contained cytochalasin B, 6 µg/ml) served as negative controls. Blood samples were also incubated with quercetin (1.3 µg/ml) for comparative purposes. Test results consisted of the number of micronuclei-containing cells per 1000 scored cells and as the incidence of micronuclei formation relative to the incidence of micronuclei formation in the control sample. The incidence (21%, mean of 5 measurements) of micronucleus formation in the sample treated with the extract was higher than that of the control sample. This incidence of micronucleus formation was also comparable to that caused by quercetin alone (20% incidence).

In Vivo

Equisetum arvense

Equisetum arvense (doses, plant part, and method of derivation not stated) was evaluated for genotoxicity potential in the rat (strain not stated) micronucleus test.[34] Details relating to the test protocol were not included. The incidence of micronucleated polychromatic erythrocytes (MNPCEs) was not significantly increased, and *Equisetum arvense* was classified as non-genotoxic in this assay.

CARCINOGENICITY STUDIES

Data on the carcinogenicity of the *Equisetum arvense*-derived ingredients reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

OTHER RELEVANT STUDIES

Hepatotoxicity

Equisetum Arvense Extract

The hepatotoxicity of an *Equisetum arvense* extract (aqueous extract of shade-dried and powdered *Equisetum arvense*) was evaluated using groups of 10 adult male Wistar rats.[40] The animals received graded doses of the extract (30 mg/kg, 50 mg/kg, and 100 mg/kg; 1 dose per group) by gavage for 14 d. The control group was dosed with distilled water. Blood samples were collected (schedule not stated) to determine hepatic enzyme (aspartate amino transferase (AST), alanine amino transferase (ALT), and gamma glutamyl transferase (γ-GT)) activities in the serum. Hepatic tissue fragments were obtained for histological analysis. None of the animals in either of the 3 dose groups died. Additionally, when compared to the control group, dosing did not change

serum activities of hepatic enzymes. Only benign changes in the hepatic morphology were observed in the 3 dose groups as well as in the control group. Centrilobular steatosis and cellular tumefaction (hydropic degeneration) were observed in the 3 dose groups. However, only centrilobular steatosis was observed in the control group. The authors concluded that oral treatment with graded doses of *Equisetum arvense* extract was not able to produce significant hepatic changes, when compared to the control group. They also noted that further studies are necessary in order to evaluate the chronic hepatotoxicity of *Equisetum arvense* in rats. In another study, an *Equisetum arvense* extract (methanol extract) had a hepatoprotective effect in human hepatoma Hep G2 cells incubated with tacrine (hepatotoxin).[32]

Cytotoxicity

Equisetum Arvense Extract

An *Equisetum arvense* extract (water and ethanol extract) was evaluated for anti-proliferative activity using mouse melanoma B16 cells.[41] This cell line is derived from a spontaneous skin tumor in C57BI/6 mice. Test concentrations ranged from < 0.25 mg/ml to > 0.5 mg/ml. After a 2-d incubation period with the extract, cell counts were made with a hemocytometer and cell viability was assessed by trypan blue exclusion. Each test was performed 6 times, and the extract concentration that caused 50% growth inhibition (IC_{50}) was determined. A cytotoxic effect was not observed (i.e., no effect on cell proliferation) at low concentrations (< 0.25 mg/ml). This *Equisetum arvense* extract caused a significant (statistical significance not stated) cytotoxic (antiproliferative) effect at high concentrations (> 0.5 mg/ml). An IC_{50} of 1.5 mg/ml was reported for *Equisetum arvense* extract.

The cytotoxicity of an *Equisetum arvense* extract (water extract; drug extract ratio[DER] 1:20) against human leukemia cells (U 937 cells) in vitro was evaluated.[42] Cultures were incubated with the extract at concentrations of 124, 248, and 496 µg dry matter/ml for 48 h. Cytotoxicity was increased in a dose-dependent manner. Whether or not the cell death was due to apoptosis was investigated. Test material concentrations of 124 µg/ml and 248 µg/ml did not influence the apoptotic process. However, the highest concentration of this *Equisetum arvense* extract (496 µg/ml) induced early and late apoptosis, when compared to the control (cells cultured without the extract).

The growth inhibitory activities of several different *Equisetum arvense* extracts (aerial parts; ethyl acetate, chloroform, petroleum ether, n-butanol, and water extracts) were evaluated using 3 histologically different human cancer cell lines (HeLa (human cervix epidermoid tumor cell line), MCF7 (human breast adenocarcinoma cell line), and HT-29 (human colon adenocarcinoma cell line)).[31] The extracts (20 µl per well) were added in order to achieve final concentrations for each extract of 0.0625 to 1 mg/ml. The HeLa human cervix epidermoid tumor cells were found to be the most sensitive to all of the extracts. Ethyl acetate, chloroform, and petroleum ether extracts exhibited a statistically significant ($p < 0.01$) antiproliferative effect in the HeLa cell line (in 0.125 to 1 mg/ml concentration range), with IC_{50} values ranging from 0.23 to 0.76 mg/ml. The *n*-butanol extract did not induce 50% inhibition of HeLa cell growth in the 0.0625 to 1 mg/ml concentration range, but growth inhibition effects at 0.5 to 1 mg/ml were statistically significantly different ($p < 0.01$) when compared to the control (not stated). Except for the *n*-butanol extract, all of the extracts statistically significantly decreased MCF-7 cell growth over the entire concentration range. The effects of the ethyl acetate and chloroform extracts were most prominent ($p < 0.01$) in the 0.125 to 0.5 mg/ml concentration range. However, in this concentration range, no extract caused 50% inhibition of MCF-7 cell growth. Both ethyl acetate and petroleum ether extracts caused a statistically significant ($p < 0.01$) antiproliferative effect in the HT-29 cell line, with IC_{50} values ranging from 0.32 to 0.53 mg/ml. Based on the IC_{50} values, the antiproliferative activity of the extracts decreased in the following order: ethyl acetate > chloroform > petroleum ether. Morphological changes that resembled necrosis were observed in all cell lines. The most prominent morphological changes were observed in HeLa cells treated with ethyl acetate, chloroform, and n-butanol extracts of *Equisetum arvense*.

Equisetum Arvense Leaf Extract

The ability of an *Equisetum arvense* leaf extract (ethanol extract) to induce apoptosis was studied using A549 lung carcinoma cells.[43] The extract was evaluated at concentrations of 100 µg/ml and 150 µg/ml using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay. Acridine orange staining was used to assess apoptosis. The development of an orange or orange-red color was indicative of disruption of the cell membrane. Following treatment with both concentrations, the cells were floating (sign of early apoptosis). Additionally, the edges of many cells were not clear and the cytoplasm was not as transparent when compared to untreated control cells. Overall, the cell structure was completely desegregated, with a hard-shelled appearance. Fifty percent of the cells treated with 100 µg/ml developed orange fluorescence. More than 70% of the cells developed orange florescence after treatment with 150 µg/ml. The results of this study indicated that this *Equisetum arvense* leaf extract manifested cytotoxicity and decreased the cell viability of A549 cells in a concentration-dependent manner.

Antimicrobial Activity

Equisetum Arvense Extract

The in vitro antimicrobial activity of an *Equisetum arvense* extract (stem; hydro-alcoholic (20:80, v/v) extract) against the following bacterial/fungal strains was evaluated: *Staphylococcus aureus*, *E. coli* 95, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *S. enteritidis* (all bacterial strains), and the fungal strains *Aspergillus niger* and *Candida albicans*.[16] Disks containing the extract (5 µg per disk) and a bacterial strain were incubated for 24 h. The incubation period for plates containing a fungal strain and the extract (5 µg per disk) was 48 h. For each disk, the diameter (mm) of the inhibition zone was measured.

Disks containing ampicillin and nystatine (30 µg per disk) served as positive controls, and disks containing methanol served as negative controls. *Staphylococcus aureus* was found to be the strain that was most resistant to this *Equisetum arvense* extract. The most sensitive strain was *Pseudomonas aeruginosa.* Results indicated that the antimicrobial activity of this *Equisetum arvense* extract (5 µg per disk) was comparable to the antimicrobial activity of the positive controls (30 µg per disk).

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

Equisetum Arvense Extract

In a non-occlusive, skin irritation test involving 4 rabbits (strain not stated), Equisetum Arvense Extract (hydroglycolic extract containing ~2% dry extract) was classified as non-irritating.[6] Data on the composition of this extract are included in the section on Composition/Impurities. Details relating to the test protocol are not included.

The skin irritation potential of Equisetum Arvense Extract (100%) was evaluated using 3 rabbits (strain not stated).[7] Details relating to the test protocol were not included. Skin irritation was not observed in the animals tested. Equisetum Arvense Extract (in butylene glycol) was evaluated for skin irritation potential using 3 rabbits (strain not stated).[7] The test material was applied to the skin at a concentration of 100%. Details relating to the test protocol were not included. Equisetum Arvense Extract (in butylene glycol) was slightly irritating to the skin.

Sensitization

Animal

Equisetum Arvense Extract

The skin sensitization potential of Equisetum Arvense Extract was evaluated in a maximization test involving 5 guinea pigs (strain not stated).[7] The first and second induction concentrations were 12.5% and 100%, respectively. The challenge concentration was 100%. Additional details relating to the test protocol were not included. The test substance did not induce skin sensitization in any of the animals tested. Equisetum Arvense Extract (in butylene glycol) was evaluated in another maximization test involving 5 guinea pigs (strain not stated).[7] The first and second induction concentrations were 25% and 100%, respectively. The challenge concentration was 100%. Additional details relating to the test protocol were not included. Test results were negative.

Human

Equisetum Arvense Extract

A human repeated insult patch test (HRIPT) on a nail polish containing 0.000049% Equisetum Arvense Extract was performed using 209 subjects.[44] During induction, the test substance (~ 0.2 ml) was applied for 24 h to the upper back (between the scapulae) using a 1" x 1" semi-occlusive patch. The application frequency was 3 times per week (Mondays, Wednesdays, and Fridays) for a total of 9 applications. Tuesday and Thursday patch removals were followed by a 1-d non-treatment period, and Saturday removals were followed by a 2-d non-treatment period. After induction, the challenge phase was preceded by a 2-wk non-treatment period. A challenge patch was applied for 24 h to a new site that was adjacent to the induction site. Reactions were scored at days 1 and 3 post-application. Results indicated no potential for dermal irritation or allergic contact sensitization.

The skin sensitization potential of a product (mask) containing 0.6% Equisetum Arvense Extract was evaluated in an HRIPT involving 100 subjects.[45] During induction, the test substance (0.02 ml) was applied for 48 h to the lower or upper back using a 0.64 cm^2 occlusive patch. The test substance was applied over a 3-wk period, which consisted of nine 48-h exposures. Following a 2-wk non-treatment period, the challenge phase was initiated. Challenge patches were applied to a new site and to the induction site. Challenge reactions were evaluated at 30 min and at 48 h, 72 h, and 96 h post-removal. Results indicated no evidence of skin irritation or sensitization.

OCULAR IRRITATION STUDIES

Equisetum Arvense Extract

In an ocular irritation test involving 4 rabbits (strain not stated), Equisetum Arvense Extract (hydroglycolic extract containing ~2% dry extract) was classified as slightly irritating.[6] Data on the composition of this extract are included in the section on Composition/Impurities. Details relating to the test protocol are not included.

CLINICAL STUDIES

Case Reports

Equisetum arvense

A dermatitis patient was regularly in contact with *Equisetum arvense* (plant part(s) not stated) in the proximity of his house.[19] In the hour after exposure, he developed dermatitis (resembled seborrheic dermatitis) of the right hand and face after passive inhalation of tobacco smoke. Additionally, a fresh exposure to *Equisetum arvense* induced a more rapid reaction, which necessitated local application of epinephrine and oral antihistamines. The authors noted that this patient's history of atopic reactions with nicotine as a hapten in tobacco smoke correlated with the possible presence of nicotine in *Equisetum arvense*. It was also noted that nicotine has been detected in British species of *Equisetum arvense*.

A woman with no history of atopy developed rhinoconjunctivitis symptoms (dyspnea and general malaise) and contact dermatitis after inhaling steam while cooking green beans, potatoes, and carrots.[46] The patient also used *Equisetum arvense* to lose weight. Prick test results for *Equisetum arvense* (1/1 (w/v) concentration in isotonic saline solution) were positive; the same was true for celery and carrots. Conjunctival challenge with *Equisetum arvense* (1/10 dilution) also yielded a positive response. Additionally, conjunctival challenge with celery (1/10 dilution) and carrot (1/1000 dilution) yielded positive responses. The authors noted that *Equisetum arvense* contains a protein that is similar to a protein that is found in carrots.

Hair loss and fragile nails were observed in a male consumer who took *Equisetum arvense* (3 units/d) for 12 mo.[47] It was noted that the hair loss could have been associated with the reported effect of *Equisetum arvense* in reducing the bioavailability of thiamine after chronic consumption.

Equisetum Arvense Extract

Hand and facial swelling were observed in a female patient after 2 d of oral consumption of an herbal diuretic containing an *Equisetum arvense* extract (ethanol extract).[15] The diuretic was taken 3 times daily and consisted of 225 mg of *Equisetum arvense* dry extract (DER 7.5 - 10.5:1; extraction solvent = ethanol (70% v/v)). Recovery was noted after treatment of symptoms.

In-Use Safety

Equisetum Arvense Extract

An in-use safety evaluation of 3 nail polish products (different shades) containing 0.000049% Equisetum Arvense Extract was performed using 31 female subjects.[48] They were instructed to use a product daily for 4 wk. Nail polish remover was used between applications. Nail plates and cuticles were examined by a trained clinical technician. No adverse reactions were observed after repeated use.

SUMMARY

The safety of 5 *Equisetum arvense*-derived ingredients as used in cosmetics is reviewed in this safety assessment. *Equisetum arvense* (horsetail) has been described as a non-flowering weed that is found throughout parts of Europe, Asia, the Middle East, and North America.

The method of production of an Equisetum Arvense Extract in ethanol and water (0.7% solids w/v) tradename mixture involves extraction of dried raw material with an unknown concentration of ethanol. Preparation of another Equisetum Arvense Extract in ethanol and water tradename mixture involves the extraction of dried raw material with 30 vol% ethanolic solution. In the production of an Equisetum Arvense Extract in butylene glycol (0.54% solids w/v) tradename mixture, the dried raw material is extracted with 50 vol% 1,3-butylene glycolic solution.

The preparations of different extracts of *Equisetum arvense* have also been described in the published literature. Air dried and powdered plant material was macerated with petroleum ether overnight, and afterwards with 70% methanol. After filtration, the methanolic extract was concentrated to dryness. The dry residue was dissolved in hot water and then separated by liquid-liquid extraction into the chloroform, ethyl acetate, and *n*-butanol extracts.

According to a cosmetics industry source, a tradename mixture Equisetum Arvense Extract has the following composition: Equisetum Arvense Extract (~ 2% dry extract), propylene glycol (~ 66%), phenoxyethanol (~ 0.36%), methylparaben (~ 0.08%), ethylparaben (~ 0.02%), propylparaben (~ 0.04%), and water (qsp 100%). Data on flavonoid composition reveal the existence of 2 chemotypes of *Equisetum arvense*, one in Asia and North America, and the other in Europe. *Equisetum arvense* from Asia and North America contains luteolin-5-*O*-glucoside and its malonyl ester, but these compounds are not found in *Equisetum arvense* from Europe. The dominant compounds in *Equisetum arvense* from Europe are quercetin 3-*O*-glucoside, apigenin 5-*O*-glucoside, and dicaffeoyl-*meso*-tartaric acid. Di-*E*-caffeoyl-*meso*-tartaric acid is a marker for both chemotypes. Whether or not flavonoids or phenolic acids are the predominant compounds in *Equisetum arvense* extracts is dependent upon the extractant that is used.

According to 2021 VCRP data, Equisetum Arvense Extract is reported to be used in 186 cosmetic products (125 leave-on products, 59 rinse-off products, and 2 products that are diluted for (bath) use). Of the *Equisetum arvense*-derived ingredients that are being reviewed in this safety assessment, this is the greatest reported use frequency. The results of a concentration of use survey submitted by the Council in 2019 indicate that Equisetum Arvense Extract is being used at maximum use concentrations up

to 0.4% in leave-on products (body and hand products (not spray)), and at maximum use concentrations up to 0.00078% in rinse-off products (skin cleansing products). Equisetum Arvense Extract is the only *Equisetum arvense*-derived ingredient in this safety assessment for which use concentration data were provided in response to the Council survey. According to VCRP and Council survey data, Equisetum Arvense Juice and Equisetum Arvense Leaf Powder are not being used in cosmetic products.

In Asian traditional medicine, the aerial parts of *Equisetum arvense* have been used to treat hemorrhage, urethritis, jaundice, and hepatitis. According to the US FDA, *Equisetum arvense* is among the ingredients that have been present in OTC drug products for use as a digestive aid. However, based on evidence currently available, there are inadequate data to establish general recognition of safety and effectiveness of this ingredient for this specified use.

In an acute oral toxicity study involving 10 mice (strain not stated), an LD_0 of ≥ 20 ml/kg was reported for Equisetum Arvense Extract (hydroglycolic extract containing ~2% dry extract). A single-dose, oral toxicity study on *Equisetum arvense* was performed using groups of male and female rats (strain and number per group not stated). The approximate LD_{50} value was > 5000 mg/kg. None of the animals died and there were no signs of toxicity at necropsy.

An *Equisetum arvense* extract (hydroalcoholic extract) was evaluated for acute toxicity using groups of 8 male Wistar rats. The groups received i.p. doses of 1000 mg/kg, 2000 mg/kg, and 5000 mg/kg. Mortalities were observed in the 2000 mg/kg group (12.5% of the animals) and in the 5000 mg/kg group (37.5% of the animals). Transitory respiratory depression and elevated sedation (dose-dependent) were observed in all 3 dose groups.

In a short-term study, male Sprague-Dawley rats (groups of 6) were fed an *Equisetum arvense* powder (0.4% or 4%) in a 20% casein diet with and without cholesterol (0.5% cholesterol and 0.15% sodium cholate) for 14 d. At a concentration of 0.4% or 4% in either diet, *Equisetum arvense* powder did not influence food intake or growth, or have an effect on serum or liver lipids. However, on days 9 to 12 of feeding with 4% *Equisetum arvense* powder in the cholesterol diet, 4 of 6 rats lost their hair and dermatitis was observed on the neck, head, nose, and back. At microscopic examination, these changes were diagnosed as nonspecific inflammatory lesion of the skin. Reversal of the dermatitis was noted when the diet was changed to a commercial diet. In 2 additional experiments, rats were fed an *Equisetum arvense* powder (concentration not stated) in a cholesterol diet (composition not stated) for 4 wk and 6 wk. Six of 21 rats from the 2 experiments had dermatitis on the neck and back. The incidence of dermatitis after feeding for 4 wk and 6 wk was approximately 20% and 30%, respectively.

An *Equisetum arvense* extract (dried stem, ethanol and water extract) was administered to 10 male Wistar rats, at a daily i.p. dose of 50 mg/kg for 8 wk. Signs of toxicity were not observed.

The subchronic oral toxicity of *Equisetum arvense* (powder extracted with hot water; plant part not stated) was evaluated in a study involving groups of 10 male and female F344 rats fed *Equisetum arvense* at a concentration of 0.3%, 1%, or 3% in the diet (no further information on test substance composition or plant part(s) included) for 13 wk. None of the animals died, and no obvious clinical signs were observed in any of the animals during the study. Statistically significant alterations in hematological parameters (e.g., mean corpuscular hemoglobin and platelet count) were observed, but no dose dependence was apparent. Histopathological changes (not treatment-related) were observed in the liver, pancreas, kidneys, and ovaries. The NOAEL for *Equisetum arvense* was determined to be more than 3% in male and female rats (> 1.79 g/kg bw/d, males; > 1.85 g/kg bw/d, females).

The results of an in vitro teratogenicity assay on *Equisetum arvense* involving zebrafish (*Danio rerio*, AB strain) embryos demonstrated no teratogenic potential of this botanical (test concentrations of 0.00625 mg/l, 0.0625 mg/l, and 0.625 mg/l) during development of the zebrafish.

Equisetum arvense (plant part, method of preparation, and doses not stated) was evaluated in a reverse mutation test using *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, and *E. coli* strain WP2uvrA. Results were negative. A chromosomal aberration test on *Equisetum arvense* (plant part, method of preparation, and doses not stated) was performed using Chinese hamster lung cells. It was concluded that *Equisetum arvense* did not have any potential for inducing chromosomal aberrations. The acquired micronucleus formation in unirradiated and irradiated samples of human blood lymphocytes cultured with *Equisetum arvense* extract (ethanol extract, 0.025, 0.05, 0.1, and 0.2 mg/ml) was evaluated using the cytochalasin block micronucleus test in vitro. *Equisetum arvense* extract (ethanol extract) had weak clastogenic properties in this test. The genotoxicity of an *Equisetum arvense* extract (stem hydro-alcoholic (20:80, v/v) extract) was evaluated in another in vitro micronucleus test. Human blood samples were cultured with the extract (62.5 µg/ml). The incidence (21%, mean of 5 measurements) of micronucleus formation in the sample treated with the extract was higher than that of the control sample.

Equisetum arvense (plant part, method of preparation, and doses not stated) was evaluated for genotoxicity potential in the rat (strain not stated) micronucleus test in vivo. The incidence of MNPCEs was not significantly increased, and *Equisetum arvense* was classified as non-genotoxic in this assay.

The hepatotoxicity of an *Equisetum arvense* extract (aqueous extract of shade-dried and powdered *Equisetum arvense*) was evaluated using groups of 10 adult male Wistar rats. The animals received graded doses of the extract (30 mg/kg, 50 mg/kg, and 100 mg/kg; 1 dose per group) by gavage for 14 d. None of the animals died, and significant hepatic changes were not observed.

An *Equisetum arvense* extract (water and ethanol extract) caused a significant (statistical significance not stated) cytotoxic (antiproliferative) effect in mouse melanoma B16 cells at high concentrations (> 0.5 mg/ml). An IC_{50} of 1.5 mg/ml was reported.

The cytotoxicity of an *Equisetum arvense* extract (water extract; DER 1:20) against human leukemia cells (U 937 cells) in vitro was evaluated. Concentrations of 124 µg/ml and 248 µg/ml did not influence the apoptotic process. However, the highest concentration of *Equisetum arvense* extract (496 µg/ml) induced early and late apoptosis, when compared to the control (cells cultured without *Equisetum arvense* extract). The growth inhibitory activity of *Equisetum arvense* extracts (aerial parts; ethyl acetate, chloroform, and petroleum ether, *n*-butanol, and water extracts) was evaluated using 3 histologically different human cancer cell lines (HeLa, MCF7, and HT-29 cells). The HeLa human cervix epidermoid tumor cells were found to be the most sensitive to all of the extracts. Ethyl acetate, chloroform, and petroleum ether extracts exhibited a statistically significant ($p < 0.01$) antiproliferative effect in the HeLa cell line (in 0.125 to 1 mg/ml concentration range), with IC_{50} values ranging from 0.23 to 0.76 mg/ml.

The ability of an *Equisetum arvense* leaf extract (ethanol extract) to induce apoptosis was studied using A549 lung carcinoma cells.[43] The extract was evaluated at concentrations of 100 µg/ml and 150 µg/ml using the MTT cytotoxicity assay. *Equisetum arvense* leaf extract manifested cytotoxicity and decreased the cell viability of A549 cells in a concentration-dependent manner.

The in vitro antimicrobial activities of an *Equisetum arvense* extract (stem hydro-alcoholic (20:80, v/v) extract) against *Staphylococcus aureus*, *E. coli* 95, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *S. enteritidis* (all bacterial strains), and the fungal strains *Aspergillus niger* and *Candida albicans* were evaluated. Results indicated that the antimicrobial activities of *Equisetum arvense* extract (5 µg per disk) were comparable to the antimicrobial activities of the positive controls (ampicillin and nystatine, 30 µg per disk).

In a non-occlusive, skin irritation test involving 4 rabbits (strain not stated), Equisetum Arvense Extract (hydroglycolic extract containing ~2% dry extract) was classified as non-irritating. Skin irritation was not observed in a study in which Equisetum Arvense Extract (100%) was applied to the skin of 3 rabbits (strain not stated). Equisetum Arvense Extract (in butylene glycol) was also evaluated for skin irritation potential in a study involving 3 rabbits (strain not stated). The extract was applied at a concentration of 100%, and was classified as slightly irritating to the skin.

The skin sensitization potential of Equisetum Arvense Extract was evaluated in a maximization test involving 5 guinea pigs (strain not stated). Prior to the challenge phase, the first and second induction concentrations were 12.5% and 100%, respectively. A challenge concentration of 100% did not induce skin sensitization in any of the animals tested. Equisetum Arvense Extract (in butylene glycol) was evaluated in another maximization test involving 5 guinea pigs (strain not stated). Prior to the challenge phase, the first and second induction concentrations were 25% and 100%, respectively. Results were negative for a challenge concentration of 100%. An HRIPT on a nail polish containing 0.000049% Equisetum Arvense Extract was performed using 209 subjects. Results indicated no potential for dermal irritation or allergic contact sensitization. The skin sensitization potential of a product (mask) containing 0.6% Equisetum Arvense Extract was evaluated in an HRIPT involving 100 subjects. Results indicated no evidence of skin irritation or sensitization.

In an ocular irritation test involving 4 rabbits (strain not stated), Equisetum Arvense Extract (hydroglycolic extract containing ~2% dry extract) was classified as slightly irritating.

Hand and facial swelling were observed in a female patient after 2 d of oral consumption of an herbal diuretic containing an *Equisetum arvense* extract (225 mg of *Equisetum arvense* dry extract (DER 7.5 - 10.5:1; extraction solvent = ethanol (70% v/v)). A dermatitis patient who was regularly in contact with *Equisetum arvense* developed dermatitis of the right hand and face after passive inhalation of tobacco smoke; a fresh exposure to *Equisetum arvense* induced a more rapid reaction. A female patient with rhinoconjunctivitis and contact dermatitis had inhaled steam while cooking vegetables and also consumed *Equisetum arvense* for weight loss. Prick test results for *Equisetum arvense* (1/1 (w/v) concentration in isotonic saline solution) were positive. Conjunctival challenge with *Equisetum arvense* (1/10 dilution) also yielded a positive response. Hair loss and fragile nails were observed in a male consumer who took *Equisetum arvense* (3 units/d) for 12 mo. An in-use safety evaluation of 3 nail polish products (different shades) containing 0.000049% Equisetum Arvense Extract was performed using 31 female subjects. After 4 wk of daily use, no adverse reactions were observed.

DISCUSSION

This assessment reviews the safety of 5 *Equisetum arvense*-derived ingredients as used in cosmetic formulations. The Panel concluded that these ingredients are safe in cosmetics in the present practices of use and concentration. Non-specific ulcerative dermatitis (noted as hair loss) was observed in an oral dosing study in which Sprague-Dawley rats were fed 4% *Equisetum arvense* powder in a cholesterol diet for 14 d. However, concern over this finding was mitigated due to no obvious clinical signs in another study in which F344 rats were fed *Equisetum arvense* (hot water extract of powder) at concentrations up to 3% in a basal diet for 13 wk. The Panel acknowledged that *Equisetum arvense* was not definitively identified as the causative agent in the 14-d study. They also agreed that the negative results in the 13-wk rat oral toxicity study on *Equisetum arvense* (aqueous extract) obviate concerns relating to the systemic toxicity of *Equisetum arvense*-derived ingredients.

Based on negative HRIPT data on products containing 0.000049% (209 subjects) and 0.6% (100 subjects) Equisetum Arvense Extract and a negative in-use safety evaluation (31 subjects) on nail products containing 0.000049% Equisetum Arvense Extract, the Panel agreed that the skin irritation and sensitization potential of this ingredient at the maximum reported use

concentration of 0.4% in cosmetics is not a concern. Slight ocular irritation was observed in a study in which Equisetum Arvense Extract (hydroglycolic extract containing ~2% dry extract) was instilled into the eyes of rabbits. However, the Panel noted that this test concentration is greater than the maximum reported use concentration of 0.4% for *Equisetum arvense*-derived ingredients in cosmetics. Furthermore, the Panel stated that, in the absence of an NOAEL for ocular irritation and use concentration data on products applied near the eye, manufacturers should assure that products containing *Equisetum arvense*-derived ingredients are formulated to be non-irritating.

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

Finally, the Panel discussed the issue of incidental inhalation exposure that could possibly result with use of *Equisetum arvense*-derived ingredients (e.g. Equisetum Arvense Extract in cologne and toilet waters, and in other fragrance preparations (concentrations unknown)). Inhalation toxicity data were not available. However, the Panel noted that, in aerosol products, 95% - 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the following 5 ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

Equisetum Arvense Extract
Equisetum Arvense Juice*
Equisetum Arvense Leaf Extract
Equisetum Arvense Leaf Powder*
Equisetum Arvense Powder

**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.*

TABLES

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

Ingredient/CAS No.	Definition & Structures	Function(s)
Equisetum Arvense Extract 71011-23-9	Equisetum Arvense Extract is the extract of the whole herb, *Equisetum arvense*.	Skin-Conditioning Agents - Miscellaneous
Equisetum Arvense Juice 71011-23-9 (generic)	Equisetum Arvense Juice is the juice expressed from *Equisetum arvense*.	Skin-Conditioning Agents - Miscellaneous
Equisetum Arvense Leaf Extract 71011-23-9	Equisetum Arvense Leaf Extract is the extract of the leaves of *Equisetum arvense*.	Skin-Conditioning Agents - Miscellaneous
Equisetum Arvense Leaf Powder	Equisetum Arvense Leaf Powder is the powder obtained from the dried, ground leaves of *Equisetum arvense*.	Skin-Conditioning Agents - Miscellaneous
Equisetum Arvense Powder	Equisetum Arvense Powder is the powder obtained from the dried, ground whole plant, *Equisetum arvense*.	Skin-Conditioning Agents - Humectant

Table 2. Dominant compounds in *Equisetum arvense* (native to Vojvodina, Serbia) extracts , based on solvent.[8]

Phenolic Compounds	Quantity (mg/g dry extract) in Ethyl Acetate Extract*	Quantity (mg/g dry extract) in *n*-Butanol Extract*	Quantity (mg/g dry extract) in Aqueous Extract*
Isoquercitrin	152	382	----
Apigenin 6-*O*-glucoside	22.40	----	----
Kaempferol 3-*O*-glycoside	26.20	----	----
di-*E*-caffeoyl-meso-tartaric acid	----**	100	10
Phenolic Acid 1 (unnamed)	----	----	3
Phenolic Acid 2 (unnamed)	----	----	6

*HPLC analysis with detection and quantification carried out at 350 nm
**not detected at 350 nm

Table 3. Composition data on Equisetum Arvense Extract from Asia and Europe.[17]

Components	*Equisetum arvense* (methanol and water extract) from Asia	*Equisetum arvense* (methanol and water extract) from Europe
Apigenin 4'-*O* glucoside	Detected (quantity not stated)	Detected (quantity not stated)
Apigenin 5-*O*-(6"-O-malonylglucoside)	Detected (quantity not stated)	Detected (quantity not stated)
Apigenin 5-*O*-glucoside	>500 µg/g dry weight	>500 µg/g dry weight
5-*O*-Caffeoylshikimic acid	Detected (quantity not stated)	Detected (quantity not stated)
Chlorogenic acid	Detected (quantity not stated)	Detected (quantity not stated)
Dicaffeoyl-*meso*-tartaric acid	>500 µg/g dry weight	>500 µg/g dry weight
Equisetumpyrone	Detected in fertile sprouts only	Detected in fertile sprouts only (quantity not stated)
Genkwanin 4'-*O*-glucoside	Detected (quantity not stated)	Detected (quantity not stated)
Genkwanin 5-*O*-glucoside	Detected (quantity not stated)	Detected (quantity not stated)
Genkwanin 5-O-(6"-*O*-malonyglucoside)	Detected (quantity not stated)	Detected (quantity not stated)
Gossypetin 7-*O*-glucoside	Detected in fertile sprouts only (quantity not stated)	Detected in fertile sprouts only (quantity not stated)
Kaempferol 3-*O*-glucoside	Detected (quantity not stated)	Detected (quantity not stated)
Kaempferol 3-*O*-rutinoside-7-*O*-glucoside	Detected (quantity not stated)	Detected (quantity not stated)
Kaempferol 3-*O*-sophoroside	Detected (quantity not stated)	Detected (quantity not stated)
Kaempferol 3-O-(6"-*O*-malonylglucoside)	Detected (quantity not stated)	Detected (quantity not stated)
Kaempferol 3-O-(6"-O-malonylglucoside)-7-O-glucoside	Detected (quantity not stated)	Detected (quantity not stated)
Kaempferol 3,7-*O*-diglucoside	Detected (quantity not stated)	Detected (quantity not stated)
Luteolin 5-*O*-glucoside	>500 µg/g dry weight	Not detected
Luteolin 5-*O*-(6"-O-malonylglucoside)	Detected (quantity not stated)	Not detected
Monocaffeoyl-*meso*-tartaric acid	Detected (quantity not stated)	Detected (quantity not stated)
Protoapigenin 4-*O*-glucoside	Detected in fertile sprouts only (quantity not stated)	Detected in fertile sprouts only (quantity not stated)
Protogenkwanin 4'-*O*-glucoside	Detected in fertile sprouts only (quantity not stated)	Detected in fertile sprouts only (quantity not stated)
Quercetin 3-*O*-glucoside	Detected (quantity not stated)	>500 µg/g dry weight
Quercetin 3-*O*-sophoroside	Detected (quantity not stated)	>500 µg/g dry weight
Quercetin 3-*O*-(6"-*O*-malonylglucoside)	Detected (quantity not stated)	Detected (quantity not stated)
Quercetin 3,7-*O*-diglucoside	Detected (quantity not stated)	Detected (quantity not stated)

Table 4. Frequency (2021) and concentration of use (2019) according to duration and type of exposure.[20,21]

	Equisetum Arvense Extract		Equisetum Arvense Leaf Extract		Equisetum Arvense Powder	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals*	**186**	**0.0000011-0.4**	**12**	**NR**	**1**	**NR**
Duration of Use						
Leave-On	*125*	*0.01-0.4*	*8*	*NR*	*1*	*NR*
Rinse off	*59*	*0.0000011-0.00078*	*4*	*NR*	*NR*	*NR*
Diluted for (bath) Use	*2*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	11	NR	1	NR	NR	NR
Incidental Ingestion	NR	0.0002	NR	NR	NR	NR
Incidental Inhalation - Sprays	1;33[a];62[c]	0.0002[a]	1;3[a];1[c]	NR	NR	NR
Incidental Inhalation - Powders	2;62[c]	0.01-0.4[b]	1[c]	NR	NR	NR
Dermal Contact	138	0.00078-0.4	5	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	46	0.0000011-0.0006	6	NR	1	NR
Hair-Coloring	1	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	9	0.0002	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
	equisetum arvense (horsetail)**					
	# of Uses	Conc. (%)				
Totals/Conc. Range	**3**	**NS**				
Duration of Use						
Leave-On	*2*	*NS*				
Rinse off	*1*	*NS*				
Diluted for (bath) Use	*NR*	*NS*				
Exposure Type						
Eye Area	1	NS				
Incidental Ingestion	NR	NS				
Incidental Inhalation - Sprays	NR	NS				
Incidental Inhalation - Powders	NR	NS				
Dermal Contact	1	NS				
Deodorant (underarm)	NR	NS				
Hair - Non-Coloring	2	NS				
Hair-Coloring	NR	NS				
Nail	NR	NS				
Mucous Membrane	NR	NS				
Baby Products	NR	NS				

NR – not reported
NS = Not Surveyed
* Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
**Not an International Nomenclature Cosmetic Ingredient (INCI) name, but uses under this name are in the VCRP
[a]It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays
[b]It is possible that these products may be powders, but it is not specified whether the reported uses are powders
[c]Not specified that these products are sprays or powders, but it is possible the use can be as a spray or powder, therefore the information is captured in both categories

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