Safety Assessment of *Paeonia suffruticosa*-Derived Ingredients as Used in Cosmetics

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All interested persons are provided 60 days from the above release date (i.e., by February 11, 2025) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party and may be cited in a peer-reviewed scientific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 Executive Director, Dr. Bart Heldreth.

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ABBREVIATIONS

CO_2	carbon dioxide
CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook*
DMEM	Dulbecco's modified Eagle medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DOPA	dihydroxyphenylalanine
ECVAM	European Centre for the Validation of Alternative Methods
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
GLP	good laboratory practices
HRIPT	human repeated-insult patch test
IC_{50}	half maximal inhibitory concentration
IL	interleukin
KFDA	Korea Food and Drug Administration
MDM2	mouse double minute 2 homolog
mLIF	murine leukemia inhibitory factor
MoCRA	Modernization of Cosmetics Regulation Act
α-MSH	α-melanocyte stimulating hormone
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NOEL	no-observed-effect-level
OECD	Organisation for Economic Co-operation and Development
p53	tumor protein p53
Panel	Expert Panel for Cosmetic Ingredient Safety
PARP	poly(adenosine diphosphate-ribose) polymerase
PBS	phosphate-buffered saline
Rac1	Ras-related C3 botulinum toxin substrate 1
RCF	relative centrifugal force
RhE	reconstructed human epidermis
RLD	Registration and Listing Data
RPMI	Roswell Park Memorial Institute
TG	test guideline
TNF-α	tumor necrosis factor alpha
US	United States
VCRP	Voluntary Cosmetic Registration Program
VEGFR-3	vascular endothelial growth factor receptor-3

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 5 *Paeonia suffruticosa*-derived ingredients, most of which are reported to function as skin conditioning agents in cosmetic products. Industry should minimize impurities that could be present in cosmetic formulations, such as heavy metals and pesticide residues, according to limits set by the US Food and Drug Administration (FDA) and Environmental Protection Agency (EPA). The Panel reviewed the available data to determine the safety of these ingredients. The Panel concluded that Paeonia Suffruticosa Seed Oil is safe in cosmetics in the present practices of use and concentration described in this safety assessment. The Panel also concluded that the available data are insufficient to make a determination of safety for the other 4 ingredients (i.e., Paeonia Suffruticosa Bark Extract, Paeonia Suffruticosa Extract, Paeonia Suffruticosa Root Extract and Paeonia Suffruticosa (Tree Peony) Root Bark Extract) under the intended conditions of use in cosmetic formulations.

INTRODUCTION

This assessment reviews the safety of 5 *Paeonia suffruticosa*-derived ingredients as used in cosmetic formulations:

Paeonia Suffruticosa Bark Extract
Paeonia Suffruticosa Extract
Paeonia Suffruticosa Root Extract
Paeonia Suffruticosa Seed Oil
Paeonia Suffruticosa (Tree Peony) Root Bark Extract

Paeonia Suffruticosa (Tree Peony) Root Bark Extract is not included in the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*); however, it had reported uses in 2023 in the US FDA Voluntary Cosmetic Registration Program (VCRP) database and thus is included in this review. According to the *Dictionary*, the other 4 ingredients are all reported to function in cosmetics as skin-conditioning agents; Paeonia Suffruticosa Seed Oil is also reported to function as a hair conditioning agent and a skin protectant (Table 1).[1]

Natural complex substances, such as *Paeonia suffruticosa*, may contain hundreds of constituents. Thus, in this assessment, the Expert Panel for Cosmetic Ingredient Safety (Panel) is evaluating the safety of each of the *Paeonia suffruticosa*-derived ingredients as a whole, complex substance; toxicity from single components may not predict the potential toxicity of botanical ingredients.

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 extensive search of the world's literature; a search was last conducted in October 2024. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

The cosmetic ingredient names, according to the *Dictionary*, are written as listed above, without italics. When referring to the plant from which these ingredients are derived, the standard scientific practice of using italics will be followed (i.e., *Paeonia suffruticosa*). Often in the published literature, a general name (e.g., *Paeonia suffruticosa* extract) is used. If it is not known whether the substance being discussed is equivalent to the cosmetic ingredient, the test substance will be identified by the name used in the publication that is being cited. However, if it is known that the substance is a cosmetic ingredient, the *Dictionary* nomenclature (e.g., Paeonia Suffruticosa Extract) will be used. For some studies, the genus and species of the test article is not specified and it is referred to by the common name, peony; in these instances the common name is used (e.g., peony seed oil). Additionally, the root bark of *Paeonia suffruticosa* can be referred to as moutan cortex, or cortex moutan, in traditional Chinese medicine. However, this term may not be exclusive to the genus and species being reviewed in this report. Thus, test articles have been presented as described in the literature and data potentially referring to *Paeonia suffruticosa* root bark extract has been placed under the Paeonia Suffruticosa (Tree Peony) Root Bark Extract heading herein.

CHEMISTRY

Definition and Plant Identification

The definitions of 4 of the 5 *Paeonia suffruticosa*-derived ingredients reviewed in this assessment are presented in Table 1.[1] (Paeonia Suffruticosa (Tree Peony) Root Bark Extract is not in the *Dictionary*.) Paeonia Suffruticosa Bark Extract, Paeonia Suffruticosa Extract, Paeonia Suffruticosa Root Extract, and Paeonia Suffruticosa Seed Oil all share the generic CAS No. 223747-88-4.

Generally, the bark is the tough protective covering of the woody stems and roots of trees and other woody perennial plants, consisting of cells produced by a cork cambium.[2] Many secondary metabolites with important biological activities biosynthesized by the plants are also stored in the bark. In woody plants, the cortex is a layer of undifferentiated parenchyma cells located between the outer bark and vascular tissues. The root is the organ of a plant that absorbs and transports water

and nutrients, lacks leaves and nodes, and is usually underground. In the roots of the vascular plants, the cortex occupies a larger volume than in herbaceous stems.

The seed is a propagating sexual structure resulting from the fertilization of an ovule, formed by embryo, endosperm, or seed coat; seeds can also result from non-sexual reproduction through apomixis and similar processes. Peony seeds are aggregate, oblong follicles with dense, yellowish-brown bristles that can be obtained after the peony follicles are cracked.[3] Peony seed is comprised of a hard shell and seed kernel.

Paeonia suffruticosa is commonly known as tree peony, moutan, or moutan peony, and has historically been cultivated in China.[4,5] It grows as a shrub, up to 4 m in height, has oval leaves, and its flowers are white, pink, red, or reddish-purple in color.[4] The root extends over 1 m into the ground and is 5 - 12 mm in diameter. The outer surface of the root is grayish or yellowish-brown, and pink when the bark falls off.[5]

Chemical Properties

Paeonia suffruticosa bark extract, *Paeonia suffruticosa* extract, *Paeonia suffruticosa* root bark extract, and *Paeonia suffruticosa* root extract are crude solid extracts, and *Paeonia suffruticosa* seed oil is a liquid.[6-10] Peony seed oil is semi-transparent and orange-yellow in color.[11] Further data on the chemical properties of the ingredients being reviewed were not found.

Method of Manufacture

Most of the methods below are general to the development of *Paeonia suffruticosa*-derived ingredients, and it is unknown if they apply to cosmetic ingredient manufacturing. In some cases, the definition of the ingredients, as given in the *Dictionary*, provides insight as to the method of manufacture.

Paeonia Suffruticosa Bark Extract

A methanolic *Paeonia suffruticosa* bark extract was prepared using 370 g of dried *Paeonia suffruticosa* bark.[6] The dried bark was pulverized and extracted with methanol under reflux.

Paeonia Suffruticosa Extract

According to a submission from a manufacturer (personal communication), the whole plant parts were dried, sliced and extracted with water and butylene glycol at room temperature. Subsequently, the mixture was filtered with membrane filters and the filtrate was separated.

Paeonia Suffruticosa Root Extract

According to a supplier, Paeonia Suffruticosa Root Extract was produced via extraction of dried raw material with 90 vol% ethanolic solution, followed by filtration, concentration, adjustment, sedimentation, secondary filtration and adjustment prior to packaging.[12]

Paeonia Suffruticosa Seed Oil

A *Paeonia suffruticosa* seed oil was obtained via cold press extraction.[10] *Paeonia suffruticosa* seeds (1000 g) were pressed at room temperature, using a screw press. The expressed liquid was centrifuged at 8000 relative centrifugal force (RCF) for 10 min at 4°C, and the resulting *Paeonia suffruticosa* seed oil was collected and stored.

Paeonia suffruticosa seed oil was also extracted from dried ground seed powder via supercritical carbon dioxide (CO_2) extraction, Soxhlet extraction, and screw press expression methods.[13] For the CO_2 extraction, ground *Paeonia suffruticosa* seeds (100 g) were added to an extraction vessel. Liquid CO_2 was then transferred to the vessel via a high-pressure pump under optimized conditions (24 MPa, at a rate of 21 l/h, at 46 °C for 124 min screw press expression method is also a method where solvents are not used. *Paeonia suffruticosa* seed powder (1000 g) was fed from the hopper to the screw press on demand by an expeller and the oil was collected at the oil outlet. The oils obtained from each method were separated by centrifuging at 9000 rpm for 10 min and kept at 4°C.

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

A *Paeonia suffruticosa* root bark extract was prepared by mixing cortex moutan powder with Roswell Park Memorial Institute (RPMI) 1640 medium and placing in an ultrasonic bath for 60 min.[14] The solution was filtered and concentrated resulting in a stock concentration of 50 mg/ml. An 80% ethanolic *Paeonia suffruticosa* root bark extract was prepared in an ultrasonic bath, filtered, concentrated under reduced pressure, and freeze-dried.[15] The lyophilized extract yielded 20.5 g of root bark extract in powder form.

Composition and Impurities

In a phytochemical analysis, flavonoids, tannins, terpenoids and steroids, paeonols (a group of phenols), and the other phenols were identified as the main constituents present in *Paeonia suffruticosa*.[16] The most important groups of secondary metabolites present in this plant are these phenolic compounds and monoterpenoids glycosides.[5] Among the compounds that are most significant are paeonol (2-hydroxy-4-methoxyacetophenone), paeoniflorin (monoterpenoid glycoside) and 1,2,3,4,6-pewnta-*O*-*β*-D-glucopyranose. The presence of various constituents by *Paeonia suffruticosa* plant part is outlined in Table 2.

Paeonia Suffruticosa Extract

Essential oil obtained from hydro-distilled *Paeonia suffruticosa* flowers was analyzed via gas chromatography-mass spectroscopy.[17] The main constituents in the *Paeonia suffruticosa* flower oil were identified as alkanes, alkenes, terpenes, aliphatic alcohols, aliphatic aldehydes, 'benzoids" terpene alcohols, and other oxygenated terpenes.

Paeonia Suffruticosa Root Extract

According to a supplier, Paeonia Suffruticosa Root Extract is composed of tannins, paeonol, and saccharides (amounts not specified).[12] It also contained not more than 20 ppm heavy metals and not more than 2 ppm arsenic.

Paeonia Suffruticosa Seed Oil

A nutritional study on peony seeds indicated the presence of crude oil (34.35%).[18] In another compositional analysis of *Paeonia suffruticosa*, seed oil, fatty acids accounted for 98.46% of the total weight. Interestingly, 89.34% of this was comprised of unsaturated fatty acids.[10,18,19] Polyunsaturated fatty acids were found in the following amounts: n-3 α-linolenic acid (38.86%), n-6 linoleic acid (26.74%), and oleic acid (23.74%). The fairly low ratio of n-3 to n-6 fatty acids (0.69), uncommonly higher levels of α-linolenic acid, and much higher levels of γ-tocopherol compared to other conventional seed oils were the unique features observed in peony oil.

These fatty acids are present in the form of 12 triacylglycerol components in peony seed oil.[10] The major triacylgycerols identified were dilinolyl-linolenoyl-glycerol + dilinolenoly-oleoyl-glycerol (21.69-25.89%), dilinolenoly-linoleoyl-glycerol (14.27-18.01%), oleoyl-linoleoyl-linolenoly-glycerol (13.33-16.03%), dioleoyl-linolenoyl-glycerol + oleoyl-dilinoleoyl-glycerol (14.08-16.3%) and trilinolenoyl-glycerol (11.24-15%). As is often observed with botanical extracts, the percent yield and resulting phytochemical composition of *Paeonia suffruticosa* seed oil is affected by the utilized solvent and method of extraction.[10,13]

Paeonia Suffruticosa (Tree Peony) Root Bark Extract; Paeonia Suffruticosa Root Extract

It has been reported that about 119 secondary metabolites have been isolated and characterized from the root bark or the moutan cortex (root bark) of *Paeonia suffruticosa*.[5] Phenolic compounds and monoterpenoid glycosides have been identified as the major chemical groups present in this extract. Amongst them, the main characteristic compounds were paeonol and its glycosides such as paeoniside, paeonolide, apiopaeonoside and suffruticosides A-D. The total phenolic content found in 8 extracts of *Paeonia suffruticosa* root bark ranged from 63.81 ± 3.96 to 112.95 ± 3.97 mg gallic acid equivalents/g extract.[20]

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 *Paeonia suffruticosa*-derived ingredients in cosmetics. Data included herein were obtained from the FDA and in response to a survey of maximum use concentrations conducted by the Personal Care Products Council (Council), and it is these values that define the present practices of use and concentration. Frequencies of use obtained from the FDA include data from the Voluntary Cosmetic Registration Program (VCRP) database as well as Registration and Listing Data (RLD). As a result of the Modernization of Cosmetics Regulation Act (MoCRA) of 2022, the VCRP was terminated in 2023, and as of 2024, manufacturers and processors have been mandated to register and list their products (and ingredients therein) with the FDA (i.e., RLD). However, because there are numerous differences in the ways the data for the VCRP and the RLD were collected and processed, and because reporting frequency of use is now mandatory (as opposed to the past practice of voluntary reporting), at this time it is not appropriate to contrast data from the VCRP and RLD to determine a trend in frequency of use. Although the VCRP program is now defunct, trends in frequency of use from the RLD alone are not yet possible in that a baseline is currently not available.

According to 2023 VCRP survey data, Paeonia Suffruticosa Root Extract was reported to be used in 213 formulations, 173 of which are leave-on formulations[21] (Table 3). RLD submitted in 2024 indicate that this ingredient is used in 736 total formulations.[22] The results of the concentration of use survey reported by the Council in 2024 indicate Paeonia Suffruticosa Root Extract also has the highest maximum reported concentration of use at up to 0.5% in paste masks and mud packs.[23]

Paeonia Suffruticosa Bark Extract, Paeonia Suffruticosa Extract, and Paeonia Suffruticosa Root Extract are reported to be used in products applied near the eye (concentrations of use not reported). Additionally, most of the ingredients are used in formulations that could come in contact with mucous membranes (e.g., Paeonia Suffruticosa Seed Oil at up to 0.0025% in bath soaps and detergents). Some of these ingredients are used in cosmetic powders and possibly cosmetic sprays, and can possibly be inhaled; for example, Paeonia Suffruticosa Root Extract is reported to be used at 0.05% in face powders. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetics would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. 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.

Some products containing *Paeonia suffruticosa*-derived ingredients may be marketed for use with airbrush delivery systems. With the advent of MoCRA and the current product categories outlined by the FDA, it is now mandatory that cosmetic products used in airbrush delivery systems be reported as such in the RLD. In other words, a reliable source of frequency of use data regarding the use of cosmetic ingredients in conjunction with airbrush delivery systems is now available. Additionally, the Council currently surveys the cosmetic industry for maximum reported use concentrations of ingredients in products which may be used in conjunction with an airbrush delivery system; thus, this type of data may also be available when submitted. However, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety. Without information regarding the consumer habits and practices data or product particle size data (or other relevant particle data, e.g., diameter) related to this use technology, the data profile is incomplete, and the Panel is not able to determine safety for use in airbrush formulations. Accordingly, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

All of the *Paeonia suffruticosa*-derived ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[24]

Non-Cosmetic

The root bark of *Paeonia suffruticosa* is often referred to as moutan cortex, cortex moutan, mockdanpi, or mu dan pi, and is extensively used in traditional Chinese medicine for its anti-inflammatory, antioxidant, anti-tumor, anti-diabetic, cardiovascular protective, neuroprotective, and hepatoprotective effects.[14,15,25-27] Traditionally, the raw material from the root bark is administered to treat fever and its alcoholic solutions are used to improve circulation and remove stasis.[5] Fresh *Paeonia suffruticosa* flowers are also considered edible in China.[28] In 2011, the Chinese Ministry of Health acknowledged the high level of α-linolenic acid (≥ 38%) present in peony seed oil and approved the oil as a new resource food.[29]

TOXICOKINETIC STUDIES

No relevant toxicokinetic studies on *Paeonia suffruticosa*-derived ingredients were found in the published literature, and unpublished data were not submitted. In general, toxicokinetic data are not expected to be found on natural complex substances because they are a complex mixture of constituents.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

Paeonia Suffruticosa Seed Oil

Kunming mice (10/sex) were administered a single oral dose of 15,000 mg/kg bw peony seed oil, via gavage.[18,30] All of the animals survived and the acute LD_{50} was determined to be > 15,000 mg/kg bw. Further details could not be gleaned (original article is in Chinese).

In another acute oral toxicity study, ICR mice (10/sex/group) were given 0, 30, or 60 ml/kg peony seed oil in 2 doses, 6 h apart, via gavage.[11] Controls received water. On the first day of dosing, mice showed reduced food intake and decreased activity; oily feces and anal oil staining were more pronounced in the 60 ml/kg group. By the second and third day of dosing, activity levels in all groups normalized. No deaths occurred during the 7-d observation period and no statistically significant pathological changes occurred in the heart, liver, spleen, lungs, kidneys, and gastrointestinal organs of treated mice, compared to controls. Further details were not provided (article is in Chinese).

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

In an acute oral toxicity study, the LD_{50} for an herbal mixture containing 14.29% moutan cortex was determined to be > 5000 mg/kg.[26] The mixture comprised a total of 2100 g, including 28.57% (600 g) *Rehmannia radix preparata*, 14.29% (300 g) moutan cortex, 14.29% (300 g) *Schisandrae fructus*, 14.29% (300 g) *Asparagi tuber*, 10.71% (225 mg) *Armeniacae semen*, 10.71% (225 mg) *Scutellariae radix*, and 7.14% (150 mg) *Stemonae radix*.

The acute oral toxicity of *Paeonia suffruticosa* tree peony bark extract was evaluated as part of a developmental toxicity study in mice. The LD_{50} was determined to be 3400 mg/kg. No further details were provided for either study.

Short-Term Toxicity Studies

Oral

Paonia Suffruticosa Seed Oil

Healthy rats (12/sex) were administered 1250, 2500, or 5000 mg/kg bw/d peony seed oil, via gavage, for 30 d.[18,30] Vegetable oil (5000 mg/kg bw/d) was given to controls. No abnormal changes in health status, biochemical indexes, hematological and blood biochemical indexes or immune organ indexes were observed at the end of dosing. Based on these results, the maximum non-effective dosage, which is equivalent to the no-observed-effect-level (NOEL), was estimated to be > 5000 mg/kg bw. Further details could not be gleaned (articles in Chinese).

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

The short-term oral toxicity of an herbal mixture containing 14.29% (300 of 2100 g) moutan cortex was evaluated in accordance with Korea Food and Drug Administration (KFDA) Notification no. 2005-60 "The Standards of Toxicity Study for Medicinal Products" and KFDA Notification no. 2005-79 "Good Laboratory Practice (GLP)."[26] Other components of the herbal mixture included: 28.57% (600 g) *Rehmannia radix preparata*, 14.29% (300g) *Schisandrae fructus*, 14.29% (300 g) *Asparagi tuber*, 10.71% (225 g) *Armeniacae semen*, 10.71% (225 g) *Scutellariae radix*, and 7.14% (150 g) *Stemonae radix*. In a 4-wk study, groups of rats were dosed with 800, 2000, or 5000 mg/kg/d of the herbal mixture, via gavage. A decrease in serum sodium was observed in 5000 mg/kg/d females was considered test article-related. Increased liver weights were observed in the 2000 and 5000 mg/kg/d groups, although the statistical significance was not confirmed (no further details provided).

Subchronic Toxicity Studies

Oral

Paeonia Suffruticosa Seed Oil

Groups of Sprague-Dawley rats (10/sex/group) were administered 0, 5, or 10 ml/kg/d peony seed oil, via gavage, for 90 d.[11] Controls received water. Body weights were measured every 10 d. After 90 d, the heart, liver, spleen, lungs, kidneys, brain, adrenal glands, testes, uterus, and ovaries were removed, weighed, and organ:body weight ratios were calculated. Blood was collected and analyzed for hematological analyses (hemoglobin, red blood cell and white blood cell counts, neutrophils, lymphocytes, and platelets) and biochemical markers (serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea nitrogen, total protein, albumin, total cholesterol, total bilirubin, creatinine, blood sugar, triglycerides, and uric acid). Besides lower blood sugar levels in treated rats, no other statistically significant differences were observed in treated rats and controls. No significant histopathological findings, such as tissue degeneration, inflammation, bleeding, or necrosis, were observed upon necropsy. (No further details provided; article is in Chinese).

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

In a 13-wk oral toxicity study, groups of male and female Sprague-Dawley rats (10/sex/group) were administered 0, 750, 1500, or 3000 mg/kg of the previously described herbal mixture (containing 14.29% moutan cortex), dissolved in saline, via gavage.[26] No mortality, clinical changes related to test article administration, or statistically significant differences in body weight or food consumption between treated and control animals were observed. A statistically significant increase in white blood cell values was observed in both male and female rats in the 750 and 3000 mg/kg/d groups; a statistically significant decrease was observed in hematocrit and mean corpuscular hemoglobin values for 750 mg/kg/d female rats, compared to controls. Hemoglobin distribution width and hemoglobin concentrations were notably lower for 3000 mg/kg/d females, compared to controls. However, these values were within the normal range and were not considered to be test-article related. Similarly, notably increased alkaline phosphatase and total bilirubin levels in female rats from the 3000 mg/kg/d group and increased relative liver weight in males from the 3000 mg/kg/d treatment group were within the normal range and occurred in the absence of histopathological effects in the liver, indicating that these changes were not test article-related. No systemic or toxicologically significant changes related to the test article were observed. The no-observed-adverse-effect-level (NOAEL) of the herbal mixture was determined to be 3000 mg/kg/d.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

In Vitro

Paeonia Suffruticosa Bark Extract

The embryotoxic potential of an aqueous *Paeonia suffruticosa* tree peony bark extract was evaluated in an embryonic stem cell test, consisting of differentiation and cytotoxicity experiments, validated by the European Centre for Validation of Alternative Methods (ECVAM).[27,31] For the cardiomyocyte differentiation experiment, undifferentiated mouse embryonic stem cell line was maintained in complete medium containing Dulbecco's modified Eagle medium (DMEM) with 20% fetal bovine serum, 2 mM L-glutamine, 0.5% penicillin/streptomycin, 1% non-essential amino acids, 0.1 mM β-mercaptoethanol, and 103 U/ml murine leukemia inhibitory factor (mLIF). For generation of mouse embryonic stem cell line embryoid bodies, cells were cultured in DMEM without mLIF, and were seeded in the complete medium as hanging drops (20 µl each) in the presence of the aqueous extract at concentrations of 0.01, 0.1, 1, 10, 100, 1000, or 10,000 µg/ml for 3 d. Subsequently, embryoid bodies formed at each concentration were plated onto a non-adhesive petri dish for 2 d and then transferred to 24-well plates (1 embryoid body/well) for 5 d. The beat rate of cardiomyocytes from treated-cells was compared with that from untreated cells. These ratio values and corresponding concentrations were used to calculate ID_{50} values, expressed as the concentration of test materials that inhibited differentiation of cardiomyocytes in comparison to the DMEM solvent control. The cytotoxicity of test materials (ranging from $1 \times 10^{-1} - 1 \times 10^{6}$ µg/ml) were determined using mouse embryonic stem cells and mouse fibroblast cell lines in a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay after 10 d of treatment. The *Paeonia suffruticosa* bark extract exerted a growth inhibition IC_{50} of 316.7 µg/ml and a cardiomyocyte differentiation inhibition ID_{50} of 342.8 µg/ml in the embryonic mouse stem cell line, both of which were considered non-embryotoxic. In mouse fibroblast cells treated with the *Paeonia suffruticosa* bark extract, cytotoxicity was observed before

stem cell cytotoxicity or inhibition of differentiation (IC_{50} = 113.8 µg/ml), suggesting a lack of embryotoxicity. These results were confirmed by an in vitro prediction model and *Paeonia suffruticosa* bark extract was classified as non-embryotoxic.

Animal

Paeonia Suffruticosa Seed Oil

The effect of peony seed oil on sperm abnormality was evaluated in male rats.[18,30] Sexually mature male rats were administered 1250, 2500, or 5000 mg/kg bw/d peony seed oil, via gavage, for 30 d. Vegetable oil (5000 mg/kg bw) was given to negative controls and cyclophosphamide (40 mg/kg bw) was given to positive controls. On day 35, animals were killed and both epididymides were collected, sperm specimens were prepared, and eosin staining was performed. Sperm deformity rates were in the normal range (0.8 – 3.4%) and no significant difference in the abnormality rate was observed between each dose group and the negative controls. In an embryonic development study, pregnant rats were orally administered 0.55, 0.75, or 1.1 ml/kg bw/d peony seed oil for 20 d. No significant differences in maternal weight gain, early embryonic development, live fetal development, live fetal bone development, or organ development were observed, compared to controls, suggested that peony seed oil did not have embryotoxic or teratogenic effects on maternal and fetal rats. No further details were provided or could be gleaned (articles are in Chinese).

GENOTOXICITY STUDIES

Genotoxicity studies were not found in published literature, and unpublished data were not submitted.

CARCINOGENICITY STUDIES

In Vitro Cell Transformation

Paeonia Suffruticosa Extract

The antimigration and antiproliferative effects of an aqueous *Paeonia suffruticosa* extract upon 786-O renal carcinoma cells were evaluated in several tests.[7] In MTT and cell migration assays, the aqueous *Paeonia suffruticosa* extract exhibited an inhibitory effect on cancer cell growth (IC_{50} growth = 1.5 mg/ml) and a cancer cell proliferation and migration ratio that indicated the same effect on (IC_{50} growth/$IC_{50\ migration}$ = 5.0). Polymerization of the actin filament was suppressed and the ratio of F-actin to G-actin was significantly reduced in *Paeonia suffruticosa* extract-treated cells, compared to controls. Cells treated with *Paeonia suffruticosa* extract had inhibited expression of vascular endothelial growth factor receptor-3 (VEGFR-3) and remarkably reduced phosphorylation of focal adhesion kinase, both of which are involved in the activation of Ras-related C3 botulinum toxin substrate 1 (Rac -1), a modulator of cytoskeletal dynamics.

Paeonia Suffruticosa Root Extract

The oncolytic activity of an aqueous *Paeonia suffruticosa* root extract was investigated in a triple negative breast cancer cell line, MDA-MB-231.[9] Human keratinocyte cells and MDA-MB-231 cells were treated with 0.6, 2.5, or 4 mg/ml aqueous *Paeonia suffruticosa* root extract for 48 h. Cell viability was measured using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. A biphasic dose-response with cell proliferation at low concentrations (0.6 mg/ml) and reduced cell viability at concentrations greater than 2 mg/ml was observed. Notably, for human keratinocyte cells, 2.5 and 4 mg/ml aqueous *Paeonia suffruticosa* root extracts did not reduce cell viability, which was indicative of a selective oncolytic effect. Cytokine production in MDA-MB-321 cells after 48-h treatment with aqueous *Paeonia suffruticosa* root extracts was examined in an enzyme-linked immunosorbent assay (ELISA). A statistically significant decrease in interleukin-6 (IL-6), interleukin-2 (IL-2), and tumor necrosis factor-alpha (TNF-α) levels were observed in cells treated with 0.6 mg/ml aqueous extract, but subsequently increased at concentrations greater than 2.5 mg/ml. Levels of interleukin-24 (IL-24) were notably increased at the 2.5 and 4 mg/ml concentrations, when measured by an indirect ELISA, compared to controls; this increase of IL-24 was considered an up-regulation caused by increased IL-2 production. Caspase-Glo assays were performed to measure caspase 3/7, 8, and 9 and to analyze anti-apoptotic effects of the *Paeonia suffruticosa* root extracts. Caspase 3/7 and 9 activities decreased at the 0.6 mg/ml concentration but increased in a dose-dependent fashion in cells treated with 2.5 and 4 mg/ml aqueous extracts; caspase-8 activity was observed to decrease or remain at vehicle-control levels at every concentration. The increase in caspase-9 activity coupled with a decrease in caspase-8 activity indicated a mechanism of action of apoptosis that is intrinsic and possibly mediated through IL-24.

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

The ability of a *Paeonia suffruticosa* root bark extract (root bark powder extracted with RPMI 1640 medium to affect cell viability, cell cycle stage, apoptosis, and cell invasion in human bladder papillary transitional cell carcinoma 5637 cells and mouse bladder carcinoma MB49 cells was examined.[14] MB49, 5637, and SV-HUC1 (human normal epithelium) cells were incubated with 0, 0.5, 1, 2, 3, or 3.5 mg/ml *Paeonia suffruticosa* root bark extract for 24 and 48 h. The IC_{50} values of *Paeonia suffruticosa* root bark extract were 1.6 mg/ml at 24 h and 1.3 mg/ml at 48 h in mouse bladder cancer cells, and 2.0 mg/ml at 24 h and 1.4 mg/ml at 48 h in human bladder cancer cells; the IC_{50} value in human normal epithelium at 24 h was 3.5 mg/ml. In the cell cycle analysis, exposure to *Paeonia suffruticosa* root bark extract increased the number of cells in the G1 and S phase in mouse bladder cells and human bladder carcinoma cells, showing that the *Paeonia suffruticosa* root bark extract induced the activation of caspase-3, and -8 (via extrinsic apoptosis) in a dose-dependent manner. The invasive

activity of the *Paeonia suffruticosa* root bark extract was examined in 5637 cells in the cell assay. The *Paeonia suffruticosa* root bark extract inhibited cell invasion in a dose dependent manner; the inhibition percentage was higher than that of cell growth at the same dose, suggesting anti-invasive activity.

Several tests were performed to investigate whether an ethanolic *Paeonia suffruticosa* root bark extract displays growth suppressive activity and induces apoptosis in human gastric cancer cells.[15] The viability of human gastric cancer cells treated with 0, 0.01, 0.05, 0.1, 0.25, or 0.5 mg/ml *Paeonia suffruticosa* root bark extract for 48 or 72 h, was tested in an MTT assay. Untreated human gastric cancer cells served as negative controls. The *Paeonia suffruticosa* root bark extract inhibited cell growth in both a dose- and time-dependent manner; compared to controls, the IC_{50} values of *Paeonia suffruticosa* root bark extract were approximately 220 and 200 µg/ml at 48 and 72 h, respectively. The lethal concentration (LC_{50}) values of human gastric cancer cells treated with 0, 0.01, 0.05, 0.1, 0.25, or 0.5 mg/ml ethanolic *Paeonia suffruticosa* root bark extract for 48 or 72 h, in a cell cytotoxicity test, were approximately 140 and 190 µg/ml at each time point. To further study the cytotoxic effects of the extract, human gastric cancer cells were treated with 200 µg/ml ethanolic *Paeonia suffruticosa* root bark extract for 12 - 36 h and then analyzed for cell cycle stage and deoxyribonucleic acid (DNA) content using flow cytometry. At this concentration, the *Paeonia suffruticosa* root bark extract increased the sub-G1 apoptotic fraction from 3.81% at 12 h to 18.75% at 36 h in a time-dependent manner; neither untreated controls or positive controls (DMSO-treated cells) showed statistically significant changes in apoptotic fractions. Furthermore, results from a DNA fragmentation ladder analysis showed that ethanolic *Paeonia suffruticosa* root bark extract decreased monolayer cell growth and changed cell morphology in a similar manner to cells treated with cisplatin, an anti-cancer agent. Additionally, the ethanolic *Paeonia suffruticosa* root bark extract was found to cause apoptotic cell death via the extrinsic caspase-dependent apoptosis pathway, due to its activation of the Fas death receptor protein and cleaving of caspase-8, caspase-3, and poly (adenosine diphosphate-ribose) polymerase (PARP). The extract was also shown to increase the expression of the active, phosphorylated form of tumor protein p53 (p53), and to decrease the expression of the active form of phosphorylated mouse double minute 2 homolog (MDM2), a negative regulator of p53. To confirm that p53 is implicated in the apoptosis induced by the *Paeonia suffruticosa* root bark extract, cells were treated with p53 inhibitor, pifithrin-α, and Western blot analysis was performed. Cleavage of caspase-8, caspase-3, and PARP were inhibited by the p53 inhibitor, suggesting that the ethanolic *Paeonia suffruticosa* root bark extract induced apoptosis via the MDM2-p53-dependent pathway in human gastric cancer cells.

Inhibition of Tumor Growth

Paeonia Suffruticosa Extract

The effects of an aqueous *Paeonia suffruticosa* extract upon tumor growth was evaluated using renal carcinoma cells in a mouse model.[7] Mice were subcutaneously inoculated with 786O renal carcinoma cells in the flank; 2 days after injection, mice (4/group) were orally administered either water or aqueous *Paeonia suffruticosa* extract (290 mg/kg) 5 d/wk and tumors were measured every 5 d till necropsy at 45 d. Statistically significant lower tumor weights were observed in treated mice compared to controls (234.8 vs. 437.5 mg; $p < 0.05$). For pulmonary tumor metastasis experiments, 8 female NOD-SCID mice were intravenously inoculated with 786O renal carcinoma cells (2×10^6) in the lateral tail vein. Two days after injection, mice were randomly divided into 2 groups (4/group) and orally administered water or aqueous *Paeonia suffruticosa* extract (290 mg/kg) 5 d/wk and body weight was measured every 5 d, for 48 d. Lungs of the mice were excised and metastatic nodules were counted to evaluate the approximate pulmonary tumor content. There were a statistically significant lower number of pulmonary nodules in treated mice compared to controls (10 ± 1.2 vs 18 ± 3.3 nodules/lung; $p < 0.01$). No statistically significant effect on the body weight of the mice was observed, suggesting low oral toxicity of the *Paeonia suffruticosa* extract.

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

In a tumor promotion study, MB49 mouse bladder cancer cells were implanted in female C57BL/6 mice (age 6 wk).[14] After MB49 inoculation, mice were randomly assigned to 2 groups (8 mice/group). One group was intravesically treated with RPMI 1640 medium, and the other group received 2.5 mg/mouse *Paeonia suffruticosa* root bark extract intravesically every other day from day 16 to 24. On day 26, the mice were killed and bladder volumes were measured before formalin fixation. After cutting the paraffin-embedded bladder tissues into 4 µm sections, slides of each mouse bladder were examined under a microscope in histological analysis by hematoxylin and eosin staining. No statistically significant differences between the body weights of control and treated mice were observed. Treatment with *Paeonia suffruticosa* root bark extract caused a statistically significant decrease in bladder volume and retarded the invasion of tumor tissue into the muscle layer. No notable differences in the blood urea nitrogen, serum creatinine, serum glutamic-oxaloacetic transaminase, or serum glutamic pyruvic aminotransferase levels were observed between both groups. The researchers considered that these results may suggest that intravesical treatment with the *Paeonia suffruticosa* root bark extract decreased bladder tumor size without adversely affecting the liver or kidney.

OTHER RELEVANT STUDIES

Tyrosinase Inhibition

Paeonia Suffruticosa (Tree Peony) Root Bark Extract

The anti-melanogenesis properties of several *Paeonia suffruticosa* root cortex extracts were tested in murine melanoma B16 cells.[32] Plant material was extracted with 95% ethanol (extract 1) and the resulting extract was partitioned between ethyl acetate (extract 2) and water (extract 3). The ethyl acetate layer was partitioned with n-hexane (extract 4) and 90% methanol (extract 5). Subsequently, the 90% methanol layer was subjected to a Sephadex LH-20 column and eluted with methanol to obtain three fractions (extract 6, extract 7, and extract 8). Based on results from an MTT assay, extract 1, extract 3, extract 4, and extract 6 did not induce observable morphological changes in human skin fibroblast Hs68 and B16 cells and were chosen for further anti-melanogenesis analyses. To measure cellular tyrosinase activity, B16 cells were treated with 1 μM α-melanocyte-stimulating hormone (α-MSH) alone and with 50 or 100 μg/ml of the extracts, arbutin, or ascorbic acid for 72 h. Extract 1 and extract 6 inhibited cellular tyrosinase activity by 79.6 and 65%, respectively, compared to controls. Extract 1 and extract 6 also decreased dihydroxyphenylalanine (DOPA)quinone and melanin content in melanoma B16 cells as compared to controls. Notably, extract 6 had an inhibitory effect on melanin formation similar to that of arbutin and ascorbic acid, but with lower cytotoxicity. Extract 3 and extract 4 did not reduce tyrosinase activity, DOPA quinone content, or melanin formation, and were, thus, not included in further tests.

In a fluorescence staining quantitative analysis, melanoma B16 cells were treated with α-MSH alone or with 100 μg/ml of extract 1 or extract 6 for 72 h to determine melanogenesis-related protein expression and nuclei content. Both extracts did not reduce the percentage DNA content or change cell nuclear morphology. Cells treated with 100 μg/ml of either extract showed markedly lower expressions of melanocortin-1 receptor, microphthalmia-associated transcription factor, tyrosinase, and tyrosinase-related protein-1 (tyrosinase-related protein-2 levels were not affected). The researchers surmised that extract 1 and extract 6 may inhibit melanin synthesis through the downregulation of these associated enzymes.

The inhibitory effect of 2 *Paeonia suffruticosa* root bark extracts (aqueous and ethanolic) upon tyrosinase activity was evaluated in A2058 human melanoma cells. First, cells were incubated with 0.5, 1, 2, 2.5, or 5 mg/ml of the extracts, paeonol (a bioactive component of the extract), or arbutin (positive control) for 24 h and followed by ultraviolet (UV) irradiation, in a cellular tyrosinase assay. The ethanolic *Paeonia* suffruticosa root bark extract and paeonol were both found to be noncompetitive inhibitors in a kinetic analysis of tyrosinase inhibition. Furthermore, the ethanolic *Paeonia suffruticosa* root bark extract exhibited a greater tyrosinase inhibition rate compared to the aqueous extract ($p < 0.01$) and was used for additional studies. The ethanolic extract (6.25, 12.5, 25, or 50 μg/ml) showed a moderate and consistent reduction in the melanin content of A2058 melanoma cells when incubated for 24 h in a melanin synthesis assay; no statistically significant difference in melanin content was observed when compared to paeonol and arbutin-treated cells. In an L-DOPA oxidation assay, cells were treated with 6.25, 12.5, or 25 μg/ml of the ethanolic *Paeonia suffruticosa* root bark extract, paeonol, or arbutin for 24 h; paeonol exhibited the greatest tyrosinase inhibition compared to the ethanol extract and arbutin, but these differences were not statistically significant. Tyrosinase activity was downregulated in a dose-dependent manner by the ethanolic *Paeonia suffruticosa* root bark extract.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

In Vitro

Paeonia Suffruticosa Bark Extract

The skin irritation potential of an aqueous *Paeonia suffruticosa* bark extract was predicted in an EpiDerm™ skin irritation test, as outlined by the European Centre for Validation of Alternative Methods (ECVAM) and Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 439.[27] A previously incubated reconstructed human epidermis (RhE) tissue sample was moistened with 25 μl of sterile Dulbecco's phosphate-buffered saline (PBS), followed by application of 100 ml aqueous *Paeonia suffruticosa* bark extract. Two separate solutions containing 1% (v/v) sodium dodecyl sulfate in either sesame seed oil or saline solution were used as positive controls and Dulbecco's PBS-treated epidermis was used as the negative control, respectively. The tissue sample was incubated for 3 h in an MTT reduction assay. Compared to the negative control, cell viability of the skin tissue sample exposed to *Paeonia suffruticosa* bark extract was within the range of 87.5 – 101.1% (> 50%) indicating that the tested extract did not produce irritation.

Human

Paeonia Suffruticosa Root Extract

Undiluted Paeonia Suffruticosa Root Extract (extracted with a 90% ethanolic solution) was tested neat in a 24-h closed patch dermal irritation test using 20 subjects.[12] The test article was deemed non-irritating. No further details were provided.

Sensitization

Human

Paeonia Suffruticosa Root Extract

A human repeated-insult patch test (HRIPT) was completed in 52 subjects with a lotion containing 0.0015% Paeonia Suffruticosa Root Extract.[33] Occlusive patches containing approximately 25 - 38 mg/cm^2 of the test material (0.375 - 0.57 µg/cm^2 Paeonia Suffruticosa Root Extract) were applied to the back of each subject for 24 h, and the test sites were evaluated 24 or 48 h after patch removal. This procedure was repeated 3 times/wk for 3 wk, for a total of 9 induction applications. After a 2-wk non-treatment period, challenge applications were made to a previously untreated test site, and the site was evaluated 24 and 72 h after application. No reactions were observed during induction or challenge; accordingly, the lotion containing 0.0015% Paeonia Suffruticosa Root Extract was not an irritant or sensitizer.

A face mask formulation containing 0.5% Paeonia Suffruticosa Root Extract was tested in a an HRIPT using 106 subjects.[34] During induction, nine, 24-h occlusive applications containing approximately 0.2 g of the undiluted test article (0.64 µg root extract/cm^2) were applied over a 3-wk period. The test article was applied to a 0.6 in^2 absorbent pad, which was then placed on the upper back to form an occlusive patch. At least 10 d following the final induction patch application, a challenge application was applied to a virgin test site, adjacent to the original induction patch site, following the same induction procedure. No adverse reactions were observed during the induction or challenge phases; the test article did not cause dermal irritation or sensitization.

OCULAR IRRITATION STUDIES

Ocular irritation studies were not found in the published literature, and unpublished data were not submitted.

SUMMARY

The safety of the following 5 *Paeonia suffruticosa*-derived ingredients as used in cosmetics is reviewed in this safety assessment: Paeonia Suffruticosa Bark Extract, Paeonia Suffruticosa Extract, Paeonia Suffruticosa Root Extract, Paeonia Suffruticosa Seed Oil, and Paeonia Suffruticosa (Tree Peony) Root Bark Extract. Paeonia Suffruticosa (Tree Peony) Root Bark Extract is not included in the *Dictionary*; however, it has reported uses in the 2023 VCRP database and in 2024 RLD. Thus, it is included in this review. According to the *Dictionary*, the other 4 ingredients are reported to function as skin-conditioning agents in cosmetics. Paeonia Suffruticosa Seed Oil is also reported to function as a hair conditioning agent and a skin protectant.

Paeonia Suffruticosa Root Extract is reported to have the greatest frequency of use, in 213 formulations, 173 of which are leave-on formulations; RLD submitted in 2024 indicates that this ingredient is used in 736 total formulations. Results reported in 2024 for a concentration of use survey conducted by the Council indicate that Paeonia Suffruticosa Root Extract also has the highest reported concentration of use at up to 0.5% in paste masks and mud packs.

Kunming mice (10/sex) were administered a single oral dose of 15,000 mg/kg bw peony seed oil, via gavage. The acute oral LD_{50} was determined to be > 15,000 mg/kg bw. No mortality or statistically significant pathological changes occurred in ICR mice (10/sex/group) administered an oral dose of up to 60 ml/kg peony seed oil. In another acute oral toxicity study, the LD_{50} for a herbal mixture (2100 mg) containing 14.29% moutan cortex (300 g) was determined to be > 5000 mg/kg. The acute oral LD_{50} of a *Paeonia suffruticosa* tree peony bark extract was determined to be 3400 mg/kg in mice.

Healthy rats (12/sex) were administered up to 5000 mg/kg bw/d peony seed oil, via gavage, for 30 d. No abnormal changes in health status, biochemical indexes, hematological and blood biochemical indexes or immune organ indexes were observed; the maximum non-effective dosage, which is equivalent to the NOEL was estimated to be > 5,000 mg/kg bw.

The oral toxicity of an herbal mixture containing 14.29% moutan cortex (300 g of total 2100 g) was evaluated in 4-wk and 13-wk studies in rats, in accordance with KFDA standards for a toxicity study and GLP practices. In the 4-wk study, rats were dosed with 800, 2000, or 5000 mg/kg/d of the herbal mixture; a decrease in the serum sodium levels of 5000 mg/kg/d females was considered test article-related. The statistical significance of increased liver weights in the 2000 and 5000 mg/kg/d groups was not confirmed. In the 13-wk study, male and female Sprague-Dawley rats (10/sex/group) were administered 0, 750, 1500, or 3000 mg/kg/d of the herbal mixture, dissolved in saline, via gavage. No clinical abnormalities related to the test article administration were observed. A statistically significant increase in white blood cell values was observed in both male and female rats in the 750 and 3000 mg/kg/d groups; a statistically significant decrease in hematocrit and mean corpuscular hemoglobin values for female rats in the 750 mg/kg/d group was observed. Hemoglobin distribution width and hemoglobin concentrations were notably lower in female rats from the 3000 mg/kg/d group. However, these values, in addition to notable increases in alkaline phosphatase and total bilirubin levels in the female rats from the 3000 mg/kg/d group and in relative liver weight in males from the 3000 mg/kg/d group, were within the normal range and were not considered to be test article-related. The NOAEL of the herbal mixture was determined to be 3000 mg/kg/d. Groups of Sprague-Dawley rats (10/sex/group) were administered 0, 5, or 10 ml/kg/d peony seed oil, via gavage, for 90 d. Besides lower blood sugar levels in treated rats, no other statistically significant differences were observed between treated rats and controls.

An embryonic stem cell test, validated by ECVAM, was used to evaluate the developmental toxicity of an aqueous *Paeonia suffruticosa* bark extract. Cultured, undifferentiated mouse embryonic stem cells were treated with the aqueous extract at concentrations of 0.01 0.1, 10, 100, 1000, or 10,000 µg/ml for 3 d. The beat rate of cardiomyocytes from the resultant embryoid bodies in treated embryonic stem cells was compared to those in untreated cells and these ratio values and corresponding concentrations were used to calculate differentiation ID_{50} values. In the cytotoxicity portion of the test, mouse embryonic stem cell and mouse fibroblast cell lines were treated with the test materials (in concentrations ranging from 1 x 10^{-1} – 1 x 10^{6} µg/ml) and evaluated in an MTT assay after 10 d of treatment. For cells treated with the aqueous *Paeonia suffruticosa* bark extract, cytotoxicity was observed in mouse fibroblast cell lines prior to stem cell cytotoxicity or inhibition of differentiation, suggesting a lack of embryotoxicity. These results were confirmed by an in vitro prediction model and *Paeonia suffruticosa* bark extract was classified as non-embryotoxic. Sperm deformity rates were within a normal range for male rats administered up to 5000 mg/kg bw/d peony seed oil, via gavage, for 30 d; no significant differences in sperm abnormality rates were observed between each dose group and the negative controls. No embryotoxic or teratogenic effects were seen in an embryonic development study in which pregnant rats were orally dosed with up to 1.1 ml/kg bw/d peony seed oil for 20 d.

An aqueous extract of *Paeonia suffruticosa* exhibited an inhibitory effect on 786O renal carcinoma cell growth ($IC_{50\ growth}$ = 1.5 mg/ml), which was reflected in the ratio between inhibitory effects on cancer cell proliferation and migration ($IC_{50\ growth}/IC_{50\ migration}$ = 5.0). Cells treated with aqueous *Paeonia suffruticosa* extract had inhibited expression of VEGFR-3 and remarkably reduced phosphorylation of focal adhesion kinase, both of which are involved in the activation of Rac -1.

The oncolytic activity of an aqueous *Paeonia suffruticosa* root extract was investigated using multiple tests in a triple negative breast cancer line, MDA-MB-231. In an MTS assay, a biphasic dose-response with cell proliferation at low concentrations and reduced cell viability at concentrations greater than 2 mg/ml was observed in triple negative breast cancer cells treated with up to 4 mg/ml aqueous *Paeonia suffruticosa* root extract. Notably, for human keratinocyte cells, 2.5 and 4 mg/ml aqueous *Paeonia suffruticosa* root extracts did not reduce cell viability, which was indicative of a selective oncolytic effect. A statistically significant decrease in IL-6, IL-2, and TNF-α levels occurred at the 0.6 mg/ml concentration, but subsequently increased at concentrations greater than 2.5 mg/ml in an ELISA assay. IL-24 levels were notably increased in MDA-MB-231 cells treated with 2.5 and 4 mg/ml aqueous *Paeonia suffruticosa* root extracts, compared to controls; this increase of IL-24 was considered an up-regulation caused by increased IL-2 production. In Caspase-Glo assays, caspase 3/7 and 9 activity increased in a dose-dependent fashion in cells treated with 2.5 and 4 mg/ml aqueous extracts; caspase-8 activity was observed to decrease or remain at vehicle-control levels at every concentration. The increase in caspase-9 activity coupled with a decrease in caspase-8 activity indicated a mechanism of action of apoptosis that is intrinsic and possibly mediated through IL-24.

The IC_{50} values of a *Paeonia suffruticosa* root bark extract were 1.6 mg/ml and 2.0 mg/ml in mouse bladder and human bladder cancer cells, respectively, compared to a 3.5 mg/ml IC_{50} value in human normal epithelium at 24 h. Exposure to *Paeonia suffruticosa* root bark extract increased the number of cells in the G1 and S phase in MB49 mouse bladder carcinoma and 5637 human bladder papillary transitional cell carcinoma cells, showing that *Paeonia suffruticosa* root bark extract induced the activation of caspase-3, and -8 (via extrinsic apoptosis) in a dose-dependent manner. *Paeonia suffruticosa* root bark extract inhibited cell invasion in a dose-dependent manner and a higher percentage than that of cell growth at the same dose, suggesting anti-invasive activity.

An ethanolic *Paeonia suffruticosa* root bark extract inhibited cell growth in human gastric cancer cells in both a dose- and time-dependent manner; compared to controls, the IC_{50} values of the *Paeonia suffruticosa* root bark extract were approximately 220 and 200 µg/ml at 48 and 72 h, respectively. In a cell cytotoxicity test, the LC_{50} values of human gastric cancer cells treated with up to 0.5 mg/ml ethanolic *Paeonia suffruticosa* root bark extract were approximately 140 and 190 µg/ml at 48 or 72 h, respectively. In a cell cycle stage and DNA fragmentation analysis, 200 µg/ml *Paeonia suffruticosa* root bark extract increased the sub-G1 apoptotic fraction from 3.81% at 12 h to 18.75% at 36 h in a time-dependent manner. The extract also decreased monolayer cell growth and changed cell morphology, similar to cells treated with cisplatin, an anti-cancer agent. Additionally, the ethanolic *Paeonia suffruticosa* root bark extract was suggested to induce apoptosis via the MDM2-p53-dependent pathway, an extrinsic caspase-dependent apoptosis pathway, in human gastric cancer cells.

To investigate the effects of aqueous *Paeonia suffruticosa* extract on tumor growth, female NOD-SCID mice were subcutaneously injected with 786O renal carcinoma cells; the animals (4/group) were orally administered either water or *Paeonia suffruticosa* extract (0.29 g/kg) 5 d/wk, and tumors were measured every 5 d till necropsy at 45 d. Tumor weights of the *Paeonia suffruticosa* extract-treated mice were remarkably lower than that of the control group (234.8 mg vs. 437.5 mg). In a pulmonary metastasis test, there were a statistically lower number of pulmonary nodules found in the mice intravenously inoculated with aqueous *Paeonia suffruticosa* extract compared to controls.

MB49 mouse bladder cancer cells were implanted in female C57BL/6 mice and mice (8/group) that were intravesically treated with either RPMI 1640 medium or 2.5 mg/mouse *Paeonia suffruticosa* root bark extract every other day from day 16 to day 24. Mice were killed and bladder volumes were measured on day 26. Treatment with *Paeonia suffruticosa* root bark extract caused a statistically significant decrease in bladder volume and retarded the invasion of tumor tissue into the muscle layer. No statistically significant differences in the blood urea nitrogen, serum creatinine, serum glutamic-oxaloacetic

transaminase, or serum glutamic pyruvic aminotransferase levels were observed between both groups. The researchers considered that intravesical treatment with the *Paeonia suffruticosa* root bark extract may decrease bladder tumor size without adversely affecting the liver or kidney.

The anti-melanogenesis properties of 8 *Paeonia suffruticosa* root cortex extracts (including sequential subfractions) were tested in murine melanoma B16 cells. Cells were treated with 1 μM α-MSH, alone, and with 50 or 100 μg/ml of the extracts, arbutin, or ascorbic acid for 72. The extract obtained with 95% ethanol (extract 1) and a methanolic subfraction obtained from the ethyl acetate layer of the ethanolic extract (extract 6) inhibited cellular tyrosinase activity by 79.6 and 65%, respectively, and decreased DOPAquinone and melanin content in B16 cells compared to controls. Notably, extract 6 had an inhibitory effect on melanin formation similar to that of arbutin and ascorbic acid, but with lower cytotoxicity. In a fluorescence staining quantitative analysis, DNA content or nuclear morphology were not altered in B16 cells treated with 100 μg/ml of extract 1 or extract 6, in the presence of α-MSH; treated cells showed markedly lower expressions of melanocortin-1 receptor, microphthalmia-associated transcription factor, tyrosinase, and tyrosinase-related protein-1 (tyrosinase-related protein-2 levels were not affected). Thus, the researchers surmised that extract 1 and 6 may inhibit melanin synthesis through downregulation of these associated enzymes.

The inhibitory effects of aqueous and ethanolic extracts of *Paeonia suffruticosa* root bark were evaluated in A2058 human melanoma cells in a tyrosinase assay. The ethanolic *Paeonia suffruticosa* root bark extract exhibited a greater tyrosinase inhibition rate compared to the aqueous extract. In subsequent studies, the ethanolic extract (tested at 6.25, 12.5, 25, or 50 μg/ml) showed a moderate and consistent reduction in the melanin content of human melanoma cells; no statistically significant difference in melanin content was observed when compared to cells treated with paeonol or arbutin. In an L-DOPA oxidation assay, paeonol exhibited the greatest tyrosinase inhibition compared to the ethanol extract and arbutin, but these differences were not statistically significant. Tyrosinase activity was downregulated in a dose-dependent manner by the ethanolic *Paeonia suffruticosa* root bark extract.

A reconstructed human epidermis tissue sample was treated with 100 ml of an aqueous *Paeonia suffruticosa* bark extract in an EpiDerm™ skin irritation test (measured as percent viability in the MTT reduction assay), in accordance with OECD TG 439. Compared to negative controls, cell viability of skin tissue samples exposed to aqueous *Paeonia suffruticosa* bark extract was within the range of 87.5 – 101.1% (> 50%); the tested extract was not considered irritating. Undiluted Paeonia Suffruticosa Root Extract (extracted with a 90% ethanolic solution) was not irritating in a 24-h closed patch dermal irritation using 20 subjects. A lotion containing 0.0015% Paeonia Suffruticosa Root Extract and a face mask formulation containing 0.5% Paeonia Suffruticosa Root Extract were not irritating or sensitizing when tested neat in an HRIPTs completed with 52 and 106 subjects, respectively.

DISCUSSION

This assessment reviews the safety of 5 *Paeonia suffruticosa*-derived ingredients as used in cosmetic formulations, in accordance with the product categories and concentrations of use identified in the Use section and Use table; one ingredient included in this report, Paeonia Suffruticosa (Tree Peony) Root Bark Extract, is not named in the *Dictionary*, but was reported in 2023 in the VCRP database. The Panel concluded that the available data are sufficient to determine that Paeonia Suffruticosa Seel Oil is safe in cosmetics in the present practices of use and concentration, but are insufficient to determine the safety of the remaining 4 ingredients. For those 4 ingredients, the Panel requires the following information to determine safety:

- For Paeonia Suffruticosa Root Bark Extract
  - Clarification on the definition, method of manufacture, and composition, as applicable to cosmetic use
  - Clarification as to whether Paeonia Suffruticosa Root Extract includes the root bark of the plant
- For Paeonia Suffruticosa Bark Extract, Paeonia Suffruticosa Extract, and Paeonia Suffruticosa Root Extract
  - Maximum concentrations of use
  - Ocular irritation data (in vitro) at the maximum reported concentrations of use for uses near the eye
- For all 4 ingredients:
  - 28-d dermal toxicity assay
    - if positive, data on systemic toxicity endpoints (e.g., developmental and reproductive toxicity)
  - Genotoxicity data
- For all except Paeonia Suffruticosa Root Extract
  - Dermal irritation and sensitization data

The Panel considered the composition of the Paeonia Suffruticosa Seed Oil with 98.46% fatty acids and the absence of other undesirable components as a significant factor contributing to its decision to consider it as safe as used in cosmetic products. The fact that the maximum use concentration reported in cosmetic product formulations is 0.0025% further supported this conclusion. Also, the absence of any harmful events in the toxicological data included in this report, and the fact that this ingredient has been used as an edible oil favored this conclusion.

Data included in this report indicate that the root bark of *Paeonia suffruticosa* may have a skin lightening effect. The Panel noted that skin lightening is considered a drug effect, and should not occur during the use of cosmetic products.

Because of that caveat, the Panel's knowledge of the mechanism of action (i.e., inhibition of tyrosinase activity resulting in reduced melanin synthesis), and clinical experience, concern for this effect in cosmetics was mitigated. Nevertheless, cosmetic formulators should only use this ingredient in products in a manner that does not cause depigmentation.

The Panel also expressed concern about heavy metals, pesticide residues, and other plant species that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to minimize impurities in cosmetic formulations according to limits set by the US FDA and EPA.

The Panel discussed the issue of incidental inhalation exposure resulting from these ingredients. Inhalation toxicity data were not available. However, the Panel noted that in aerosol products, the majority of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial 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 low concentrations at which these ingredients are used (or expected to be used) in potentially inhaled products, 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.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern. Although frequency and/or concentration of use data are now available (and in some cases mandated) for ingredients marketed for use with airbrush delivery systems in certain product categories, no data are available for consumer habits and practices thereof, product particle size, or other relevant particle data (e.g., diameter). As a result of deficiencies in these critical data needs, the data profile is incomplete, and the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Accordingly, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Paeonia Suffruticosa Seed Oil is safe in cosmetics in the present practices of use and concentration described in this safety assessment. The Panel also concluded that the available data are insufficient to make a determination of safety for Paeonia Suffruticosa Bark Extract, Paeonia Suffruticosa Extract, Paeonia Suffruticosa Root Extract and Paeonia Suffruticosa (Tree Peony) Root Bark Extract under the intended conditions of use in cosmetic formulations.

TABLES

Table 1. Definitions and functions of *Paeonia suffruticosa*–derived ingredients[1*]

Ingredient/CAS No.	Definition	Function
Paeonia Suffruticosa Bark Extract 223747-88-4 (generic)	Paeonia Suffruticosa Bark Extract is the extract of the bark of *Paeonia suffruticosa*.	Skin-conditioning agents - miscellaneous
Paeonia Suffruticosa Extract 223747-88-4 (generic)	Paeonia Suffruticosa Extract is the extract of the whole plant, *Paeonia suffruticosa*.	Skin-conditioning agents - miscellaneous
Paeonia Suffruticosa Root Extract 223747-88-4 (generic)	Paeonia Suffruticosa Root Extract is the extract of the roots of *Paeonia suffruticosa*.	Skin-conditioning agents - miscellaneous
Paeonia Suffruticosa Seed Oil 223747-88-4 (generic)	Paeonia Suffruticosa Seed Oil is the fixed oil expressed from the seeds of *Paeonia suffruticosa*.	Hair conditioning agent Skin protectants Skin-conditioning agents – emollient Skin conditioning agents – humectant Skin conditioning agents - miscellaneous

*Paeonia Suffruticosa (Tree Peony) Root Bark Extract is not included in this table because it is not an INCI ingredient

Table 2. Constituents in *Paeonia suffruticosa*, by plant part[16]

Constituent*; **	Flower	Fresh leaves	Root	Root Cortex	Seed
Monoterpenoid Glycosides					
α-(benzolyloxy)paeoniflorin				•	
β-(benzoyloxy)paeoniflorin			•	•	
(-)-paeonisuffrone				•	
(galloyloxy)paeoniflorin				•	
6-*O*-vanillyloxypaeoniflorin				♦	
albiflorin			•	•	
benzoylpaeoniflorin			•	•	
deoxypaeonisuffrone				•	
galloylpaeoniflorin			•	•	
isopaeonisuffral				•	
mudanpioside A				•	
mudanpioside B				•	
mudanpioside C				•	
mudanpioside D				•	
mudanpioside E				•	
mudanpioside F				•	
mudanpioside G				•	
mudanpioside H				•	
mudanpioside I				•	
mudanpioside I				•	
mudanpioside J				•	
oxypaeoniflorin			•	•	
paeoniflorigenone				•	
paeoniflorin			•	•	•
paeonisothujone				•	
paeonisuffral			•		
paeonisuffrone			•		
Flavonoids					
5,6,4'-trihydroxy-7,3'-dimethoxyflavone					•
apigenin 7-neohesperidoside	•				
apigenin 7-rhamnoside	•				
astragalin	•				
catechin				•	•
chalcone (flower)	•				
cosmosin	•				
cyanidine 3,5-glucoside	•				
cyanidine-3-glucoside	•				
kaempferol				•	
kaempferol 3,7-*β*-D-diglucoside	•				
kaempferol 7-rhamnoglucoside	•				
luteolin					•
luteolin 7-glucoside					
pelargonin	•				
peonidin 3,5-di-*O*-*β*-D-glucopyranoside	•				
peonin chloride	•				

Table 2. Constituents in *Paeonia suffruticosa*, by plant part[16]

Constituent*; **	Flower	Fresh leaves	Root	Root Cortex	Seed
populnin	•				
quercetin				•	
Phenols and their glycosides					
apiopaeonoside				•	
paenol				•	
paeonolide				•	
paeonoside				•	
suffruticoside A				•	
suffruticoside B				•	
suffruticoside C				•	
suffruticoside D				•	
suffruticoside E				•	
2,3-dihydroxy-4-methoxyacetophenone				•	
2,5-dihydroxy-4-methoxyacetophenone				•	
3-hydroxy-4-methoxyacetophenone				•	
3-hydroxy-4-methoxybenzoic acid				•	
4-hydroxyacetophenone				•	
4-hydroxybenzoic acid				•	
acetovanillone				•	
gallacetophenone				•	
gallic acid				•	
methyl 3-hydroxy-4-methoxybenzoate				•	
methyl gallate				•	
mudanoside A				•	
resacetophenone				•	
trans-caffeic acid stearyl ester				•	
Tannins					
mudanoside B				•	
1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose				•	
1,2,3,6-tetra-*O*-galloyl-*β*-D-glucose		•			
6-*O*-(*m*-galloyl)galloyl-1,2,3,4-tetra-*O*-galloyl-*β*-D-glucose		•			
(-)-epigallochatechin gallate				•	
Stilbenes					
(*Z*)-resveratrol					•
suffruticosol A					•
suffruticosol B					•
suffruticosol C					•
Terpenoids and Steroids					
β-sitosterol				•	
betulinic acid				•	
campesterol				•	
daucosterol				•	
oleanolic acid				•	
Others					
adenosine				•	

*quantities of chemicals not provided; • Specific compound detected

**Blank cells indicate specific compounds were not detected

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[22]	VCRP (2023)[21]	% (2024)[23]	RLD (2024)[22]	VCRP (2023)[21]	% (2024)[23]	RLD (2024)[22]	VCRP (2023)[21]	% (2024)[23]
	Paeonia Suffruticosa Bark Extract			Paeonia Suffruticosa Extract			Paeonia Suffruticosa Root Extract		
Totals*	**1**	**8**	**NR**	**49**	**18**	**NR**	**736**	**213**	**0.000029 – 0.5**
summarized by likely duration and exposure**									
Duration of Use									
Leave-On	***	*6*	*NR*	***	*14*	*NR*	***	*173*	*0.00009 - 0.05*
Rinse-Off	***	*2*	*NR*	***	*4*	*NR*	***	*40*	*0.000029 - 0.5*
Diluted for (Bath) Use	***	*NR*	*NR*	***	*NR*	*NR*	***	*NR*	*NR*
Exposure Type									
Eye Area	***	1	NR	***	3	NR	***	9	NR
Incidental Ingestion	***	NR	NR	***	NR	NR	***	2	NR
Incidental Inhalation-Spray	***	4[a]	NR	***	4[a]; 5[b]	NR	***	84[a]; 46[b]	0.0011[b]
Incidental Inhalation-Powder	***	4[a]	NR	***	4[a]	NR	***	84[a]; 2[c]	0.05; 0.0014 - 0.005[c]
Dermal Contact	***	8	NR	***	16	NR	***	193	0.000029 - 0.5
Deodorant (underarm)	***	NR	NR	***	NR	NR	***	1[b]	NR
Hair - Non-Coloring	***	NR	NR	***	2	NR	***	12	0.00009 - 0.0011
Hair-Coloring	***	NR	NR	***	NR	NR	***	2	NR
Nail	***	NR	NR	***	NR	NR	***	NR	NR
Mucous Membrane	***	2	NR	***	1	NR	***	14	0.0025
Baby Products	***	NR	NR	***	NR	NR	***	3	NR
as reported by product category									
Baby Products									
Baby Shampoos							NR	1	NR
Baby Lotions/Oils/Powders/Creams							NR	2	NR
Bath Preparations							***3***		
Bath Oils, Tablets, and Salts							1	NR	NR
Other Bath Preparations							2	NR	NR
Eye Makeup Preparations (not children's)				***1***			***16***		
Eyebrow Pencil							NR	1	NR
Eye Shadow				NR	1	NR	NR	1	NR
Eye Lotion				1	NR	NR	2	2	NR
Eye Makeup Remover							3	NR	NR
Mascara							NR	2	NR
Eyelash and Eyebrow Adhesives, Glues, and Sealants							2	NA	NA
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)							5	NA	NA
Eyelash Cleansers							1	NA	NA
Other Eye Makeup Preparations	NR	1	NR	NR	2	NR	3	3	NR
Fragrance Preparations				***2***			***7***		
Cologne and Toilet Water							1	NR	NR
Perfumes				1	NR	NR	5	NR	NR
Other Fragrance Preparation				1	NR	NR	1	NR	NR
Hair Preparations (non-coloring)				***1***			***75***		
Hair Conditioners							3 (l.o.); 17 (r.o.)	3	0.00009
Rinses (non-coloring)							1	3	NR
Shampoos (non-coloring)							42 (r.o.)	5	0.0009
Tonics, Dressings, and Other Hair Grooming Aids				1	NR	NR	11	NR	0.0011

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[22]	VCRP (2023)[21]	% (2024)[23]	RLD (2024)[22]	VCRP (2023)[21]	% (2024)[23]	RLD (2024)[22]	VCRP (2023)[21]	% (2024)[23]
Other Hair Preparations				1 (l.o.)	2	NR	3 (l.o.); 2 (r.o.)	NR	0.00009
Hair Coloring Preparations							**1**		
Hair Dyes and Colors (all types requiring caution statements and patch tests)							NR	2	NR
Hair Shampoos (coloring)							1 (r.o.)	NR	NR
Makeup Preparations (not eye; not children's)				***14***			***22***		
Blushers and Rouges (all types)									
Face Powders							2	NR	0.05
Foundations				11 (traditional application)	NR	NR	2 (traditional application)	NR	NR
Lipsticks and Lip Glosses				1	NR	NR	11	NR	NR
Makeup Bases				1 (traditional application)	NR	NR	4 (traditional application)	3	NR
Makeup Fixatives				1	NR	NR		1	NR
Other Makeup Preparations							4 (l.o.)	1	NR
Manicuring Preparations							***1***		
Cuticle Softeners									
Nail Polish and Enamel Removers									
Other Manicuring Preparations							1	NR	NR
Oral Products							***4***		
Dentifrices							4	NR	NR
Other Oral Products							NR	2	NR
Personal Cleanliness				***3***			***16***		
Bath Soaps and Body Washes				2	NR	NR	10	7	0.0025
Deodorants (underarm)							NR	1	NR
Douches							1	2	NR
Feminine Deodorants							2	NR	NR
Other Personal Cleanliness Products				1 (r.o.)	1	NR	5 (r.o.)	3	NR
Skin Care Preparations	***1***			***26***			***570***		
Cleansing				NR	2	NR	49	9	NR
Depilatories							5	NR	NR
Face and Neck (excluding shaving preps)	NR	4	NR	10 (l.o.); 1 (r.o.)	4	NR	349 (l.o.); 27 (r.o.)	55	0.0014 (not spray)
Body and Hand (excluding shaving preps)							25 (l.o.); 8 (r.o.)	29	0.005 (not spray)
Foot Powders and Sprays							2	NR	NR
Moisturizing	1	NR	NR	3	4	NR	200	55	0.0014 (not spray)
Night				2	1	NR	12	29	0.005 (not spray)
Paste Masks (mud packs)				11	1	NR	24	55	0.0014 (not spray)
Skin Fresheners							21	29	0.005 (not spray)
Other Skin Care Preparations	NR	1	NR	1 (l.o.); 1 (r.o.)	NR	NR	52 (l.o.); 26 (r.o.)	55	0.0014 (not spray)
Suntan Preparations							***1***		
Suntan Gels, Creams, and Liquids							1	NR	NR
Tattoo Preparations							***2***		
Other Tattoo Preparations							2	NA	NA

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[22]	VCRP (2023)[21]	% (2024)[23]	RLD (2024)[22]	VCRP (2023)[21]	% (2024)[23]	RLD (2024)[22]	VCRP (2023)[21]	% (2024)[23]
Other Preparations (i.e., those that do not fit another category)				***2***	NA	NA	***29***	NA	NA
	Paeonia Suffruticosa Seed Oil			**Paeonia Suffruticosa (Tree Peony) Root Bark Extract**					
Totals*	**21**	**4**	**0.0025**	**NR**	**2**	**NR**			
summarized by likely duration and exposure**									
Duration of Use									
Leave-On	***	*NR*	*NR*	***	*1*	*NR*			
Rinse-Off	***	*1*	*0.0025*	***	*1*	*NR*			
Diluted for (Bath) Use	***	*3*	*NR*	***	*NR*	*NR*			
Exposure Type									
Eye Area	***	NR	NR	***	NR	NR			
Incidental Ingestion	***	NR	NR	***	NR	NR			
Incidental Inhalation-Spray	***	NR	NR	***	NR	NR			
Incidental Inhalation-Powder	***	NR	NR	***	NR	NR			
Dermal Contact	***	4	0.0025	***	1[b]	1[b]			
Deodorant (underarm)	***	NR	NR	***	NR	NR			
Hair - Non-Coloring	***	NR	NR	***	NR	NR			
Hair-Coloring	***	NR	NR	***	NR	NR			
Nail	***	NR	NR	***	2	2			
Mucous Membrane	***	4	0.0025	***	NR	NR			
Baby Products	***	NR	NR	***	NR	NR			
as reported by product category									
Baby Products									
Baby Shampoos									
Baby Lotions/Oils/Powders/Creams									
Bath Preparations (diluted for use)									
Bath Oils, Tablets, and Salts									
Other Bath Preparations	NR	3	NR						
Eye Makeup Preparations									
Eyebrow Pencil									
Eye Shadow									
Eye Lotion									
Eye Makeup Remover									
Mascara									
Eyelash and Eyebrow Adhesives, Glues, Sealants									
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)									
Eyelash Cleansers									
Other Eye Makeup Preparations									
Fragrance Preparations									
Cologne and Toilet Water									
Perfumes									
Other Fragrance Preparation									
Hair Preparations (non-coloring)	***1***								
Hair Conditioner									
Rinses (non-coloring)									
Shampoos (non-coloring)									
Tonics, Dressings, and Other Hair Grooming Aids	1	NR	NR						

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use	# of Uses		Max Conc of Use
	RLD (2024)[22]	VCRP (2023)[21]	% (2024)[23]	RLD (2024)[22]	VCRP (2023)[21]	% (2024)[23]	RLD (2024)[22]	VCRP (2023)[21]	% (2024)[23]
Other Hair Preparations									
Hair Coloring Preparations									
Hair Dyes and Colors (all types requiring caution statements and patch tests)									
Hair Shampoos (coloring)									
Makeup Preparations (not eye; not children's)	***1***								
Blushers and Rouges (all types)	1	NR	NR						
Face Powders									
Foundations									
Lipsticks and Lip Glosses									
Makeup Bases									
Makeup Fixatives									
Other Makeup Preparations									
Manicuring Preparations (Nail)	***1***								
Cuticle Softeners	1	NR	NR						
Nail Polish and Enamel Removers	1	NR	NR						
Other Manicuring Preparations									
Oral Products									
Dentifrices									
Other Oral Products									
Personal Cleanliness Products	***4***								
Bath Soaps and Body Washes	4	1	0.0025						
Deodorants (underarm)									
Douches									
Feminine Deodorants									
Other Personal Cleanliness Products									
Skin Care Preparations	***14***								
Cleansing	2	NR	NR						
Depilatories									
Face and Neck (excluding shaving preps)	7 (l.o.)	NR	NR						
Body and Hand (excluding shaving preps)	1 (l.o.)	NR	NR						
Moisturizing	5	NR	NR	NR	1	NR			
Night									
Paste Masks (mud packs)				NR	1	NR			
Skin Fresheners									
Other Skin Care Preparations									
Suntan Preparations									
Suntan Gels, Creams, and Liquids									
Tattoo Preparations									
Other Tattoo Preparations									
Other Preparations (i.e., those that do not fit another category)									

NR – not reported; NA – not applicable (this category was not part of the VCRP)
l.o. – leave-on; r.o. – rinse-off
*The total FOU provided for RLD refers to the ingredient count supplied by FDA, and is not a summation of the number of uses per category because each product may be categorized under multiple product categories. For data supplied via the VCRP or by the Council survey, the sum of all exposure types may not equal the sum of total uses because each ingredient may be used in cosmetics with multiple exposure types.
**Likely duration and exposure are derived from VCRP and survey data based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

***Because RLD are product-centric and not ingredient-centric, each ingredient may be reported under several product categories, making a summation of RLD misleading in comparison to VCRP data. Accordingly, RLD are presented below by product category (as supplied by FDA), but are not summarized by likely duration and exposure.)

[a] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

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

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

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