
Safety Assessment of *Punica granatum* -Derived Ingredients as Used in Cosmetics

Status: Scientific Literature Review for Public Comment
Release Date: January 24, 2019
Panel Meeting Date: April 8-9, 2019

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date to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a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

The 2019 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A. Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer.

INTRODUCTION

Most of the *Punica granatum*-derived ingredients detailed in this safety assessment are reported to function in cosmetics as skin conditioning agents, while some are reported to have other functions, such as abrasives and antioxidants, according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*; see Table 1).¹ This assessment of the safety of the following 18 *Punica granatum*-derived ingredients is based on the data contained in this report:

Punica Granatum Extract	Punica Granatum Fruit Water
Punica Granatum Bark Extract	Punica Granatum Juice Extract
Punica Granatum Bark/Fruit Extract	Punica Granatum Leaf Cell Extract
Punica Granatum Callus Culture Extract	Punica Granatum Peel Extract
Punica Granatum Flower Extract	Punica Granatum Pericarp Extract
Punica Granatum Fruit Extract	Punica Granatum Seed
Punica Granatum Fruit Juice	Punica Granatum Seed Cell Culture Lysate
Punica Granatum Fruit/Root/Stem Powder	Punica Granatum Seed Extract
Punica Granatum Fruit/Sucrose Ferment Filtrate	Punica Granatum Seed Powder

Punica granatum, commonly referred to as pomegranate, has been used as a source of Unani and Chinese medicines.² Investigations into the antioxidant activity of various extracts derived from parts of *Punica granatum* are numerous; however, CIR is not evaluating these claims as these are not related to the safety of the use of these ingredients in cosmetic products.³⁻⁸ There are no publicly available toxicity data that corresponds to any one of these cosmetic ingredients, specifically.

The pomegranate ingredients in this assessment are found in foods, and daily exposure from food use would result in much larger systemic exposures than those from use in cosmetic products. Additionally, essential oils, oleoresins (solvent free), and natural extracts (including distillates) derived from *Punica granatum* L. are generally recognized as safe (GRAS) for their intended use in foods for human and animal consumption according to the US Food and Drug Administration (FDA). The focus of this safety assessment will be on data relevant to the use of *Punica granatum*-derived ingredients in cosmetics, with specific focus on topical exposure when available.

Botanicals, such as *Punica granatum*-derived ingredients, may contain hundreds of constituents, some of which may have the potential to cause toxic effects. In this assessment, CIR is reviewing the potential toxicity of each of the *Punica granatum*-derived ingredients as a whole, complex mixture. CIR is not reviewing the potential toxicity of the individual constituents, except wherein such constituents are also ingredients under review.

This safety assessment includes relevant published and unpublished data 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 CIR typically evaluates, is provided on the 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.

Note: In many of the published studies, it is not known how the substance being tested compares to the cosmetic-grade ingredient. Therefore, if it is not known whether the ingredient being discussed is a cosmetic ingredient, the test substance will be identified as "pomegranate..." (e.g. pomegranate seed extract); if it is known that the substance is a cosmetic ingredient, the terminology "Punica Granatum..." (e.g. Punica Granatum Seed Extract) will be used.

CHEMISTRY

Definition and Plant Identification

The definitions and functions of the *Punica granatum*-derived ingredients included in this report are provided in Table 1. The raw materials for the ingredients in this report are obtained from the deciduous shrub or small tree, *Punica granatum*.⁹ These trees can grow to 6 to 10 m (20 to 30 ft) tall. *Punica granatum* are native to Afghanistan, Iran, Iraq, Turkey, the Russian Federation, Tajikistan, Turkmenistan, and India.¹⁰ In the United States (US), the trees are cultivated in Arizona and California.⁹

The fruit produced by the tree are nearly round and are 2.5 to 5 inches wide with a tough, leathery skin or rind, and are light to deep pink or red in color.⁹ The fruit interior is separated by membranous walls and white spongy tissue into compartments filled with transparent sacs containing fleshy and tart pulp, known as arils, that are red, pink or white in color. The seeds in the arils represent approximately half of the weight of the whole fruit.

Physical Properties

No physical properties were found in the published literature, and unpublished data were not submitted.

Methods of Manufacturing

No methods of manufacturing for the cosmetic ingredients derived from *Punica granatum* were found in the published literature, and unpublished data were not submitted.

Composition/Impurities

The main classes of phytochemicals identified from pomegranate (various plant parts) are as follows: ellagitannins, gallotannins, and derivatives; flavonoids; lignans; triterpenoids and phytosterols; fatty acids and lipids; organic acids and phenolic acids; and other compounds, such as catechol and coumestrol.¹¹ Specifically, the triterpenes ursolic acid and oleanolic acid are reported to be constituents of pomegranate leaves, bud, fruits, flowers and seeds.¹² Gallic acid is reported to be a constituent of pomegranate peel, pomegranate juice, pomegranate fruit, and pomegranate flowers. Analyses of methanol extracts of a patented pomegranate fruit extract and a flower extract characterized a total of 71 phenolic compounds, including 64 tannins.¹³ Yields of constituents have been found to be dependent on solvent types, with polar solvents having a greater ability to extract antioxidants when compared to non-polar solvents.^{4,5,14} Pomegranates grown in different conditions and locations may have varying composition levels in different plant parts.⁶ Table 2 describes the total phytochemical contents in different parts of pomegranate extracts.

Punica Granatum Flower Extract

The tannin content of a pomegranate flower extract used in a wound healing efficacy study was 48.7%.¹⁵ The test material was extracted with ethanol.

The gallic acid and ellagic acid contents of an ethyl acetate soluble fraction of a methanolic extract of pomegranate flower extract were 2.00 mg/g and 68.80 mg/g, respectively.² A methanolic extract, and the water-soluble fraction of the methanolic extract, quantified ellagic acid content as 18.85 mg/g and 10.88 mg/g, respectively.

Punica Granatum Fruit Extract

A food-grade pomegranate fruit extract that was produced from whole pomegranate fruit was standardized to contain 70% polyphenols total, including 30% punicalagins.¹⁶ Other constituents of the extract included not more than 5% ellagic acid and 0.3% gallic acid.

Punica Granatum Leaf Extract

A chromatogram of an acetyl acetate extract of pomegranate leaves identified the following constituents: punicalin, ellagic acid derivate, galloyl-HHDP-glucose, castalagin derivatives, granatin B, ellagic acid rhamnoside, kaempferol-3-*O*-glucoside, kaempferol-arbinoside, and a kaempferol derivative.¹⁷

Punica Granatum Peel Extract

The major constituents of aqueous pomegranate peel extract were reported as punicalagin, punicalin, ellagic acid, gallic acid, quercetin, luteolin, kaempferol, and naringenin.¹⁸ Ellagic acid, punicalagin α , and punicalagin β contents of a methanolic pomegranate peel extract were 2.75 mg/g, 3.52 mg/g, and 5.04 mg/g, respectively.² A methanolic extract of pomegranate peel used in a wound healing efficacy study contained 34.03% gallic acid and 3.31% catcechin.¹⁹

Punica Granatum Seed Extract

The fatty acid composition of an ethanol extract of pomegranate seed is described in Table 3.³

An ethanolic extract of pomegranate seeds was found to contain triterpenoids, steroids, glycosides, saponins, tannins, alkaloids, and flavonoids.²⁰ No further details were provided.

Total phenolic content of pomegranate seed extracts were dependent on the solvent type used during extraction.⁵ Methanol and water yielded the highest amount of phenolic compounds (27.93 and 22.61 mg/l seed extract, respectively), followed by acetone (3.41 mg/l), butanol (0.57 mg/l), ethyl acetate (0.37 mg/l), and hexane (0.29 mg/l).

USE

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the 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 the FDA Voluntary Cosmetic Registration Program (VCRP) database. 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.

According to 2018 VCRP survey data, *Punica Granatum* Extract has the most reported uses in cosmetic products, with a total of 299; the majority of the uses are in leave-on skin care products (Table 4).²¹ *Punica Granatum* Fruit Extract has

the second greatest number of reported uses in this safety assessment with 133 uses; the majority of these uses are also in leave-on skin care products. The results of the concentration of use survey conducted in 2018 by the Council indicated that Punica Granatum Seed Extract is used at up to 0.3% in leave-on cuticle softeners.²² Punica Granatum Extract and Punica Granatum Fruit Extract are used at up to 0.13% and 0.1%, respectively, in leave-on skin care products. Punica Granatum Fruit Juice is used at up to 0.1% in makeup preparations. Ingredients with no reported uses in the VCRP or by the Council are listed in Table 5.

Punica granatum-derived ingredients may be used in products that can be incidentally ingested or come into contact with mucous membranes; for example, Punica Granatum Seed Extract is reported to be used in a lipstick at up to 0.11%.²² Additionally, some ingredients have been reported to be used in products that may come into contact with the eyes; for example, Punica Granatum Extract is used at up to 0.018% in eye shadows. Moreover, some ingredients have been reported to be used in spray and powder products that could possibly be inhaled; for example, Punica Granatum Extract is used in a face and neck spray at 0.001% and in a face powder at 0.005%. 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 spray.²³⁻²⁶ 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.^{23,24} 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.²⁷⁻²⁹

The *Punica granatum*-derived ingredients described in this report are not restricted from use in any way under the rules governing cosmetic products in the European Union.³⁰

Non-Cosmetic

According to 21CFR182.20 and 21CFR582.20, the essential oils, oleoresins (solvent-free) and natural extractives (including distillates) from *Punica granatum* L. (pomegranate) are GRAS for their use in food intended for human consumption and in animal drugs, feeds, and related products.

Because of antioxidant and anti-inflammatory properties, the extracts of various parts of *Punica granatum* have been researched for use as alternative or therapeutic treatments (as herbal medicines or dietary supplements) for burn injuries and other dermal wounds, canker sores and oral hygiene, neurodegenerative conditions, convulsions, management of diabetes and weight, acute pancreatitis, acute lung injury, myocardial infarctions and other cardiovascular protection, and various cancers.^{3,4,8,12,15,17,19,20,31-42} The juice and peel extracts have also been researched for use as antifungal and antibacterial treatments.⁴³⁻⁴⁷

TOXICOKINETIC STUDIES

No relevant toxicokinetics studies on *Punica granatum*-derived ingredients were found in the published literature, and unpublished data were not submitted. 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

Punica Granatum Fruit Extract

In separate experiments by the same researchers, groups of 6 male and 6 female Wistar rats and Swiss albino mice received a single dose of pomegranate fruit extract at 0, 50, 500, or 5000 mg/kg body weight via oral gavage.¹⁶ The oral LD₅₀ was determined to be greater than 5000 mg/kg body weight for both species. No adverse effects were observed during the 14-day observation period, and no gross pathological abnormalities were observed during necropsy in both the rats and mice.

Punica Granatum Seed Extract

An ethanolic extract of pomegranate seeds was administered orally to 5 groups of 6 fasted NMRI male mice.²⁰ Doses were 2, 3, or 5 g/kg. No mortalities were observed in any dose level. No further details were provided.

Short Term Toxicity Studies

Oral

Punica Granatum Peel Extract

In a 15-day study investigating the prospect of the use of pomegranate peel extract in oral fungal treatments, male rats received the extract by oral gavage at 250 mg/kg/d body weight as a control and at up to 500 mg/kg/d body weight in the disease-induced treatment groups.⁴⁵ No adverse effects were observed in Wistar rats.

Intranasal

Punica Granatum Fruit Extract

The toxic effects of a pomegranate fruit extract was studied in a 35 day intranasal study in groups of 10 male Wistar rats.⁴⁸ The rats received 0, 0.4, 1.2, or 7 mg/kg lyophilized extract in each nasal cavity with a microsyringe. The controls received saline solution. The rats were weighed and feed consumption was measured every 7 days. At the end of the treatment period, biochemical and histopathology samples were analyzed and organs were weighed. No statistically significant differences in mean animal weight or feed consumption were observed. There were no clinical signs of toxicity. The only biochemical effect noted was an increase in creatinine values in the highest dose group (7 mg/kg), but there was no indication of kidney damage in the histopathology samples. No other treatment-related effects were observed in any dose group.

Subchronic Toxicity Studies

Oral

Punica Granatum Fruit Extract

The toxicity of a pomegranate fruit extract was investigated in a 90-day oral toxicity study in Wistar rats in accordance with the Organization for Economic Co-operation and Development (OECD) test guideline (TG) 408.¹⁶ Groups of 10 male and 10 female rats received 0, 60, 240, or 600 mg/kg body weight/day pomegranate fruit extract via gavage. Two additional groups of animals that received 0 and 600 mg/kg/day of the extract were recovery groups that were observed for 28 days after the initial 90-day treatment period. Clinical observations, body weight and feed consumption measurements, clinical pathology, and macroscopic and microscopic examinations of tissues from over 40 sites (including ovaries and uteri in females and testes and epididymides in males) were performed on all animals.

All animals survived until scheduled necropsies in both the 90-day study group and the recovery group. No adverse effects were observed during clinical observations. No treatment-related biologically significant effects were noted on body weight or body weight gain, feed consumption, in urinalysis parameters, in hematology parameters, in serum chemistry parameters, in absolute or relative organ weights, or in macroscopic or microscopic findings at any dose tested. No treatment-related effects were reported in the recovery groups. The no-observed-adverse-effect-level (NOAEL) for pomegranate fruit extract was determined to be 600 mg/kg/day.¹⁶

Chronic Toxicity Studies

No relevant chronic toxicity studies were found in the published literature, and unpublished data were not submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

Punica Granatum Fruit Extract

Abnormal sperm were observed 5 weeks after male Balb/C mice were treated with a hydroalcoholic pomegranate fruit extract in a sperm-shape abnormality assay.⁴⁹ Route of exposure was not defined. The extract was tested at concentrations up to 700 mg/kg body weight in groups of 5 mice. There was a dose-dependent increase in sperm with amorphous and hookless head. The frequency of abnormal sperm was significant ($p < 0.05$) at doses ≥ 70 mg/kg body weight.

Oral

Punica Granatum Fruit Juice Extract and Punica Granatum Seed Extract

The potential effects of pomegranate seed extract (described as husk extract) and pomegranate juice extract on chondrogenesis and osteogenesis on developing embryos was investigated in Balb/c mice.⁵⁰ Groups of 10 pregnant mice received the seed extract (1.0 g/kg suspended in 0.2 ml distilled water), the juice extract (3.3 ml/kg suspended in 0.2 ml distilled water), a mixture of both extracts, or distilled water daily in an oral dietary supplement between days 8 and 18 of gestation. On day 19 of gestation, the embryos were weighed and the length of the femur, tibia, and the ossification zones were measured by stereomicroscopy. The bone calcium content of the femurs of the pregnant mice was also measured.

Body weight gains of the pregnant mice were not affected by the test material. The pregnant mice that received the pomegranate extracts had an increase in bone calcium content, with a statistically significant increase ($P < 0.05$) in the group that received pomegranate juice extract. The fetuses from the mixed extract group did have significantly reduced body

weights and crown-rump lengths: these effects were not observed in the pomegranate seed extract only and pomegranate juice extract only treatment groups. Significantly increased femur lengths and osteogenesis index were observed in all extract-exposed groups. No craniofacial abnormalities or limb defects were reported during gross observations; and no pathological changes, including necrosis, abnormal cells, or congestion in longitudinal section of fetuses were observed. The liver and kidneys of the fetuses and the dams were within normal parameters.⁵⁰

Punica Granatum Fruit Juice

The effects of pomegranate juice on sperm quality, spermatogenic cell density, antioxidant activity, and testosterone levels were studied in male Wistar rats.⁷ Groups of 7 rats received 0.25 ml pomegranate juice with 0.75 ml distilled water, 0.50 ml pomegranate juice with 0.50 ml distilled water, 1 ml pomegranate juice, or 1 ml distilled water via gavage daily for 7 weeks. Body weights, reproductive organ weights, spermatogenic cell density, sperm characteristics, levels of antioxidant vitamin, testosterone, lipid peroxidation, and antioxidant enzyme activities were recorded. Analyses were done only once at the end of the study. There were no statistically significant effects on body weights in the treated groups when compared to the control group. Weights of testes, epididymides, seminal vesicles, prostate glands, and Coper glands were higher in the treated groups when compared to the controls, but the differences were not statistically significant. A significant ($P < 0.05$) decrease in malondialdehyde level and marked increases in glutathione, glutathione peroxidase and catalase activities, and vitamin C levels were observed in rats treated with different doses of pomegranate juice. Increases in epididymal sperm concentration, sperm motility, spermatogenic cell density, diameter of seminiferous tubules, germinal cell layer thickness, and a decreased abnormal sperm rate were observed with pomegranate juice consumption when compared to the controls.

GENOTOXICITY

In Vitro

Punica Granatum Fruit Extract

A hydroalcoholic extract of pomegranate fruit (including peel) induced significant increases of revertants (2 mg/plate $p < 0.05$; 4 mg/plate $p < 0.01$) in an Ames study using *Salmonella typhimurium* strain TA100, with and without S9 metabolic activation.⁴⁹ The extract was tested at 0, 0.45, 1, 2, or 4 mg/plate. The positive control yielded expected results.

The same pomegranate fruit extract described above did not induce gene-conversion events in *Saccharomyces cerevisiae* strain D7, but an increased frequency of reverse mutations was observed, with and without S9 metabolic activation.⁴⁹ The yeast cells were treated with the extract at concentrations up to 18 mg/ml.

In Chinese hamster ovary cell assays, a dose-dependent and statistically significant increase in sister chromatid exchanges per cell was observed after treatment with a hydroalcoholic pomegranate fruit extract at concentrations up to 450 $\mu\text{g/ml}$ in the absence of S9 metabolic activation.⁴⁹ Significant increases in the percentage of chromosomal aberrations were also observed following treatment with the extract at concentration up to 225 $\mu\text{g/ml}$ without S9 metabolic activation.

In Vivo

Punica Granatum Fruit Extract

In a mouse bone marrow micronucleus assay studying the genotoxic effects of a hydroalcoholic extract of pomegranate fruit, a dose-dependent increase in the number of polychromatic erythrocytes with micronuclei was observed.⁴⁹ The extract was administered intraperitoneally at concentrations up to 700 mg/kg body weight in 5 Balb/C mice/sex/group. The genotoxicity index was statistically significant at doses ≥ 70 mg/kg bodyweight in both sexes. The cytotoxicity index was significantly increased at doses ≥ 70 and 184 mg/kg body weight in males and females, respectively.

Punica Granatum Leaf Extract

In a mouse bone marrow micronucleus assay studying antigenotoxicity effects of an aqueous pomegranate leaf extract, groups of 6 male Swiss mice received 0, 400, 600, or 800 mg/kg body weight of the extract in distilled water by gavage for 7 days before exposure to the genotoxin cyclophosphamide (CPH).⁵¹ Another two groups of 6 mice served as genotoxin and test material (800 mg/kg extract) controls. Prior to the final treatment with the extract, the mice received 40 mg/kg CPH and all mice were killed after 24 h and the micronucleus assay was performed. Antigenotoxic effects were observed in a non-dose dependent manner in all 3 extract dose levels. The maximum reduction was observed in mice that received 800 mg/kg of the extract. There was no reduction in the percentage of polychromatic erythrocytes following treatment with the extract and CPH. No genotoxic effects were observed to the pomegranate leaf extract alone.

CARCINOGENICITY

No relevant carcinogenicity studies were found in the published literature, and unpublished data were not submitted.

OTHER RELEVANT STUDIES

Skin Lightening

In Vitro

Punica Granatum Fruit Extract

The potential for pomegranate fruit extract to inhibit melanin production has been studied in vitro using the Melan-a melanocyte cell culture model.⁵² The Melan-a cells were treated with pomegranate fruit extract that was standardized to 20% punicalagins. The test material was produced from fruit (with peel) that was macerated and extracted with a 75% - 80% ethanol solution at a ratio of 1:4 (fruit:solvent) before filtration and vacuum processing. Melanin content was reduced by approximately 40% to 60% at test concentrations of 50 µg/ml and 100 µg/ml, respectively. Further testing with the purified punicalagins isolated from pomegranate fruit found that this constituent reduced melanin production by 60%, 70%, and 75% of control levels at test concentrations of 20 µg/ml, 60 µg/ml, and 100 µg/ml, respectively.

Punica Granatum Peel Extract

An aqueous pomegranate extract of the rind containing 90% ellagic acid showed inhibitory activity against mushroom tyrosinase (IC₅₀ 182.2 µg/ml) in vitro.⁵³ The inhibition effects were comparable to arbutin (IC₅₀ 162.2 µg/ml), but was about ten times weaker than L-ascorbic acid (IC₅₀ 18.4µg/ml).

Animal

Punica Granatum Peel Extract

Ultraviolet (UV) light-induced skin pigmentation was inhibited in female brownish guinea pigs after the animals received the same aqueous pomegranate extract orally for 35 days.⁵³ There were 6 animals per dose group that received either 100 mg/kg/day of the extract diluted in water at 100 mg/ml, 1000 mg/kg/day of the extract diluted in water at 100 mg/ml, water, or 600 mg/kg/day L-ascorbic acid diluted in water at 60 mg/ml. The animals were irradiated on days 7, 9, and 11. The number of L-3,4-dihydroxyphenylalanine (DOPA)-positive melanocytes in the epidermis of the UV-irradiated guinea pigs were reduced in the animals that received the pomegranate extract. The researchers of this in vitro and in vivo study concluded that the skin-whitening effects were likely due to inhibition of the proliferation of melanocytes and melanin synthesis by tyrosinase in melanocytes.

Human

Punica Granatum Juice

Significant decreases (details not provided) in skin melanin content were observed in an efficacy study of a water/oil emulsion containing 4% concentrated pomegranate juice.⁵⁴ The test material was applied daily to the cheeks of 25 healthy volunteers for 60 days. A mexameter was used to measure the melanin on the cheeks of the volunteers on the day prior to application and on weeks 1, 2, 3, 4, 6, and 8.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Human

Punica Granatum Juice

No dermal irritation was observed in a 60-day efficacy study of a water/oil emulsion containing 4% concentrated pomegranate juice in 25 healthy volunteers.⁵⁴ The test material was applied daily to the cheeks.

No other dermal irritation studies for *Punica granatum*-derived ingredients were found in the published literature, and unpublished data were not submitted.

Sensitization

No dermal sensitization studies were found in the published literature, and unpublished data were not submitted.

OCULAR IRRITATION STUDIES

No ocular irritation studies were found in the published literature, and unpublished data were not submitted.

SUMMARY

According to the *Dictionary*, most of the *Punica granatum*-derived ingredients detailed in this safety assessment are reported to function in cosmetics as skin conditioning agents, while some are reported to have other functions, such as abrasives and antioxidants. Investigations into the antioxidant activity of various extracts derived from parts of *Punica*

granatum are numerous. The available toxicity data that correspond to specific use of these ingredients in cosmetics are extremely limited. There are no publicly available toxicity data that corresponds to any one of these cosmetic ingredients, specifically. The focus of this safety assessment will be on data relevant to the use of *Punica granatum*-derived ingredients in cosmetics, with specific focus on topical exposure when available.

According to 2018 VCRP survey data, Punica Granatum Extract has the most reported uses in cosmetic products, with a total of 299; the majority of the uses are in leave-on skin care products. Punica Granatum Fruit Extract has the second greatest number of reported uses in this safety assessment with 133 uses; the majority of these uses are also in leave-on skin care products. The results of the concentration of use survey conducted in 2018 by the Council indicated that Punica Granatum Seed Extract is used at up to 0.3% in leave-on cuticle softeners. Punica Granatum Extract and Punica Granatum Fruit Extract are used at up to 0.13% and 0.1%, respectively, in leave-on skin care products.

The essential oils, solvent-free oleoresins, and natural extractives from *Punica granatum* L. (pomegranate) are GRAS for their use in food intended for human consumption and in animal drugs, feeds, and related products. Extensive research has been performed on the extracts of various parts of *Punica granatum* for use as alternative or therapeutic treatments for various conditions.

The oral LD₅₀ in mice and rats for a pomegranate fruit extract was greater than 5000 mg/kg body weight. No mortalities were observed in mice that received an ethanolic extract of pomegranate seeds at up to 5000 mg/kg.

In repeated dose studies, no adverse effects were reported in a 15-day oral rat study of pomegranate peel extract at up to 500 mg/kg/day. The NOAEL for an oral study of a pomegranate fruit extract in rats was 600 mg/kg/day, the maximum dose tested. No adverse effects were noted in rats that received lyophilized pomegranate fruit extract at up to 7 mg/kg intranasally for 35 days. The only biochemical effect observed was an increase in creatinine values in the high dose group, but there was no kidney damage noted histopathologically.

Abnormal sperm were observed in male mice treated with a hydroalcoholic pomegranate fruit extract at concentrations up to 700 mg/kg body weight. Route of exposure was not defined. No adverse effects were observed in an oral DART study in female mice that received pomegranate seed extract (1.0 g/kg suspended in 0.2 ml distilled water) or pomegranate juice extract (3.3 ml/kg suspended in 0.2 ml distilled water) separately or as a mixture, and there was no effect on the fetuses. In a rat sperm study, increases in epididymal sperm concentration, sperm motility, spermatogenic cell density, diameter of seminiferous tubules, germinal cell layer thickness, and a decreased abnormal sperm rate were observed with pomegranate juice consumption when compared to the controls.

Positive genotoxic effects to a hydroalcoholic extract of pomegranate fruit were observed in an Ames test, a reverse mutation study in *S. cerevisiae*, and in Chinese hamster ovary cell assays, with and without metabolic activation. The same extract was associated with a dose-dependent increase in the number of polychromatic erythrocytes in a mouse micronucleus assay. No genotoxic effects were observed to a pomegranate leaf extract in a mouse micronucleus assay.

In vitro and in vivo studies indicate that a pomegranate fruit extract and pomegranate juice, and a pomegranate peel extract may inhibit melanin production. In a 60-day efficacy study of an emulsion containing 4% concentrated pomegranate juice, no dermal irritation was observed.

No relevant chronic toxicity, carcinogenicity, dermal sensitization, or ocular irritation studies on *Punica granatum*-derived ingredients were found in the published literature, and no unpublished data were provided. No relevant toxicokinetic studies were found in the published literature; however, in general, toxicokinetics data are not expected to be found on botanical ingredients because each botanical ingredient is a complex mixture of constituents.

DATA NEEDS

The CIR is seeking information regarding physical properties, method of manufacturing, and additional data on the composition and impurities of the *Punica granatum*-derived ingredients as used in cosmetic formulations, as there may be a difference in constituent levels of different extracts. Additional toxicological data, specifically dermal irritation and sensitization data on these cosmetic ingredients at use concentrations, are especially being sought in order to help the CIR Expert Panel assess the safety of the use of these ingredients in cosmetics.

TABLES

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

Ingredient/CAS No.	Definition & Structure	Function
Punica Granatum Extract 84961-57-9 (generic)	Punica Granatum Extract is the extract of the whole plant, <i>Punica granatum</i> .	Fragrance Ingredient; Skin-Conditioning Agent – Misc.
Punica Granatum Bark Extract 84961-57-9 (generic)	Punica Granatum Bark Extract is the extract of the bark of <i>Punica granatum</i> .	Fragrance Ingredient; Skin-Conditioning Agent – Misc.
Punica Granatum Bark/Fruit Extract 84961-57-9 (generic)	Punica Granatum Bark/Fruit Extract is the extract of the bark and fruit of <i>Punica granatum</i> .	Antimicrobial Agent; Antioxidant; Cosmetic Astringent
Punica Granatum Callus Culture Extract 84961-57-9 (generic)	Punica Granatum Callus Culture Extract is the extract of a culture of the callus of <i>Punica granatum</i> .	Skin-Conditioning Agent – Misc.
Punica Granatum Flower Extract 84961-57-9 (generic)	Punica Granatum Flower Extract is the extract of the flowers of <i>Punica granatum</i> .	Skin-Conditioning Agent – Misc.
Punica Granatum Fruit Extract 84961-57-9 (generic)	Punica Granatum Fruit Extract is the extract of the fruit of <i>Punica granatum</i> .	Skin-Conditioning Agent – Misc.
Punica Granatum Fruit Juice 84961-57-9 (generic)	Punica Granatum Fruit Juice is the juice expressed from the fruit of the pomegranate, <i>Punica granatum</i> .	Flavoring Agent; Skin-Conditioning Agent – Misc.
Punica Granatum Fruit/Root/Stem Powder 84961-57-9 (generic)	Punica Granatum Fruit/Root/Stem Powder is the powder obtained from the finely ground fruit, roots, and stems of <i>Punica granatum</i> .	Antioxidants; Hair Conditioning Agent; Skin-Conditioning Agent – Misc.
Punica Granatum Fruit/Sucrose Ferment Filtrate	Punica Granatum Fruit/Sucrose Ferment Filtrate is a filtrate of the product obtained by the spontaneous fermentation of the fruit of <i>Punica granatum</i> and sucrose.	Antioxidants
Punica Granatum Fruit Water 84961-57-9 (generic)	Punica Granatum Fruit Water is an aqueous solution of the steam distillates obtained from the fruit of <i>Punica granatum</i> .	Flavoring Agent; Fragrance Ingredient; Skin-Conditioning Agent – Misc.
Punica Granatum Juice Extract 84961-57-9 (generic)	Punica Granatum Juice Extract is the extract of the juice of <i>Punica granatum</i> .	Skin-Conditioning Agent – Misc.
Punica Granatum Leaf Cell Extract 84961-57-9 (generic)	Punica Granatum Leaf Cell Extract is the extract of a culture of the leaf cells of <i>Punica granatum</i> .	Antioxidant; Skin Protectant
Punica Granatum Peel Extract 84961-57-9 (generic)	Punica Granatum Peel Extract is the extract of the peel of <i>Punica granatum</i> .	Antimicrobial Agent; Antioxidant; Cosmetic Astringent; Preservative; Skin-Conditioning Agent – Misc.
Punica Granatum Pericarp Extract 84961-57-9 (generic)	Punica Granatum Pericarp Extract is the extract of the pericarp of <i>Punica granatum</i> .	Skin-Conditioning Agent – Misc.
Punica Granatum Seed 84961-57-9 (generic)	Punica Granatum Seed is the seed of <i>Punica granatum</i> .	Abrasive; Bulking Agent; Skin-Conditioning Agent – Misc.
Punica Granatum Seed Cell Culture Lysate	Punica Granatum Seed Cell Culture Lysate is a lysate of a suspension of the cultured seed cells of <i>Punica granatum</i> .	Skin-Conditioning Agent – Misc.
Punica Granatum Seed Extract 84961-57-9 (generic)	Punica Granatum Seed Extract is the extract of the seeds of <i>Punica granatum</i> .	Skin-Conditioning Agent – Misc.
Punica Granatum Seed Powder 84961-57-9 (generic)	Punica Granatum Seed Powder is the powder obtained from the dried, ground seeds of <i>Punica granatum</i> .	Abrasive

Table 2. Phytochemical constituents of pomegranate extracts (mg/g of dry extract)^{3,6,37,51,55,56}

	Flower Extract	Peel Extract	Seed Extract	Juice Extract	Leaf Extract	Stem Extract
Total phenolic content	336.51 (M)	276-413 (E) 190.27-298 (M) 185 (A)	2.57-73 (E) 0.65 (M)	12.4-23.8 (E) 0.094 (A) 0.057 (B)	87.81 (M) 70.00 (A)	52.92 (M)
Total flavonoid content	213.54 (M)	36-54 (E) 49.8-80.10 (M) 23.05 (A)	7.55-38.0 (E) 0.33 (M)	1.8-8.7 (E) 0.46 (A) 0.22 (B)	63.89 (M) 50.43 (A)	41.36 (M)
Total flavonol content		25-45 (E) 0.39-0.44 (A)	3.4-22 (E)	1.5-2.0 (E)		
Total proanthocyanidin content	1.46 (M)	2.48-14.09 (M) 9.09 (A)	0.13 (M)		0.21 (M)	0.32 (M)

Solvents: M = methanol, E = ethanol, A = water/aqueous, B = n-butanol

Table 3. Fatty acid composition (%) for pomegranate seed extract (ethanolic)³

Palmitic Acid	4.7
Stearic Acid	2.2
Oleic Acid	5.3
Vaccenic Acid	0.8
α -Linoleic Acid	8.8
α -Linolenic Acid	0.5
Gondoic Acid	0.5
Punicic Acid	73.7
α -Eleostearic Acid	1.6
Catalpic Acid	1.2

Table 4. 2018 frequency and concentration of use according to duration and type of exposure for *Punica granatum* (Pomegranate)-derived ingredients^{21,22}

	<i># of Uses</i>	<i>Max Conc of Use (%)</i>	<i># of Uses</i>	<i>Max Conc of Use (%)</i>	<i># of Uses</i>	<i>Max Conc of Use (%)</i>	<i># of Uses</i>	<i>Max Conc of Use (%)</i>
	Punica Granatum Seed Extract		Punica Granatum Seed Powder					
Totals[†]	NR	0.01-0.3	3	0.01				
<i>Duration of Use</i>								
Leave-On	NR	0.01-0.3	3	NR				
Rinse Off	NR	NR	NR	0.01				
Diluted for (Bath) Use	NR	NR	NR	0.01				
<i>Exposure Type</i>								
Eye Area	NR	NR	NR	NR				
Incidental Ingestion	NR	0.11	NR	NR				
Incidental Inhalation-Spray	NR	NR	1 ^a ; 2 ^b	NR				
Incidental Inhalation-Powder	NR	NR	2 ^b	NR				
Dermal Contact	NR	0.01	3	0.01				
Deodorant (underarm)	NR	NR	NR	NR				
Hair - Non-Coloring	NR	NR	NR	NR				
Hair-Coloring	NR	NR	NR	NR				
Nail	NR	0.3	NR	NR				
Mucous Membrane	NR	0.11	NR	0.01				
Baby Products	NR	NR	NR	NR				

NR = Not reported.

[†] 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.^a It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.^b Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.^c It is possible these products may be powders, but it is not specified whether the reported uses are powders.

Table 5. Ingredients not reported in use.

Punica Granatum Bark/Fruit Extract
Punica Granatum Callus Culture Extract
Punica Granatum Fruit/Root/Stem Powder
Punica Granatum Fruit/Sucrose Ferment Filtrate
Punica Granatum Leaf Cell Extract
Punica Granatum Peel Extract
Punica Granatum Seed
Punica Granatum Seed Cell Culture Lysate

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