Safety Assessment of
Silk Proteins as Used in Cosmetics

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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date to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a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 Director, Dr. Lillian J. Gill.

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INTRODUCTION

The safety of the following 10 silk proteins as used in cosmetics is reviewed in this safety assessment:

Fibroin	Sericin
Hydrolyzed Fibroin	Silk
Hydrolyzed Sericin	Silk Extract
Hydrolyzed Silk	Silk Powder
MEA-Hydrolyzed Silk	Silkworm Cocoon Extract

These ingredients function as skin and hair conditioning agents and bulking agents in cosmetic products.[1]

Silk is a fibrous protein that is a product of insects that belong to the Lepidoptera order. The silks that have been most extensively characterized are from the domesticated silk worm, *Bombyx mori*, and from spiders (*Nephila clavipes* and *Araneus diadematus*). Silk proteins are usually produced within specialized glands. These proteins are biosynthesized in epithelial cells and secreted into the lumen of these glands, where the proteins are stored prior to being spun into silk fibers by the silk-producing animal.[2]

CHEMISTRY

Definition and Structure

The silkworm, *Bombyx mori*, produces silk proteins during the final stage of larval development, and two silk proteins, fibroin and sericin, have been distinguished as major components of silk cocoons.[3] The definitions and functions of fibroin, sericin, and other silk proteins reviewed in this safety assessment are presented in Table 1.[1]

Fibroin

The structure of *Bombyx mori* (*B. mori*) silk fibroin was determined to be a repeated type II β-turn structure. The conformation of one chain is a repeated β-turn type II that is capable of forming intra-molecular hydrogen bonds.[4]

Sericin

A circular dichroism spectrum and infrared absorption spectrum show that the molecular configuration of sericin is mainly random crimp. The secondary structure of sericin varies depending on the ways in which it is prepared. It can remain in a partially unfolded state, with 35% β-sheet, 63% random coil, and no α-helix content.[5]

Chemical and Physical Properties

Properties of silk proteins, sericin and fibroin, are summarized in Table 2. Polarization microscopy shows that, in silk, sericin forms three layers surrounding a fibroin fiber.[4]

Method of Manufacture

Silk

Fibroin (main protein of silk) and sericin (another silk protein) are secreted by insect silk glands. Fibroin, in aqueous solution, is converted into silk fibers by a process that is called spinning.[6,7] According to another source, in the process of manufacturing lustrous silk from the dried cocoons of silkworms, fibroin is separated from sericin, the other major component of the cocoon, by a degumming process, and the sericin is mostly discarded in the wastewater.[5]

There are several methods for removing sericin in the so-called degumming process of cocoons. However, practically all industrial removal methods involve extraction with soaps and detergents. Heat and acid extraction are other methods. Sericin extracted by different methods can yield different amino acid compositions.[5]

Composition/Impurities

Fibroin

Silk derived from the silkworm *Bombyx mori* contains two major proteins, fibroin and sericin. Fibroin is a fibrous protein, present as a delicate twin thread that is linked by disulfide bonds and enveloped by successive sticky layers of sericin.[5] It is a large molecule (3700 amino acids).[8] Fibroin has also been described as a glycoprotein composed of two equimolar protein subunits that are covalently linked by disulfide bonds. Fibroin filaments have both crystalline and amorphous domains.[2] The amorphous domains are characterized by the presence of amino acids with bulkier side chains.[9] The crystalline domains are characterized by high percentages of alanine, glycine and serine.[2] Fibroin is a highly insoluble protein containing, as a whole, up to 90% of the amino acids glycine, alanine and serine.[10] According to another source, fibroin contains 46% glycine, 29% alanine, and 12% serine.[8]

More detailed information on the composition of fibroin, from the cocoon of the *Bombyx mori* caterpillar, indicates that it consists of 2 polypeptide chains, i.e., heavy and light chains of 391 kDa and 25 kDa, respectively; a disulfide bridge links the heavy chain to the light chain.[11] The heavy chains contain 5263 residues, composed of 45.9% glycine, 30.3% alanine, 12.1% serine, 5.3% tyrosine, 1.8% valine, and only 4.7% of the other 15 amino acid types.

The fibroins produced in the spider's major ampullate gland contain multiple repeats of motifs that include an 8- to 10-residue poly-alanine block and a 24- to 35-residue glycine-rich block.[12] The chemical composition consists of up to 90% of the amino acids glycine, alanine, and serine.

Sericin

Sericin, also referred to as silk glue, is a globular protein that constitutes 25% to 30% of silk proteins. It contains 18 amino acids, most of which have strong polar side chains, such as hydroxyl, carboxyl and amino groups. The highly hydrophilic nature of sericin is due to the high content of serine and aspartic acid, approximately 33.4% and 16.7% of sericin, respectively.[5] The predominant amino acid groups comprising sericin are serine, glycine, and glutamic acid, and it consists of a polar side-chain made of hydroxyl, carboxyl, and amino groups that enable easy cross-linking, copolymerization and blending with other natural or synthetic polymers.[13] According to another source, sericin contains 37% serine, 17% glycine, and 16% aspartate.[8]

Depending on the solubility, sericin can be separated into three fractions:[2] A, B and C. Sericin A consists of nitrogen (17.2%) and amino acids. It is the outermost layer and is insoluble in hot water. Sericin B is the middle layer and, on acid hydrolysis, it yields the same amino acids as sericin A and tryptophan. It contains 16.8% nitrogen. Sericin C is the innermost layer that is adjacent to fibroin.

Because the method of production of sericin involves extraction from cocoons using soaps and detergents, alkali soaps and detergents are typically present as impurities.[5]

USE

Cosmetic

The safety of silk proteins is evaluated based on the expected use of these ingredients in cosmetics. The Cosmetic Ingredient Review (CIR) Expert Panel uses data received from the Food and Drug Administration (FDA) and the cosmetics industry to determine expected cosmetic use. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by Industry in response to surveys of maximum reported use concentrations, by product category, that are conducted by the Personal Care Products Council (Council). Collectively, the use frequency and use concentration data indicate that 7 of the 10 silk proteins are used in cosmetic products. According to these data, the following 3 silk proteins are not being used in cosmetics:

Fibroin
MEA-Hydrolyzed Silk
Silkworm Cocoon Extract

This categorization, i.e., not being used in cosmetics, is based on the absence of use frequency data from FDA's VCRP, a voluntary reporting system, and the absence of use concentration data in an industry survey.

According to the 2015 VCRP survey, the greatest reported use frequency is for hydrolyzed silk (675 formulations, mostly rinse-off), followed by silk powder (177 formulations, mostly leave-on) (Table 3).[14] Lower use frequencies are being reported for the remaining silk ingredients. The results of a concentration of use survey provided in 2015 indicate that silk powder has the highest maximum concentration of use; it is used at concentrations up to 1.4% in leave-on products (face powders) (Table 3).[15] In some cases, reported uses appear in the VCRP database, but concentrations of use data were not provided. For example, hydrolyzed sericin is reported as used in 4 cosmetic formulations, but use concentration data were not submitted.

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

Hydrolyzed silk and silk extract are used in hairspray at maximum concentrations up to 0.024% and 0.0036%, respectively. Silk powder is also used in hairspray (maximum concentration 0.02%). Hydrolyzed fibroin and silk powder are used in perfume at maximum concentrations up to 0.000047% and 0.1%, respectively. Maximum use concentrations for the following ingredients in face powders are reported: sericin (0.00047%), silk (0.1%-0.2%), and silk powder (0.1%-1.4%). In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm, compared with pump sprays.[16,17,18,19] 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.[16,17]

The silk proteins reviewed in this safety assessment do not appear on the list of ingredients prohibited from use in cosmetic products marketed within the European Union.[20]

Noncosmetic

Fibroin

Silk fibers made from fibroin have many uses in textiles (medical and industrial applications) mainly because of the unique properties of fibroin, such as water absorbency, dying affinity, thermo-tolerance, luster and insulation properties. Fibroin is also a raw material for producing precious fabrics, parachutes, tire lining materials, artificial blood vessels and surgical sutures.[5]

Natural, nonabsorbable silk surgical suture containing the organic protein fibroin is an FDA-approved medical device.[21]

TOXICOKINETICS

Toxicokinetics studies of the silk proteins reviewed in this safety assessment were not found in the published literature.

TOXICOLOGY

Single Dose (Acute) Toxicity

Dermal

Silk Protein Film

An acute dermal toxicity study on silk protein film (protein components not stated) was performed using adult Wistar albino rats (groups of 6; males and females), according to the OECD Guideline 402 protocol.[22] Each film was moistened with physiological saline and applied, using a porous gauze dressing, to the shaved skin of the dorsal trunk of each rat for 24 h. Gauze moistened with physiological saline served as the control. The application of silk protein film did not result in any abnormal clinical signs during the 14-day observation period, and body weights, biochemical parameters and gross pathological observations were not substantially different from those of the control group. None of the animals died, and there were no notable gross lesions in any of the vital organs examined.

Repeated Dose Toxicity

Repeated dose (oral and dermal) toxicity studies of the silk proteins reviewed in this safety assessment were not found in the published literature.

Cytotoxicity

Sericin obtained via urea extraction was slightly toxic to mouse fibroblasts in vitro at concentrations as low as 60 µg/ml, and toxicity was substantial (i.e., severely harmful) at concentrations greater than100 µg/ml. When using other extraction methods, sericin yielded less toxicity, as measured by % viability of fibroblasts.[23]

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Reproductive and developmental toxicity studies of the silk proteins reviewed in this safety assessment were not found in the published literature.

GENOTOXICITY

Genotoxicity studies of the silk proteins reviewed in this safety assessment were not found in the published literature.

CARCINOGENICITY

Sericin

Sericin accelerated proliferation of the rat insulinoma cell line RIN-5F.[24]

Photocarcinogenicity

Sericin

A study was performed to assess the protective effect of sericin on UVB-induced acute damage and tumor promotion in HR-1 hairless mouse skin.[25] Three groups of 10 mice were treated with sericin, bovine serum albumin (BSA), and vehicle (ethanol), respectively, in the first experiment. One group of mice was treated with 180 mJ/cm^2 UVB light once daily for 7 days, after which red sunburn lesions of the skin were observed. Both the area and the intensity of the redness of these lesions were reduced by the topical application of 5 mg sericin immediately after UVB treatment. The differences (area and intensity of the redness) between the vehicle and sericin groups were statistically significant ($p < 0.01$). This was not true when the group treated with BSA (5 mg), rather than sericin, was compared to the vehicle control. The results of immunohistochemical analyses indicated that the application of sericin suppressed UVB-induced elevations in 4-hydroxynonenal (4-HNE), expression of cyclooxygenase-2 (COX-2) protein, and proliferating cell nuclear antigen (PCNA)-labeling index in the UVB-exposed epidermis.

Three groups of 15 mice of the same strain were treated with sericin, bovine serum albumin (BSA), and vehicle (ethanol), respectively, in the second experiment. One group of mice was treated (dermal application) with 200 nmol 7,12-dimethylbenz[a]anthracene (DMBA), followed by a 1-week non-treatment period. DMBA-treated skin was then irradiated with 180 mJ/ cm^2 of UVB twice weekly, and each irradiation was followed by topical treatment with sericin (5 mg). Another group of mice was treated similarly with BSA (5 mg), rather than sericin. Treatments (UVB dosing, followed by topical treatment) were repeated for 22 weeks. A statistically significant reduction in both tumor incidence and multiplicity was noted at a dose of 5 mg, indicative of a suppressive effect of sericin. When compared to 100% animals in the vehicle and

BSA groups having skin tumors 22 weeks after the topical application of DMBA, only 6% of the DMBA-exposed mice in the sericin-treated group exhibited skin tumors, indicating 94% ($p < 0.001$) reduction in tumor incidence. Similarly, when the tumor data were evaluated for tumor multiplicity (i.e., the number of tumors per mouse), from the first tumor appearance to the termination of the experiment, sericin produced statistically significant ($p < 0.05$) protection against UVB-induced tumor promotion in DMBA-exposed mouse skin. The results of this study (including the first and second experiments) suggest that sericin possesses a photoprotective effect against UVB-induced acute damage and tumor promotion by reducing oxidative stress, COX-2, and cell proliferation in mouse skin.[25]

The antitumor activity of sericin has also been reported in two other studies, which will summarized in the next versioin of this safety assessment.[26,27]

IRRITATION AND SENSITIZATION

Skin Irritation and Sensitization

Animal

Silk Protein Film

The dermal irritation potential of silk protein film (protein names not stated; tested as supplied) was examined in a Draize test (OECD Guideline 404) using male New Zealand White rabbits (number not stated).[22] Three test patches were applied sequentially to clipped dorsal skin of the trunk of each rabbit for 3 minutes, 1 h, and 4 hours, respectively. Negative findings for irritation of the exposed skin were confirmed using two additional animals, each tested with one patch, for an exposure period of 4 hours. The responses were scored 1 h, 24 h, 48 h, and 72 h after patch removal. There were no signs of erythema, edema, or eschar in any of the animals during the study.

The skin sensitization potential of silk protein film (protein names not stated; tested as supplied) was performed according to the Buehler test method using 2 groups of 6 guinea pigs.[22] The test material was moistened with physiological saline and applied on days 7 and 14 to clipped skin of the left flank (2 x 2 cm area) using an occlusive patch. The patch application period was 6 h. Sterile gauze moistened with physiological saline served as the control. On day 28, an occlusive challenge patch was applied for 24 h to a new test site on the flank. There were no skin reactions at 24 h or 48 h after removal of challenge patch.

Human

Silk

The relationship between silk sensitization and asthma incidence was evaluated in 871 children living in China. Skin testing was performed using a slightly modified version of the semiquantitative puncture method. The results of multivariate analyses of asthma incidence and skin test reactivity to aeroallergens were presented. Individual skin test results were not provided. Children who were sensitized to silk had 2.6 times higher odds of having asthma than did nonreactors, after adjustment for age, gender, familial correlations, and skin test reactivity to other aeroallergens using generalized estimating equations. This association between sensitization to silk and asthma yielded lower statistical p values when the eosinophil counts of the participants were included as either a categorical variable or a linear term in the multivariate model.[28]

Sixty-four children (< 15 years old; males and females) with silk-induced asthma in China were studied. The diagnosis was based on a history of wheezing, positive skin tests to silk, positive nasal or conjunctival provocation tests, or serum IgE-silk waste. Severely broken silk threads, used only as filling for bed quilts or clothes and mattresses, are known as silk waste. The average age of asthma onset was 4 years 2 months. Conjunctival provocation tests were performed on 80% of the cases. The first symptom was observed an average of 10 months after initial exposure to silk. Asthma was accompanied by allergic rhinitis in 61% of the patients, and was accompanied by conjunctivitis in 14% of the cases. In most cases, asthma occurred during the winter, due to the seasonal use of bed quilts or clothes filled with silk. The average mean wheal diameter elicited by silk in prick tests was greater than the diameters measured from 2 histamine equivalent prick tests per silk-sensitive subject.[29]

Case Reports

According to one case report, recurrent granulomas with remarkable infiltration of eosinophils may have resulted from an IgE-mediated hypersensitivity reaction to silk fibroin, a component of the braided silk suture used. [30] In this report, a lateral skin flap technique had been performed to correct tracheostomal stenosis, using silk sutures, after a total laryngectomy.

Adverse reactions to virgin silk sutures in 12 cataract surgery patients have also been reported.[31] Nodular episcleritis, peripheral corneal ulceration, and wound necrosis with dehiscence were observed, sometimes resulting in endophthalmus or epithelial down-growth. Conjunctival and scleral histopathologic studies in 4 eyes showed acute and chronic inflammation with multinucleated giant cells. Type I allergic responses and up-regulated levels of specific IgE were reported to occur in patients after repeated surgical procedures.[30,32]

OTHER EFFECTS

Skin Depigmentation

Sericin was formulated as an 8% cream and applied to one side of the extremity (arm and leg) of renal patients who normally experienced dry and itchy skin.[33] A cream base was applied to the other extremity and served as the control. From 47 subjects who completed the study, the skin hydration of the patients' extremities increased after receiving both sericin cream and the cream base, but the changes in skin hydration were much greater on the side receiving the sericin cream than on the side receiving the cream base. Additionally, at the end of the study, the skin pigmentation level was significantly reduced on both the arms ($p = 0.032$) and legs ($p = 0.021$) of the sericin-treated side compared with the side treated with cream base.

The degree of inhibition of tyrosinase (i.e., the rate-limiting enzyme for melanin production) activity by sericin depends upon the extraction method and silk strain source.[34] For example, colored silk cocoons, which contain flavonoids and carotenoids, exhibit higher anti-tyrosinase activity than white-shelled cocoons.

Immunological Responses

Sericin

Non-human

The effect of a sericin cream on wound healing was evaluated using 18 male Sprague-Dawley rats (8 weeks old).[35] The composition of the cream was described as follows: 8% sericin, white petrolatum, mineral oil, lanolin, glycerin, bisabolol, propylparaben, and methylparaben. Except for sericin, the concentration of each cream component was not stated. The cream was applied topically to full-thickness skin wounds on the dorsum of each animal, and wound surfaces were observed for 15 days post-application. Cream base without sericin served as the control. Wounds treated with the sericin cream healed almost completely, without any allergic rash, while inflammation persisted in wounds not treated with sericin cream.

In a similar study involving 45 Sprague-Dawley rats (8 weeks old), 8% sericin cram was applied to full-thickness wounds on the dorsum of each animal. Cream base without sericin served as the control. Excised rat tissue was prepared for cytokine determination. IL-1β and TNF-α are proinflammatory cytokines that are involved in a variety of immunological functions. Wounds treated with sericin cream did not yield significantly high levels of IL-1β and TNF-α on day 7, which suggests that the cream did not induce an inflammatory or immunological response.[36]

In Vitro

Soluble sericin proteins extracted from native silk fibers did not induce significant macrophage activation.[37] Macrophages exposed *in vitro* to the silk preparations failed to respond with consistently elevated levels of tumor necrosis factor (TNF) in either short- or long-term cultures. However, the suspension of the crystalline particles prepared by enzymatic digestion of silk fibroin was the only silk preparation that yielded significant TNF release, which was probably a non-specific response to insoluble physical particulates, rather than a specific, chemically-induced response to silk. Whether or not the statistical signifance of this finding was determined was not stated. However, it was noted that the average TNF release (corrected for volume and expressed as total release from specified cell count) and standard error of the mean were determined.

Silk sericin increased the amounts of inflammatory mediators and proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), which are involved in the modulation of skin growth, repair and scarring during inflammation.[38] However, the maximum levels of TNF-α and IL-1β released from monocytes and macrophage cells after silk sericin exposure were 500 and 350 pg/ml, respectively. It was noted that these levels of cytokines would not be sufficient to cause an inflammatory response or prevent cellular proliferation.

The suppression of inflammation by sericin has been reported.[39] Sericin solution applied topically to the top of the hind paw of rats prior to a carrageenan subcutaneous injection under the plantar surface of the hind paw exhibited anti-inflammatory activity, similar to the effect of indomethacin (a non-steroidal anti-inflammatory drug used as a control). The amount of mast cells in rat tissue treated with sericin or indomethacin was much lower compared to the amount of cells found in tissue treated with water (control). Further investigation indicated that sericin did not cause a hypersensitivity reaction. On the contrary, it inhibited cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) production (monitored by total RNA and real-time polymerase chain reaction (RT-PCR)) in fibroblast cell culture, resulting in lowering the inflammation of the carrageenan induction.

Regarding the latter finding in the preceding paragraph, similarly, topical application of sericin solution inhibited the expression of epidermal TNF-α , a pro-inflammatory cytokine that is produced by a number of different cell types, including keratinocytes, under a variety of inflammatory conditions and is known to prime inflammatory cells to produce enhanced levels of reactive oxygen in mouse skin.[40]

Wound Healing

In Vitro

Sericin

Living human skin fibroblasts were incubated with sericin *in vitro* for 72 h.[41] The living cell count in treated cultures after 72 h was enhanced to 250% of the untreated (i.e., no-sericin) control cultures. In another study, replacing the culture medium with sericin solution in the mouse L929 fibroblastic cell line in culture increased the percentage of cell proliferation significantly, especially at a high sericin concentration (1.0 mg/ml).[38] The amount of NF-1α and IL-1β released from alveolar macrophage NR8383 and mouse J774.2 monocyte cell lines after the addition of sericin (0.2 - 1.0 mg/ml) to the culture media was negligible, indicating that sericin did not cause severe damage to the cells.

Non-human

Sericin

The effect of a sericin cream on wound healing was evaluated using 18 male Sprague-Dawley rats (8 weeks old).[35] The composition of the cream was described as follows: 8% sericin, white petrolatum, mineral oil, lanolin, glycerin, bisabolol, propylparaben, and methylparaben. Except for sericin, the concentration of each cream component was not stated. The cream was applied topically to full-thickness skin wounds on the dorsum of each animal, and wound surfaces were observed for 15 days post-application. Cream base without sericin served as the control. Histological examination of wounds after 15 days of treatment with 8% sericin cream revealed complete healing, no ulceration, and an increase in collagen, as compared to treatment with the control cream. Wounds treated with the control cream had some ulceration and acute inflammatory exudative materials.

SUMMARY

The safety of the following 10 silk proteins in cosmetics is reviewed in this safety assessment: fibroin, hydrolyzed fibroin, hydrolyzed sericin, hydrolyzed silk, MEA-hydrolyzed silk, sericin, silk, silk extract, silk powder, and silkworm cocoon extract. These ingredients function as skin and hair conditioning agents and bulking agents in cosmetic products. Frequency of use data from FDA VCRP and the results of an industry survey indicate that 7 of the 10 silk proteins are being used in cosmetic products. Silk powder has the highest reported maximum concentration of use; it is used at concentrations up to 1.4% in leave-on products (face powders).

The silkworm, *Bombyx mori*, produces silk proteins during the final stage of larval development, and two silk proteins, fibroin and sericin, have been distinguished as major components of silk cocoons. In the process of manufacturing silk, fibroin is separated from sericin by a degumming. There are several methods for removing sericin in the degumming process of cocoons. However, practically all industrial removal methods involve extraction with soaps and detergents. Alkali soaps and detergents are present as impurities in sericin.

In an acute dermal toxicity study on silk protein film involving rats, none of the animals died and there were no notable gross lesions in any of the vital organs examined.

Sericin obtained via urea extraction was toxic to mouse fibroblasts *in vitro* at concentrations as low as 60 µg/ml.

Sericin accelerated proliferation of the rat insulinoma cell line RIN-5F. The results of a study in mice suggested that sericin possesses a photoprotective effect against UVB-induced damage and tumor promotion by reducing oxidative stress and cell proliferation in mouse skin.

In a study using rabbits, the application of silk protein film (tested as supplied) to the skin did not cause erythema, edema, or eschar. This silk protein film (tested as supplied) also did not induce sensitization when applied to the skin of guinea pigs.

The suppression of inflammation by sericin was reported in a study on rats, and a hypersensitivity reaction was not observed.

An association between sensitization to silk and asthma incidence was found in a study of 871 children. In another study of 64 children, the average mean wheal diameter elicited by silk in prick tests was greater than 2 histamine equivalent prick tests.

In a case report, recurrent granulomas with remarkable infiltration of eosinophils may have resulted from an IgE-mediated hypersensitivity reaction to silk fibroin. Additionally, type I allergic responses and up-regulated levels of specific IgE have been reported in patients after repeated surgical procedures that involved the use of silk sutures. Skin depigmentation has been observed in renal patients after application of an 8% sericin cream for treatment of dry and itchy skin.

Histological examination of wounds in rats after 15 days of treatment with 8% sericin cream revealed complete healing, no ulceration, and an increase in collagen, as compared to treatment with the control cream.

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

Ingredient/CAS No.	Definition	Function
Fibroin 9007-76-5	Fibroin is a protein filament produced by the silkworm, *Bombyx mori* which together with Sericin composes Silk.	Bulking Agents
Hydrolyzed Fibroin	Hydrolyzed Fibroin is the hydrolysate of Fibroin derived by acid, enzyme or other method of hydrolysis.	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous
Hydrolyzed Sericin 870616-36-7 73049-73-7	Hydrolyzed Sericin is the hydrolysate of Sericin derived by acid, enzyme or other method of hydrolysis.	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous
Hydrolyzed Silk 73049-73-7 96690-41-4	Hydrolyzed Silk is the hydrolysate of silk protein derived by acid, enzyme or other method of hydrolysis.	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous
MEA-Hydrolyzed Silk	MEA-Hydrolyzed Silk is the monoethanolamine salt of Hydrolyzed Silk (q.v.).	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous
Sericin 60650-88-6 60650-89-7	Sericin is a protein isolated from the silk produced by the silk worm, *Bombyx mori.*	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous
Silk	Silk is the fibrous protein obtained from cocoons of the silk worm.	Bulking Agents
Silk Extract 91079-16-2	Silk Extract is the extract of silk fiber.	Skin-Conditioning Agents - Miscellaneous
Silk Powder 9009-99-8	Silk Powder is finely pulverized silk.	Bulking Agents; Skin-Conditioning Agents - Miscellaneous; Slip Modifiers
Silkworm Cocoon Extract 91079-16-2	Silkworm Cocoon Extract is the extract of the cocoon of the silkworm, *Bombyx mori*.	Skin-Conditioning Agents - Humectant

Properties of Silk Proteins

Property	Value	Background Information
	Sericin	
Form	Gelatinous;[13] sol-gel.[2]	Easily dissolves in water at 50°C to 60°C; returns to gel form on cooling.[2] Gelation is rapid at 108°C and pH ≈ 6 to 7.[5]
	Powder; amorphous structure.[5]	Transforms into a β-structure in presence of water.[5]
Molecular Weight	10 to > 400 kDa.[5]	Depending on extraction methods, temperature, pH, and processing time.[5]
	35 to 150 kDa.[5]	Heat and acid extraction.[5]
	15 to 75 kDa.[5]	Alkaline solution extraction.[5]
	10 to > 225 kDa.[5]	Urea extraction.[5]
	< 20 kDa.[5]	Recovered during early stages of raw silk production.[5]
	> 20 kDa.[5]	Obtained from later stages of raw silk production.[5]
Solubility	Highly soluble in water.[13]	Decreases when molecules are transformed from random coil into the ß-sheet structure.[2]
Isoelectric Point	≈ 4.[9]	Because there are more acidic than basic amino acids in sericin.[9]
	Fibroin	
Form	Pale yellow mass.[6]	
Molecular Weight	300 to 420 kDa.[42]	
Solubility	Soluble in concentrated alkalies, concentrated mineral acids, and in ammoniacal nickel oxide solution. Insoluble in water, alcohol, ether, and dilute alkalies.[6]	
	Hydrolyzed Fibroin	
Form	Yellow solution.[43]	Acid hydrolysis usually causes fibroin solution to turn yellow, and chemical changes in amino acids such as tryptophan and tyrosine are generally considered as the main reason for yellowing. Trytophan and tyrosine become yellow upon hydrolysis, and the same is true for serine and glycine. Serine and threonine beak down easily during hydrolysis, and other amino acids in fibroin decompose in the following order: tyrosine, methionine, cysteine, phenylalanine, and tryptophan.[43]

Table 2. Frequency and Concentration of Use According to Duration and Type of Exposure.[14,15]

	Hydrolyzed Silk		Hydrolyzed Fibroin		Sericin	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	675	0.0000007-0.5	1	0.001-0.045	44	0.00047
Duration of Use						
Leave-On	97	0.0000007-0.23	1	0.001	16	0.00047
Rinse off	321	0.0000007-0.5	NR	0.045	14	0.00047
Diluted for (bath) Use	5	0.0001-0.0003	NR	NR	NR	NR
Exposure Type						
Eye Area	36	0.0000007-0.23	NR	NR	10	0.00047
Incidental Ingestion	3	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	NR	0.0005-0.024	1***	NR	1	0.000047
Incidental Inhalation- Powders	NR	0.00023-0.11**	1***	0.001**	1	0.000047
Dermal Contact	213	0.0000007-0.11	1	NR	12	0.000047
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	189	0.00002-0.5	NR	NR	NR	0.000047
Hair-Coloring	6	0.00022-0.008	NR	0.045	12	NR
Nail	1	0.01-0.03	NR	NR	NR	NR
Mucous Membrane	126	0.0001-0.035	NR	NR	1	NR
Baby Products	4	0.0003	NR	NR	NR	NR
	Silk		**Silk Extract**		**Silk Powder**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	27	0.000005-0.2	13	0.0013-0.015	177	0.0001-1.4
Duration of Use						
Leave-On	18	0.000005-0.2	2	0.0036-0.015	85	0.0001-1.4
Rinse off	4	NR	6	0.0013-0.015	14	0.0001-0.1
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Exposure Type						
Eye Area	1	0.000005	1	NR	33	0.05-1.1
Incidental Ingestion	NR	NR	NR	NR	16	0.05-1.1
Incidental Inhalation- Sprays	NR	NR	3*	0.0036	1	0.005-0.1
Incidental Inhalation- Powders	4	0.1-0.2	NR	0.1-0.2	60	0.1-1.4
Dermal Contact	17	0.000005-0.2	1	0.0013	67	0.0001-1.4
Deodorant (underarm)	NR	NR	NR	NR	NR	0.005
Hair - Non-Coloring	NR	NR	7	0.0036-0.015	6	0.01-0.048
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	5	0.001-0.05	NR	NR	7	0.0001-0.025
Mucous Membrane	3	NR	NR	NR	19	0.01-1.1
Baby Products	NR	NR	NR	NR	NR	NR

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

Table 2. Current Frequency and Concentration of Use According to Duration and Type of Exposure.[14,15]

	Hydrolyzed Sericin			
	# of Uses	Conc. (%)		
Totals/Conc. Range	4	NR		
Duration of Use				
Leave-On	3	NR		
Rinse off	NR	NR		
Diluted for (bath) Use	NR	NR		
Exposure Type				
Eye Area	1	NR		
Incidental Ingestion	NR	NR		
Incidental Inhalation- Sprays	1*	NR		
Incidental Inhalation- Powders	NR	NR		
Dermal Contact	1	NR		
Deodorant (underarm)	NR	NR		
Hair - Non-Coloring	2	NR		
Hair-Coloring	NR	NR		
Nail	NR	NR		
Mucous Membrane	NR	NR		
Baby Products	NR	NR		

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

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