Safety Assessment of Silk Protein Ingredients as Used in Cosmetics

Status: Draft Tentative Report for Panel Review
Release Date: August 28, 2015
Panel Date: September 21-22, 2015

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

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Cosmetic Ingredient Review

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: August 28, 2015
Subject: Draft Tentative Report on Silk Proteins

At the June 15-16, 2015 CIR Expert Panel (Panel) meeting, the Panel issued an Insufficient Data Announcement with the following data requests on MEA-Hydrolyzed Silk and Silkworm Cocoon Extract: (1) Method of manufacture and impurities, (2) Concentration of use, (3) 28-day dermal toxicity study; if absorbed, genotoxicity and reproductive and developmental toxicity data may be needed, and (4) Skin irritation and sensitization data. To date, these data have not been received. The Panel also agreed that the available data are sufficient for evaluating the safety of the following ingredients: Fibroin, Hydrolyzed Fibroin, Hydrolyzed Sericin, Hydrolyzed Silk, Sericin, Silk, Silk Extract, and Silk Powder. The draft tentative report on these ingredients (*slkprt092015rep*) has been revised to include data received from the Council, and the Council's comments (*slkprt* 092015pcpc1) have been addressed.

Included in this package for your review is the draft tentative safety assessment (*slkprt092015rep*), the CIR report history (*slkprt 092015hist*), Literature Search Strategy (*slkprt092015strat*), Ingredient Data Profile (*slkprt092015prof*), 2015 FDA VCRP data (*slkprt092015FDAdata*), and minutes from the June 15-16, 2015 Panel meeting (*slkprt092015min*). Data relating to the allergenicity of silk proteins (*slkprt092015data1*), received from the Council, are also included. These data as well as a case report on the potential for silk to induce contact urticaria are enclosed within vertical borders in the report text. Per the Panel's deliberations at the June meeting, a draft report discussion addressing issues relating to sericin-induced hypopigmentation and the potential for silk-induced type I allergy is included for the Panel's review.

After considering the data included in this safety assessment, the Panel will need to determine whether to issue a tentative report with a safe or safe with qualifications conclusion on all ingredients or issue a tentative report with an insufficient data conclusion on MEA-Hydrolyzed Silk and Silkworm Cocoon Extract and a safe as used or safe with qualifications conclusion on the remaining ingredients.

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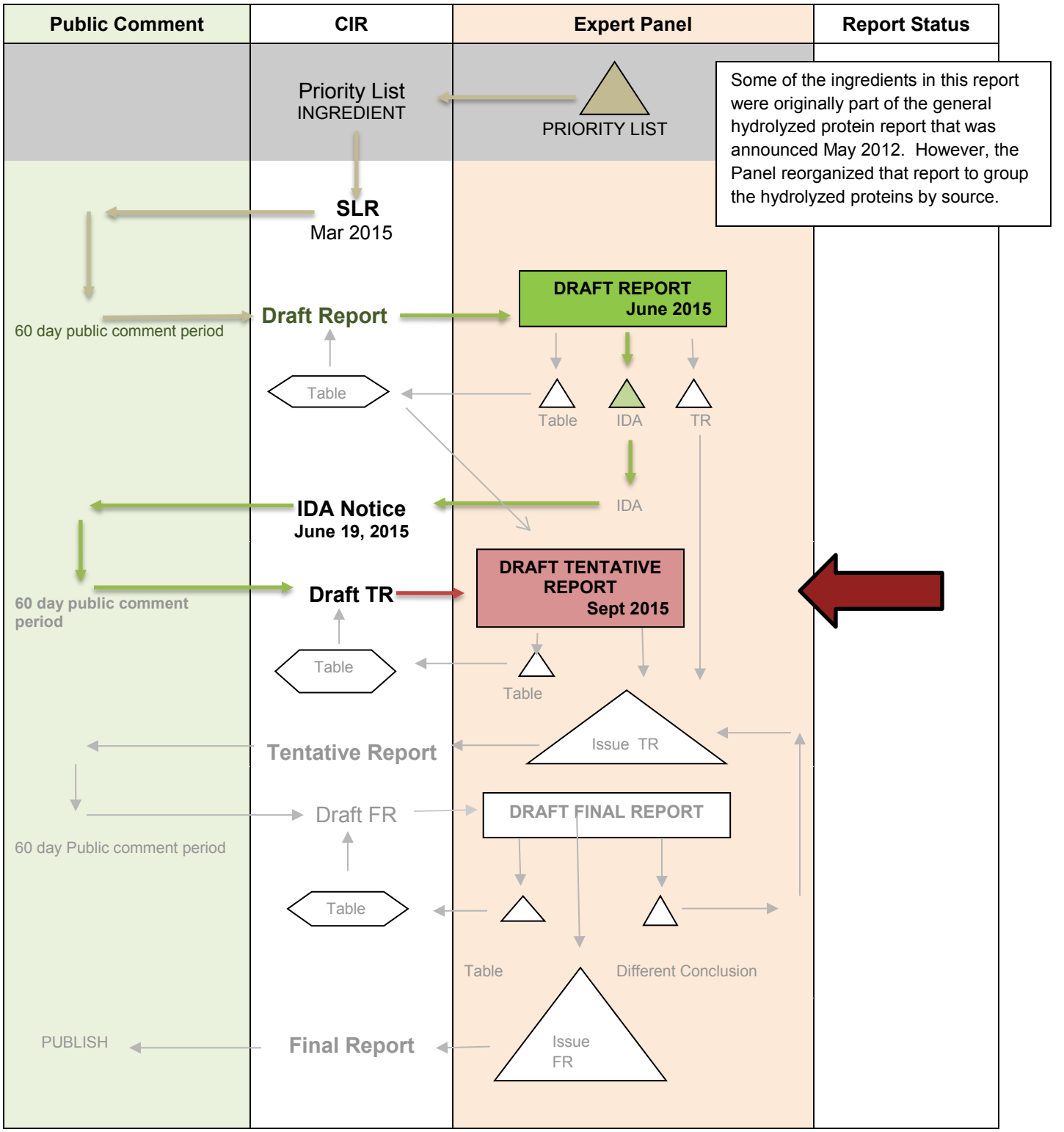
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Silk Proteins

MEETING Sept 2015



CIR History of:

Silk Proteins

A scientific literature review (SLR) on Silk Proteins was issued on March 2, 2015. Unpublished data were received during the 60-day comment period.

Draft Report, Belsito and Marks Teams/Panel: June 15-16, 2015

Comments received from the Council have been addressed and the following unpublished data were added to the draft report:

- Use concentration data
- Sensitization data on silk powder *(slkprt062015data2* pdf file)
- Method of manufacture and chemistry data on hydrolyzed silk (*slkprt 062015data 3* pdf file)
- Chemistry and toxicity data on silk (*slkprt062015data4* pdf file)
- Method of manufacture, chemistry, and toxicity data on hydrolyzed silk (*slkprt062015data5* pdf file)
- Method of manufacture, chemistry, and toxicity data on hydrolyzed silk (*slkprt062015data6* pdf file)

The Panel agreed that the available data are sufficient for evaluating the safety of the following 8 silk protein ingredients.

fibroin
hydrolyzed fibroin
hydrolyzed sericin
hydrolyzed silk
sericin
silk
silk extract
silk powder

However, the Panel issued an insufficient data announcement on two silk protein ingredients, MEA-Hydrolyzed Silk and Silkworm Cocoon Extract:

The data that are needed to evaluate the safety of these two ingredients are:

- Method of manufacture and impurities
- Concentration of use
- 28-day dermal toxicity study; if absorbed, genotoxicity and reproductive and developmental toxicity data may be needed
- Skin irritation and sensitization data

Draft Tentative Report, Belsito and Marks Teams/Panel: September 21-22, 2015

To date, the data requested in the insufficient data announcement have not been received.

Silk Proteins Check List for September, 2015. Analyst – Wilbur Johnson																				
			Acute toxicity					Repeated dose toxicity				Irritation			Sensitization					
	Skin Penetration	Penetration Enhancement	ADME	Oral	Parenteral	Dermal	Inhale	Oral	Parenteral	Dermal	Inhale	Ocular Irritation	Dermal Irr. Animal	Dermal Irr Human	Sensitization Animal	Sensitization Human	Repro/Devel toxicity	Genotoxicity	Carcinogenicity	Phototoxicity
Fibroin																				
Hydrolyzed Fibroin																				
Hydrolyzed Sericin																				
Hydrolyzed Silk				X								X	X	X	X	X		X		X
MEA-Hydrolyzed Silk																				
Sericin																			X	
Silk				X		X						X	X		X	X				
Silk Extract																				
Silk Powder				X						X		X	X	X	X					X
Silkworm Cocoon Extract																				

Literature Searches on Silk Proteins (11/21/2014)

SciFinder/PubMed Searches

Search Terms

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

Search Updates

8/1/2015

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Abstract: The CIR Expert Panel (Panel) reviewed the safety of 10 silk protein ingredients, which function primarily as as skin and hair conditioning agents and bulking agents in cosmetic products. The Panel reviewed relevant data relating to the safety of these ingredients and concluded that the ingredients in this report are [*To be Determined at the September, 2015, Panel meeting*].

INTRODUCTION

The safety of the following 10 silk protein ingredients 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 are reported to function as skin and hair conditioning agents and bulking agents in cosmetic products.[1]

Silk is a fibrous protein that is a product of the silk worm, *Bombyx mori*, and 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.[2] The silk worm (*Bombyx mori*) is the source of silk from which cosmetic ingredients are derived.[1]

Safety test data on a product identified as silk protein film (protein names not stated; tested as supplied) are included in this safety assessment. This material is not a cosmetic ingredient, but the data may be useful in assessing the safety of the silk proteins that are being reviewed.

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] All of the ingredients in this report are related because they are derived from the silk fibers produced by *Bombyx mori.* The definitions and functions of fibroin, sericin, and other silk protein ingredients reviewed in this safety assessment are presented in Table 1.[1]

Fibroin

Bombyx mori (*B. mori*) silk fibroin was determined to exist as a repeated type II β-turn structure, wherein the conformation of one chain enables the formation of intra-molecular hydrogen bonds.[4]

Sericin

Circular dichroism and infrared absorption spectra 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 from the raw silk. 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 fibroin, sericin, and other silk protein ingredients, 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]

Several methods have been reported for removing sericin in the degumming process of cocoons. Practically all industrial removal processes 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]

Additional information indicates that some commercial silk is prepared from natural silk by removing sericin, and that the purified aqueous fibroin is dried and pulverized into a powder.[8]

Hydrolyzed Silk

Hydrolyzed silk has been reported to be prepared from the cocoon of the silkworm moth (*Bombyx mori*).[9] The silk thread is isolated from the cocoon and the fibers are cleaned and degummed. The individual silk fiber is then wound with other silk fibers to create one long thread. The threads are then combed to remove noils, which are short fibers considered to be by-products of the textile industry. The noils are used in the production of hydrolyzed silk proteins through carefully controlled hydrolysis. The resultant material is a 5% solution of a water-soluble silk protein.

It has been reported that hydrolyzed silk protein (mw = 300 Da) may be prepared by acid, alkaline, or enzyme-catalyzed hydrolysis; hydrolyzed silk protein (650 Da) may be prepared by alkaline or enzymatic hydrolysis.[10,11] These processes occur for several hours until the desired molecular weight is reached. The final product is a 20% water solution of hydrolyzed silk protein (mw = 300 Da) or a 6.5% water solution of hydrolyzed silk protein (mw = 650 Da). Furthermore, another supplier has reported that hydrolyzed silk is prepared by acid and enzyme hydrolysis until the molecular weight reaches the target range.[12]

According to other sources, hydrolyzed silk is produced according to the following procedures:[13,14,15] **Procedure 1**: (1) hydrolysis, (2) inactivation of hydrolytic agent, (3) filtration, (4) treatment, (5) concentration, and (6) sterilization. **Procedure 2**: (1) proteins hydrolyzed in water at specific pH and temperature for specific duration, (2) filtration to isolate desired components, (3) addition of quaternium-15, EDTA, and methylparaben, or just EDTA and methylparaben, and (4) make batch adjustments if needed (refiltration).

Composition/Impurities

Hydrolyzed Silk

Data on the composition of hydrolyzed silk (mostly amino acids) are presented in Table 3.[16,17] Hydrolyzed silk is marketed as an amino peptide concentrate that is rich in the 2 proteins that comprise natural silk, sericin and fibroin.[18] It consists of ~ 19% hydrolyzed silk and also contains the preservatives phenoxyethanol (0.4%) and potassium sorbate (0.2%).[19] Other preservatives in hydrolyzed silk include quaternium-15, EDTA, and methylparaben.[14,15]

Another source indicates that hydrolyzed silk (mw = 300 Da) is marketed as a 20% water solution and that hydrolyzed silk protein (mw = 650 Da) is marketed as a 6.5% water solution.[12] Hydrolyzed silk protein (mw = 300 Da) from one source was reported to contain heavy metals and arsenic at levels of $\leq$ 4 ppm and 0.4 ppm, respectively.[10] Hydrolyzed silk protein from another source (mw = 650 Da) was reported to contain heavy metals and arsenic at $\leq$ 10 ppm and 1 ppm, respectively.[11]

According to another supplier, their hydrolyzed silk ingredients are marketed as aqueous solutions, two of which are 20-30% hydrolyzed silk and 27-32% hydrolyzed silk.[20]

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 in which the two strands are linked by disulfide bonds and enveloped by successive sticky layers of sericin.[5] Individual filaments are large molecules (3700 amino acids).[21] Fibroin has also been described as a glycoprotein composed of two comparably composed protein subunits 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,[22] whereas 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.[23] According to another source, fibroin contains 46% glycine, 29% alanine, and 12% serine.[21]

More-detailed information on the composition of fibroin, from the cocoon of the *Bombyx mori* caterpillar, indicates that it consists of 2 polypeptide chains, or, more specifically, heavy and light chains of 391 kDa and 25 kDa, respectively; a disulfide bridge links the heavy chain to the light chain.[24] 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.

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 highly polar side chains, containing hydroxyl, carboxyl or 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 side-chain hydroxyl, carboxyl, and amino groups enable easy cross-linking, copolymerization and blending with other natural or synthetic polymers.[25] According to another source, sericin contains 37% serine, 17% glycine, and 16% aspartate.[21]

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

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]

Silk

Silk contains nitrogen (13% to 20%) and, for material from one supplier, the reported or specified maximum concentration of heavy metals is 20 ppm.[8]

USE

Cosmetic

The safety of the silk protein ingredients included in this safety assessment is evaluated based on the expected use of these ingredients in cosmetics. The Cosmetic Ingredient Review (CIR) Expert Panel uses data received from the U.S. 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 protein ingredients are currently being used in cosmetic products. According to these data, the following 3 silk protein ingredients are not being used in cosmetics:

Fibroin
MEA-Hydrolyzed Silk
Silkworm Cocoon Extract

According to the 2015 VCRP, the greatest reported use frequency is for hydrolyzed silk (675 formulations, mostly rinse-off), followed by silk powder (177 formulations, mostly leave-on) (Table 4).[26] Lower use frequencies are being reported for the remaining silk ingredients. The results of a concentration-of-use survey conducted in 2014 indicated that silk powder had the highest maximum concentration of use; it was used at concentrations up to 1.4% in leave-on products (face powders) (Table 4).[27] 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.[28,29,30,31] 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.[28,29]

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 (Annex II of the Council of the European Communities Council Directive), or on the list of ingredients with use restrictions in products marketed within the European Union (Annex III).[32]

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.[33]

TOXICOKINETICS

Toxicokinetics studies of the silk proteins reviewed in this safety assessment were not found in the published literature, and unpublished data were not submitted.

TOXICOLOGY

Single Dose (Acute) Toxicity

Oral

Hydrolyzed Silk

The acute oral toxicity of hydrolyzed silk (further details on its nature unavailable) was evaluated using rats (5 males, 5 females; strain not stated).[34] A single dose of 10 g/kg was administered orally to each animal. No signs of toxicity were observed during the 14-day observation period after dosing.

In another study, the acute oral toxicity of hydrolyzed silk protein (mw ~ 1,000 Da; produced via alkali hydrolysis) was evaluated using albino rats (5 males, 5 females).[35] A single dose of the test material (5 g/kg body weight) was administered using an intragastric feeding needle. Signs of toxicity were not observed during the study and none of the animals died. The LD_{50} was > 5 g/kg.

The acute oral toxicity of hydrolyzed silk protein (15% to 25% in water; specific gravity = 1.10) was studied using 10 albino rats (5 males, 5 females).[35] The test substance was administered orally at a dose of 5 g/kg, and dosing was followed by a 14-day observation period. Gross necropsy was performed on all animals. Only 1 animal (male) died, and thoracic cavity filled with fibrous tissue was noted at necropsy. Gross changes were not observed in the remaining animals. The test substance was classified as non-toxic.

Silk

Ten male Sprague-Dawley rats were dosed orally (16 g/kg) with silk.[36] The form of silk administered, test concentration, vehicle, and dosing method were not stated. Dosing was followed by a 14-day observation period. None of the animals died, and, except for slight lethargy, there were no signs of toxicity during the observation period. The oral LD_{50} was > 16 g/kg, and silk was considered nontoxic in this study.

Silk Powder

A 30% solution of silk powder in distilled water was administered orally (feeding tube) to 12 female DD-strain mice.[37] The animals received doses up to 12,000 mg/kg body weight (dose volume = 40 ml/kg). Dosing was followed by a 7-day observation period. Toxic signs were not observed during the study, and the test substance was classified as practically non-toxic (LD_{50} > 12,000 mg/kg body weight).

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.[38] 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

Hydrolyzed Silk

In a cumulative skin irritation study involving 8 Hartley guinea pigs, hydrolyzed silk protein was applied to the back once daily for 35 days.[39] Details relating to the composition of the test material or method of preparation were not included. The animals were killed and necropsied at the end of the dosing period. Body weight gain was normal, and no abnormalities were noted at necropsy. Results relating to skin irritation potential are included in Table 5.

Silk Powder

In a skin sensitization study, silk powder (50% in sterile water; 0.5 ml on 20 x 20 mm occlusive patch) was applied for 6 h to the left flank of 20 young adult female guinea pigs of the Dunkin-Hartley strain.[40] Ten guinea pigs served as controls. This procedure was repeated at weekly intervals for a total of 3 weeks. Following a 2-week non-treatment period, challenge patches were applied to the right flank for 6 h. Two animals died during the study, and necropsy results did not indicate a test substance-related effect.

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 (heat, acid, or alkaline), sericin yielded less toxicity, as measured by the percentage of viable fibroblasts.[41]

Skin Depigmentation

Sericin

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.[42] 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.[43] For example, colored silk cocoons, which contain flavonoids and carotenoids, exhibit higher anti-tyrosinase activity than white-shelled cocoons.

Allergenicity

Human

Silk

The relationship between silk sensitization and asthma incidence was evaluated in 871 children living in China.[44] 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.

Sixty-four children (< 15 years old; males and females) with silk-induced asthma in China were studied.[45] 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 (serum antibodies against silk waste [severely broken silk threads, used only as filling for bed quilts or clothes and mattresses]). 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.

In relation to the preceding study, it should be noted that allergenic proteins have been extracted from one silk batch that was imported to be used as filling material for bed mattresses and rugs.[46] IgE and IgG antibodies to the extracted silk proteins were measured by radioallergosorbent (RAST) in sera of 9 silk-sensitive subjects and in sera of healthy control subjects. IgE and IgG antibodies to the individual silk polypeptides were detected using the immunoblot technique. The sera of silk-sensitive subjects contained high titers of IgE and low titers of IgG antibodies to the separated silk polypeptides. Low IgG antibody titers to a limited number of these polypeptides were detected in sera of control subjects. In another study involving 10 patients with a positive skin prick test to silkworm crude extract, arginine kinase (42-kda protein) was identified as a major allergen in this crude extract.[47]

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, and unpublished data were not submitted.

GENOTOXICITY

Hydrolyzed Silk

The genotoxicity of hydrolyzed silk protein (10% aqueous) was evaluated in the Ames test using the following *Salmonella typhimurium* strains, with and without metabolic activation: TA98, TA100, TA1535, TA1537, and TA1538.[48] Results were negative in all bacterial strains. The positive and negative controls performed as expected in this assay.

CARCINOGENICITY

Sericin

Male CD-1 (ICR): Crj mice (2 groups of 11 and 12 respectively) were fed diets supplemented with 1.5% or 3% sericin for 5 weeks.[49] The animals also received weekly injections of 1,2-dimethylhydrazine (DMH) during the initial 3 weeks of the study. The feeding of sericin in the diet resulted in a dose-dependent decrease in the development of colonic aberrant crypt foci. In a second experiment, mice were fed a diet supplemented with 3% sericin for 115 days. The animals were also injected with DMH weekly during the initial 10 weeks. Both the incidence and number of colon tumors were suppressed by sericin consumption.

Cell Proliferation

Sericin

The effect of sericin on the rat insulinoma cell line (seeded on ASF104 culture medium [serum-free medium containing insulin and transferrin]) was evaluated. Bovine serum albumin (BSA) served as the control protein. The RIN-5F cell cultures were identified as follows: ASF104 (1 ml), ASF104 with 0.1% sericin, and ASF104 with 0.1% BSA. The cells were cultured for 22 h. Viable and non-viable cell numbers were determined using the trypan blue exclusion method. The cells in the control culture failed to proliferate. However RIN-5F cells propagated significantly in the presence of sericin ($p < 0.05$) or BSA ($p < 0.01$). Therefore, sericin and BSA were efficient inducers of RIN-5F cell proliferation.[50]

Photocarcinogenicity

Sericin

A study was performed to assess the protective effect of sericin on ultraviolet B light (UVB)-induced acute damage and tumor promotion in HR-1 hairless mouse skin.[51] Three groups of 10 mice were treated dermally with sericin, 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, 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 all of the 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.[51]

IRRITATION AND SENSITIZATION

Skin Irritation and Sensitization

Skin irritation and sensitization studies on silk protein ingredients are summarized in Table 5. These ingredients are, at most, mild skin irritants and lack skin sensitization potential. In the only available human skin irritation/sensitization tests in which test concentrations were reported, 20% aqueous hydrolyzed silk was a non-irritant and a non-sensitizer and 6.5% aqueous hydrolyzed silk was a non-irritant (skin irritation only evaluated).

Phototoxicity

Hydrolyzed Silk

The phototoxicity of 6.5% aqueous hydrolyzed silk protein (mw = 650 Da; produced by alkaline and enzyme hydrolysis) was evaluated using groups of 6 Hartley guinea pigs.[52] The 3 groups were identified as test, positive control, and negative control groups. 8-Methoxypsoralen (1%) served as the positive control. The negative control was not stated. The test material was applied topically (dose = 0.05 ml/2 x 2 cm) to 2 sites on dorsal skin. One site was irradiated once with a FL-40S lamp and BLP lamp (wavelength range not stated), and the other site was covered. The sites were examined macroscopically at 24 h, 48 h, and 72 h. Phototoxicity was evaluated based on the difference in severity of skin reactions between the irradiated and non-irradiated sites. Hydrolyzed silk protein was classified as non-phototoxic. Positive responses were observed in all of the guinea pigs treated with 8-methoxypsoralen + light.

Silk Powder

Silk powder (0.1 g) was applied to the back (2 sites [2 areas per site]) of each of 6 female guinea pigs of the Hartley strain.[37] The test site (cm^2 area not stated) was covered with a patch plaster for 4 h, after which the sites were irradiated with UV light (minimal erythema dose, 15-minute exposure) from a 20-watt lamp. The test sites were evaluated for erythema and eschar formation after 24 h and 48 h. Positive reactions were not observed in this study.

Photoallergenicity

Hydrolyzed Silk

The photoallergenicity of 6.5% aqueous hydrolyzed silk protein (mw = 650 Da; produced by alkaline and enzyme hydrolysis) was evaluated using groups of 6 Hartley guinea pigs.[53] The 3 groups were identified as test, positive control, and negative control groups; the positive control was 3,5,4'-tribromosalicylanilide (2% in 85% dimethylsulfoxide),but the negative control was not stated. The test material was applied transdermally (0.05 ml /2 x 2 cm), with or without UV irradiation, 5 times per week (2 h per day) for a total of 10 applications. Applications were made on both sides of the dorsal area, symmetrically. One side was irradiated for 2 h, and the other side was covered. The photochallenge phase was initiated after a 2-week non-treatment period. The test material was applied to 2 sites. One side was irradiated for 2 h, and the other side was covered. The application site was examined macroscopically after the challenge and 24 h, 48 h, and 72 h later. No effects were observed in negative controls or in guinea pigs treated with the test material (with or without exposure to light). Hydrolyzed silk protein was considered non-photosensitizing in this study. The expected results were achieved with the positive control.

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. [54] 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.[55] 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.[54,56]

A female patient with a history of severe atopic dermatitis and various allergies, and a family history of atopic eczema and asthma, presented with exacerbation of eczema on her hands and wrists and urticarial papules on the flexor aspects of both forearms.[57] The same types of lesions had been observed on both arms after wearing the same silk shirt, but quickly disappeared after the shirt was taken off. RAST tests yielded positive results for silk waste (k73) of 2.08 universal arbitrary units (Ua)/ml and positive results for silk (k74) of 3.62 Ua/ml. The total IgE count was > 5000 kilo units (kU)/l. These results confirmed the diagnosis of immunological contact urticarial caused by silk.

Ocular Irritation

Animal

Hydrolyzed Silk

The ocular irritation potential of hydrolyzed silk protein (15% to 25% in water) was evaluated in the Draize test using 6 New Zealand white rabbits.[58] The test substance (0.1 ml) was instilled into one eye of each animal, and eyes were not rinsed. Contralateral eyes served as controls. Observations for ocular reactions were made up to 72 h post-instillation. The test substance was practically non-irritating to the eyes of rabbits.

The ocular irritation potential of 6.5% aqueous hydrolyzed silk (mw ~ 300 Da) was studied using 6 New Zealand white rabbits.[34] The test material (0.1 ml) was instilled into the right eye of each animal, and the left eye served as ghe untreated control. Reactions were scored at 24 h, 48 h, and 72 h post-instillation. Slight conjunctival rednesss, the only reaction reported, was observed in one rabbit. It was concluded that it is not likely that hydrolyzed silk would be classified as an ocular irritant, according to the definitions of the U.S. Federal Hazardous Substances Act. The ocular irritation potential of a higher molecular weight hydrolyzed silk (mw = 650 Da; test concentration not stated) was evaluated in New Zealand white rabbits according to the same test procedure, and the results were negative.[59]

Hydrolyzed silk (mw ~ 1,000 Da; produced by alkali hydrolysis) was placed (0.1 ml) in the right eye of each of 6 New Zealand white rabbits.[35] Observations for any signs of corneal opacity, iritis, or conjunctivitis were made at 24 h, 48 h, and 72 h post-instillation. The authors concluded that hydrolyzed silk was practically non-irritating to the eyes of rabbits.

In a cumulative ocular irritation test, 6.5% aqueous hydrolyzed silk protein (mw = 650 Da; 0.1 ml; produced by acid, alkaline, and enzyme hydrolysis), was instilled into the eyes of 6 New Zealand white rabbits three times per day for 4 days continuously.[60] Conjunctival redness was observed in 5 of 6 animals at 3 or 4 days. Reactions in the cornea or iris were not observed. The authors concluded that hydrolyzed silk protein was practically non-irritating to the eyes of rabbits.

Silk

Silk (0.1g) was instilled into one eye of each of 9 adult albino rabbits.[36] The eyes of 3 rabbits were rinsed immediately after instillation. Untreated eyes served as controls. The eyes were examined at 24 h, 48 h, and 72 h post-instillation. Transient conjunctival redness (unrinsed eyes) was observed in 5 of 6 rabbits. However, no effects on the cornea or iris were observed. Ocular irritation was not observed in the 3 rabbits subjected to ocular rinsing. Silk was classified as a non-irritant in this study.

Silk Powder

The ocular irritation potential of a silk powder solution (10% in saline) and the supernatant fluid from this solution (filtered after 24 h) was evaluated using a total of 6 white rabbits.[37] Four rabbits received the 10% in saline solution and 6 rabbits received the supernatant. Either test substance (0.1 ml) was instilled into the right eye, and eyes were rinsed. Untreated left eyes served as controls. The eyes were examined for reactions for up to 168 h post-instillation. The silk powder solution (10% in saline) was classified as slightly positive. The supernatant from this solution was not an ocular irritant.

In Vitro

Hydrolyzed Silk

The ocular irritation potential of hydrolyzed silk protein (mw = 300 Da; 2% active solution
n) was evaluated in the *in vitro* hen's egg test on the chorioallantoic membrane (HET-CAM).[10,61] The material (0.3 ml) was tested on the choroallantoic membrane of fertilized Leghorn hens' eggs that had been incubated for 10 days. Results were negative (score = 0.3).

Hydrolyzed silk protein (mw = 300 Da; 10% active solution) was tested for ocular irritation potential in the *in vitro* red blood cell aggregation test (RBCA), which evaluates effects on the cytoplasmic membrane.[10,62] A total irritation classification was obtained by determining the hemolysis/denaturation (L/D) ratio. A substance with an L/D of > 100 was classified as a non-irritating. Hydrolyzed silk caused neither hemolysis nor denaturation, and was classified as non-irritating.

The Irritection® assay was used to evaluate the ocular irritation potential of hydrolyzed silk.[63] The test material was applied to the Irritection® system at dose volumes of 25 µl, 50 µl, 75 µl, 100 µl, and 125 µl. The samples remained at room temperature for 24 h and were then analyzed by spectrophotometry. Over the range of dose volumes tested, ocular Irritection scores for hydrolyzed silk ranged from 2.5 to 3.5. Scores in this range corresponded to a classification of minimally irritating.

The ocular irritation potential of hydrolyzed silk was studied using the EpiOcular™ model assay.[64] The test material was applied to a reconstructed human corneal epithelial model for 30 minutes, and cell viability was measured by dehydrogenase, present in the cell mitochondria, reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), into blue, MTT formazan salt. The irritation potential of the test material is dictated by the reduction in tissue viability of exposed tissues, compared to the negative control (sterile deionized water). Methyl acetate served as the positive control. Hydrolyzed silk was classified as a non-irritant. The negative and positive controls were non-irritating and irritating, respectively.

OTHER EFFECTS

Immunological Responses

Sericin and Fibroin

In Vitro

Soluble sericin proteins (2 µg of sericin per well tissue culture plate) extracted from native silk fibers did not induce significant macrophage activation.[65] 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 significance 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 (0.2 to 1.0 mg/ml) 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.[66] 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.[67] Sericin solution (0.004 to 0.080 mg/ml) 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.

Topical application of sericin solution (2.5 mg or 5 mg in acetone) 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.[68]

Wound Healing

Non-human

Sericin

The effect of a sericin cream on wound healing was evaluated using 18 male Sprague-Dawley rats (8 weeks old).[69] 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.

In a similar study involving 45 Sprague-Dawley rats (8 weeks old), 8% sericin cream 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.[70]

In Vitro

Sericin

Human skin fibroblasts were incubated with sericin *in vitro* for 72 h.[71] The 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).[66] 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.

SUMMARY

The safety of the following 10 silk protein ingredients 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 the FDA's VCRP and the results of an industry survey indicate that 7 of the 10 silk protein ingredients 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 process. 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 typically present as impurities in sericin.

Hydrolyzed silk is prepared from the cocoon of *Bombyx mori*. Hydrolyzed silk protein (mw = 300 Da) may be prepared by acid, alkaline, or enzyme hydrolysis; hydrolyzed silk protein (650 Da) may be prepared by alkaline and enzyme hydrolysis.

In acute oral toxicity studies involving rats, LD_{50} values of > 10 g/kg body weight (hydrolyzed silk protein, mw ~ 300 Da), > 5 g/kg (hydrolyzed silk protein, mw ~ 1,000 Da), > 16 g/kg (silk), and > 12 g/kg (30% aqueous silk powder) were reported. Signs of toxicity were not observed.

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.

In a study (RIPT, occlusive patches) involving repeated dermal applications of silk powder (50% in sterile water) to 20 guinea pigs over a 3-week period, 2 animals died. However, necropsy results were not indicative of a test substance-related effect.

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

In the Ames test, results for 10% aqueous hydrolyzed silk protein were negative.

Sericin and BSA was an efficient inducer of rat RIN-5F cell proliferation. 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 another study involving mice, the feeding of diets supplemented with 1.5% or 3% sericin for 5 weeks resulted in a dose-dependent decrease in the development of colonic aberrant crypt foci.

Undiluted hydrolyzed silk protein (mw ~ 300 Da; dose = 0.5 ml/2.5 cm^2) caused reactions ranging from very slight to well-defined erythema on intact and abraded skin of rabbits. However, a PII of 1.1 was reported, and the test material was not classified as a primary skin irritant. Hydrolyzed silk protein (mw ~ 1,000 Da; dose = 0.5 ml/ 2.5cm^2; PII = 0.65) and hydrolyzed silk protein (mw = 650 Da; dose = 0.5/2.5 cm^2; PII = 0.05) were also classified as non-irritating to the skin of rabbits. Hydrolyzed silk protein (15% to 25% aqueous) was non-irritating to the skin of rabbits. In a cumulative skin irritation study involving guinea pigs, hydrolyzed silk protein (mw = 650 Da) was considered non-irritating to the skin and no abnormalities were observed at necropsy. Hydrolyzed silk protein (mw = 650) did not induce skin sensitization in a study involving guinea pigs. Silk powder (0.5 g) was non-irritating to abraded or intact skin of rabbits, and silk powder (0.1 g) was non-irritating and non-sensitizing to the skin of guinea pigs.

There was no evidence of skin irritation in 6 rabbits after the application of silk (0.5 g) under a 2 cm^2 patch for 24 h. Silk powder (0.5% w/v in distilled water) did not induce sensitization in an RIPT involving 20 guinea pigs. Also, the results of a preliminary skin irritation test at concentrations up to 10% in distilled water were negative. Similarly, silk powder (50% in sterile water) did not induce sensitization in an RIPT involving 20 guinea pigs. Furthermore, the results of a preliminary skin irritation test indicated that 75% silk powder was reasonably tolerated by the 5 guinea pigs tested.

Negative results were reported for hydrolyzed silk protein (mw = 300 Da) in a skin irritation study involving 20 subjects, and for hydrolyzed silk protein (mw = 650) in a study involving 24 subjects. In two RIPT's involving 57 subjects and 49 subjects, respectively, hydrolyzed silk protein (mw ~ 1,000 Da; dose ~ 0.2 ml/1" x 3/4" area and dose ~ 2 ml/4 cm^2 area, respectrively) was not classified as a skin irritant or sensitizer. In an HRIPT involving 48 subjects, the results relating to the skin irritation and sensitization potential of hydrolyzed silk protein (mw ~ 300 Da; dose = 20 µl/40 mm^2 Finn chamber) were negative. Silk powder (0.05 g) was non-irritating to the skin of 30 subjects.

Results for hydrolyzed silk were negative for skin irritation potential in the Irritection® (dose volumes up to 125 µl) and Epiderm™ (aqueous solution containing 27% to 32% hydrolyzed silk) *in vitro* assays. The mouse local lymph node assay yielded negative results relating to the sensitization potential of 20% aqueous hydrolyzed silk protein (mw = 300 Da).

Hydrolyzed silk protein (mw = 650 Da; dose = 0.05 ml/2 x 2 cm) was neither phototoxic nor photoallergenic to the skin of guinea pigs. Silk powder (0.1 g) also was not phototoxic when applied to the skin of guinea pigs.

Hydrolyzed silk protein of molecular weight ~ 300 Da, 650 Da, or ~ 1,000 Da did not induce ocular irritation when instilled into the eyes of rabbits. The tests involving hydrolyzed silk protein (mw ~ 300 Da or ~ 1,000 Da) were single-instillation tests, whereas, hydrolyzed silk protein (mw = 650 Da) was instilled 3 times per day for 4 days. In another test, hydrolyzed silk protein (15% to 25% aqueous, single instillation) was non-irritating to the eyes of rabbits. Hydrolyzed silk protein (mw = 300 Da; 2% active solution) was also negative for ocular irritation potential in the HeT-CAM, Irritection®, and Epiderm™ *in vitro* assays. Silk was also classified as a non-irritant when instilled into the eyes of rabbits, and the results for 10% aqueous silk powder were slightly positive when instilled into the eyes of rabbits.

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.

DRAFT DISCUSSION

The Panel is aware of a study in which an 8% sericin cream applied to dry/itchy skin of renal patients caused significant skin depigmentation of the arms and legs. Sericin is known to inhibit the enzyme tyrosinase, rate-limiting enzyme for melanin production, leading to skin depigmentation. However, the Panel noted that given the low use concentration of sericin in cosmetics (0.00047 %), hypopigmentation would not be an issue.

The Panel discussed studies in which associations were found between asthma and dermal allergies to silk in children in China. The Panel noted that the reported results of these studies are not sufficient to demonstrate that there is a cause-and-effect relationship between silk exposure and asthma. Alternate explanations for the association are plausible, including the likelihood that the children studied were atopic and, thus, prone to IgE-mediated hypersensitivities.

The Panel also discussed the potential for incidental inhalation exposures to apple-derived ingredients in products that are sprayed or in powder form and agreed that, based on likely airborne particle size distributions and concentrations in the breathing zone and ingredient use, incidental inhalation would not lead to local respiratory effects or systemic effects.

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

Table 2. Properties of Silk Proteins

Property	Value	Background Information
	Sericin	
Form	Powder; amorphous structure.[5]	Transforms into a β-sheet structure in presence of water.[5]
		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]
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.[25]	Decreases when molecules are transformed from random coil into the ß-sheet structure.[2] IsoelectricPoint ≈ 4, because there are more acidic than basic amino acids in sericin.[22]
	Fibroin	
Form	Pale yellow mass.[6]	
Molecular Weight	300 to 420 kDa.[72]	
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.[73]	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.[73]
	Hydrolyzed Silk	
Form	Amber liquid.[18]	
Odor	Characteristic.[18]	
Molecular Weight	< 10,000 Da.[19]; 2,000-4,000 Da.[20]; 300 Da and 650 Da[10,11]	
Solubility	Soluble in water.[19]	
Density (at room temperature)	1.05 to 1.11 g/ml.[18]	
	Silk	
Appearance	White to slightly gray powder.[8]	
Particle Size	5 to 15 μm.[8]	

Table 3. Composition Data on Hydrolyzed Silk.[16,17]

	Silk Hydrolysate (g/100 g protein)
Cysteic Acid	0
Hydroxpyroline	0
Aspartic Acid	7.4
Threonine	3.9
Serine	17.4
Glutamic Acid	3.6
Proline	0.9
Glycine	20.6
Alanine	21
Cystine	0
Valine	3.7
Methionine	0.4
Isoleucine	0.8
Leucine	1.2
Tyrosine	12.2
Phenylalanine	2
Lysine	1.6
Histidine	0.9
Arginine	2.4
Tryptophan	NR
Lysinoalanine	NR

NR = Not Reported

Table 4. Frequency and Concentration of Use According to Duration and Type of Exposure.[26,27]

	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 4. Current Frequency and Concentration of Use According to Duration and Type of Exposure.[26,27]

	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.

Table 5. Skin Irritation/Sensitization Potential of Hydrolyzed Silk Ingredients

Skin Irritation and Sensitization - Non-Human

Hydrolyzed Silk

6 female New Zealand albino rabbits. Undiluted test material applied (0.5 ml) for 24 h to abraded or intact skin using 2.5 cm^2 occlusive patch. Reactions (scored 1 h after patch removal) ranged from very slight to well-defined erythema at both intact and abraded test sites. Primary irritation score (6 rabbits) = 1.1. According to Draize system, combined averages (primary irritation scores) of 2 or less classified as mildly irritating. Mild skin irritant.[74]

6 New Zealand white rabbits. 15% to 25% aqueous solution (0.5 ml) applied for 24 h to abraded and intact sites on trunk (2.5 cm^2 area per site) using occlusive patch. The test sites examined at 24 h and 72 h post-application. Non-irritant (PII = 0.65).[35]

6 New Zealand white rabbits. 6.5% aqueous solution applied to back (2.5 x 2.5 cm area) according to preceding test procedure. Very slight erythema in one rabbit. Non-irritant (PII = 0.05).[75]

8 Hartley guinea pigs. Test material applied to back once daily for 35 days. After 31 days, the only reaction observed was very slight erythema in 3 animals. Non-irritant.[39]

Groups of 6 Hartley guinea pigs. 6.5% aqueous solution. Maximization test. Subcutaneous injection and dermal application during induction. Dose per cm^2 not stated. 24-h occlusive challenge patch (0.2 ml test material) applied to dorsal skin on day 14. Reactions scored at 24 h and 48 h after challenge patch removal. 1 of 6 animals had moderate erythema and 2 of 6 animals had slight erythema at 24 h after patch removal. Two of 6 animals had slight erythema at 48 h after patch removal. Non-sensitizer. Positive control (0.1% 4-dinitrochlorobenzene) induced sensitization.[76]

Silk

6 adult new Zealand albino rabbits. Test material (0.5 g in solvent [unnamed]) applied to intact area and abraded area on the back for 24 h; each site covered with 2 cm^2 occlusive patch. Reactions scored at 24 h and 72 h. No evidence of erythema, eschar, or edema at abraded or intact sites. Silk classified as non-irritant.[36]

Silk Powder

6 female albino rabbits. Test material (0.5 g, under an occlusive patch) was applied for 4 h to abraded and intact sites (1 $inch^2$ area) on the back. Sites examined at 24 h. No skin irritation reactions at intact or abraded skin sites.[37]

Female guinea pigs (number of animals and strain not stated). Test material (0.1 g) applied to back (cm^2 area not stated) 5 times per week for 13 weeks. No irritation or sensitization reactions.[37]

20 Hartley albino guinea pigs (10 males, 10 females). Test material (5% w/v in distilled water; 0.5 ml on occlusive patch [Hill Top Chamber®]) applied for 6 h to left shoulder once per week for a total of three 6-h applications. Dose per cm^2 not stated. After 2-week non-treatment period, challenge patch applied for 24 h to new site. After patch removal, depilatory applied to challenge site for 30 minutes. Reactions scored at ~ 2 h after depilation. Ten guinea pigs (controls) tested with distilled water. Non-irritant and non-sensitizer (test and controls).[77]

20 female Dunkin-Hartley guinea pigs. Test material (50% in sterile water; 0.5 ml on 20 x 20 mm occlusive patch) applied repeatedly for 6 h to left flank (induction). Reactions were scored at 24 h after patch removal. This procedure repeated at weekly intervals (Days 8 to 9 and 15 to 16 of study). At dfay 29, challenge patch applied to right flank for 6 h. Reactions scored 24 h and 48 h after patch removal. Sterile water applied to 10 control guinea pigs. Non-sensitizer (test and control animals). Silk powder (75%) reasonably tolerated in preliminary skin irritation test involving 5 guinea pigs.[40]

Silk Protein Film

New Zealand White rabbits (male; number not stated). Silk protein film (protein names not stated; tested as supplied) evaluated in Draize test (OECD Guideline 404). 3 test patches applied sequentially to clipped dorsal skin of the trunk for 3 minutes, 1 h, and 4 h, respectively. Dose per cm^2 not stated. Negative findings for irritation confirmed using two additional animals, each tested with one patch for 4 h. Reactions scored at 1 h, 24 h, 48 h, and 72 h after patch removal. No signs of erythema, edema, or eschar.[38]

Guinea pigs (2 groups of 6). Silk protein film (protein names not stated; tested as supplied) evaluated in Buehler test. Occlusive patch with test material (moistened with physiological saline) applied for 6 h (on days 7 and 14) to clipped skin of left flank (2 x 2 cm area; 6 animals). Dose per cm^2 not stated. Control (6 animals): Sterile gauze moistened with physiological saline. On day 28, occlusive challenge patch applied for 24 h to new test site on flank. Non-sensitizer.[38]

Skin Irritation and Sensitization - Human

Hydrolyzed Silk

20 subjects (2 men, 18 women). 20% aqueous hydrolyzed silk (~ 3 mg on occlusive patch [Finn chamber]) applied to back for 48 h. Dose per cm^2not stated. At 30 minutes after patch removal, mild erythema observed in 3 subjects. Mild skin irritation in only 1 of the 3 subjects at 24 h post-removal. No skin irritation in remaining 17 subjects.[10,78]

24 subjects (10 men, 14 women). 6.5% aqueous solution (0.2 ml on occlusive patch) applied to upper back for 24 h. Dose per cm^2 not stated. Classified as a non-irritant.[79]

57 male and female subjects. HRIPT. During induction, hydrolyzed silk (~ 0.2 ml; concentration not stated) applied to upper back repeatedly using a 1" x 3/4" semi-occlusive patch. Challenge patch applied to original site and to new site on forearm. Application sites evaluated at 24 h and 48 h post-application. Non-irritant and non-sensitizer.[58]

Table 5. Skin Irritation/Sensitization Potential of Hydrolyzed Silk Ingredients

49 male and female subjects. HRIPT. Semi-occlusive patch (2 cm x 2 cm) containing ~ 2 ml hydrolyzed silk (concentration not stated) applied to back repeatedly during induction. Challenge patch applied for 24 h to new site. Application sites evaluated at 24 h and 48 h post-application. Transient erythema (non-irritant and non-allergic in nature) observed during induction; 1 subject with cumulative skin irritation reaction after removal of 9th induction patch. Subject also had barely perceptible erythema at challenge site (48-h reading). No clinically significant irritation or evidence of allergic contact dermatitis.[80]

48 subjects. HRIPT. During induction, 20% aqueous hydrolyzed silk (20 µl on occlusive patch [8 mm diameter Finn chamber; 40 mm^2 surface]) applied for 48 h to the back repeatedly. 48-h challenge patch applied to new site on back. Non-irritant and non-sensitizer.[10,81]

Silk Powder

30 male and female subjects. Silk powder (0.05 g) applied for 48 h, under a closed patch, to the arm of each subject. Examinations for dermal reactions after 1 h and 24 h. No skin irritation.[37]

Skin Irritation and Sensitization - In Vitro

Hydrolyzed Silk

The Irritection® assay. *In vitro* system that involves use of a proprietary solution containing both proteins and macromolecules in a well that is covered by a membrane. Hydrolyzed silk applied to the Irritection® system at dose volumes of 25 µl, 50 µl, 75 µl, 100 µl, and 125 µl. Irritation measured quantitatively using a spectrophotometer. Non-irritant (Ocular Irritection® scores: 0.25 to 0.40).[63]

EpiDerm™ model assay. Test material (30 µl of aqueous solution containing 27% to 32% hydrolyzed silk) applied to human epidermal-derived keratinocytes (cultured to form a multilayer). Non-irritant.[64]

Mouse local lymph node assay (OECD Guideline No. 429). 20% aqueous hydrolyzed silk was non-sensitizer.[10,82]

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2015 FDA VCRP Data

Hydrolyzed Fibroin

12C - Face and Neck (exc shave)	1
Total	**1**

Hydrolyzed Sericin

03G - Other Eye Makeup Preparations	1
05I - Other Hair Preparations	2
12G - Night	1
Total	**4**

Hydrolyzed Silk

01A - Baby Shampoos	2
01B - Baby Lotions, Oils, Powders, and Creams	2
01C - Other Baby Products	2
02B - Bubble Baths	1
02D - Other Bath Preparations	4
03A - Eyebrow Pencil	1
03B - Eyeliner	2
03C - Eye Shadow	3
03D - Eye Lotion	12
03E - Eye Makeup Remover	2
03F - Mascara	11
03G - Other Eye Makeup Preparations	5
04E - Other Fragrance Preparation	4
05A - Hair Conditioner	74
05B - Hair Spray (aerosol fixatives)	7
05C - Hair Straighteners	6
05D - Permanent Waves	3
05E - Rinses (non-coloring)	2
05F - Shampoos (non-coloring)	72
05G - Tonics, Dressings, and Other Hair Grooming Aids	52
05H - Wave Sets	2
05I - Other Hair Preparations	28
06A - Hair Dyes and Colors (all types requiring caution statel	5
06H - Other Hair Coloring Preparation	1
07B - Face Powders	6
07C - Foundations	2
07D - Leg and Body Paints	3
07E - Lipstick	3
07F - Makeup Bases	1
07G - Rouges	9
07I - Other Makeup Preparations	3
08E - Nail Polish and Enamel	1
10A - Bath Soaps and Detergents	102
10B - Deodorants (underarm)	1
10E - Other Personal Cleanliness Products	16

11E - Shaving Cream	1
11G - Other Shaving Preparation Products	6
12A - Cleansing	22
12B - Depilatories	2
12C - Face and Neck (exc shave)	24
12D - Body and Hand (exc shave)	40
12F - Moisturizing	67
12G - Night	5
12H - Paste Masks (mud packs)	3
12I - Skin Fresheners	1
12J - Other Skin Care Preps	11
13B - Indoor Tanning Preparations	41
13C - Other Suntan Preparations	2
Total	**675**

Sericin

03D - Eye Lotion	1
03F - Mascara	9
04E - Other Fragrance Preparation	1
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	12
07B - Face Powders	1
07C - Foundations	4
10E - Other Personal Cleanliness Products	1
12C - Face and Neck (exc shave)	3
12D - Body and Hand (exc shave)	4
12F - Moisturizing	5
12H - Paste Masks (mud packs)	1
12J - Other Skin Care Preps	2
Total	**44**

Silk

03G - Other Eye Makeup Preparations	1
07A - Blushers (all types)	1
07B - Face Powders	4
07C - Foundations	9
07G - Rouges	1
07I - Other Makeup Preparations	1
08A - Basecoats and Undercoats	2
08G - Other Manicuring Preparations	3
10A - Bath Soaps and Detergents	3
12A - Cleansing	1
12F - Moisturizing	1
Total	**27**

Silk Extract

03B - Eyeliner	1

05A - Hair Conditioner	3
05F - Shampoos (non-coloring)	1
05G - Tonics, Dressings, and Other Hair Grooming Aids	3
05H - Wave Sets	2
05I - Other Hair Preparations	1
12F - Moisturizing	2
Total	**13**

Silk Powder

03A - Eyebrow Pencil	1
03B - Eyeliner	2
03C - Eye Shadow	17
03D - Eye Lotion	1
03F - Mascara	10
03G - Other Eye Makeup Preparations	2
04C - Powders (dusting and talcum, excluding aftershave talc)	26
05A - Hair Conditioner	3
05B - Hair Spray (aerosol fixatives)	1
05E - Rinses (non-coloring)	1
05F - Shampoos (non-coloring)	2
05G - Tonics, Dressings, and Other Hair Grooming Aids	2
07A - Blushers (all types)	12
07B - Face Powders	34
07C - Foundations	10
07E - Lipstick	16
07H - Makeup Fixatives	1
07I - Other Makeup Preparations	4
08E - Nail Polish and Enamel	6
08G - Other Manicuring Preparations	1
10A - Bath Soaps and Detergents	2
10E - Other Personal Cleanliness Products	1
12A - Cleansing	4
12C - Face and Neck (exc shave)	7
12D - Body and Hand (exc shave)	2
12F - Moisturizing	6
12H - Paste Masks (mud packs)	1
12J - Other Skin Care Preps	2
Total	**177**

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Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: CIR Science and Support Committee of the Personal Care Products Council

DATE: July 22, 2015

SUBJECT: Additional Information Concerning the Allergenic Potential of Silk Proteins

We have identified two additional articles concerning the allergenic potential of silk proteins which we believe are informative. Abstracts of the listed references are attached.

Dewair M, Baur X, Ziegler K. 1985. Use of immunoblot technique for detection of human IgE and IgG antibodies to individual silk proteins. *J Allergy Clin Immunol* 76(4): 537-542.

Liu Z, Xia L, Wu Y, et al. 2009. Identification and characterization of an arginine kinase as a major allergen from silkworm (Bombyx mori) larvae. *Int Arch Allergy Immunol* 150(1): 4-14.

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Abstract

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ELSEVIER FULL-TEXT ARTICLE

J Allergy Clin Immunol. 1985 Oct;76(4):537-42.

Use of immunoblot technique for detection of human IgE and IgG antibodies to individual silk proteins.

Dewair M, Baur X, Ziegler K.

Abstract

Allergenic proteins were extracted from one **silk** batch that was imported to be used as filling material for bed mattresses and rugs. IgE and IgG antibodies to the extracted **silk** proteins were measured by RAST in sera of nine **silk**-sensitive persons as well as in sera of healthy control donors. **Silk** proteins were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis into 12 polypeptides of molecular weights between 14 and 70 kilodaltons. By means of the immunoblot technique, IgE and IgG antibodies to the individual **silk** polypeptides could be detected. Sera of **silk**-sensitive persons contained high titers of IgE and low titers of IgG antibodies to the separated **silk** polypeptides. Sera of control donors contained low IgG antibody titers to a limited number of these polypeptides.

PMID: 4056241 [PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Substances

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Abstract

Int Arch Allergy Immunol. 2009;150(1):8-14. doi: 10.1159/000210375. Epub 2009 Apr 2.

Identification and characterization of an arginine kinase as a major allergen from silkworm (Bombyx mori) larvae.

Liu Z[1], Xia L, Wu Y, Xia Q, Chen J, Roux KH.

Author information

Abstract

BACKGROUND: The **silkworm**, **Bombyx** mori, is an important insect in the textile industry and its pupa are used in Chinese cuisine and traditional Chinese medicine. The silk, urine and dander of silkworms is often the cause of allergies in sericulture workers and the pupa has been found to be a food allergen in China. Recent studies have focused on reporting cases of **silkworm** allergies, but only a few studies have addressed the specific allergens present in the B. mori **silkworm**.

METHODS: We collected sera from 10 patients with a positive skin prick test to **silkworm** crude extract (SCE) and analyzed these samples by Western blot and ELISA. The cDNA of arginine kinase from the B. mori **silkworm** was also cloned and expressed in high yield in Escherichia coli. Allergenicity and cross-allergenicity of the recombinant B. mori arginine kinase (rBmAK) were investigated by ELISA inhibition assay.

RESULTS: Collected sera all reacted to a 42-kDa protein in a Western blot with SCE as the antigen. Preincubation of sera with rBmAK eliminated the reactivity of the patients' sera to this 42-kDa band. All patient sera also exhibited positive reactivity to SCE in an ELISA assay. BmAK also demonstrated cross-reactivity with a recombinant AK from cockroach.

CONCLUSION: Arginine kinase from the B. mori **silkworm** is a major allergen and crossreacts with cockroach AK.

PMID: 19339797 [PubMed - indexed for MEDLINE]

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Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Lange, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: June 10, 2015

SUBJECT: Comments on the Draft Report Prepared for the June 2015 CIR Expert Panel Meeting: Safety Assessment of Silk Protein Ingredients as Used in Cosmetics

Key Issue

All of the ingredients in this report are from the silk of silk worms, *Bombyx mori*. The information about spider silk in the Introduction and the Composition/Impurities section needs to be deleted from the report.

Additional Comments

Introduction - If the information about spider silk is not removed from the Introduction, it needs to be revised. The Introduction currently implies that spiders are insects that belong to the order Lepidoptera. This is not correct. Spiders belong to the class Arachnida, not the class Insecta.

Cosmetic Use - The VCRP is a registration program. It should not be called a survey.

It is not clear why only the European prohibited list of ingredients is mentioned in the Cosmetic Use section. What about the restricted list of ingredients or other Annexes?

Carcinogenicity - Please correct "arerrant" and "iojected"

Skin Irritation and Sensitization - Although the details of each study does not need to be in the text, the text should include a few more details such as the maximum concentration of the ingredients that was not found to be a sensitizer. The references should also be included in the text.

Photoallergenicity - "Two percent of 3,5,4'-tribromosalicylanilide (in 85% dimethylsulfoxide)." is not a complete sentence.

Ocular Irritation - Please correct "obvserved"

Did they really observe the rabbit eyes (reference 36) at 38 hours after treatment?

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Please define what is meant by an L/D ratio.

Immunological Responses - Please include the concentrations of Sericin tested.

Summary - It is not necessary to state that Hydrolyzed Silk may be prepared by acid, alkaline or enzyme hydrolysis and alkaline and enzyme hydrolysis. It would be more appropriate to state the different methods of hydrolysis and indicate that different suppliers provide preparations of different molecular weights and provide examples of the ranges of molecular weights.

The guinea pig repeat insult patch test is described on both the first and second pages of the Summary.

Please correct : " x3/4 " area

Table 2, Solubility, Sericin - Please correct "serine" (it should be sericin)

Table 5 -As Skin Irritation/Sensitization is in the Table title, it does not need to be in the subheading.

Non-Human, Hydrolyzed Silk - Please correct "Non-irritatnt"

What concentration of Hydrolyzed Silk was tested in the EpiDerm assay?