Safety Assessment of
Silk Protein Ingredients as Used in Cosmetics

Status: Draft Report for Panel Review
Release Date: May 22, 2015
Panel Date: June 15-16, 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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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: May 22, 2015
Subject: Draft Report on Silk Proteins

A Scientific Literature Review (SLR) on silk proteins was issued on March 2, 2015, and the SLR (now a draft report, *slkprt062015rep*) has been revised to include unpublished data that were received during the 60-day comment period. Comments that were received from the Council (*slkprt 062015pcpc1*) have been addressed.

Included in this package for your review is the draft safety assessment (*slkprt062015rep*), the CIR report history (*slkprt 062015hist*), Literature Search Strategy (*slkprt062015strat*), Ingredient Data Profile (slkprt062015prof), 2015 FDA VCRP data (*slkprt062015FDAdata*). The following unpublished data that were received are also included:

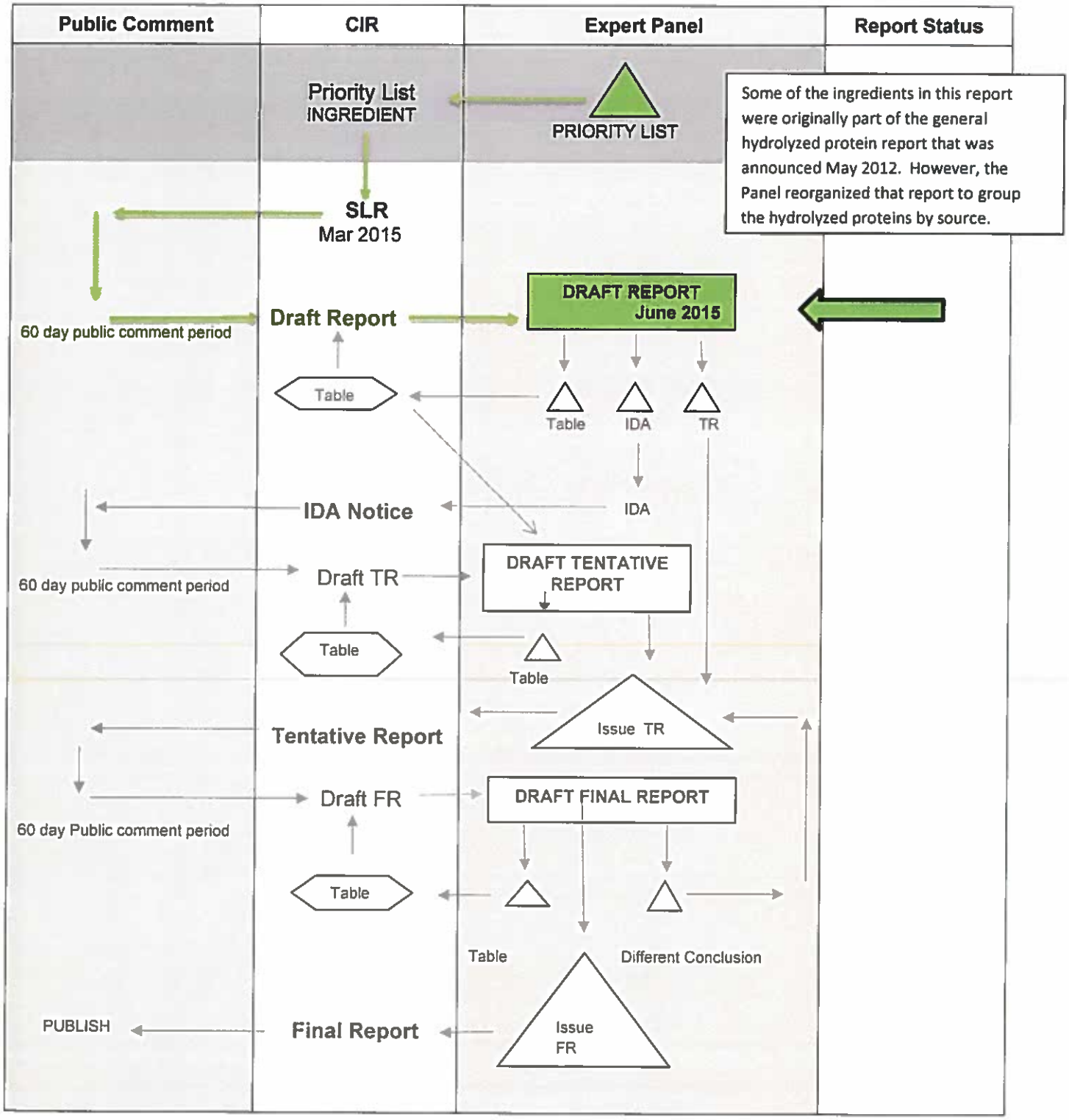
- Use concentration data memo (*slkprt062015data1*) and data (s*lkprt062015data1A*)
- Sensitization data on silk powder *(slkprt062015data2*)
- Method of manufacture and chemistry data on hydrolyzed silk (*slkprt 062015data 3*)
- Chemistry and toxicity data on silk (*slkprt062015data4*)
- Method of manufacture, chemistry, and toxicity data on hydrolyzed silk (*slkprt062015data5*)
- Method of manufacture, chemistry, and toxicity data on hydrolyzed silk (*slkprt062015data6*)

After considering the data included in this safety assessment, the Panel will need to determine whether the available data are sufficient for issuing a tentative report with a safe as used, safe with qualifications, or unsafe conclusion on these ingredients, or whether an insufficient data announcement should be issued.

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Silk Proteins

MEETING June 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)

Silk Proteins Check List for June, 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
MEA-Hydrolyzed Silk																				
Sericin																			X	
Silk				X		X						X	X		X	X				
Silk Extract																				
Silk Powder										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

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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 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]

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] The definitions and functions of fibroin, sericin, and other silk protein ingredients 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 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]

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. Heat and acid extraction are other methods. Sericin extracted by different methods can yield different amino acid compositions.[5]

Additional information indicates that 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 (m.w. = 300 Da) may be prepared by acid, alkaline, or enzyme hydrolysis; hydrolyzed silk protein (650 Da) may be prepared by alkaline or enzyme 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 (m.w. = 300 Da) or a 6.5% water solution of hydrolyzed silk protein (m.w. = 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 that are being reported include quaternium-15, EDTA, and methylparaben.[14,15]

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

According to another supplier, 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 that is linked by disulfide bonds and enveloped by successive sticky layers of sericin.[5] It is a large molecule (3700 amino acids).[21] 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.[22] 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, i.e., 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.

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.[25] 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.[26] 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 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]

Silk

Silk contains nitrogen (13% to 20%) and the maximum concentration of heavy metals is 20 ppm.[8]

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 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 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

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 4).[27] 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).[28] 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.[29,30,31,32] 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.[29,30]

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.[33]

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.[34]

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 was evaluated using rats (5 males, 5 females; strain not stated).[35] 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 (m.w. ~ 1,000 Da; produced via alkali hydrolysis) was evaluated using albino rats (5 males, 5 females).[36] 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.

Silk

Ten male Sprague-Dawley rats were dosed orally (16 g/kg) with silk.[37] 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.

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] 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. Results relating to skin sensitization potential are included in Table 5.

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 % viability of 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.

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

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

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.[48] 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 arerrant crypt foci. In a second experiment, mice were fed a diet supplemented with 3% sericin for 115 days. The animals were also iojected 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.[46]

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.[47] Three groups of 10 mice were treated 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.[47]

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.

Phototoxicity

Hydrolyzed Silk

The phototoxicity of 6.5% aqueous hydrolyzed silk protein (m.w. = 650 Da; produced by alkaline and enzyme hydrolysis) was evaluated using groups of 6 Hartley guinea pigs.[49] 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.

Photoallergenicity

Hydrolyzed Silk

The photoallergenicity of 6.5% aqueous hydrolyzed silk protein (m.w. = 650 Da; produced by alkaline and enzyme hydrolysis) was evaluated using groups of 6 Hartley guinea pigs.[50] The 3 groups were identified as test, positive control, and negative control groups. Two percent of 3,5,4'-tribromosalicylanilide (in 85% dimethylsulfoxide). 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.

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. [51] 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.[52] 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.[51,53]

Ocular Irritation

Animal

Hydrolyzed Silk

The ocular irritation potential of 6.5% aqueous hydrolyzed silk (m.w. ~ 300 Da) was studied using 6 New Zealand white rabbits.[35] 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 obvserved 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 (m.w. = 650 Da; test concentration not stated) was evaluated in New Zealand white rabbits according to the same test procedure, and the results were negative.[54]

Hydrolyzed silk (m.w. ~ 1,000 Da; produced by alkali hydrolysis) was placed (0.1 ml) in the right eye of each of 6 New Zealand white rabbits.[36] Observations for any signs of corneal opacity, iritis, or conjunctivitis were made at 24 h, 38 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 (m.w. = 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.[55] 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.[37] 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.

In Vitro

Hydrolyzed Silk

The ocular irritation potential of hydrolyzed silk protein (m.w. = 300 Da; 2% active solution) was evaluated in the *in vitro* hen's egg test on the chorioallantoic membrane (HET-CAM).[10,56] 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 (m.w. = 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.[10,57] A total irritation classification was obtained by determining the 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.[58] 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.[59] The test material was applied to a reconstructed human corneal epithelial model for 30 minutes, and cell viability was measured by dehydrogenase conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, present in the cell mitochondria, into blue 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 extracted from native silk fibers did not induce significant macrophage activation.[60] 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.[61] 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.[62] 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.

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.[63]

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).[64] 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.[65]

In Vitro

Sericin

Human skin fibroblasts were incubated with sericin *in vitro* for 72 h.[66] 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).[61] 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 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 typically present as impurities in sericin.

Hydrolyzed silk is prepared from the cocoon of *Bombyx mori*. hydrolyzed silk protein (m.w. = 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, m.w. ~ 300 Da), > 5 g/kg (hydrolyzed silk protein, m.w. ~ 1,000 Da), and > 16 g/kg (silk) 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.

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 (m.w. ~ 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 (m.w. ~ 1,000 Da; dose = 0.5 ml/ 2.5cm^2; PII = 0.65) and hydrolyzed silk protein (m.w. = 650 Da; dose = 0.5/2.5 cm^2; PII = 0.05) were also classified as non-irritating to the skin of rabbits. In a cumulative skin irritation study involving guinea pigs, hydrolyzed silk protein (m.w. = 650 Da) was considered

non-irritating to the skin and no abnormalities were observed at necropsy. Hydrolyzed silk protein (m.w. = 650) did not induce skin sensitization in a study involving guinea pigs.

Negative results were reported for hydrolyzed silk protein (m.w. = 300 Da) in a skin irritation study involving 20 subjects, and the same was true for hydrolyzed silk protein (m.w. = 650) in study involving 24 subjects. In two RIPT's involving 57 subjects and 49 subjects, respectively, hydrolyzed silk protein (m.w. ~ 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, on hydrolyzed silk protein (m.w. ~ 300 Da; dose = 20 µl/40 mm^2 Finn chamber) was also negative for skin irritation and sensitization potential.

Results for hydrolyzed silk were negative for skin irritation potential in the Irrection® and EpidermTM *in vitro* assays. The mouse local lymph node assay yielded negative results relating to the sensitization potential of 20% aqueous hydrolyzed silk protein (m.w. = 300 Da).

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 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.

Hydrolyzed silk protein (m.w. = 650 Da; dose = 0.05 ml/2 x 2 cm) was neither phototoxic nor photoallergenic 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 (m.w. ~ 300 Da or ~ 1,000 Da) were single-instillation tests, whereas, hydrolyzed silk protein (m.w. = 650 Da) was instilled 3 times per day for 4 days. Hydrolyzed silk protein (m.w. = 300 Da; 2% active solution) was also negative for ocular irritation potential in the HeT-CAM, Irrection®, and Epiderm™ *in vitro* assays. Silk was also classified as a non-irritant 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.

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-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.[26]	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 serine.[22]
	Fibroin	
Form	Pale yellow mass.[6]	
Molecular Weight	300 to 420 kDa.[67]	
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.[68]	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.[68]
	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.[27,28]

	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.[27,28]

	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.[69]

6 New Zealand white rabbits. Test material applied (0.5 ml) for 24 h to intact and abraded sites on opposite sides of vertebral column using 2.5 cm^2 occlusive patch. Reactions scored at patch removal and at 72 h. Non-irritatnt (primary irritation index (PII) = 0.65.[36]

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).[70]

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 aplication 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.[71]

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.[37]

Silk Powder

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).[72]

20 female Dunkin-Hartley guinea pigs. RIPT. 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). 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.[10,73]

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.[74]

57 male and female subjects. HRIPT. During induction, hydrolyzed silk (~ 0.2 ml) 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.[75]

49 male and female subjects. HRIPT. Semi-occlusive patch (2 cm x 2 cm) containing ~ 2 ml hydrolyzed silk applied toback 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.[76]

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.[10,77]

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

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).[58]
EpiDerm™ model assay. Test material applied to reconstructed human epidermis model. Non-irritant.[59]
Mouse local lymph node assay (OECD Guideline No. 429). 20% aqueous hydrolyzed silk was non-sensitizer.[10,78]

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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 statem	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**

Personal Care Products Council
Committed to Safety, Quality & Innovation

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: January 6, 2015

SUBJECT: Concentration of Use by FDA Product Category: Silk

Concentration of Use by FDA Product Category*

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

Ingredient	Product Category	Maximum Concentration of Use
Hydrolyzed Silk	Baby shampoo	0.0003%
Hydrolyzed Silk	Other bath preparations	0.0001-0.0003%
Hydrolyzed Silk	Eye lotion	0.00071-0.02%
Hydrolyzed Silk	Eye makeup remover	0.0000007-0.0021%
Hydrolyzed Silk	Mascara	0.02-0.23%
Hydrolyzed Silk	Hair conditioner	0.00002-0.41%
Hydrolyzed Silk	Hair sprays Aerosol Pump spray	 0.0005-0.024% 0.0018-0.01%
Hydrolyzed Silk	Hair straighteners	0.01%
Hydrolyzed Silk	Permanent waves	0.002%
Hydrolyzed Silk	Rinses (noncoloring)	0.1%
Hydrolyzed Silk	Shampoos (noncoloring)	0.0023-0.5%
Hydrolyzed Silk	Tonics, dressings and other hair grooming aids	0.00002-0.1%
Hydrolyzed Silk	Other hair preparations (noncoloring)	0.005-0.02%
Hydrolyzed Silk	Hair dyes and colors	0.00022-0.005%
Hydrolyzed Silk	Hair bleach	0.008%
Hydrolyzed Silk	Foundations	0.0000007-0.02%
Hydrolyzed Silk	Lipstick	0.039%
Hydrolyzed Silk	Makeup bases	0.0000007-0.02%
Hydrolyzed Silk	Basecoats and undercoats (manicuring preparations)	0.01-0.03%
Hydrolyzed Silk	Bath soaps and detergents	0.0029-0.035%
Hydrolyzed Silk	Shaving cream	0.002%
Hydrolyzed Silk	Other shaving preparations	0.0022%
Hydrolyzed Silk	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.000007-0.0045%
Hydrolyzed Silk	Face and neck products Not spray	 0.001-0.11%
Hydrolyzed Silk	Body and hand products Not spray	 0.00023-0.1%
Hydrolyzed Silk	Moisturizing products Not spray	 0.001-0.02%
Hydrolyzed Silk	Night products Not spray or powder	 0.02%
Hydrolyzed Silk	Skin freshener	0.0045%

Hydrolyzed Silk	Other skin care preparations	0.0045%
Hydrolyzed Fibroin	Hair dyes and colors	0.045%
Hydrolyzed Fibroin	Face and neck products Not spray	0.001%
Sericin	Eye lotion	0.00047%
Sericin	Perfume	0.000047%
Sericin	Hair conditioners	0.00047%
Sericin	Rinses (noncoloring)	0.00047%
Sericin	Shampoos (noncoloring)	0.00047%
Sericin	Tonics, dressings and other hair grooming aids	0.00047%
Sericin	Wave sets	0.00047%
Sericin	Face powders	0.00047%
Sericin	Foundations	0.00047%
Sericin	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.00047%
Sericin	Face and neck products Not spray	0.00047%
Sericin	Body and hand products Not spray	0.00047%
Sericin	Moisturizing products Not spray	0.00047%
Silk	Eyeliner	0.000005%
Silk	Face powder	0.1-0.2%
Silk	Foundation	0.0025%
Silk	Other makeup preparations	0.0025%
Silk	Basecoats and undercoats (manicuring preparations)	0.001-0.05%
Silk	Nail polish and enamel	0.001-0.01%
Silk	Body and hand products Not spray	0.01%
Silk Extract	Hair conditioners	0.015%
Silk Extract	Hair sprays Pump spray	0.0036%
Silk Extract	Shampoos (noncoloring)	0.015%
Silk Extract	Tonics, dressings and other hair grooming aids	0.0036%
Silk Extract	Other hair preparations (noncoloring)	0.015%
Silk Extract	Depilatories	0.0013%
Silk Powder	Eyebrow pencil	1%
Silk Powder	Eyeliner	0.1-0.26%
Silk Powder	Eye shadow	0.1-1.1%
Silk Powder	Mascara	0.05-0.5%
Silk Powder	Perfume	0.1%
Silk Powder	Other fragrance preparations	0.005%
Silk Powder	Hair conditioners	0.02-0.048%
Silk Powder	Hair sprays Aerosol	0.02%

Silk Powder	Shampoos (noncoloring)	0.01-0.02%
Silk Powder	Tonics, dressings and other hair grooming aids	0.01%
Silk Powder	Blushers (all types)	0.1-1.1%
Silk Powder	Face powders	0.1-1.4%
Silk Powder	Foundations	0.0001-1%
Silk Powder	Lipstick	0.05-1.1%
Silk Powder	Rouges	0.01%
Silk Powder	Makeup fixatives	0.1-0.6%
Silk Powder	Basecoats and undercoats (manicuring preparations)	0.0001%
Silk Powder	Nail polish and enamel	0.001-0.025%
Silk Powder	Other manicuring preparations	0.01%
Silk Powder	Bath soaps and detergents	0.01%
Silk Powder	Deodorants Not spray	 0.005%
Silk Powder	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0001-0.001%
Silk Powder	Face and neck products Not spray	 0.01-0.1%
Silk Powder	Body and hand products Not spray	 0.5%
Silk Powder	Moisturizing products Not spray	 0.01%
Silk Powder	Pastes masks and mud packs	0.1%
Silk Powder	Other skin care preparations	0.01%

*Ingredients found in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.

Information collected in 2014

Table prepared January 5, 2015

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: March 11, 2015

SUBJECT: Silk Powder: Guinea Pig Sensitization Studies

Springborn Institute for Bioresearch, Inc. 1985. Guinea pig sensitization screen: Silk Powder.

Research Toxicological Centre S.p.A. 1995. Silk Powder: Delayed dermal sensitisation study in the guinea pig.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

SPRINGBORN INSTITUTE FOR BIORESEARCH, INC.
SPENCERVILLE, OHIO 45887
PHONE 419 647 4196

GUINEA PIG SENSITIZATION SCREEN

[redacted]

Lab Study No.: [redacted]
Report Date: 4/10/85

Protocol: Guinea Pig Sensitization Screen (No. C4A)

Sponsor: [redacted] Silk Powder

Enclosed:

Signed: Mary Nickson
Laboratory Technician

Richard A. Hiles
Richard A. Hiles, Ph.D.
President and Technical Director
(Study Director)

James T. F. Liao
James T. F. Liao, DVM., Ph.D.
Director of Toxicology
(Management)

[redacted] 4/10/85

SPRINGBORN GROUP

DELAYED CONTACT HYPERSENSITIVITY IN GUINEA PIG - SUMMARY

Report of biological test performed at:

Springborn Institute for Bioresearch, Inc.
553 North Broadway
Spencerville, Ohio 45887

During the period: 1/18/85 to 4/10/85
According to the attached protocol and addenda (if any).
Deviations from Protocol: Sixteen guinea pigs over 400 g. Irritation animals were originally patched incorrectly.

Lab Study No.: [redacted]

Description: [redacted] White powder

Storage Condition(s): Room temperature

Strain and Source of Animals: [redacted] Hartley Albino Guinea Pigs Laboratory Supply Company

silk powder is only test material in this study

	Irritation Screen	Induction	Challenge
Concentration	10%, 5%, 2.5% w/v	5% w/v	5% w/v
Amount Dosed	0.4 ml	0.4 ml	0.4 ml
Solvent	Distilled water	a	a

[a]Distilled water

Results

Phase	Level	Incidence	Severity (Avg) 24 hr	Severity (Avg) 48 hr	Max. Score 24 hr	Max. Score 48 hr
CHALLENGE - Test	5%	0/20	0.0	0.0	0	0
- Control	5%	0/10	0.0	0.0	0	0

Conclusions:

There were no responders (score > 1) in a group of 20 guinea pigs previously exposed to 5% [redacted] when challenged with 5% [redacted]. There were no responders in the control group.

redacted section refers to 5% silk powder

(2a)

Study No. [redacted]

Incidence	Number of Animals 24 hr Test/Control	48 hr Test/Control
0	20/10	20/10
±	0/0	0/0
1	0/0	0/0
2	0/0	0/0
3	0/0	0/0

Study No. [redacted] (3)

GLP REPORT REQUIREMENTS

GLP Requirement	Page
Name and Address of Test Facility	2
Date Study Initiated	2
Date Study Completed	2
Objective and Procedures	8
Statistical Methods Used	NA
Test and Control Article Identification	9
Stability of Test and Control Articles	19
Description of Test System	9
Species, Strain, Substrain, Source of Supply, Age, Sex, Body Weight Range, Number of Animals Used, and Procedure Used for Identification	
Dosage Information	10
Description of Circumstances Which May Have Affected the Data	2,5
Name of the Study Director	1
Names of Other Scientists, Professionals, and Personnel Involved in this Study	1
Description of Operations Performed on the Data	14
A Summary and Analysis of the Data	2
A Statement of Conclusions Drawn from the Analysis	2
Signed and Dated Reports of Individual Scientists or Other Professionals Involved in the Study	1
Locations Where All Specimens, Raw Data, and Final Report are to be Stored	4
Quality Assurance Statement (signed)	4

NA = Not Applicable

QUALITY ASSURANCE STATEMENT

Study No. [redacted] Test Substance [redacted]

Type of Study Guinea Pig Sensitization Screen (No. C4A)

Listed below are dates that this study was inspected by the Quality Assurance Unit and the dates findings were reported to the Study Director and to Management.

Phase Inspected	Dates of Inspection
Data Audit	3/19/85
Report Review	4/08/85

Dates Findings Reported to Study Director	Dates Findings Reported to Management
4/02/85	4/10/85
4/10/85	4/10/85

Location of Raw Data Storage Statement

The original copy of the final report and all raw data will be on file at the testing facility.

Anita M Bosau Date 4/10/85

Anita M. Bosau
Director
Quality Assurance Unit

PRIMARY IRRITATION OBSERVATIONS—SCREEN

Study No. [redacted] Test Article [redacted]

Solvent Distilled water

	Animal No. G- - 85	Sex	Exposure Level 10% w/v 24 hr	Exposure Level 10% w/v 48 hr	Exposure Level 5% w/v 24 hr	Exposure Level 5% w/v 48 hr	Exposure Level 2.5% w/v 24 hr	Exposure Level 2.5% w/v 48 hr
1	G-0080-85	M	0	0	0	0	0	0
2	G-0081-85	M	0	0	0	0	0	0
3	G-3126-85	F	0	0	0	0	0	0
4	G-3127-85	F	0	0	0	0	0	0
5								
6								

Test Article Level:	10%	5%	2.5%
Incidence:	0/4	0/4	0/4
Severity:			
Sum (24 hr/48 hr)	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
Avg (24 hr/48 hr)	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0

NOTE: These animals were originally patched using Hill Top Chamber® patches. When error was caught, animals were immediately re-patched with Parke-Davis Readi-bandage.

OBSERVATIONS--TEST

Study No. [redacted] Test Article [redacted] silk powder Phase Challenge

Induction at 1/31/85, 2/06/85, 2/15/85 - 5% w/v Solvent Distilled water

Challenge at 2/27/85 - 5% w/v Solvent Distilled water

Animals were clipped as per Protocol. (Date) 2/26/85

	Animal No. G- -85	Sex	Exposure Level 5% w/v 24 hr	48 hr
1	G-001-85	M	0	0
2	G-012-85	M	0	0
3	G-023-85	M	0	0
4	G-024-85	M	0	0
5	G-079-85	M	0	0
6	G-155-85	M	0	0
7	G-156-85	M	0	0
8	G-157-85	M	0	0
9	G-158-85	M	0	0
10	G-159-85	M	0	0
11	G-3010-85	F	0	0
12	G-3011-85	F	0	0
13	G-3012-85	F	0	0
14	G-3013-85	F	0	0
15	G-3024-85	F	0	0
16	G-3025-85	F	0	0
17	G-3080-85	F	0	0
18	G-3095-85	F	0	0
19	G-3140-85	F	0	0
20	G-3155-85	F	0	0

Test Article Level: 5%

Incidence: 0/20

Severity:
Sum 24 hr/48 hr 0.0/ 0.0
Avg 24 hr/48 hr 0.0/ 0.0

OBSERVATIONS—CONTROL

Study No. [redacted] Test Article [redacted] silk powder Phase Challenge

Challenge at 2/27/85 - 5% w/v Solvent Distilled water

Animals were clipped as per Protocol. (Date) 2/26/85

	Animal No. G- -85	Sex	Exposure Level 5% w/v 24 hr	48 hr
1	G-294-85	M	0	0
2	G-295-85	M	0	0
3	G-296-85	M	0	0
4	G-297-85	M	0	0
5	G-298-85	M	0	0
6	G-3550-85	F	0	0
7	G-3551-85	F	0	0
8	G-3552-85	F	0	0
9	G-3553-85	F	0	0
10	G-3554-85	F	0	0

Test Article Level: 5%

Incidence: 0/10

Severity:
Sum 24 hr/48 hr 0.0/ 0.0
Avg 24 hr/48 hr 0.0/ 0.0

[redacted]

(8)

PROTOCOL NO. CBA

Guinea Pig Sensitization Screen

JAN 18 1985

Issue Date: June 16, 1983
Supersedes Issue Dated: May 20, 1983

~~Dr. Hugh GA Bruce: Therapy Final Disposition Hirsch~~ not required Hirsch

[redacted]

Sponsor: [redacted]

Testing Facility:
(To be filled in by Operations Section)

Springborn Institute for Bioresearch, Inc.
Spencerville, OH 45887

Study # [redacted]
(To be filled in by Testing Facility)

Purpose:

To determine if a test substance, under the conditions of this protocol, causes an increased reaction in guinea pigs at challenge when compared to appropriate controls.

Justification for Selection of Test System:

The guinea pig is the classical animal for determining delayed contact hypersensitization.

Route of Administration of Test Substance and Reason for Choice:

Closed patch on clipped area of intact skin of the restrained guinea pig. Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Diet and/or Water Analyses Required:

None (no known contaminants expected which would interfere with this study)

Records to be Maintained:

All records that would be required to reconstruct the study and demonstrate adherence to protocol.

[redacted]

PROTOCOL NO. C4A (Cont'd)

Guinea Pig Sensitization Screen

Issue Date: June 16, 1983

[redacted]

Description		Expiration Date
Color	Physical Form	
White	Powder	12/1/85

Storage Conditions: (Check one)

[✓] Room temperature [ ] Refrigerator [ ] Freezer
[ ] Other

Hazards: (Check one)

[✓] None known. Take ordinary precautions in handling.
[ ] As follows:

Animals: Use Hartley outbred guinea pigs of a size sufficient to easily fit in the restrainers while the experiment is in progress. Use a minimum of 20 test animals, 10 control animals and sufficient numbers of animals for primary irritation for each test substance. Whenever possible, use equal numbers of males and females.

Animal Care: Follow the approved Standard Operating Procedures of the Test Facility. (Acclimation period must be a minimum of four (4) days.)

Environmental Conditions: Follow the approved Standard Operating Procedures of the Test Facility.

Animal Identification: Mark each cage and restrainer or restraining rubber dental dam with an identifying number. Careful attention must be given to see that the proper animal goes into the proper restrainer during each treatment and back to its original cage after treatment.

[redacted]

PROTOCOL NO. C4A (Cont'd)

Guinea Pig Sensitization Screen

00015

Issue Date: June 16, 1983

Special Instructions:

[ ] None
[✓] As follows:

Conduct the primary irritation screen and contact the toxicologist for dose to be used in the study.
[redacted] is insoluble. In order to prepare the dosing solutions, weigh the appropriate amt. of material and add distilled water & stir vigorously. [redacted] is a fine suspension. Sample the dosing solution while the material is still on the stirrer

Dose Preparation and Test Concentrations:

[ ] As indicated
[✓] All solutions should be freshly prepared.

1. Induction [ ] As indicated below

Test Substance(s)	Concentration(s)	Vehicle(s)

[✓] To be determined by Primary Irritation Screen

2. Primary Irritation (designate number of guinea pigs 4 per group)

Test Substance(s)	Concentration(s)	Vehicle(s)
[redacted]	10%, 5%, 2.5% w/v suspension in distilled water	Suspension in distilled water

3. Challenge [ ] As follows:

[✓] To be determined by the [redacted] Toxicologist after Primary Irritation Screen (p. 5 and 6)
[ ] Highest non-irritating concentration

PROTOCOL NO. C4A (Cont'd)

Guinea Pig Sensitization Screen

U9617

Issue Date: June 16, 1983

Dose Preparation (Cont'd):

Note

A concentration analysis of the test substance - vehicle mixture(s) will [ ]; will not [✓] be required.

If a concentration analysis is required:

Prepare a sufficient quantity of the test substance - vehicle mixture(s) so that a portion can be returned to the Sponsor's Divisional Toxicologist. Store solution/mixture at [ ] room temperature; [ ] refrigerator; [ ] freezer; [ ] other _____

Shipping Instructions

Send approximately _____ml. Send [ ] frozen; [ ] under ambient conditions; [ ] other_____

Dose Level:

The dose level chosen for the induction of sensitization may be greater than the anticipated level of human exposure (if too irritating, this dose level may be decreased during the induction phase). If necessary, determine the dose level for elicitation of responses at primary challenge by pre-screening various doses on naive animals as described under "Primary Irritation" on page 5.

Procedure:

Use 30 test animals and 10 control animals in each study unless otherwise specified. Provide about equal numbers of males and females in both test and control groups.

Testing conditions will vary from study to study. Therefore, it should be clearly indicated in the final report which test sites were used for induction, primary challenge, and rechallenge. Use the appended Format for Sensitization Studies (Appendix A) for this purpose (e.g. if the test animals were exposed to two (2) substances at primary challenge and three (3) substances at rechallenge, the reference figure would be #8). When the animals are challenged with several substances at one time, alternate the test substances on the patch sites to prevent bias due to site-to-site variation. Expose control animals for primary challenge and rechallenge at the sites corresponding to those of the test animals.

[redacted]

PROTOCOL NO. C4A (Cont'd)

Guinea Pig Sensitization Screen

Issue Date: June 16, 1983 [illegible]19

Special Instructions:

Procedure for Primary Sensitization:

Induction of Sensitization - Clip the left shoulder of each animal with a small animal clipper the day before exposure. The area shaved should be approximately one-fourth of the animal's back and side. Apply closed patches to the animals in the test group(s) in the following manner:

[ ] Apply 0.3 ml of a test substance or freshly prepared solution in a 25 mm Hill Top Chamber.

[✓] Apply 0.4 ml of a test substance or freshly prepared solution on a Parke-Davis Readi-Bandage.

Put the animal in the restrainer and apply the appliance to the clipped surface as quickly as possible after the substance has been applied. Occlude the appliance with a rubber dental dam pulled taut and fastened to the bottom of the restrainer with binder clips or holders.

Adjust the restrainer to minimize movement of the animal during the exposure period. Both edges of the dental dam should be under the front and back adjustable braces of the restrainer. About six (6) hours later, remove the dental dam and chamber, take the animal from the restrainer, and place it in its cage. Remove extremely viscous substances by a gentle rinse with warm water (35-42°C) or other appropriate solvent as specified under special instructions before returning the animals to their cages. Repeat the procedure at the same site once a week for the next two (2) weeks for a total of three 6-hour exposures (the interval between induction exposures may vary from 5 to 9 days). After the last induction exposure, leave the animals untreated for approximately two (2) weeks (12-16 days) before primary challenge:

Primary Irritation #1 - If requested (see page 3), determine the primary irritancy of the substance in question: Treat the naive animals by the above-described patching technique to the designated concentrations of the test substance in the prescribed vehicle. Clearly indicate in the final report which test sites were used. Use the appended Format for Primary Irritation Studies (Appendix B) for this purpose. In this portion of the

[redacted]

PROTOCOL NO. CHA (Cont'd)

Guinea Pig Sensitization Screen

00021

Issue Date: June 16, 1983

Procedure for Primary Sensitization (Cont'd):

Primary Irritation #1 (Cont'd)

study, clip the entire back and both sides of the animal the day before application and then expose each animal for one 6-hour period to the various concentrations of the substance. Alternate the different concentrations of the test substance on the various test sites among the animals in order to minimize the site-to-site variation in responsiveness. Grade the responses at 22-26 hours and at 46-50 hours according to the procedure described below for primary challenge.

The concentration of test substance to be used for challenge will be selected by verbal communication with the Divisional Toxicologist.

Primary Irritation #2 - An additional screen may be required after reporting the results of the #1 screen to the Divisional Toxicologist by telephone. Levels will be determined by the Divisional Toxicologist.

[ ] Special Instructions:

Primary Challenge - Challenge the animals previously exposed during the induction period as well as the previously untreated control animals approximately two (2) weeks after the last induction exposure (the time between the last induction exposure and the primary challenge may vary from 12 to 16 days) using the dose as prescribed by the Divisional Toxicologist. Use the same patching procedure as for the induction, but apply the patches to a naive skin site. The left posterior quadrant of the side and back of the animal should be clipped for single or double challenge techniques; the entire right side should be clipped for a triple challenge. The site for primary challenge may be varied, if necessary, to achieve the objectives of the experiments (e.g., using multiple samples at primary challenge will require using several sites).

Note any gross skin reactions during induction phase. Notify the Divisional Toxicologist of any unusual or severe inflammatory skin reactions.

[redacted]

PROTOCOL NO. C4A (Cont'd)

Guinea Pig Sensitization Screen

Issue Date: June 16, 1983

Observations: Approximately twenty-four (24) hours after primary challenge patches have been removed, depilate all animals with Neet Cream or Lotion Hair Remover (Whitehall Laboratories, Inc., New York). Place the depilatory on the test sites and surrounding areas, and leave it on for no more than thirty (30) minutes. Thoroughly wash off the depilatory with a stream of warm, running water, dry the animals with a towel, and return them to their cages.

A minimum of two (2) hours after depilation, grade the test sites on a scale of 0 to 3 (0 = no reaction, ± = slightly patchy erythema, 1 = slight, but confluent or moderate, patchy erythema, 2 = moderate erythema, 3 = severe erythema with or without edema). Repeat the grading 24 hours later (48-hour grades).

Rechallenge: The Sponsor will be notified of primary challenge results. Verbal instructions for rechallenge will be given by the Divisional Toxicologist followed by written confirmation from the Study Director. At study termination, sacrifice all surviving animals following agreement with the Sponsor's Divisional Toxicologist.

Protocol Changes: If it becomes necessary to change the approved protocol, verbal agreement to make this change should be made between the Study Director and the Divisional Toxicologist. As soon as practical, this change and the reasons for it should be put in writing and signed by both the Study Director and the Sponsor's Divisional Toxicologist. This document is then attached to the protocol as an addendum.

Report: Report should include how the study was conducted, the dates the study was initiated and terminated, and the results of both the primary challenge and the rechallenge in terms of the severity of responses of individual animals. This report shall conform to all requirements outlined in Section 58.185, Subpart J, Good Laboratory Practices Regulations.

[redacted]

PROTOCOL NO. CAR (Cont'd)

Guinea Pig Sensitization Screen

Issue Date: June 15, 1983

Sponsor: [redacted] Telephone No. [redacted]

Date Approved by Sponsor's [redacted] 1/16/85

Proposed Starting Date: 1-18-85

Defined as PROTOCOL SIGNED

Proposed Completion Date: 4-12-85

Defined as FINAL REPORT ISSUED

To be completed by the Test Facility

Study Director: James F. Zeis for RAH

Date: 1/18/85

1/22/85

Study Cost:

[redacted]

Issue Date: 6/16/83

Appendix A

Format for Sensitization Studies

1

Induction
Primary Challenge

2

1: Induction
2: Primary Challenge
3: Rechallenge

3

1: Induction
2: Primary Challenge
3,4: Rechallenge

4

1: Induction
2: Primary Challenge
3-5: Rechallenge

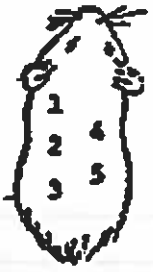
5

Induction
Primary Challenge

6

1: Induction
2,3: Primary Challenge
4: Rechallenge

7



1: Induction
2,3: Primary Challenge
4,5: Rechallenge

8

1: Induction
2,3: Primary Challeng
4-6: Rechallenge

9

Induction
Primary Challenge

10

1: Induction
2-4: Primary Challenge

11

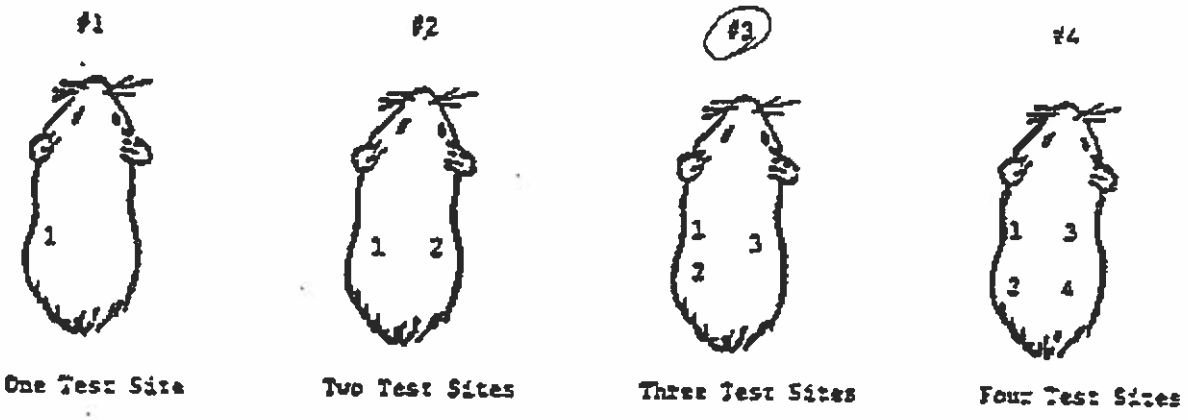
1: Induction
2-4: Primary Challenge

[redacted]

Issue Date: 6/16/83

Protocol - Appendix B

Format for Primary Irritation Studies



[redacted]

Appendix C
Special Instructions

1) [redacted]

2) Page 2 of 10 under Animals; starting body weight should be 300-350 grams.

3) Page 3 of 10 under Challenge; highest non-irritating concentration, i.e. a concentration which gives no scores of "1" or greater, and which results in <50% "±" reactions in the primary irritation screen (p. 5 and 6).

4) Clipping the fur from the animals for induction (p. 5), primary irritation screen (p. 6), primary challenge (p. 6) and rechallenge (p. 7) is to be done with a fine clipper blade #80, size 40 Oster or equivalent.

5) Page 5 of 10 under Procedure for Primary Sensitization; the volume of test substance is to be delivered to the designated appliance with a push button adjustable pipet. The pipet should be calibrated every three months (90 days) to assure accurate delivery (±1%) of 0.3 and 0.4 ml volumes.

6) Page 5 of 10 under Procedure for Primary Sensitization; appliance designated 20x20 mm Webril pad affixed to adhesive tape; appliance should be purchased from Professional Medical Product, Inc., Greenwood, South Carolina, product #33-4022-1.

7) Page 5 of 10 under Procedure for Primary Sensitization; the rubber dam used for occlusion of the patches should be of medium gauge 5 or 6 inches wide depending on the animal size and the number of patches to be covered. The rubber dam must be pulled snug on each side of the animal and secured with at least one clip on each side of the restrainer. The rubber dam must be placed under the front and back metal restraining bands, and should make snug contact with the animal over the entire dorsal surface.

8) Page 7 of 10 under Observations; Neet cream or lotion unscented is to be used for hair removal.

9) Page 7 of 10 under Observations; a four tube fluorescent type light of 160 watts, or equivalent, suspended 3 ft above a flat black background is to be used for scoring of all animals.

10) Page 8 of 10 under Report; in addition to severity, the incidence should be reported.

Incidence is defined as the number of animals in each group showing each of the five (5) grades (i.e., the number of animals showing the grade "0", the number showing the grade "±", the number showing the grade "1", etc.).

st/AMEND

RESEARCH TOXICOLOGY CENTRE S.p.A.

SILK POWDER

DELAYED DERMAL SENSITISATION STUDY IN THE GUINEA PIG

FINAL REPORT

RTC Study Number: [redacted]
RTC Report Number: [redacted]

Seen and approved by:

A. Nunziata
Responsible for Toxicological Experimentation as Authorized by the Italian Ministry of Health

A. Marzoli
President

Sede: Via Tito Speri, 12 - 00040 POMEZIA (Roma) - ITALIA Telefono: + 39.6.910951 - Fax. 06/9105737 - C.P. 15301 - 00143 Roma Eur Laurentino
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Capitale Sociale: 10.000.000.000 - C.C.I.A.A. n. 375376 - Reg. Soc. Trib. di Roma n. 2828/72 - Cod. Fisc.: 00653120584 - Partita IVA: 00920611001
Autoriz. G.L.P.: DL 120 del 27/1/92 - D.M. ex Art. 6 del D.P.R. nº 927/81 del 20/5/89

RTC Report Number: [redacted]

COMPLIANCE STATEMENT

We, the undersigned, hereby declare that this report is a true and faithful account of the procedures adopted and the results obtained in the performance of this study. The aspects of this study conducted by Research Toxicology Centre S.p.A. were performed in accordance with:

A. "Good Laboratory Practice Standards" of the U.S. Environmental Protection Agency, Code of Federal Regulations, 40, Part 792, U.S. Federal Register, Vol. 54, No. 158, 17th August 1989.

B. Decreto Legislativo 27 gennaio 1992 n.120 published in the Gazzetta Ufficiale della Repubblica Italiana 18 febbraio 1992.

(Adoption of the Commission Directive of 18th December 1989 adapting to technical progress the Annex to Council Directive 88/320/EEC on the inspection and verification of Good Laboratory Practice (90/18/EEC)).

(G. Haynes, B.Sc., C.Biol., M.I.Biol.)
Study Director:

Date: 07 JUNE 1995

(J. Brightwell, Ph.D.)
Scientific Director:

Date: 7.06.95

RTC Report Number: [redacted]

QUALITY ASSURANCE STATEMENT

Study phases monitored by RTC's QAU according to current relevant Standard Operating Procedures	Quality Assurance Inspections (Day Month Year)		
	Inspection	Report to Study Director	Report to Company Management
STUDY PROTOCOL	14-02-95	14-02-95	14-02-95
PROCEDURES, DATA AND FACILITIES RELEVANT TO THIS TYPE OF STUDY	16-01-95 30-01-95 24-02-95 02-03-95 14-03-95 21-03-95 23-03-95 23-03-95 12-04-95 20-04-95 26-04-95 27-04-95		13-02-95 01-03-95 01-03-95 15-03-95 03-05-95 24-03-95 28-03-95 31-03-95 05-05-95 26-04-95 05-05-95 09-05-95
FINAL REPORT Review of this report by RTC's QAU found the reported methods and procedures to describe those used and the results to constitute an accurate representation of the recorded raw data.		Review completed 7 June, 1995	

V. Sforza, B.S.
(Head of Quality Assurance)

07/06/95
Date

RTC Report Number: [redacted]

CONTENTS

TABLES

1. SUMMARY

The potential of the test substance, Silk powder, to induce and elicit delayed dermal sensitisation was assessed by a guinea pig model using the methods of Buehler.

The concentrations of the test substance used in the main study were determined by the results of a preliminary screening test.

The main sensitisation test was undertaken using a test group of twenty animals and a control group of ten animals. In an attempt to induce sensitisation test animals were treated by topical application of the test substance at 50% concentration. This was repeated at weekly intervals for a total of three weeks. Control group animals were treated in the same manner but the selected vehicle (sterile water) was used in place of the test substance. Two weeks after the third and final induction exposure, all animals were challenged by topical application of both the vehicle and the test substance at 50% concentration.

One animal from the control group and one from the test group died during the study.

At challenge, no response to the test substance was observed in any of the nineteen surviving animals in the test group or any of the nine surviving animals of the control group. No response to the vehicle was observed in any animal of test or control groups.

Changes in body weight during the period of the study were generally similar in animals from both test and control groups.

These results indicate that the test substance does not elicit a sensitisation response in the guinea pig. Classification based on these results would indicate the following:-

Classification: Not required
Symbol: None indicated
R phrase: None indicated

2. INTRODUCTION

The purpose of the study was to assess the ability of the test substance to induce and elicit delayed dermal hypersensitivity (skin sensitisation) by use of a guinea pig model. This allowed an indication of hazard classification required by EC Directives on the Classification, Packaging and Labelling of Dangerous Substances.

The procedures were designed to meet the requirements of the Buehler test for skin sensitisation described in Commission Directive 92/69/EEC of 31st July 1992. (Adapting to technical progress for the seventeenth time, Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances).

The species and route of administration were those stated in the regulations, giving a valid model for the assessment of sensitisation.

The test was designed to assess the potential for delayed dermal sensitisation to occur on exposure to the test substance during manufacturing, handling or use.

The study was carried out at: Research Toxicology Centre S.p.A.
Via Tito Speri, 12
00040 Pomezia (Roma)
Italy

On behalf of: [redacted]

The study started on 9th February 1995 with Study Director signature of the protocol. The in-life phase of the study commenced on 22nd February 1995 with allocation to treatment of animals for the preliminary dose-ranging screen and was completed on 6th April 1995. The study was completed on the date shown against the Study Director signature at the front of this report.

3. TEST SUBSTANCE

Details of the test substance received at the testing facility were as follows:

Name	: SILK POWDER
Alternative names	:
Raw Material Number	: [redacted]
Lot or Batch Number	:
Expiry date	:
Received from	:
Date received	: 8th September 1994
Amount shipped	: 52.73 grams gross
Description	: White powder
Container	: Transparent plastic container
Storage at RTC	: Ambient conditions, in a dessicator
RTC reference number	: [redacted]

Detailed characterisation of the test substance was not undertaken at the testing facility. The determination of the identity, strength, purity, composition, stability and method of derivation and/or synthesis of the test substance was the responsibility of the Sponsor. An aliquot of the test substance was taken and will be retained within the RTC archives for a minimum period of five years.

The test substance was prepared for dosing by mixing with sterile water. A range of concentrations was selected for the preliminary tolerance phase of the study and the results of this indicated that the test substance at 50% concentration would be suitable for use in the the induction procedure, being tolerated by the test system. The test substance at 50% concentration was used at challenge, being a concentration judged to be non-irritant.

4. METHODS

Any deviations from the study protocol are detailed within the text of the report. No deviations occurred that were considered to have compromised the purpose or conduct of the study.

Dated and signed records of all activities relating to the day by day conduct and maintenance of the study were made.

4.1 Animal management

4.1.1 Animal supply

Young adult female guinea pigs of the Dunkin-Hartley strain were ordered from Charles River Italia S.p.A., Calco (Como), Italy. Animals were ordered nulliparous and non-pregnant, within the weight range of 300 to 350 grams and 5 to 6 weeks of age.

The animals were delivered to the testing facility, in batches for the different phases of the study, on 26th January and 23rd February 1995. Animals appeared to be in an acceptable condition when they arrived and an acclimatisation period of at least five days was permitted before undertaking any dosing procedure. Animals were identified by temporary markings during the acclimatisation period.

4.1.2 Animal husbandry

Animals for the preliminary screen were housed, in groups of five animals, in stainless steel cages measuring 69 x 45 x 51 cm with a grid floor (Techniplast Gazzada S.a.r.l., Buguggiate, Varese, Italy). Animals for the main phase of the study were housed in similar cages measuring 63 x 48 x 41 cm. Cages were suspended over metal trays which held an absorbent material. This was inspected daily and changed as necessary. Throughout the study, each cage was identified by a label, colour-coded according to group, recording the study number, animal numbers and details of treatment.

Controls for the animal room were set to maintain temperature within the range of 17 to 23°C and relative humidity within the range of 30 to 70%. Actual conditions achieved were recorded daily.

The room was lit by fluorescent light to give an artificial cycle of twelve hours light/twelve hours dark.

4.1.3 Water and diet

Animals were offered drinking water supplied to each cage via a water bottle and a commercially available laboratory diet (Altromin MSK, A. Rieper S.p.A., Bolzano, Italy) ad libitum throughout the study.

There was no information to indicate that any component was present in either diet or drinking water at a level likely to interfere with the purpose or conduct of the study.

4.1.4 Allocation to groups

Animals were selected from available stock and randomly allocated to treatment groups prior to each phase of the study. Animals were then identified by tattoo in the ear with an individual number.

4.2 Experimental Procedure

The study was divided into two distinct phases. The first of these was a preliminary screen which was used to determine suitable test substance concentrations for use in the second phase. This second phase formed the main study, a determination of the sensitisation potential of the test substance.

4.2.1 Preliminary screen

Five animals were selected from those available and the flanks clipped free of hair. Each animal was dosed with two concentrations of the test substance, one on either flank. A gauze patch measuring at least 20 x 20 mm was soaked with 0.5 ml of the selected concentration of the test substance. This was then placed onto the selected treatment site. When both sites of the animal had been treated, they were secured in position by wrapping the trunk with a length of adhesive strapping.

All animals were treated in this manner such that a total of five concentrations (75%, 50%, 20%, 10% and 5% in sterile water) of the test substance were each dosed in duplicate. The adhesive strapping and patches were removed after six hours contact with the skin. The treated sites were washed with water at approximate body temperature to remove any remaining test substance.

Approximately twenty one hours after removing the patches, the treated sites were again clipped free of hair. Twenty four and forty eight hours after removal of the patches, the treated sites were examined for signs of reaction to treatment. Each site was assessed and scored on the following scale:-

Score	Reaction observed
0	No visible response (change)
1	Discrete or patchy erythema
2	Moderate and confluent erythema
3	Intense erythema and swelling

4.2.2 Main study - Induction

Animals were allocated to treatment to give a test group of twenty animals and a control group of ten animals.

On the day of dosing (Day 1) the hair was clipped from the left flank of each animal. Animals of the test group were treated with the test substance at 50% concentration. A gauze patch measuring 20 x 20 mm was covered with 0.5 ml of the test substance and placed onto the selected skin site. This was secured in position by encircling the trunk of the animal with a length of adhesive strapping.

All animals of the test group were treated with the test substance in this manner and animals of the control group were similarly treated with the vehicle alone (sterile water).

After an exposure period of six hours the dressings were removed. The treated sites were cleaned of remaining test substance by washing with warm water.

Approximately twenty four hours after removal of the patches, the treated sites were examined for signs of reaction to treatment. Each site was assessed and scored on the following scale:-

Score	Reaction observed
0	No visible response (change)
1	Discrete or patchy erythema
2	Moderate and confluent erythema
3	Intense erythema and swelling

These procedures were repeated at weekly intervals (Days 8 to 9 and 15 to 16 of the study).

4.2.3 Main study - Challenge

On Day 29, the hair was removed with electric clippers from both the anterior and posterior regions of the right flank of all animals of both test and control groups.

A 0.5 ml aliquot of the test substance at a concentration of 50% was spread evenly over an absorbent patch measuring approximately 20 x 20 mm. This was placed onto the skin of the posterior region of the prepared site on the right flank. A similar patch, this containing 0.5 ml of the vehicle alone (sterile water), was placed onto the anterior region of the prepared site. The patches were secured in position by encircling the trunk of the animal with a length of adhesive strapping.

All animals of both the test and control groups were treated with both the test substance and the vehicle in this manner.

After an exposure period of six hours the dressings were removed and the treated sites cleaned of remaining test substance or vehicle by washing with warm water.

Approximately twenty one hours after removing the patches, the treated sites were again clipped free of hair. Approximately three hours later (thirty hours after first dosing and twenty four hours after removal of the patches) reaction at the treated sites was assessed for any lesions and other toxic effects. If found, these were fully described. The degree of skin reaction was scored according to the following scheme:

Score	Reaction observed
0	No visible response (change)
1	Discrete or patchy erythema
2	Moderate and confluent erythema
3	Intense erythema and swelling

Skin reaction at the treated sites was again assessed approximately twenty four hours after the first examination (approximately forty eight hours after removal of the patches).

4.2.4 Body weight

Animals used in the main sensitisation assessment were weighed at the start of treatment and on termination of the study.

4.2.5 Termination and necropsy

All animals found dead during the study, or humanely killed, underwent a necropsy examination to establish, where possible, the cause of death or ill health.

Other animals were killed by carbon dioxide narcosis following the end of the experimental procedure but were not subjected to any necropsy procedure.

4.3 Classification

The results obtained on testing were used to classify the test substance according to the requirements of the classification, packaging and labelling of dangerous substances regulations (Commission Directive 83/467/EEC of 29th July 1983, updated by Commission Directive 91/325/EEC of 1st March 1991).

The test would be considered positive if 15% or more of animals in the test group exhibit erythema or dermal swelling following challenge with a non-irritant concentration of the test substance. The non-irritant nature of the test substance at the concentration used at challenge would be demonstrated by the lack of dermal responses in the control group.

Should the test have been considered positive, the test substance would require labelling with the risk phrase (R 43) "May cause sensitisation by skin contact" and symbol "Xi".

4.4 Archives

The raw data and documentation generated during the course of this study will be retained at the testing facility for five years after which the Sponsor will be contacted regarding despatch or disposal of the material.

5. RESULTS

5.1 Preliminary screen (Table 1)

The preliminary screen to establish a suitable concentration for use in the main sensitisation test indicated that the test substance at 75% concentration (the powdered substance mixed to a paste to bind the particles) was reasonably tolerated by the test system. A lower concentration of 50% was selected for use during the induction phase of the main study, there being a limited amount of the test substance available for use. The screen also indicated that the test substance at the same concentration should exhibit no irritant response and this was selected for use at challenge.

5.2 Induction (Table 2)

No response was observed at the treated skin site following six hours topical exposure to either the test substance (test group) or the vehicle (control group).

5.3 Challenge (Table 3)

No response to the test substance was observed in any of the nineteen surviving animals of the test group or any of the nine surviving animals of the control group after the end of the challenge dosing procedure.

No reaction to the vehicle was noted in any animal of either test or control group.

5.4 Body weight (Table 4)

Changes in body weight during the period of the study were generally similar in animals from both test and control groups.

5.5 Necropsy (Table 5)

Examination of the animals that died during the study revealed no abnormalities to indicate that death was caused as a result of administration of the test substance.

6. CONCLUSION

The results of this study indicate that the test substance, Silk powder, does not act as a sensitiser in the guinea pig, there being no response to challenge following an induction procedure with the substance.

Evaluation of sensitisation required by regulations on the classification, packaging and labelling of dangerous substances would indicate the following :-

Classification: Not required
Symbol: None indicated
R phrase: None indicated

TABLE 1 - PRELIMINARY SCREEN - INDIVIDUAL RESULTS

This table details the results of examination of treated sites following topical application of a range of concentrations of the test substance (Silk powder) in the selected vehicle (sterile water).

Animal number	Observation time	Test substance concentration 75%	50%	20%	10%	5%
203	24 hours	0	0			
	48 hours	0	0			
205	24 hours		0	0		
	48 hours		0	0		
207	24 hours			0	0	
	48 hours			0	0	
209	24 hours				0	0
	48 hours				0	0
211	24 hours	0				0
	48 hours	0				0

KEY

0 = No visible change
1 = Discrete or patchy erythema
2 = Moderate and confluent erythema
3 = Intense erythema and swelling

TABLE 2 - MAIN STUDY - INDUCTION - INDIVIDUAL RESULTS

This table details the findings at the treated sites on the left flank of each animal following topical application of the test substance (Silk powder) at 50% concentration or vehicle alone (sterile water) during the induction procedure.

Group function	Animal number	Dermal response 1st induction	2nd induction	3rd induction
	221	0	0	0
	223	0	0	0
C	225	0	0	0
O	227	0	0	0
N	229	0	0	0
T	231	0	0	0
R	233	0	0	0
O	235	0	0	0
L	237	0	0	0
	239	0	0	0
	241	0	0	0
	243	0	0	0
	245	0	0	0
	247	0	0	0
	249	0	0	0
	251	0	0	0
	253	0	0	0
	255	0	0	0
T	257	0	0	0
E	259	0	0	0
S	261	0	0	0
T	263	0	0	0
	265	0	0	0
	267	0	0	0
	269	0	0	0
	271	0	0	0
	273	0	-	-
	275	0	0	0
	277	0	0	0
	279	0	0	0

KEY: 0 = No visible change
1 = Discrete or patchy erythema
2 = Moderate and confluent erythema
3 = Intense erythema and swelling
- = Decedent

RTC Report Number: [redacted]

TABLE 3 - MAIN STUDY - CHALLENGE - INDIVIDUAL RESULTS

This table details the findings at the treated sites on the right flank of each animal following topical application of the test substance (Silk powder) at 50% concentration and vehicle alone (sterile water) during the challenge procedure.

Group function	Animal number	Dermal response			
		Vehicle		Test substance	
		24 hours	48 hours	24 hours	48 hours
	221	0	0	0	0
	223	-	-	-	-
C	225	0	0	0	0
O	227	0	0	0	0
N	229	0	0	0	0
T	231	0	0	0	0
R	233	0	0	0	0
O	235	0	0	0	0
L	237	0	0	0	0
	239	0	0	0	0
	241	0	0	0	0
	243	0	0	0	0
	245	0	0	0	0
	247	0	0	0	0
	249	0	0	0	0
	251	0	0	0	0
	253	0	0	0	0
	255	0	0	0	0
T	257	0	0	0	0
E	259	0	0	0	0
S	261	0	0	0	0
T	263	0	0	0	0
	265	0	0	0	0
	267	0	0	0	0
	269	0	0	0	0
	271	0	0	0	0
	273	-	-	-	-
	275	0	0	0	0
	277	0	0	0	0
	279	0	0	0	0

KEY: 0 = No visible change
1 = Discrete or patchy erythema
2 = Moderate and confluent erythema
3 = Intense erythema and swelling
- = Decedent

RTC Report Number: [redacted]

TABLE 4 - MAIN STUDY - BODY WEIGHTS - INDIVIDUAL VALUES

This table details the body weights of animals used in the study.

Group function	Animal number	Body weight (g) on Day:- 1	31	Change in body weight (g) Day 1 to Day 31
	221	449	601	152
	223	420	-	-
	225	466	700	234
C	227	434	674	240
O	229	502	760	258
N	231	410	594	184
T	233	461	673	212
R	235	401	634	233
O	237	449	646	197
L	239	413	651	238
	Mean	440.5	659.2	216.4
	S.Dev.	31.1	50.9	33.4
	241	458	654	196
	243	459	646	187
	245	497	707	210
	247	466	673	207
	249	453	667	214
	251	414	630	216
	253	428	629	201
	255	450	580	130
	257	418	588	170
T	259	427	623	196
E	261	393	526	133
S	263	402	576	174
T	265	403	571	168
	267	451	630	179
	269	409	650	241
	271	470	640	170
	273	413	-	-
	275	410	540	130
	277	394	581	187
	279	505	751	246
	Mean	436.0	624.3	187.1
	S.Dev.	33.3	56.0	33.2

KEY: - = Decedent

TABLE 5 - MAIN STUDY - NECROPSY - INDIVIDUAL FINDINGS

This table details the necropsy findings on examination of those animals that died during the study.

Group	Animal number	Tissue/ organ	Finding
Control	223	Head	Partially cannibalised around mouth.
		Hind feet	Partially cannibalised.
Test	273	Head	Left upper lip cannibalised.
		Left eye	Cannibalised.
		Ears	Both ears cannibalised.

RTC Report Number: [redacted]

TABLE 6 - RELIABILITY CHECK - SUMMARY

This table summarises the results obtained in the most recent reliability checks.

REFERENCE SUBSTANCE:	Mercaptobenzothiazole	
CONCENTRATION:	INDUCTION	- 25% in ethanol
	1ST CHALLENGE	- 25% in ethanol
	2ND CHALLENGE	- 50% in dimethylsulphoxide
CRITICAL DATES:	INDUCTION	- 6th March 1995 13th March 1995 20th March 1995
	1ST CHALLENGE	- 3rd April 1995
	2ND CHALLENGE	- 10th April 1995
RESULTS:	15% response in test group, 0% response in control group at first challenge. 75% response in test group, 30% response in control group at second challenge.	
INTERPRETATION:	Incidence at first challenge equivocal. Incidence at second challenge (accounting for irritation) acceptable Test system regarded as valid	

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: March 13, 2015

SUBJECT: Hydrolyzed Silk

Proalan s/a. 2014. Norsilk (INCI: Hydrolyzed Silk): Technical information.

Proalan s/a. 2014. Norsilk (INCI: Hydrolyzed Silk): Manufacturing flow diagram.

Proalan s/a. 2014. Norsilk (INCI: Hydrolyzed Silk): Material safety data sheet.

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PROALAN S/A

Pol. Ind. Congost - Camí de Can Ninou, 16 - Apartat 301 - 08403 Granollers (Barcelona)
Tels. 93 846 69 38 - 93 849 53 99 - Fax 93 849 10 16 - E-mail: general@proalan.es

2014

aminoácidos

NORSILK

INCI: Hydrolyzed silk

REACH No.: 01-2119779799-06-0003

Description

Norsilk is an amino peptide concentrate with a great richness of the two proteins (Sericin and Fibroin) that compose the natural silk. Its solubility is achieved by processing the silk thread that composes the cocoon protecting the chrysalis. The liquid silk preserves the properties and the natural organic form which makes Norsilk the most appropriate ingredient in the formulation of shampoos, masks, permanents and dyes.

Physico-chemical Characteristics

- Appearance..... Amber liquid
- Odour ..... Characteristic
- Density at room temp..... 1,05 – 1,11 g/ml
- pH in 10 % aqueous solution
  at room temperature ..... 4,0 – 4,5

Guaranteed Richness

- Proteic nitrogen ..... 2,7 – 3,4 %
- Natural liquid silk (Nitrogen x 6,25)..... 16,8 – 21,2 %
- Dry matter ..... 18,0 – 22,0 %

Other Characteristics

- Mineral salts..... Max. 1,8 %

Microbiological analysis

- Total aerobic germs..... < 300
- Pathogenic germs ..... Negative

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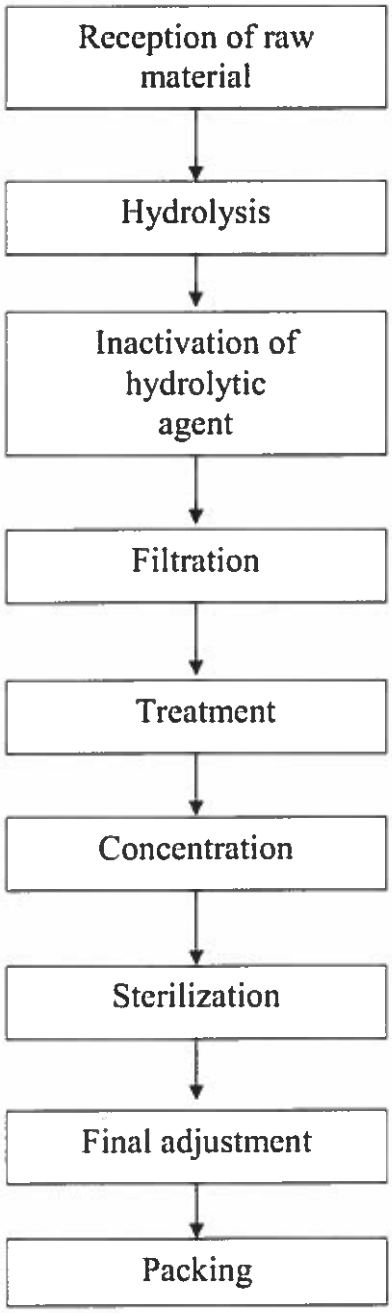
Flow diagram

Norsilk
INCI: Hydrolyzed Silk



Granollers, on 22.12.14

V. López
PROALAN

PROALAN s/A

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aminoácidos

MATERIAL SAFETY DATA SHEET

According to (UE) 453/2010 regulation.

Date of issue: 07/10/2013 *Edition: 2*
Date of review: 29/05/2014 *Review: 2*

Section 1.- Product and manufacturer identification

1.1 Product identification.

Trade Name	Norsilk
INCI Name	Hydrolyzed Silk
EINECS Number	309-203-1
CAS Number	100085-61-8
REACH Registry Number	01-2119779799-06-0003

1.2 Relevant identified uses of the substance or mixture and uses advised against.

Ingredient of cosmetic.

1.3 Identification of the supplier of the safety data sheet.

Company: Proalan, S.A.
Address: Pol. Ind. Congost, Camí de Can Ninou, 16.
08403 Granollers (Barcelona).
Phone No: 34 93 849 53 99.
E-mail: general@proalan.es.

1.4 Emergency phone number.

34 938495399 - Customer Service only. From 9 a.m. to 1 p.m.

Section 2.- Identification of hazards

Norsilk is an aqueous solution of silk protein hydrolyzate. Due to its biological origins, it does not present any hazard or risk, as it is neither toxic, nor irritant, nor explosive nor corrosive.

Section 3.- Composition/ Information on the composition

3.1 Components.

Name	Richness	EINECS No	CAS No	REACH No
Hydrolyzed silk	Approx. 19%	306-235-8	96690-41-4	05-2114578866-26-0000

3.2 Preservatives.

Name	Richness	EINECS No.	CAS No
Phenoxyethanol	0,4%	254-372-6	39236-46-9
Potassium sorbate	0,2%	246-376-1	24634-61-5

Section 4.- First Aid

4.1 First Aid description.

Contact with skin: Wash the affected area with water and soap, rinse thoroughly.

Contact with eyes: Rinse thoroughly with water.

Inhalation: Irrelevant.

Ingestion: Rinse the mouth. Consult a doctor if you feel unwell.

4.2 Most important symptoms and effects, both acute and delayed.

There is no knowledge of important symptoms and effects.

4.3 Indication of any immediate medical attention and special treatment needed

Not known.

Section 5.- Fire fighting measures

5.1 Appropriate fire extinguishing systems.
All conventional means of extinction are appropriate (water, carbon dioxide, etc.)

5.2 Special hazards.
Not known.

5.3 Specific fire extinguishing systems.
Not known.

Section 6.- Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures.
The usual precautions for the handling must be observed: gloves (CE 0072 Approval Regulation) and safety goggles (0196 CE Approval Regulation).

6.2 Environmental precautions.
Prevent releases from entering sewers, watercourses, trenches and pits.

6.3 Methods and material for containment and cleaning up.
Collect as much released product as possible. Use soil or earth to deprive the spread of the spill. Collect the maximum amount of the spilled product. Absorb residue with inert material (soil, sand).

6.4 References to other sections.
See Section 8 regarding the different personal protection systems and Section 13 regarding waste disposal.

Section 7.- Handling and storage

7.1 Precautions for safe handling.
Avoid contact with skin and eyes. Do not eat or drink during the handling of the product.

7.2 Conditions for safe storage and incompatibilities.
Store the product in their original package, perfectly sealed and avoid direct sun light and low temperature.

7.3 Specific end-uses.
See Section 1.2.

Section 8.- Exposure controls/personal protection

8.1 Control parameters.
See Section 2.

8.2 Exposure controls.
See Sections 6 and 7.

Section 9.- Physical and chemical properties

9.1 Information regarding physical and chemical properties.

Appearance:	Liquid.
Colour:	Yellowish.
Odour:	Characteristic.
Molecular weight:	Below 10.000 Dalton.
pH:	4,0 – 4,5.
Boiling point:	Not known.
Melting point:	Not applicable.
Flash point:	Non-flammable.
Flammability:	Non-flammable.
Explosive properties:	Non-explosive.
Auto-ignition Temperature:	Not applicable.
Decomposition temperature:	Information not available.
Lower explosion limit:	Not applicable.
Upper explosion limit:	Not applicable.
Oxidising properties:	Not applicable.
Relative density:	1,05 – 1,11 g/ml.
Steam pressure at 20ºC:	Information not available.
Vapour density:	Not applicable.
Partition coefficient n-octanol/water:	Information not available.
Viscosity:	Information not available.
Solubility in water:	Water-soluble.

9.2 Additional information.
Not required.

Section 10.- Stability and reactivity

10.1 Reactivity.
Unknown.

10.2 Chemical Stability.
Product is stable.

10.3 Possibility of hazardous reactions.
Unknown.

10.4 Conditions to Avoid.
See Section 7.2.

10.5 Incompatible Materials.
None known.

10.6 Hazardous decomposition products.
None under normal processing conditions.

Section 11.- Toxicological information

11.1 Information on toxicological effects.
See section 2.

Section 12.- Ecological information

12.1 Toxicity.
See Section 2.

12.2 Persistence and degradability.
There is no information available regarding the its persistence and degradability.

12.3 Potential for bioaccumulation.
This product is not bioaccumulative.

12.4 Mobility in soil.
Do not allow the product to enter the sewage system.

12.5 Results of PBT and vPvB assessment.
There is no information available regarding PBT and vPvB.

12.6 Other adverse effects.
Unknown.

Section 13.- Disposal

13.1 Waste treatment methods.
Do not pour the product to enter the sewage system. Waste must be disposed according to the law. Consult your local authorities.

Section 14.- Transport information

Product not subjected to the rules that govern the transportation of dangerous goods.

14.1 UN number.
Not applicable.

14.2 UN proper shipping name.
Not applicable.

14.3 Transportation hazard categories.
Not applicable.

14.4 Packing group.
Not applicable.

14.5 Environmental hazards.
Not applicable.

14.6 Special precautions for users.
Not applicable.

14.7 Transport in bulk according to Annex II of MARPOL73/78 and the IBC Code
Not applicable.

Section 15.- Regulatory Information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture.
Not known.

15.2 Chemical safety assessment.
Not known.

Section 16.- Other information

This information is intended to describe the product for purposes of health, safety and environmental requirements only. Therefore it should not be construed as its data sheet.
The data and recommendations described within this document are based on our current knowledge and experience.
It is the responsibility of the recipient of our products to ensure that any existing laws and legislation are observed.

Personal Care Products Council
Committed to Safety, Quality & Innovation

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: March 13, 2015

SUBJECT: Silk

A supplier reports the following information on their trade name material sold under the INCI name Silk.

The ingredient is prepared from natural silk by removing sericin. The purified, aqueous fibroin is dried and pulverized into a powder.

Appearance:	White to slightly gray powder
Particle size:	5-15 µm
Nitrogen:	13.0-20%
pH (10% aqueous slurry):	4.5-7.5
Heavy metals:	20 ppm max

A summary of safety data on this ingredient is attached.

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Applied biological Sciences Laboratory. 1980. Summary information Silk: Skin irritation, eye irritation, acute oral toxicity.

Primary Skin Irritation

Test Species (n):	Adult New Zealand albino rabbits (6)
Skin site preparation:	Clipped free of hair; two areas on the back; one area was abraded
Patches:	2.5 cm^2 gauze with plastic to retard evaporation; secured with adhesive tape
Test material:	0.5 g dissolved in solvent (not further identified)
Exposure period:	24 hours
Evaluation:	at removal of patch at 24 hours and at 72 hours
Results:	No reactions (no erythema, eschar or edema) were observed in any of the rabbits at 24 or 72 hours. Silk was classified as a non-irritant.

Modified Draize Eye Irritation Test

Test Species (n):	Adult Albino rabbits (9)
Dose:	0.1 g
Treatment:	Test material instilled into one eye of each rabbit; the other eye served as a control. The treated eye was immediately rinsed in 3 of the 9 rabbits.
Evaluation:	The eyes were examined a 24, 48 and 72 hours
Results:	No effects on the cornea or iris were observed in any of the rabbits at any time point. Conjunctival redness was observed in 5/6 rabbits with unrinsed eyes at 24 hours this effect was not consistently observed in any of the rabbits by 48 and 72 hours (one rabbit had a redness score of 1 at 48 hours; a different rabbit had a redness score of 1 at 72 hours; all other conjunctiva scores were 0 at 48 and 72 hours). No effects were noted in the 3 rabbits in which the eyes were treated immediately after treatment. Based on the results of the study, Silk was classified as non-irritant.

Acute Oral Toxicity

Test Species (n):	Sprague-Dawley rats (10 males)
Dose:	16 g/kg
Observation Period:	14 days after dosing
Results:	Directly after dosing the animals were slightly lethargic. There were no additional abnormal observations during the 14 day observation period. None of the rats died. The oral LD_{50} was greater than 16 g/kg and Silk was considered nontoxic.

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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: March 25, 2015

SUBJECT: Hydrolyzed Silk

The Active Concepts Hydrolyzed Silk ingredients (20621-AC Silk Hydrolysate and 20625 AC Silk Hydrolysate H) have molecular weights of about 2000-4000 Da. The ingredients are sold as aqueous solutions; 20621 AC Silk Hydrolysate contains 20-30% Hydrolyzed Silk and 20625 AC Silk Hydrolysate H contains 27-32% Hydrolyzed Silk. These ingredients were tested in the provided *in vitro* irritation tests without dilution.

Active Concepts. 2015. 20621-AC silk hydrolysate - manufacturing flow chart.

Active Concepts. 2009. AC silk hydrolysate irritation analysis.

Active Concepts. 2015. 20625-AC silk hydrolysate H - manufacturing flow chart.

Active Concepts. 2015. Dermal and ocular irritation tests (AC silk hydrolysate H).

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Active Concepts

20621-AC Silk Hydrolysate-Manufacturing Flow Chart

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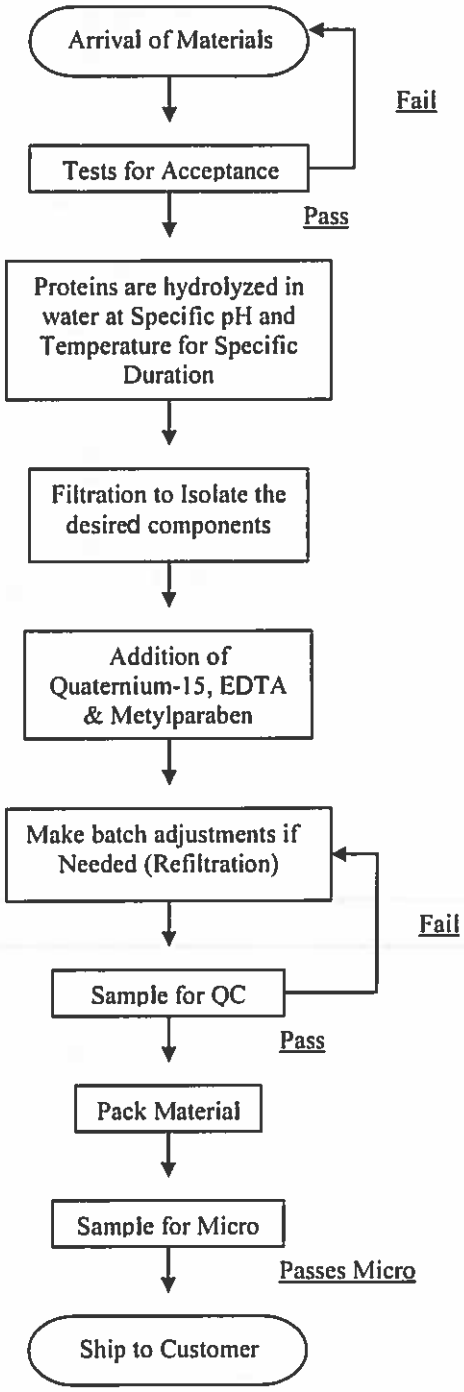

Version#2/03-20-15

Active Concepts

AC Silk Hydrolysate Irritation Analysis

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Abstract:

To confirm that **AC Silk Hydrolysate** is non-irritating, we used *in-vitro* dermal and ocular irritation assays.

The Irritection® assays purchased from InVitro International were used to determine the potential ocular and dermal irritancy of **AC Silk Hydrolysate**. The *in-vitro* system involves the use of a proprietary solution comprised of both proteins and macromolecules in a well that is covered by a membrane. Testing material is applied to the membrane and diffuses into the well. The proteins and macromolecules undergo conformational changes based on the irritancy of the diffused material, these changes are intended to mimic the biomolecular changes that occur when irritants are applied to both the eyes and skin. The conformational changes cause the solution to become turbid; there is a direct correlation between the irritancy level of the material and the solution's turbidity. Irritancy is quantitatively measured using a spectrophotometer.

Figure 1. The Ocular Irritection Model

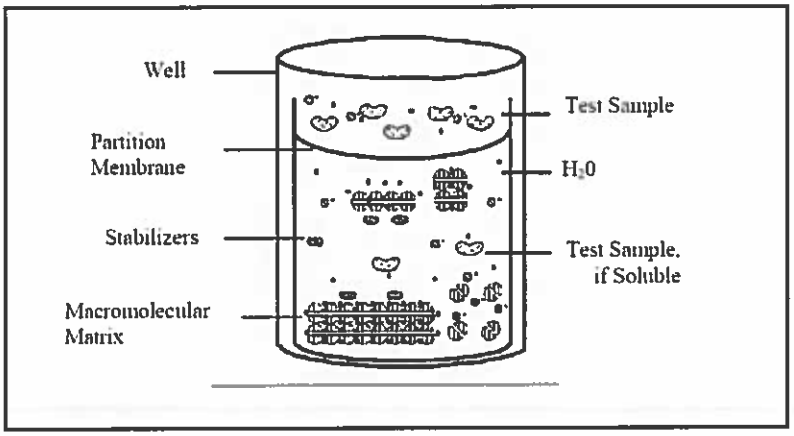
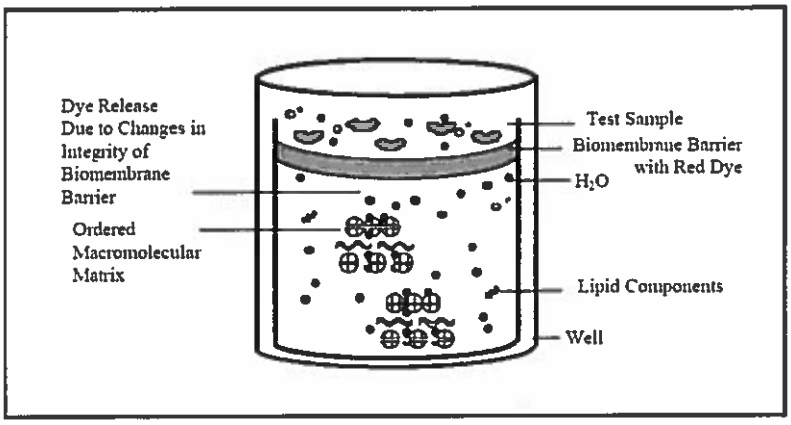


Figure 2. The Dermal Irritection Model



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Materials and Methods:

For the ocular and dermal Irritation assays, samples of **AC Silk Hydrolysate** were applied to Irritection® systems at concentrations of 25, 50, 75, 100 and 125 µl. The samples were left at room temperature for a period of 24 hours before they were analyzed with a spectrophotometer. The scales used to correlate the quantitative spectrophotometer value and potential Irritancy for both ocular and dermal analysis follows.

Table 1. Ocular Irritancy Scale

Ocular Irritection Score	Ocular Irritancy Classification
0.0 – 12.5	Minimal Irritant
12.6 – 30.0	Mild Irritant
30.1 – 51.0	Moderate Irritant
51.1 – 80.0	Severe Irritant

Table 2. Dermal Irritancy Scale

Dermal Irritection Score	Dermal Irritancy Classification
0.0 – 0.90	Non-Irritant
0.91 – 1.20	Mild Irritant
1.21 – 5.00	Irritant

Results:

Ocular Assay:

Lot #	Sample	Dose (µl)	Irritection Score	Ocular Assay Classification
SN060616-3	AC Silk Hydrolysate	25	2.5	Minimal Irritant
		50	2.7	Minimal Irritant
		75	2.9	Minimal Irritant
		100	3.1	Minimal Irritant
		125	3.5	Minimal Irritant

Dermal Assay:

Lot #	Sample	Dose (µl)	Irritection Score	Dermal Assay Classification
SN060616-3	AC Silk Hydrolysate	25	0.25	Non-Irritant
		50	0.31	Non-Irritant
		75	0.33	Non-Irritant
		100	0.38	Non-Irritant
		125	0.40	Non-Irritant

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Discussion:

Irritation is defined by the American Heritage Dictionary as a condition of inflammation, soreness or irritability of a bodily organ or part. Physical irritation is usually characterized with hyperpigmentation, dry flakey skin and watery eyes. Both the dermal and ocular assays reveal that **AC Silk Hydrolysate** is non-irritating and should not cause any of the aforementioned conditions. Although the Irritection® scores vary per dose, all the scores fall within the non-irritant range for the dermal assay, and the minimal irritant range for the ocular assay.

Active Concepts

20625-AC Silk Hydrolysate H-Manufacturing Flow Chart

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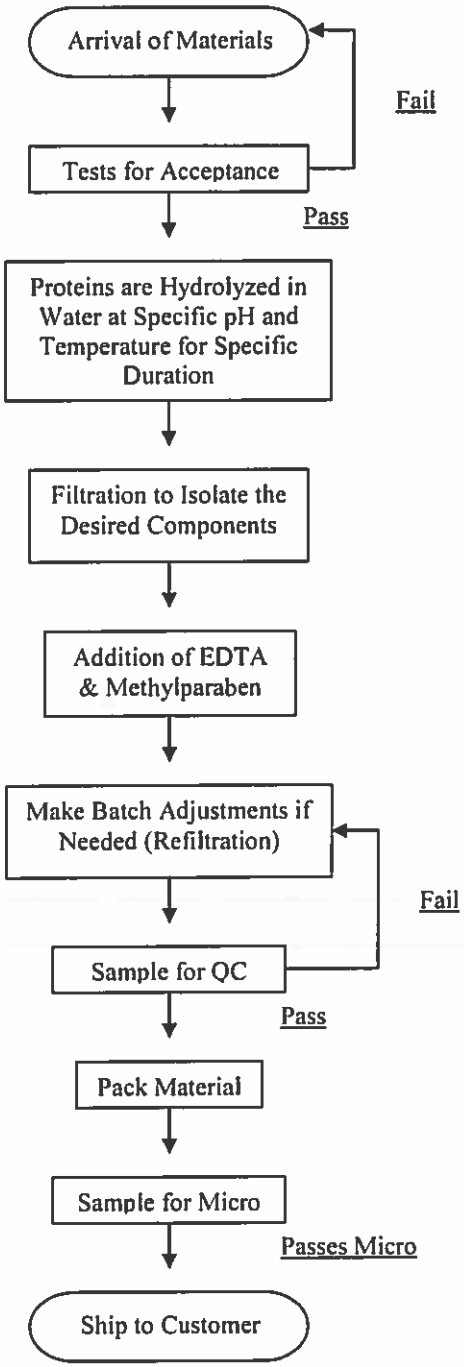

Version#1/03-20-15

Dermal and Ocular Irritation Tests

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Sample: AC Silk Hydrolysate H

Code: 20625

CAS #: 96690-41-4

Test Request Form/Submission #: 1165

Lot #: 16896P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
In Vitro EpiDerm™ Dermal Irritation Test (EPI-200-SIT)
EpiOcular™ Eye Irritation Test (OCL-200-EIT)

SUMMARY

In vitro dermal and ocular irritation studies were conducted to evaluate whether **AC Silk Hydrolysate H** would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays.

The product was tested according to the manufacture's protocol. The test article solution was found to be **non-irritating**. Reconstructed human epidermis and cornea epithelial model were incubated in growth media overnight to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substances were applied to the tissue inserts and incubated for 60 minutes for liquid and solid substances in the EpiDerm™ assay and 30 minutes for liquid substances and 90 minutes for solid substances in the EpiOcular™ assay at 37°C, 5% CO_2, and 95% relative humidity (RH). Tissue inserts were thoroughly washed and transferred to fresh plates with growth media. After post substance dosing incubation is complete, the cell viability test begins. Cell viability is measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The irritation potential of the test chemical is dictated by the reduction in tissue viability of exposed tissues compared to the negative control.

Under the conditions of this assay, the test article was considered to be **non-irritant**. The negative and positive controls performed as anticipated.

I. Introduction

A. Purpose

In vitro dermal and ocular irritation studies were conducted to evaluate whether a test article would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays. MatTek Corporation's reconstructed human epidermal and human ocular models are becoming a standard in determining the irritancy potential of test substances. They are able to discriminate between irritants and non-irritants. The EpiDerm™ assay has accuracy for the prediction of UN GHS R38 skin irritating and no-label (non-skin irritating) test substances. The EpiOcular™ assay can differentiate chemicals that have been classified as R36 or R41 from the EU classifications based on Dangerous Substances Directive (DSD) or between the UN GHS Cat 1 and Cat 2 classifications.

II. Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity
B. Equipment:	Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes
C. Media/Buffers:	DMEM based medium; DPBS; sterile deionized H_2O
D. Preparation:	Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
E. Tissue Culture Plates:	Falcon flat bottom 96-well, 24-well, 12-well, and 6-well tissue culture plates
F. Reagents:	MTT (1.0mg/mL); Extraction Solution (Isopropanol); SDS (5%); Methyl Acetate
G. Other:	Nylon Mesh Circles (EPI-MESH); Cotton tip swabs; 1mL tuberculin syringes; Ted Pella micro-spatula; 220mL specimen containers; sterile disposable pipette tips; Parafilm

III. Test Assay

A. Test System

The reconstructed human epidermal model, EpiDerm™, and cornea epithelial model, EpiOcular™, consist of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis and cornea epithelium. These models consist of organized basal, spinous, and granular layers, and the EpiDerm™ systems also contains a multilayer stratum corneum containing intercellular lamellar lipid layers that the EpiOcular™ system is lacking. Both the EpiDerm™ and EpiOcular™ tissues are cultured on specially prepared cell culture inserts.

B. Negative Control

Sterile DPBS and sterile deionized water are used as negative controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

C. Positive Control

Known dermal and eye irritants, 5% SDS solution and Methyl Acetate, were used as positive controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

D. Data Interpretation Procedure

a. EpiDerm™

An irritant is predicted if the mean relative tissue viability of the 3 tissues exposed to the test substance is reduced by 50% of the mean viability of the negative controls and a non-irritant's viability is > 50%.

b. EpiOcular™

An irritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance is reduced by 60% of the mean viability of the negative controls and a non-irritant's viability is > 40%.

IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Concepts, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for an additional 18 to 21 hours.

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B. Test Substance Exposure

a. EpiDerm™

30µL (liquid) or 25mg (solid) of the undiluted test substance is applied to 3 tissue inserts and allowed to incubate for 60 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

b. EpiOcular™

Each tissue is dosed with 20µL DPBS prior to test substance dosing. 50µL (liquid) or 50mg (solid) of the undiluted test substance is applied to 2 tissue inserts and allowed to incubate for 90 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

C. Tissue Washing and Post Incubation

a. EpiDerm™

All tissue inserts are washed with DPBS, dried with cotton tipped swab, and transferred to fresh media and culture plates. After 24 hours the inserts are again transferred into fresh media and culture plates for an additional 18 to 20 hours.

b. EpiOcular™

Tissue inserts are washed with DPBS and immediately transferred into 5mL of assay medium for 12 to 14 minutes. After this soak the inserts are transferred into fresh media and tissue culture plates for 120 minutes for liquid substances and 18 hours for solid substances.

D. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO_2, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD_{570}) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

B. Positive Control

a. EpiDerm™

The assay meets the acceptance criterion if the mean viability of positive control tissues expressed as a % of the negative control is ≤ 20%.

b. EpiOcular™

The assay meets the acceptance criterion if the mean viability of positive control tissues is < 60% of control viability.

C. Standard Deviation

Since each irritancy potential is predicted from the mean viability of 3 tissues for EpiDerm™ and 2 tissues for EpiOcular™, the variability of the replicates should be < 18% for EpiDerm™ and < 20% EpiOcular™.

VI. Results

A. Tissue Characteristics

The tissue inserts included in the MatTek EpiDerm™ and EpiOcular™ assay kits were in good condition, intact, and viable.

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B. Tissue Viability Assay

The results are summarized in Figure 1. In no case was the tissue viability ≤ 50% for EpiDerm™ or ≤ 60% for EpiOcular™ in the presence of the test substance. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited substantial loss of tissue viability and cell death.

C. Test Validity

The data obtained from this study met criteria for a valid assay.

VII. Conclusion

Under the conditions of this assay, the test article substance was considered to be **non-irritating**. The negative and positive controls performed as anticipated.

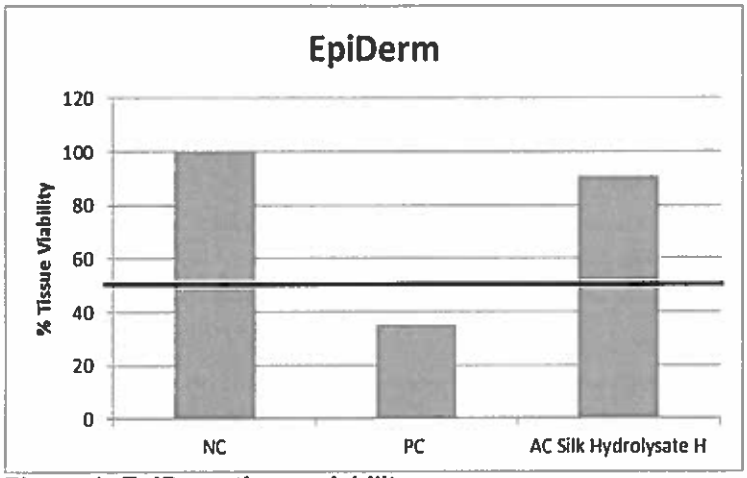

Figure 1: EpiDerm tissue viability

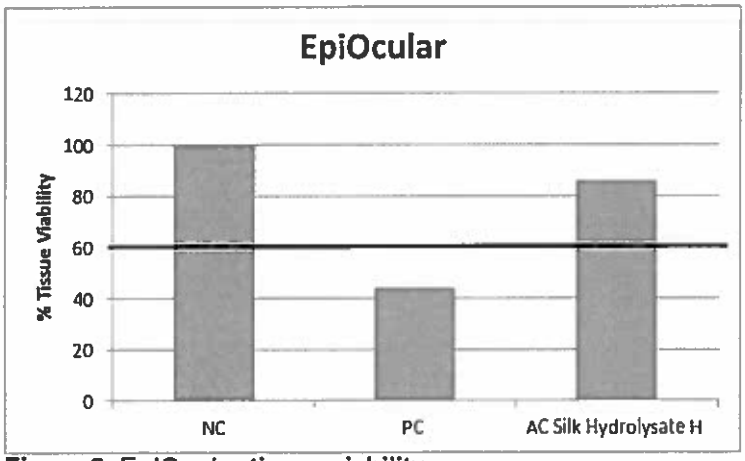

Figure 2: EpiOcular tissue viability

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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: April 3, 2015

SUBJECT: Hydrolyzed Silk

A supplier reports that their Hydrolyzed Silk is prepared by acid and enzyme hydrolysis until the molecular weight reaches the target range.

One Hydrolyzed Silk product (hydrolyzed silk protein-1) has a number average molecular weight of 300 and is sold as a 20% water solution. Heavy metals and arsenic are less than or equal to 4 ppm and 0.4 ppm, respectively.

The second Hydrolyzed Silk product (hydrolyzed silk protein-2) has a number average molecular weight of 650 and is sold as a 6.5% water solution. Heavy metals and arsenic are less than or equal to 10 ppm and 1 ppm, respectively.

Information Concerning Hydrolyzed Silk Protein-1
Anonymous. 2014. Size exclusion chromatogram of hydrolyzed silk protein-I

Ocular Irritation
Anonymous. 2003. Summary of HET-CAM Test on Hydrolyzed Silk protein-1.

Test System:	fertilized Leghorn hens' eggs incubated for 10 days
Test Concentration:	2% Hydrolyzed Silk Protein in demineralized water
Dosing Procedure:	0.3 ml of the sample was spread over the chorio-allantoic membrane using a 1 ml pipette. The membrane was rinsed with 5 ml demineralized water 20 seconds later.
Results:	The score was 0.3. The test material was classified as a non-irritant

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Anonymous. 2003. Summary of RBCA Test (tolerance on red blood cells) of Hydrolyzed Silk protein-1.

Test System:	sheep red blood cells in a phosphate buffer medium
Method:	adapted from INVITTOX protocol No. 37: the concentration of the test substance resulting in 50% lysis of the blood cells and the concentration denaturing oxyhemoglobin from lysed blood cells is determined
Results:	10% Hydrolyzed Silk (protein-1) caused neither hemolysis nor denaturation. Hydrolyzed Silk was classified as a non-irritant.

Dermal Sensitization

SafePharm Laboratories. 2003. Summary of local lymph node assay in the mouse: Hydrolyzed Silk protein-1.

Method:	OECD Guideline for the Testing of Chemicals No. 429 "Skin Sensitisation: Local Lymph Node Assay" (adopted 24 April 2002)
Test System:	CBA/Ca strain mice (groups of 4)
Protocol:	Three groups of mice were treated with 50 µl (25 µl per ear) of the undiluted test material (20% Hydrolyzed Silk Protein in water) and the test material as a solution in dimethyl formamide at concentrations of 25% and 50% v/v. A fourth group of four mice treated with 100% dimethyl formamide served as a control.
Results:	Stimulation Index (SI) values were 0.79, 0.66 and 0.73 at 25%, 50% and 100% of the test material, respectively. Hydrolyzed Silk protein-1 was considered to be a non-sensitizer under the conditions of the study.

Dermal Irritation - Human

Dermis Research Center Co., Ltd. 2003. Summary: Human patch test under occlusive patch for 48 hours: Hydrolyzed Silk protein-1.

The irritation potential of Hydrolyzed Silk protein-1 (20% Hydrolyzed Silk Protein in water) was investigated in a 48 h human patch test (occlusive) of 20 subjects (2 males and 18 females). The subjects were treated on the back using Finn chambers and scanpor tape. About 3 mg of the test sample was applied.

Thirty minutes after removal of the patch, 3 subjects had mild erythema reactions. Only one subject had mild irritation 24 hours after patch removal. The other subjects had no irritation after 30 minutes and 24 hours after patch removal.

Dermal Sensitization - Human

Aster. 2004. Summary of a human repeat insult patch test of Hydrolyzed Silk protein-1.

Objective:	To evaluate the irritation potential of a cosmetic ingredient (20% Hydrolyzed Silk Protein in water) after repeated applications during 3 weeks (induction phase) and to evaluate its sensitization potential after a single application performed 2-3 weeks after the induction phase.
Subjects:	52 subjects started the study; 4 subjects withdrew for reasons unrelated to the test product; 48 subjects completed the study
Induction Phase:	9 repeated topical applications (20% Hydrolyzed Silk Protein in water) were performed on the same skin site on the left part of the back. The test product remained in contact with the skin for 48 hours (72 hours if applied on Fridays). Scoring of the skin sites were completed 30 minutes after removal.
Rest Phase:	The induction phase was followed by a 2-3 week rest phase during which no application was completed
Challenge Phase:	The challenge phase (20% Hydrolyzed Silk Protein in water) was completed after the rest phase on the right side of the back under the same conditions as in the induction phase (48 hour contact; scored 30 minutes after removal and again 48 hours later).
Application:	Finn chamber (50 mm^2 surface area) - 20 µl of the test material was applied.
Results/Conclusion:	During the induction and challenge phases, no clinically significant objective reaction was observed in any subject. The product did not induce a sensitization reaction

Information Concerning Hydrolyzed Silk Protein-2

Anonymous. 2014. Size exclusion chromatogram of Hydrolyzed Silk protein-2.

Ocular Irritation

Hiroshima University. 1985. Summary primary eye irritation test: Hydrolyzed Silk protein-2.

A Draize eye irritation test was completed in 6 New Zealand white rabbits. The test substance (0.1 ml; 6.5% Hydrolyzed Silk Protein in water) was instilled into the conjunctival sac of one eye. The other eye served as the control. The eyes were examined 24, 48 and 72 hours after instillation.

The score of primary eye irritation was 0. The test sample was considered to be a non-irritant under the conditions of this study.

Hiroshima University. 1985. Summary of cumulative application (eye) test: Hydrolyzed Silk protein-2.

The test material (0.1 ml; 6.5% Hydrolyzed Silk in water) was instilled into the conjunctival sac of one eye of 6 male New Zealand white rabbits. The other eye served as the control. The rabbits were treated 3 times a day for 4 days.

Redness of the conjunctiva was observed in 5/6 rabbits at day 3 or 4 (average scores: day1: 0; day 2: 0; day 3: 0.7; day 4: 1.7). No reactions were seen in the cornea or iris. The test sample was considered practically non-irritating under the conditions of this study.

Skin Irritation

Hiroshima University. 1985. Summary of primary skin irritation test: Hydrolyzed Silk protein-2.

Test Animals:	6 New Zealand white rabbits
Method:	Clipped normal and abraded skin of the back (2.5 x 2.5 cm) treated with 0.5 ml of the test material (6.5% Hydrolyzed Silk Protein in water) and the test site was occluded for 24 hours.
Observation:	Macroscopic evaluation (Draize classification method) 24 and 72 hours after dosing
Results:	Very slight erythema was observed in 1/6 rabbits after 24 hours. The test sample was considered a non-irritant under the conditions of the study.

Osaka Institute of Public Health Science. 1984. Summary of human patch test: Hydrolyzed Silk protein-2.

Subjects:	24 volunteers (10 male; 14 female)
Treatment:	One 0.2 ml dose (6.5% Hydrolyzed Silk Protein in water) was placed on the upper dorsal area of the back under an occlusive patch for 24 hours
Observations:	After disappearance of transient erythema caused by removal of the patch
Results:	All of the observations were negative. Hydrolyzed Silk protein-2 was considered a non-irritant under the conditions of this study.

Dermal Sensitization

Hiroshima University. 1985. Summary of skin sensitization test of Hydrolyzed Silk protein-2.

Test animals:	6 Hartley guinea pigs/group
Test groups:	Test material group; positive control group (0.1% 2,4-dinitrochlorobenzene); negative control group
Method:	First induction: subcutaneous injection of FCA, the test material (0.05 ml) followed by the test material emulsified in FCA into clipped dorsal skin

Second induction: On day 6, the dorsal area was treated with petrolatum containing 10% sodium lauryl sulfate and on day 7 the same area was patched (24-hours occlusive) with 0.2 ml of the test substance (6.5% Hydrolyzed Silk Protein in water).
Challenge: on day 14, the same dorsal area was treated (0.2 ml; 6.5% Hydrolyzed Silk Protein in water) with a 24 hour occlusive patch.

Observations: The application sites were evaluated 24 and 48 hours after challenge patch removal

Results: In the animals treated with Hydrolyzed Silk protein-2, 1/6 guinea pigs had moderate erythema and 2/6 has slight erythema at 24 hours after challenge patch removal. At 48 hours, 2/6 had slight erythema. No reactions were observed in the negative control group and all guinea pigs treated with 2,4-dinitrobenzene reacted. The test material was considered to be a non-sensitizer under the conditions of the study.

Phototoxicity

Hiroshima University. 1985. Summary of phototoxicity test Hydrolyzed Silk protein-2.

Test animals: 6 Hartley guinea pigs per group

Test groups: Test material, positive (1% 8-methoxypsoralen) and negative controls

Method: Topical application (0.05 ml/2 x 2 cm), 6.5% Hydrolyzed Silk Protein in water to two sites on the clipped dorsal skin, followed by irradiation at one of the two sites (second site was covered to avoid irradiation)

Light source: FL-40S lamp and BLB lamp (Toshiba Corporation)

Observations: Evaluation of application sites at 24, 48 and 72 hours after the challenge application

Results: No effects were observed in negative controls or in guinea pigs treated with Hydrolyzed Silk protein-2 with or without exposure to light. Positive responses (scores of 3.8 to 5) were observed in all guinea pigs treated with 8-methoxypsoralen and light. The test sample, Hydrolyzed Silk protein-2 was considered non-phototoxic under the conditions of the test.

Photoallergenicity

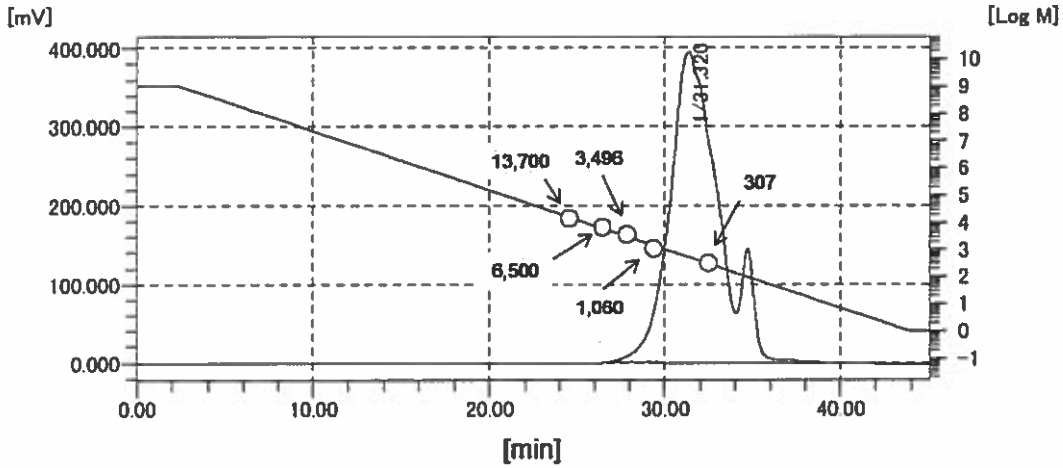
Hiroshima University. 1985. Summary of photoallergenicity test: Hydrolyzed Silk protein-2.

Test animals: 6 Hartley guinea pigs per group

Test groups: Test material, positive control (2% 3,5,4'-tribromosalicylanilide in 85% DMSO), negative control

Method:	Photoinduction: Topical application (0.05 ml/2 x 2 cm) 6.5% Hydrolyzed Silk Protein in water to two sites on the clipped dorsal skin, followed by irradiation at one of the two sites. An application and irradiation for 2 hours was completed per day, 5 times per week for 2 weeks (total of 10 applications). Photochallenge: Two weeks after completion of photoinduction, the application area was clipped and test material (0.05 ml/2 x 2 cm; 6.5% Hydrolyzed Silk Protein in water) was applied to two sites. One of the sites was irradiated for 2 hours while the other site was protected with a cover.
Light source:	FL-40S lamp and BLB lamp (Toshiba Corporation)
Observations:	Evaluation of application sites at 24, 48 and 72 hours after application
Results:	No effects were observed in negative controls or in guinea pigs treated with Hydrolyzed Silk protein-2 with or without exposure to light. Positive responses (scores of 1.5 to 2) were observed in all guinea pigs treated with 3,5,4'-tribromosalicylanilide and light). The test sample, Hydrolyzed Silk protein-2, was considered non-photosensitizing under the conditions of the test.

2014



[Conditions for analysis]

Column	: G3000PWXL
Eluent	: 0.1% TEA / acetonitrile (55/45)
Flow rate	: 0.3 ml/min
Detection	: UV at 210 nm

[Results]

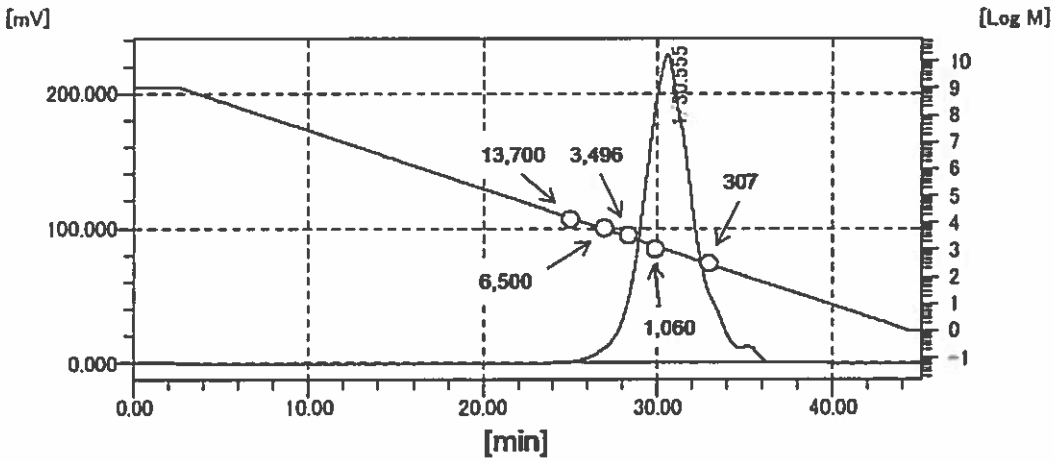
Mn	282
Mw	507
Mz	830

Figure 1 Size Exclusion Chromatogram of hydrolyzed silk protein-1

Molecular weight of hydrolyzed silk protein-1 was obtained with size exclusion chromatography, and calculated by plotting a calibration curve using the correlating relationship between the retention time (X axis) and the common logarithm of molecular weight (Y axis of the right). The calibration curve was plotting a linear function with 5 molecular markers (circle in the graph): 307 (Glutathione), 1060 (Bradykinin), 3496 (Insulin B-chain), 6500 (Aprotinin), and 13700 (Ribonuclease). The chromatogram in the graph was hydrolyzed silk protein-1.

"Results" indicates the values of number-average molecular weight (Mn), weight-average molecular weight (Mw), and Z-average molecular weight (Mz) of hydrolyzed silk protein-1.

2014



[Conditions for analysis]

Column	: G3000PWXL
Eluent	: 0.1% TEA / acetonitrile (55/45)
Flow rate	: 0.3 ml/min
Detection	: UV at 210 nm

[Results]

Mn	637
Mw	1,208
Mz	2,371

Figure 1 Size Exclusion Chromatogram of hydrolyzed silk protein-2

Molecular weight of hydrolyzed silk protein-2 was obtained with size exclusion chromatography, and calculated by plotting a calibration curve using the correlating relationship between the retention time(X axis) and the common logarithm of molecular weight(Y axis of the right). The calibration curve was plotting a linear function with 5 molecular markers (circle in the graph): 307 (Glutathione), 1060 (Bradykinin), 3496 (Insulin B-chain), 6500 (Aprotinin), and 13700 (Ribonuclease). The chromatogram in the graph was hydrolyzed silk protein-2.

"Results" indicates the values of number-average molecular weight (Mn), weight-average molecular weight (Mw), and Z-average molecular weight (Mz) of hydrolyzed silk protein-2.

Personal Care Products Council
Committed to Safety, Quality & Innovation

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: April 17, 2015

SUBJECT: Comments on the Scientific Literature Review: Safety Assessment of Silk Proteins as Used in Cosmetics

The Council has no suppliers listed for MEA-Hydrolyzed Silk.

Key Issue

As the Dictionary definitions indicate that these ingredients are from materials produced by the silkworm, *Bobmyx mori*, all the information about spider silk needs to be deleted from this report.

Additional Comments

Cytotoxicity - Please state the other extraction methods that yielded Sericin that caused less cytotoxicity.

Carcinogenicity - The study concerning proliferation of a rat cell line is not appropriate for the Carcinogenicity section. Please include the concentrations of Sericin that were tested in this study (reference 24).

Photocarcinogenicity - Please revise: "When compared to 100% animals...."

Please correct "versioin"

Based on the title, reference 27 appears to be about colon carcinogenesis and should not have been mentioned in the Photocarcinogenicity section.

Skin Irritation and Sensitization, Human - It is not clear what is meant by "serum IgE-silk waste"

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Immunological Responses and Wound Healing - The studies on Wound Healing effects should only be presented in the Wound Healing section. They should not be presented in the Immunological Response section.

Immunological Responses - The study on Fibroin (reference 37) should not be presented under Sericin.

Please revise the last paragraph of this section so that it is at least two sentences. It does not appear that it is necessary to relate this paragraph to the previous paragraph.

Wound Healing - Please delete "living" as they would not be culturing the cells if they were not alive.