International Journal of Toxicology
2021, Vol. 40(Supplement 2) 36S–51S

DOI: 10.1177/1091581821013019
journals.sagepub.com/home/ijt
SAGE

Safety Assessment of Keratin and Keratin-Derived Ingredients as Used in Cosmetics

Christina L. Burnett*, Wilma F. Bergfeld, Donald V. Belsito**, Ronald A. Hill***, Curtis D. Klaassen**, Daniel C. Liebler**, James G. Marks, Jr***, Ronald C. Shank**, Thomas J. Slaga**, Paul W. Snyder**, Lillian J. Gill****, and Bart Heldreth†**

Abstract

The Expert Panel for Cosmetic Ingredient Safety (Panel) reviewed the safety of 8 keratin-derived ingredients, which function mainly as skin and hair conditioning agents in personal care products. The Panel reviewed relevant data provided in this safety assessment and concluded that the 8 keratin-derived ingredients are safe in the present practices of use and concentration described in this safety assessment.

Keywords

safety, cosmetics, keratin

Introduction

The keratin-derived ingredients detailed in this report are described by the *International Cosmetic Ingredient Dictionary and Handbook* (INCI; *Dictionary*) to function mainly as skin and hair conditioning agents in personal care products.[1] This report assesses the safety of the following 8 keratin-derived ingredients:

Hydrolyzed Keratin
Hydrolyzed Hair Keratin
Hydrolyzed Oxidized Keratin
Hydrolyzed Sulfonated Keratin
Keratin
Oxidized Keratin
Soluble Keratin
Sulfonated Keratin

This is the first Cosmetic Ingredient Review report on ingredients derived from the keratin family of proteins. However, the safety of several hydrolyzed proteins as used in cosmetics has been reviewed by the Panel in several previous assessments. The Panel concluded that Hydrolyzed Collagen, Hydrolyzed Soy Protein, Hydrolyzed Silk, Hydrolyzed Rice Protein, and Hydrolyzed Corn Protein are safe for use in cosmetics.[2-7] Additionally, the Panel concluded that Hydrolyzed Wheat Gluten and Hydrolyzed Wheat Protein are safe for use in cosmetics when formulated to restrict peptides to a weight-average molecular weight (MW) of 3500 Da or less.[8]

Keratin occurs naturally in epithelial cells and is essential for normal tissue structure and function. Much of the available published literature evaluated the ability of cosmetic or pharmaceutical ingredients to have a desired effect on naturally occurring keratin in skin, hair, or other tissues. These studies were not considered relevant for assessing the safety of the keratin-derived ingredients as used in cosmetics and are not included in this assessment.

The main sources of Keratin ingredients are sheep wool and bovine hoof (nail) or horn.[9] Goat (cashmere) wool and bird (chicken) feathers may also be used. Human hair was once a major source of Keratin, but its use is now limited because of the European ban on human-sourced materials for cosmetics. These differing sources could potentially produce or result in keratin with unique properties, which may result in varying compositions and impurities within a single ingredient (eg, Keratin from human hair may have some impurities that are different from Keratin obtained from bird feathers).

* Cosmetic Ingredient Review Senior Scientific Analyst/Writer
** Expert Panel for Cosmetic Ingredient Safety Member
*** Expert Panel for Cosmetic Ingredient Safety Former Member
**** Cosmetic Ingredient Review Former Director
† Cosmetic Ingredient Review Executive Director

Corresponding Author:
Bart Heldreth, Executive Director, Cosmetic Ingredient Review, 1620L Street, NW, Suite 1200, Washington, DC 20036, USA.
Email: cirinfo@cir-safety.org

Table 1. Definitions, Structures, and Functions of the Ingredients in this Safety Assessment.[1]

Ingredient/CAS No.	Definition and Structure	Function
Hydrolyzed Keratin 65997-21-9 73049-73-7	Hydrolyzed Keratin is the hydrolysate of keratin derived by acid, enzyme, or other method of hydrolysis.	Hair conditioning agents; nail conditioning agents; skin-conditioning agents-misc.
Hydrolyzed Hair Keratin 69430-36-0 73049-73-7 [peptones]	Hydrolyzed Hair Keratin is the hydrolysate of human hair keratin derived by acid, enzyme, or other method of hydrolysis.	Hair conditioning agents; skin-conditioning agents-misc.
Hydrolyzed Oxidized Keratin [1142948-22-8]	Hydrolyzed Oxidized Keratin is the hydrolysate of Oxidized Keratin obtained by acid, enzyme, or other method of hydrolysis.	Hair conditioning agents; skin-conditioning agents-misc.
Hydrolyzed Sulfonated Keratin [1119233-83-8]	Hydrolyzed Sulfonated Keratin is the hydrolysate of Sulfonated Keratin derived by acid, enzyme, or other method of hydrolysis.	Hair conditioning agents; skin-conditioning agents-emollient; skin-conditioning agents-humectant
Keratin 169799-44-4 68238-35-7	Keratin is the protein derived from hair, wool, horn, nails, or other similar tissues in animals.	Hair conditioning agents; skin-conditioning agents-misc.
Oxidized Keratin [143819-61-8]	Oxidized Keratin is the material derived chemically from Keratin by oxidation with hydrogen peroxide. This reaction converts some of the sulfur atoms in Cysteine and Cystine residues in keratin to the corresponding sulfonic acid grouping (cysteic acid).	Hair conditioning agents; skin-conditioning agents-misc.
Soluble Keratin	Soluble Keratin [is] a water soluble nonhydrolyzed, native protein derived from Keratin.	Hair conditioning agents
Sulfonated Keratin [1119232-93-7]	Sulfonated Keratin is the product obtained by the oxidative sulfitolysis of wool.	Film formers; hair conditioning agents; skin protectants

When the specific cosmetic ingredient names are discussed in this report, Keratin and the related ingredients will be capitalized, which is how they are presented in the *Dictionary* (see list above). When referring generally to the naturally occurring protein from which these ingredients are derived, lower-case lettering will be used (ie, keratin).

Chemistry

Definition

The definition, structures, and functions of the keratin-derived ingredients in this report are provided in Table 1.

The general term, keratin, refers to a broad category of proteins that result in intermediate filaments that form the bulk of cytoplasmic epithelia and epidermal appendage structures (ie, hair, wool, horn, nails and similar tissues in animals).[10] Keratins can be classified into 2 distinct groups ("hard" and "soft") based on their structure, function, and regulation.

"Hard" keratin filaments form ordered arrays and are the primary contributors to the tough structure of epidermal appendages. Hard keratins (derived from hair, wool, horn, nails, or other similar tissues in animals) are utilized as cosmetic ingredients, rather than soft (derived, eg, from cytoplasmic epithelial keratins). These hard keratin proteins contain a much higher content of cysteine residues than the soft keratins in their nonhelical domains, and, thus form tougher and more durable structures via intra/intermolecular disulfide bond formation. These hard keratins may be modified/extracted (ie, hydrolyzed, oxidized, sulfonated, etc) to produce other cosmetic ingredients (eg, Hydrolyzed Oxidized Keratin). The structural subunits of these keratins comprise 2 chains, which differ by MW and amino acid residue sequence (designated types I and II), that each contain nonhelical end terminal domains, and a highly conserved, central α-helical domain.

Chemical and Physical Properties

Keratin is insoluble in water, and many keratin-derived materials have MWs between 9 and 60 kDa.[11,12] Keratin proteins extracted from hair are classified into 3 broad groups: α, β, and γ.[13] α-Keratin (found in hair fiber cortex) is low in sulfur content and has an average MW of 60 to 80 kDa. β-Keratin is protective and forms the majority of the hair cuticle; this type of keratin is difficult to extract. γ-Keratin is globular, high in sulfur content, and has a MW of ~15 kDa.

Molecular weights for Hydrolyzed Hair Keratin are reported to be around 400 Da.[14] The MWs for Hydrolyzed Keratin have been reported to be as low as 150 Da, but may be around 1000 to 3000 Da.[14-16]

Method of Manufacturing

Keratin, Oxidized Keratin, and Sulfonated Keratin

Keratins are difficult to solubilize compared to other proteins. After mechanical means of processing, such as grinding of materials like animal horn, one of the first known processes for extracting keratins involved harsh (caustic) conditions using lime. Eventually, a number of oxidative and reductive

Table 2. Properties and Specifications of Hydrolyzed Keratin Products as Reported by a Supplier.[70-72]

Specification	Hydrolyzed Protein (sheep wool source, powder)	Hydrolyzed Protein (sheep wool source, solution)	Hydrolyzed Protein (goat wool source, solution)
Appearance	Pale cream free flowing powder	Clear to hazy amber liquid	Clear to hazy amber liquid
Color	8 Gardner max (10% solution)	11 Gardner max	11 Gardner max
Odor	Slight, characteristic	Characteristic protein note	Characteristic
Moisture Content (1 g-1 h-105 °C)	8.0% max	30.0-35.0%	30.0%-35.0%
pH	5.0-7.0 (10% solution at 25 °C)	5.5-7.0 (direct)	5.5-7.0 (direct)
Ash (800 °C)	4.0% max	4.0% max	4.0% max
Nitrogen (Kjeldahl)	14.0% min	4.0% min	4.0% min
Heavy Metals	<20 ppm	<20 ppm	<20 ppm
Arsenic	<2 ppm	<2 ppm	<2 ppm
Microbial content	<100 opg, no pathogens	<100 opg, no pathogens	<100 opg, no pathogens

opg = organisms per gram.

methods were developed for extracting keratin. Production of many of the ingredients in this assessment involves extraction of keratin via acid- or enzymatic hydrolysis.[17]

Keratin may be produced by nonchemical and chemical methods. Using nonchemical methods, Keratin may be dissolved and converted from wool via steam explosion or from feathers via superheated water.[11] Under conditions of steam explosion, wool is heated under pressure at a high temperature (with steam ~220 °C) for several minutes (~10 minutes), followed by explosive decompression. The wool is disrupted into solid and liquid phases consisting of oligopeptides, water soluble peptides, and free amino acids. Feather barbs are treated with superheated water (liquid ~220 °C) for 2 hours and then cooled. The products are oligopeptides with MWs of 1.0 to 1.8 kDa.

For the chemical methods, wool Keratin is extracted by reduction or oxidation of disulfide bonds.[18] Reducing agents include thioglycolic acid, dithiothreitol, or 2-mercaptoethanol, and oxidizing agents include peracetic acid or performic acid. These agents must work in combination with a protein denaturing agent, like urea, to break disulfide bonds and disrupt hydrogen bonding. These chemical extraction methods can directly result in some of these modified ingredients, such as Oxidized Keratin or Sulfonated Keratin. Resultant keratins may have low or high sulfur content (low sulfur MW between 45 and 60 kDa, high sulfur MW between 11 and 28 kDa) or high glycine and tyrosine content (MW between 9 and 12 kDa). However, for a fully sulfonated keratin, an oxidant such as molecular oxygen is required for the complete sulfonation of protein thiol (S-H) groups by sulfite.[19] It has been reported that Keratin may be extracted from chicken feathers with reducing agents.[20]

Hydrolyzed Keratin

Hydrolyzed Keratin may be prepared from sheep wool.[21] The wool is first washed to remove soil and debris and then boiled to remove residual oils. Next, the wool is enzyme hydrolyzed under mild conditions for 4 to 6 hours. When the target MW is reached, the pH is adjusted to neutralize the enzyme. The resultant solution is a mixture of Hydrolyzed Keratin fractions with a MW of ~1000 Da. The solution may be diluted to produce a 30% active material.

A supplier reported that 3 Hydrolyzed Keratin products (MW = 310, 320, and 11,000 Da, respectively) are prepared by acidic, alkaline, and/or enzymatic hydrolysis of sheep wool until the MW reaches the target range.[22] Further properties and specifications of these products are described in Table 2.

Another supplier certified that their powdered and liquid Hydrolyzed Keratin products are derived from sheep or goat wool.[23-25]

Hydrolyzed Keratin and Hydrolyzed Hair Keratin

A supplier reported that Hydrolyzed Hair Keratin and Hydrolyzed Keratin are obtained through acid hydrolysis.[15] Another supplier reported that Hydrolyzed Keratin is manufactured by enzymatic hydrolysis for a specific duration of time and at an elevated temperature (details not provided).[26] The resultant hydrolyzed proteins have MWs in the 2000 to 4000 Da range and all contain di- and tripeptides.

Soluble Keratin

A supplier reported that a Soluble Keratin powder product (MW = 30,000 Da) is prepared by extraction from sheep wool.[27]

Composition

Keratins ubiquitously consist of central α-helical rod domains that are flanked by non-α-helical head and tail domains.[28] However, the amino sequences are not highly conserved across various source species (eg, equine versus human) or even among tissue-specific function types within 1 species (eg, hair vs nail versus skin). Indeed, the differences in amino acid sequences among keratins can be rather striking (eg, many cysteine residues in hair keratin vs a very small number in epidermal keratin, to no cysteine residues in other types). Accordingly, the species and tissues from which Keratin is derived can significantly impact the composition of the cosmetic ingredient. The amino acid sequences of some keratin

Table 3. Amino Acid Composition of Commercialized Keratin Hydrolysate (g/100 g protein).[73]

Cysteic acid	0.3
Hydroxyproline	0
Aspartic acid	7.8
Threonine	6.1
Serine	8.1
Glutamic acid	17.0
Proline	7.6
Glycine	3.9
Alanine	7.6
Cystine	7.2
Valine	5.7
Methionine	1.0
Isoleucine	3.8
Leucine	8.1
Tyrosine	2.0
Phenylalanine	2.4
Lysine	3.2
Histidine	1.0
Arginine	9.0

proteins have been elucidated. However, the definition of Keratin in the Dictionary is "the protein derived from hair, wool, horn, nails or other similar tissues in animals." Thus, the composition of a keratin-derived ingredient cannot be known without composition data from raw material suppliers.

Keratin

Cysteine residues in Keratin protein molecules make up 7% to 20% of the total amino acid residues.[11]

Hydrolyzed Keratin

Amino acid composition data on Hydrolyzed Keratin is described in Table 3.

Impurities

Product specifications for Hydrolyzed Keratin and Soluble Keratin are presented in Table 4.

As with most nonsynthetic raw materials, numerous environmental factors may profoundly affect the presence and concentration of impurities, dependent on the Keratin source. Accordingly, manufacturers should use best practices to ensure the lowest possible values of any impurities.

Hydrolyzed Keratin

A supplier has certified that their Hydrolyzed Keratin products are free of bovine spongiform encephalopathy (BSE) infectivity, or the prions that cause BSE.[23-25]

Use

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. 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, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2016 VCRP data, Hydrolyzed Keratin has the most reported uses of the ingredients listed in this safety assessment in cosmetic products, with a total of 667; more than half of the uses are in rinse-off non-coloring hair products (Table 5).[29] Keratin has the second greatest number of overall uses reported, with a total of 90; the majority of the uses are in non-coloring hair products. The results of the concentration of use survey conducted in 2015 by the Council indicate Hydrolyzed Keratin has the highest reported maximum concentration of use; it is used at up to 5% in hair tonics, dressings, and other hair grooming aids. Keratin is used at up to 0.075% in hair tonics, dressings, and other hair grooming aids.

Based on the VCRP data and the results of the Council's concentration of use survey, Hydrolyzed Oxidized Keratin, Hydrolyzed Sulfonated Keratin, Oxidized Keratin, and Sulfonated Keratin are not in use.

Some of these ingredients may be used in products that can come into contact with the eye or mucous membranes. For example, Hydrolyzed Keratin is used in mascara at up to 0.2% and in bath soaps and detergents at up to 0.028%. Additionally, some of these ingredients were reported to be used in hair sprays and could possibly be inhaled. For example, Hydrolyzed Keratin was reported to be used in hair sprays at a maximum concentration of 0.059%. 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.[30-33] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (ie, they would not enter the lungs) to any appreciable amount.[30,32]

The FDA has recently issued a new final rule on use of materials derived from cattle in cosmetics (21 CFR §700.27). Prohibited materials did not include hoofs or horns or the keratin that may be derived from these substances.

The Keratin ingredients described in this safety assessment, with the exception of Hydrolyzed Hair Keratin, are not restricted from use in any way under the rules governing cosmetic products in the European Union (EU).[34] Hydrolyzed Hair Keratin is a substance prohibited in cosmetic products in

Table 4. Specifications of Keratin-Derived Products (sheep wool source).[22,27]

	Average Molecular Weight (Da)	Concentration	Heavy Metals	Arsenic
Hydrolyzed Keratin Product 1	310	25% solution in water	Not more than 10 ppm	Not more than 1 ppm
Hydrolyzed Keratin Product 2	320	25% solution in water	Not more than 10 ppm	Not more than 1 ppm
Hydrolyzed Keratin Product 3	11,000	20% solution in water	Not more than 10 ppm	Not more than 1 ppm
Soluble Keratin Powder	30,000	NR	Not more than 20 ppm lead	Not more than 2 ppm

NR = not reported.

Table 5. Frequency and Concentration of use According to Duration and type of Exposure for Keratin Ingredients.[29,74]

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Keratin		Hydrolyzed Hair Keratin	
Totals†	**90**	**0.075**	**27**	**NR**
Duration of Use				
Leave-On	44	0.075	3	NR
Rinse Off	46	NR	24	NR
Diluted for (Bath) Use	NR	NR	NR	NR
Exposure Type				
Eye Area	7	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation—Sprays	2; 18[a]	0.075[a]	1[a]	NR
Incidental Inhalation—Powders	NR	NR	NR	NR
Dermal Contact	3	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR
Hair—Non-Coloring	80	0.075	27	NR
Hair Coloring	NR	NR	NR	NR
Nail	2	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR
	Hydrolyzed Keratin		Soluble Keratin[d]	
Totals†	**667**	**0.000034-5**	**1**	**NR**
Duration of Use				
Leave-On	249	0.000034-5	NR	NR
Rinse Off	417	0.0001-0.88	1	NR
Diluted for (Bath) Use	1	<0.01	NR	NR
Exposure Type				
Eye Area	36	0.001-0.2	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation—Sprays	26; 131[a]; 9[b]	0.000034-0.059; 0.0003-5[a]	NR	NR
Incidental Inhalation—Powders	9[b]	0.0025[c]	NR	NR
Dermal Contact	40	0.001-0.21	NR	NR
Deodorant (underarm)	1[a]	NR	NR	NR
Hair—Non-Coloring	393	0.000034-5	1	NR
Hair-Coloring	193	0.0001-0.5	NR	NR
Nail	10	0.002-0.04	NR	NR
Mucous Membrane	10	0.01-0.028	NR	NR
Baby Products	NR	NR	NR	NR

NR = Not reported; VCRP = Voluntary Cosmetic Registration Program.

† Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

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

[b]Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

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

[d]Listed in the VCRP as Soluble Animal Keratin.

Table 6. Acute Toxicity Studies.

Ingredient	Concentration/Dose	Animals	Results	Reference
		Oral		
Hydrolyzed Keratin (MW = 320 Da; sheep wool source)	5 mL/kg of a 25% solution in water	5 male and 5 female Wistar rats	$LD_{50} > 5$ mL/kg	22
Hydrolyzed Keratin (MW = 11,000 Da; sheep wool source)	2 g/kg of a 20% solution in water	3 male and 3 female Sprague-Dawley CD rats	$LD_{50} > 2$ g/kg	22
Hydrolyzed Keratin (hoof meal source)	10 g/kg of a 20% solution	5 male and 5 female Sprague-Dawley rats	$LD_{50} > 10$ g/kg	41
Hydrolyzed Keratin (hoof meal source)	2.5, 5.0, 10.0, 20.0, or 40.0 mL/kg of a 20% solution	Five groups of 3 male and 2 female Wistar rats	$LD_{50} > 40$ mL/kg	40
Hydrolyzed Keratin (hoof meal source)	5 g/kg of a 25% solution	5 male and 5 female Wistar rats	$LD_{50} > 5$ g/kg	39
		Intravenous		
Keratin (enzymatically and chemically fragmented, MW ~ 8000 Da and 33,000 Da, respectively; buffalo horn/hoof source)	300 mg/kg dissolved in 1/15 M sodium phosphate buffer	male ddY mice, number not reported	No significant changes observed in lungs, livers, kidneys, spleens; body weight changed similar to those of the control group	37
Hydrolyzed Keratin (MW = 310 Da; sheep wool source)	5 mL/kg of a 20% solution in water	15 male ddY mice	$LD_{50} > 5$ mL/kg	22

MW = molecular weight.

the EU due to its human origin; however, the Scientific Committee on Consumer Products (SCCP) concluded that "the resulting risk, based on current scientific data, of the use [of amino acids obtained by hydrolysis of human hair] in cosmetic products for topical application . . . is negligible" when the human hair has undergone hydrolysis with concentrated HCl (> 20%) for 6 hours at 100 °C followed by activated carbon filtration, crystallization, and drying.[35]

Noncosmetic

Noncosmetic uses of Keratin include use as a biopolymer in nanomaterials and in biomedical applications such as wound dressings, drug delivery, tissue engineering, and trauma and medical devices.[10-13,36,37]

Toxicokinetics

Absorption, Distribution, Metabolism, Excretion

Keratin. The tissue distribution of enzymatically and chemically fragmented Keratin (MW ~ 8000 Da and 33,000 Da, respectively) was studied in male ddY mice.[37] The fragmented Keratin was radiolabeled with Na[^{125}I]. The radiolabeled test materials were then injected via the tail vein at a dose of 2 mg/kg. The mice were killed at varying times following injection. Tissues were excised and weighed, and the radioactivity measured. The fragmented Keratin was found to be quickly eliminated from the plasma, taken up into the kidney, and gradually excreted in urine. Chemically fragmented Keratin was also observed to be taken up into the liver.

Dermal Penetration

Hydrolyzed keratin. A study of the efficacy of Hydrolyzed Keratin derived from wool stated that Hydrolyzed Keratin peptide can penetrate into the skin and increase moisturization.[38] No further details regarding the penetration properties of the Hydrolyzed Keratin were provided.

Toxicological Studies

Acute Toxicity

Acute toxicity studies are presented in Table 6.[22,37,39-41] Hydrolyzed Keratin sourced from sheep wool and hoof meal was nontoxic in oral studies in rats (ie, the LD_{50} was greater than 40 mL/kg in a 20% solution). Keratin (MW = 8000 Da and 33,000 Da; buffalo horn/hoof source) and Hydrolyzed Keratin (MW = 310 Da; sheep wool source) were nontoxic in intravenous studies in mice.

Dose Toxicity

No relevant published repeated dose toxicity studies on Keratin ingredients were identified in a literature search for these ingredients, and no unpublished data were submitted.

Reproductive and Developmental Toxicity

No relevant published reproductive and developmental toxicity studies on Keratin ingredients were identified in a literature search for these ingredients and no unpublished data were submitted.

Genotoxicity

In vitro genotoxicity studies are presented in Table 7.[22,27,42-47] Hydrolyzed Keratin (MW ranging from 320-11,000 Da), Soluble Keratin (MW = 30,000 Da), and Keratin (MW not specified) that were mainly sourced from sheep wool were not mutagenic in Ames studies. In a comet assay, a Keratin peptide (MW = 1600 Da; synthesized from human hair) was not genotoxic in aqueous solutions, but genotoxicity was observed in organic solvent solutions. However, the negative controls also displayed genotoxicity in these solutions.

Carcinogenicity

No relevant published carcinogenicity studies on Keratin ingredients were identified in a literature search for these ingredients and no unpublished data were submitted.

Irritation and Sensitization

Dermal Irritation

In vitro, animal, and human dermal irritation studies are presented in Table 8.[20,22,26,27,38,39,47-58] No irritation was predicted in in vitro studies of sheep wool-sourced Hydrolyzed Keratin tested at 100% and Keratin tested at 5%. Keratin (concentration not reported; chicken feather source) and Hydrolyzed Keratin (tested neat; source not provided) were mainly not irritating in rabbit studies. Hydrolyzed Keratin was not irritating in human studies at concentrations up to 25% (sheep wool source), nor was Soluble Keratin at 10% (sheep wool source).

Ocular Irritation

Ocular irritation studies are presented in Table 9.[22,26,27,39,47,49-53,56,59-62] No irritation was predicted in in vitro studies of Keratin (5%), Hydrolyzed Keratin (undiluted), or Soluble Keratin (5%), which were mainly sourced from sheep wool. Hydrolyzed Keratin was minimally to nonirritating in Draize ocular rabbit studies when tested neat (source not provided).

Dermal Sensitization

Dermal sensitization studies are presented in Table 10.[22,27,47,63-68] No sensitization was predicted in in vitro studies of Hydrolyzed Keratin (sheep and goat wool sources). No sensitization to Hydrolyzed Keratin was observed when tested up to 25% in a guinea pig maximization study (sheep wool source) or up to 5% in human repeat insult patch tests (HRIPTs; bovine source). No sensitization was observed in HRIPTs to Keratin (5%; sheep wool source) or Soluble Keratin (10%; sheep wool source).

Phototoxicity and Photosensitization

The potential for phototoxicity of Hydrolyzed Keratin (MW = 320 Da; sheep wool source) was studied in 6 male Hartley guinea pigs.[22] A 25% solution in water was applied to 2 clipped dorsal skin sites (2 cm^2). Each site received 0.05 mL test material. One site was covered and the other site was irradiated for 2 hours. The sites were evaluated at 24, 28, and 72 hours postirradiation. No further details were provided. No erythema or edema was observed with or without irradiation.

The potential for photosensitization of Hydrolyzed Keratin (MW = 320 Da; sheep wool source) was studied in 6 male Hartley guinea pigs.[22] The guinea pigs were induced on 2 clipped dorsal skin sites (2 cm^2) with 0.05 mL of a 25% solution in water. One site was covered and the other irradiated for 2 hours daily, 5 times per week for 2 weeks. Following a 2-week rest period, the test sites received the test material with the same site covered and the other site irradiated for 2 hours. Sites were evaluated at 24, 48, and 72 hours postirradiation. No further details were provided. No evidence of phototoxicity or photosensitization was observed.

Clinical Studies

Case Reports

Hydrolyzed keratin. A 22-year-old woman was reported to have a severe allergic reaction that included marked periorbital edema and swollen, sore, and itchy eyes and hands following use of a hair conditioner.[69] Prick testing elicited a strong positive (10 mm) wheal-and-flare response to the hair conditioner, which contained steartrimonium hydrolyzed animal protein (also known as Steartrimonium Hydroxyethyl Hydrolyzed Collagen). Additional prick testing showed further reactions to the quaternary hydrolyzed protein as well as to shampoos and conditioners that contained gelatin keratin amino acids, Hydrolyzed Keratin, and/or hydrolyzed collagen. The allergic reaction to Hydrolyzed Keratin was only observed in the prick test. Patch tests using the European standard series and a series of 15 common bases of medicines and cosmetics were negative.

Summary

The keratin-derived ingredients detailed in this report function mainly as skin and hair conditioning agents in personal care products. Keratin occurs naturally in epithelial cells and is essential for normal tissue structure and function.

According to 2016 VCRP data, Hydrolyzed Keratin has the most reported uses of the ingredients listed in this safety assessment in cosmetic formulations, with a total of 667; more than half of the uses are in rinse-off non-coloring hair formulations. Keratin has the second greatest number of overall uses

Table 7. In Vitro Genotoxicity Studies.

Ingredient	Concentration/Dose	Study Protocol	Results	Reference
Keratin (characterized as a peptide with a MW ~1600 Da, 13 amino acid sequence comprised of 2 cysteine residues; synthesized from human hair)	0.025 g/L to 0.5 g/L prepared in an aqueous solution with 100% phosphate buffer or in an organic solvent solution with 10% ethanol, 1.5% propylene glycol, 0.5% benzyl alcohol, and 88% phosphate buffer	Comet assay with human fibroblasts; cells incubated with test materials and controls for either 1 h or 72 h	In the aqueous solutions, Keratin peptide was not genotoxic; in the organic solvent solutions for the 1 h exposure, genotoxicity was observed in the negative controls as well as in the treated cells; for the 72 h exposure, genotoxicity was also observed, but the extent of DNA damage was not as great as observed in the 1 h exposure	42
Keratin (sheep wool source)	100, 333, 1000, 3333, and 5000 μg/plate	Bacterial reverse mutation assay (Ames test) *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 and *Escherichia coli* strain WP2 uvr A, with and without metabolic activation	Nonmutagenic	47
Hydrolyzed Keratin (25% solution in water; MW = 320 Da; sheep wool source)	500 to 100,000 μg/plate	Ames test using *S typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 and in *E coli* strain WP2 uvrA, with or without metabolic activation	Nonmutagenic	22
Hydrolyzed Keratin (MW = 3000 Da; 14% peptide content; source not provided)	Up to 5000 μg/plate	Ames test using *S typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 and in *E coli* strain WP2 uvrA, with or without metabolic activation	Nonmutagenic	43
Hydrolyzed Keratin (20% solution in water; MW = 11,000 Da; sheep wool source)	50 to 5000 μg/plate	Ames test using *S typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 and in *E coli* strain WP2 uvrA, with or without metabolic activation	Nonmutagenic	22
Hydrolyzed Keratin (powder product, sheep wool source; MW not provided)	1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 μg/plate	Ames test using *S typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 and *E coli* strain WP2 uvr A, with and without metabolic activation	Nonmutagenic	46
Hydrolyzed Keratin (solution, sheep wool source; MW not provided)	1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 μ/plate	Ames test using *S typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 and *E coli* strain WP2 uvr A, with and without metabolic activation	Nonmutagenic	45
Hydrolyzed Keratin (solution, goat wool source; MW not provided)	1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 μ/plate	Ames test using *S typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 and *E coli* strain WP2 uvr A, with and without metabolic activation	Nonmutagenic	44
Soluble Keratin (MW = 30,000 Da; sheep wool source)	50 to 5000 μg/plate.	Ames test using *S typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 and in *E coli* strain WP2 uvrA, with or without metabolic activation	Nonmutagenic	27

h = hours; MW = molecular weight.

Table 8. Dermal Irritation Studies.

Ingredient	Concentration	Method	Results	Reference
		In Vitro		
Keratin (sheep wool source)	5%	EpiDerm™ skin model	Nonirritating	47
Hydrolyzed Keratin (source not provided)	100% (MW = 3000 Da)	EpiDerm™ skin model	Nonirritating	54
Hydrolyzed Keratin (source not provided)	2 forms tested (MW = 2000-4000 Da), solution concentration not reported, powder undiluted	EpiDerm™ assay	Nonirritating	26
Hydrolyzed Keratin (sheep wool source)	Undiluted powder product (MW not provided)	EpiDerm™ skin model	Nonirritating	51
Hydrolyzed Keratin (sheep wool source)	25, 50, 75, 100, and 125 μL (MW not provided)	Irritection® dermal assay	Nonirritating	50
Hydrolyzed Keratin (goat wool source)	25, 50, 75, 100, and 125 μL (MW not provided)	Irritection® dermal assay	Nonirritating	49
		Animal		
Keratin (chicken feathers source)	Not reported	6 male rabbits received test material in a cream daily for 8 weeks on a 4 cm^2 area in an efficacy study (no further studies)	No irritation was observed	20
Hydrolyzed Keratin (hoof meal source)	20% solution	Primary dermal irritation study in 6 female New Zealand white rabbits; 0.5 mL occluded for 24 h; intact and abraded skin; exposure area 2.5 cm^2	Well defined erythema in both intact and abraded skin of 4 rabbits 24 h postdosing while the remaining 2 rabbits had very slight erythema; very slight edema in 3 abraded and 3 intact sites, slight edema in 1 abraded and 2 intact sites, and moderate edema in 1 abraded site; irritation response declined during study with well-defined erythema and very slight edema remaining at abraded site and very slight erythema remaining at 5 abraded and 4 intact sites at 72 h postdosing; PII = 2.0; mildly irritating	55
Hydrolyzed Keratin (hoof meal source)	20% solution	Draize primary dermal irritation study in 6 albino rabbits; 0.5 mL occluded for 24 h; intact and abraded skin; exposure area 1 in^2	Erythema in abraded skin of all 6 rabbits and edema in abraded skin of 4 rabbits; edema cleared in 2 of the rabbits by the 72 h observation period; mild irritant; PII = 0.75	58
Hydrolyzed Keratin (hoof meal source)	25% solution	Draize primary dermal irritation study in 6 New Zealand white rabbits; 0.5 mL occluded for 24 h; intact and abraded skin; exposure area 2.5 cm^2	PII = 0.43; not a primary dermal irritant	39
Hydrolyzed Keratin (sheep wool source)	25% solution in water (MW = 320 Da)	Draize primary dermal irritation study in 6 New Zealand white male rabbits; 0.5 mL occluded for 24 h; intact and abraded skin; exposure area 2.5 cm^2	Very slight erythema in abraded skin of 2/6 rabbits after 24 h and 1/6 rabbits after 72 h; mild erythema in abraded skin of 1/6 rabbits after 72 h; PII = 0.2; nonirritant	22

(continued)

Table 8. (continued)

Ingredient	Concentration	Method	Results	Reference
Hydrolyzed Keratin (sheep wool source)	20% solution in water (MW = 11,000 Da)	Draize primary dermal irritation study in 3 male New Zealand white rabbits; 0.5 mL occluded for 4 h; intact skin; exposure area 2.5 cm^2	No skin irritation noted; PII = 0.0; nonirritant	22
Hydrolyzed Keratin (source not provided)	Neat (MW = 500 Da)	Draize primary dermal irritation study in 6 New Zealand white rabbits; occluded for 24 h	PII = 2.15; not a primary irritant	53
Hydrolyzed Keratin (source not provided)	Neat (MW not provided)	Draize primary dermal irritation study in 6 male New Zealand white rabbits; occluded for 24 h	PII = 0.4; not a primary irritant	57
Hydrolyzed Keratin (source not provided)	Neat (MW = 125,000 Da)	Draize primary dermal irritation study in 6 female New Zealand white rabbits; occluded for 24 h	PII = 2.0; not a primary irritant	48
Hydrolyzed Keratin (source not provided)	Neat (MW = 600 Da)	Draize primary dermal irritation study in 6 New Zealand white rabbits; occluded for 24 h	PII = 0.0; not a primary irritant	52
Human—In Vivo				
Hydrolyzed Keratin (enzymatically hydrolyzed; nonspecific wool source)	Hand cream containing 3% Keratin peptide (as supplied, 0.3% active; MW = < 1000 Da or 6-8 amino acids)	Efficacy study in 16 female volunteers on undisturbed hand skin and on sodium lauryl sulfate disturbed hand skin; exposure was once a day for 2.5 weeks for a total of 12 applications; exposure area = 9 cm^2; amount not reported	No adverse effects	38
Hydrolyzed Keratin (sheep wool source)	25% solution in water (as supplied, MW = 310 Da)	24 h occlusive human patch test in 40 healthy subjects and 10 allergic subjects; 0.5 mL test material applied by Finn chambers	Nonirritant	22
Hydrolyzed Keratin (sheep wool source)	25% solution in water (as supplied, MW = 320 Da)	24 h occlusive human patch test in 24 health subjects; 0.2 mL test material applied by adhesive tape	Nonirritant	22
Hydrolyzed Keratin (sheep wool source)	20% solution in water (as supplied, MW = 11,000 Da)	24 h occlusive human patch test in 23 subjects; 0.03 g test material applied by Finn chambers	1 subject had a mild erythema reaction at 30-60 min postpatch removal; no erythema in any subjects at 24 h postpatch removal; nonirritant	22
Soluble Keratin (sheep wool source)	10% active in distilled water (MW = 30,000 Da)	24 h occlusive human patch test in 20 subjects; test material applied by Finn chambers	Nonirritant	27

h = hours; MW = molecular weight.

reported, with a total of 90; the majority of the uses are in non-coloring hair formulations. The results of the concentration of use survey conducted in 2014 by the Council indicate Hydrolyzed Keratin has the highest reported maximum concentration of use; it is used at up to 5% in hair tonics, dressings, and other hair grooming aids. Keratin is used at up to 0.075% in hair tonics, dressings, and other hair grooming aids.

Noncosmetic uses of Keratin include use as a biopolymer in nanomaterials and in biomedical applications such as wound dressings, drug delivery, tissue engineering, and trauma and medical devices.

In an intravenous tissue distribution study of enzymatically and chemically fragmented Keratin, fragmented Keratin was found to be quickly eliminated from the plasma, taken up into the kidney, and gradually excreted in urine. Chemically fragmented Keratin was also observed to be taken up into the liver.

Hydrolyzed Keratin peptide derived from wool may penetrate into the skin.

Hydrolyzed Keratin was nontoxic in oral studies in rats (ie, the LD_{50} was greater than 5 mL/kg in a product with MW = 320 Da). Keratin (MW = 8000 Da and 33,000 Da) and Hydrolyzed Keratin (MW = 310 Da) were nontoxic in intravenous studies in mice.

Hydrolyzed Keratin (MW ranging from 320 to 11,000 Da), Soluble Keratin (MW = 30,000 Da), and Keratin (MW not specified) were not mutagenic in Ames studies. In a comet assay, a Keratin peptide (MW = 1600 Da) was not genotoxic

Table 9. Ocular Irritation Studies.

Ingredient	Concentration	Method	Results	Reference
		In Vitro		
Keratin (sheep wool source)	5% tested at 1%, 5%, and 10% in distilled water	HET-CAM method	Practically no irritation potential	47
Hydrolyzed Keratin (sheep wool source)	25, 50, 75, 100, and 125 μL (MW not provided)	Irritection® ocular assay	Minimal irritant	50
Hydrolyzed Keratin (goat wool source)	25, 50, 75, 100, and 125 μL (MW not provided)	Irritection® ocular assay	Minimal irritant	49
Hydrolyzed Keratin (sheep wool source)	Undiluted powder product (MW not provided)	EpiOcular assay	Nonirritating	51
Hydrolyzed Keratin (source not provided)	1%, 5%, and 10% (MW = 3000 Da)	HET-CAM method	Practically no irritation potential at 1% and 5%. Slight irritation potential at 10%.	59
Hydrolyzed Keratin (source not provided)	2 forms tested (MW = 2000-4000 Da), solution concentration not reported, powder undiluted	EpiOcular assay	Nonirritating	26
Soluble Keratin (sheep wool source)	Product (MW = 30,000 Da) diluted in distilled water to 5%	HET-CAM method	Average score 0.75; nonirritant	27
		Animal		
Hydrolyzed Keratin (hoof meal source)	20% solution	0.1 mL instilled into the right eye of 6 female New Zealand white rabbits; eyes assessed 24, 48, and 72 h postdosing	Slight conjunctival redness noted in 1 rabbits at 24 h postdosing; nonirritating	61
Hydrolyzed Keratin (hoof meal source)	20% solution	Unreported amount instilled into eyes of 6 albino rabbits; eyes assessed 24, 48, and 72 h and 7 days postdosing	Conjunctival effects observed in 3 rabbits that cleared by the 48 h observation period; mild, transient irritant	56
Hydrolyzed Keratin (hoof meal source)	25% solution	0.1 mL instilled into 1 eye of 6 New Zealand white rabbits; eyes assessed 24, 48, and 72 h and 4 and 7 days postdosing	Nonirritating	39
Hydrolyzed Keratin (sheep wool source)	25% solution in water diluted by 1%, 5%, 10%, 15%, and 25% v/v in saline (MW = 310 Da)	Dilutions instilled into 1 eye each of groups of 3 male domestic rabbits while other eye received saline; eyes observed immediately after instillation and at 1 and 24 h after instillation	Redness of the eye was not observed in 1% and 5% dilutions. Redness of the eye was observed immediately after instillation, but not observed at 1 and 24 h after instillation in 10%, 15%, and 25% dilutions. Considered a nonirritant at 1.25% active and a mild irritant at 2.5% active	22
Hydrolyzed Keratin (sheep wool source)	25% solution in water (MW = 320 Da)	Ocular irritation study in 6 male New Zealand white rabbits; material instilled in 1 eye and other eye served as control, eyes observed at 24, 48, and 72 h after instillation; no further details provided	Ocular irritation score = 0; nonirritating	22
Hydrolyzed Keratin (sheep wool source)	25% solution in water (MW = 320 Da)	Cumulative ocular irritation study in 6 male New Zealand white rabbits; test material instilled into 1 eye 3 times a day for 4 days; other eye served as control	Hyperemia of the iris observed in 3/6 rabbits for 4 days and in 1/6 rabbits for 3 days; hyperemia of the conjunctivae observed in 4/6 rabbits for 4 days and in 1/6 rabbits for 1 day; minimal irritant	22

(continued)

Table 9. (continued)

Ingredient	Concentration	Method	Results	Reference
Hydrolyzed Keratin (source not provided)	Neat (MW not provided)	Draize ocular irritation study in 6 New Zealand white rabbits; unrinsed eyes	Slight ocular irritant	62
Hydrolyzed Keratin (source not provided)	Neat (MW = 500 Da)	Draize ocular irritation study in 6 New Zealand white rabbits; unrinsed eyes	Minimal ocular irritant	53
Hydrolyzed Keratin (source not provided)	Neat (MW = 125,000 Da)	Draize ocular irritation study in 6 female New Zealand White rabbits; unrinsed eyes	Nonirritating	60
Hydrolyzed Keratin (source not provided)	Neat (MW = 600 Da)	Draize ocular irritation study in 6 New Zealand white rabbits; unrinsed eyes	Nonirritating	52

h = hours; HET-CAM Egg Test-Chorioallantoic Membrane; MW = molecular weight.

in aqueous solutions, but genotoxicity was observed in organic solvent formulations. However, the negative controls also displayed genotoxicity in these formulations.

No dermal irritation was predicted in in vitro studies of Hydrolyzed Keratin tested at 100% and Keratin tested at 5%. Keratin (concentration not reported) and Hydrolyzed Keratin (tested neat) were not irritating in rabbit studies. Hydrolyzed Keratin was not irritating in human studies at concentrations up to 25%, nor was Soluble Keratin at 10%.

No ocular irritation was predicted in in vitro studies of Keratin (5%), Hydrolyzed Keratin (undiluted), or Soluble Keratin (5%). Hydrolyzed Keratin was minimally to nonirritating in Draize ocular rabbit studies when tested neat.

No sensitization was predicted in in vitro studies of Hydrolyzed Keratin. No sensitization to Hydrolyzed Keratin was observed when tested up to 25% in a guinea pig maximization study or up to 5% in HRIPTs. No sensitization was observed in HRIPTs to Keratin (5%) or Soluble Keratin (10%).

Hydrolyzed Keratin in a 25% solution was not phototoxic or photosensitizing in guinea pigs.

No relevant published repeated dose toxicity, reproductive and developmental toxicity, or carcinogenicity studies on Keratin ingredients were identified in a literature search for these ingredients and no unpublished data were submitted.

Discussion

Keratin is a protein that occurs naturally in epithelial cells and is essential for normal tissue structure and function. The main sources for Keratin and Hydrolyzed Keratin are sheep wool and bovine hoof or horn. Goat wool, bird feathers, and human hair may also be used. Keratin ingredients derived from different sources are likely to have different compositions and impurities, which may result in varying compositions and impurities within a single ingredient (eg, Keratin from human hair may have some impurities that are different from Keratin obtained from bird feathers).

Pesticide residues and heavy metals may be present in Keratin source materials. The Panel stressed that the cosmetics industry should continue to use the necessary procedures to limit these impurities in the ingredients before blending into cosmetic formulations.

The Panel was also concerned about the inherent risks of using animal- and human-derived ingredients in cosmetic products, namely the potential for transmission of infectious agents. The Panel stressed that these ingredients must be free of detectible infectious pathogens (eg, BSE, HIV, and Creutzfeld-Jacob disease). Raw material suppliers and formulators of these ingredients must assure that these ingredients are free from pathogenic viruses and other infectious agents.

The Panel discussed the issue of incidental inhalation exposure in hair sprays. There were no inhalation toxicity data available. The Panel considered other pertinent data indicating that incidental inhalation exposures to keratin-derived ingredients in such cosmetic products would not cause adverse health effects, including data characterizing the potential for keratin-derived ingredients to cause ocular or dermal irritation or sensitization, and other effects. These ingredients are reportedly used at concentrations up to 0.059% in cosmetic products that may be aerosolized. The Panel noted that 95% to 99% of droplets/particles produced in cosmetic aerosols would not be respirable to any appreciable amount. The potential for inhalation toxicity is not limited to respirable droplets/particles deposited in the lungs. In principle, inhaled droplets/particles deposited in the nasopharyngeal and thoracic regions of the respiratory tract may cause toxic effects depending on their chemical and other properties. However, coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

Table 10. Dermal Sensitization Studies.

Ingredient	Concentration	Method	Results	Reference
		In vitro		
Hydrolyzed Keratin (powder product, sheep wool source)	0.39-1600 μg/mL in DMSO	ARE-Nrf2 Luciferase Test Method	Predicted to be nonsensitizing	64
Hydrolyzed Keratin (solution, sheep wool source)	0.39-1600 μg/mL in DMSO	ARE-Nrf2 Luciferase test method	Predicted to be nonsensitizing	65
Hydrolyzed Keratin (solution, goat wool source)	0.39-1600 μg/mL in DMSO	ARE-Nrf2 Luciferase test method	Predicted to be nonsensitizing	63
		Animal		
Hydrolyzed Keratin (sheep wool source)	25% solution in water (MW = 320 Da)	Guinea pig maximization test in 3 male and 3 female Harley guinea pigs	No reactions observed in animals at 24 and 48 h after challenge patch removal; nonsensitizer	22
Hydrolyzed Keratin (sheep wool source)	25% solution in water (MW = 320 Da)	35 day cumulative exposure study in 8 Hartley male guinea pigs; exposure area 2.5 cm^2 on clipped dorsal skin	Very slight erythema in 2/8 guinea pigs after day 13 and 1/8 guinea pigs after day 16; mild erythema in 2/8 guinea pigs after day 13, 1/8 guinea pigs after day 14 and 1/8 after day 15; minimally irritant	22
		Human		
Keratin (sheep wool source)	5%	HRIPT with 51 subjects; exposure site was 1 $inch^2$	No dermal irritation or sensitization	47
Hydrolyzed Keratin (source not provided)	Not reported (MW = 3000 Da)	HRIPT with 51 subjects; semiocclusive	No dermal irritation or sensitization	66
Hydrolyzed Keratin (nonspecific bovine source)	0.1% (prick test) and 5% (patch test	HRIPT with 500 patients; prick tests in 25 subjects with scalp dermatitis to the test materials (no further details provided)	No positive patch test reactions and no positive prick test reactions	67
Multiple Hydrolyzed Proteins including Hydrolyzed Keratin (source not provided)	Not reported	Sensitization study of protein hydrolysates in hair care products in 3 groups of patients. Group 1 was comprised of 11 hairdressers with hand dermatitis, group 2 was comprised of 2160 consecutive adults with suspected allergic respiratory disease, and group 3 was comprised of 28 adults with atopic dermatitis. Subjects submitted to scratch and/or prick tests.	No adverse reactions to Hydrolyzed Keratin were observed.	68
Soluble Keratin (sheep wool source)	10% active in distilled water	HRIPT with 50 subjects; occlusive; 0.2 mL applied	Nonsensitizer	27

DMSO = dimethyl sulfoxide; h = hours; HRIPT = human repeat insult patch tests; MW, molecular weight.

Conclusion

The Panel concluded the following keratin-derived ingredients are safe in cosmetics in the present practices of use and concentration as described in this safety assessment.

Hydrolyzed Keratin
Hydrolyzed Hair Keratin
Hydrolyzed Oxidized Keratin*
Hydrolyzed Sulfonated Keratin*
Keratin
Oxidized Keratin*
Soluble Keratin
Sulfonated Keratin*

*Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.

Author's Note

Unpublished sources cited in this report are available from the Director, Cosmetic Ingredient Review, 1620L Street, NW, Suite 1200, Washington, DC 20036, USA.

Author Contributions

Burnett, C. contributed to conception and design, contributed to acquisition, analysis, and interpretation, drafted manuscript, and critically revised manuscript; Bergfeld, W., Belsito, D., Hill, R., Klaassen, C., Liebler, D., Marks, J., Shank, R., Slaga, T., Snyder, P., and Gill, L. contributed to conception and design, contributed to analysis and interpretation, and critically revised manuscript; Heldreth, B. contributed to design, contributed to analysis and interpretation, and critically revised manuscript. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The articles in this supplement were sponsored by the Cosmetic Ingredient Review. The Cosmetic Ingredient Review is financially supported by the Personal Care Products Council.

References

1. Nikitakis J, Lange B. *International Cosmetic Ingredient Dictionary and Handbook*. 16th ed. Personal Care Products Council; 2016.
2. Elder RL. Final report on the safety assessment of hydrolyzed collagen. *Am Coll Toxicol*. 1985;4(5):199-221.
3. Andersen FA, Bergfeld WF, Belsito DV, et al. Final report of the safety assessment of cosmetic ingredients derived from zea mays (Corn). *Int J Toxicol*. 2011;30(Suppl 1):17S-39S.
4. Andersen FA, ed. Annual review of cosmetic ingredient safety assessments - 2004/2005. *Int J Toxicol*. 2006;25(Suppl 2):1-89.
5. Andersen FA, ed. Amended Final Report on the Safety Assessment of Oryza Sativa (Rice) Bran Oil, Oryza Sativa (Rice) Germ Oil, Rice Bran Acid, Oryza Sativa (Rice) Bran Wax, Hydrogenated Rice Bran Wax, Oryza Sativa (Rice) Bran Extract, Oryza Sativa (Rice) Extract, Oryza Sativa (Rice) Germ Powder, Oryza Sativa (Rice) Starch, Oryza Sativa (Rice) Bran, Hydrolyzed Rice Bran Extract, Hydrolyzed Rice Bran Protein, Hydrolyzed Rice Extract, and Hydrolyzed Rice Protein. *Int J Toxicol*. 2006; 25(Suppl 2):91-120.
6. Johnson WJ, Bergfeld WF, Belsito DV, et al. *Safety Assessment of Silk Protein Ingredients as Used in Cosmetics*. Cosmetic Ingredient Review; 2015.
7. Burnett CL, Heldreth B, Belsito DV, et al. *Safety Assessment of Soy Proteins and Peptides as Used in Cosmetics*. Cosmetic Ingredient Review; 2015.
8. Burnett CL, Heldreth B, Boyer I, et al. *Safety Assessment of Hydrolyzed Wheat Protein and Hydrolyzed Wheat Gluten as Used in Cosmetics*. Cosmetic Ingredient Review; 2014.
9. Chahal S. Letter from Dr. Surinder Chahal concerning the CJR review of Keratin and Hydrolyzed Keratin. Unpublished data submitted by Personal Care Products Council; 2016.
10. Rouse JG, Van Dyke ME. A review of keratin-based biomaterials for biomedical applications. *Materials*. 2010;3(2):999-1014.
11. Tonin C, Aluigi A, Varesano A, Vineis C. *Keratin-Based Nanofibres*. Kumar A.InTech; 2010:139-158.
12. O'Neil MJ, ed. The merck index. 15th ed. The Royal Society of Chemistry; 2013.
13. Hill P, Brantley H, Van Dyke M. Some properties of keratin biomaterials: Kerateines. *Biomaterials*. 2010;31(4):585-593.
14. Personal Care Products Council. *Information on Hydrolyzed Keratin, Hydrolyzed Silk Protein, Hydrolyzed Elastin, Hydrolyzed Soy Protein, Hydrolyzed Milk Protein and Hydrolyzed Vegetable Protein*. Unpublished data submitted by Personal Care Products Council; 7-3-2012:25.
15. Personal Care Products Council. *Molecular Weights and Hydrolysis Method: Hydrolyzed Brazil Nut Protein, Hydrolyzed Hair Keratin, Hydrolyzed Keratin and Hydrolyzed Sweet Almond Protein*. Unpublished data submitted by the Personal Care Products Council; 2012.
16. Personal Care Products Council. *Method of Manufacture and Molecular Weights: Hydrolyzed Protein Ingredients*. Unpublished data submitted by Personal Care Products Council; 5-14-2012:8.
17. Wortmann FJ, Sendelbach G, Popescu C. Fundamental DSC investigations of α-keratinous materials as basis for the interpretation of specific effects of chemical, cosmetic treatments on human hair. *J Cosmet Sci*. 2007;58(4):311-317.
18. Vasconcelos A, Freddi G, Cavaco-Paulo A. Biodegradable materials based on silk fibroin and keratin. *Biomacromolecules*. 2008;9(4):1299-1305.
19. Chan WW. A method for the complete S sulfonation of cysteine residues in proteins. *Biochemistry*. 1968;7(12):4247-4254.
20. Mohd Amir SNK, Gupta A, Tuan Chik SMS, Ahmad K. Development of anti-ageing cream from chicken feathers. *Res J Pharm Biol Chem Sci*. 2014;5(6):389-393.
21. Arch Personal Care Products LP. *Hydrokeratin AL-30 (Hydrolyzed Keratin) Manufacturing Procedure*. Unpublished data submitted by Personal Care Products Council; 2012:8.
22. Personal Care Products Council. *Hydrolyzed Keratin*. Unpublished data submitted by Personal Care Products Council; 1-28-2016.
23. Active Concepts. *Certificate of Origin (AC Keratin Hydrolysate SD - Powder Hydrolyzed Keratin from Sheep Wool)*. Unpublished data submitted by Personal Care Products Council; 2014.
24. Active Concepts. *Certificate of Origin (AC Keratin Hydrolysate 30- Solution Hydrolyzed Keratin from Sheep Wool)*. Unpublished data submitted by Personal Care Products Council; 2015.
25. Active Concepts. *Certificate of Origin (AC Cashmere Hydrolysate - solution Hydrolyzed Keratin from Goat Wool)*. Unpublished data submitted by Personal Care Products Council; 2015.

26. Anonymous. *Summaries of Dermal and Ocular Irritation Tests of Hydrolyzed Protein Ingredients (Including Proteins Hydrolyzed to Amino Acids)*. Unpublished data submitted by Personal Care Products Council; 2012:4.
27. Personal Care Products Council. *Soluble Keratin*. Unpublished data submitted by Personal Care Products Council; 2016.
28. Strnad P, Usachov V, Debes C, Gräter F, Parry DAD, Omary MB. Unique amino acid signatures that are evolutionarily conserved distinguish simple-type, epidermal and hair keratins. *J Cell Sci*. 2011;124(24):4221-4232.
29. Food and Drug Administration (FDA). *Frequency of use of Cosmetic Ingredients*. *FDA Database.* FDA; 2016.
30. Bremmer HJ, Prud'homme de Lodder LCH, Engelen JGM. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. Report No. RIVM 320104001/ 2006; 2006:1-77.
31. Rothe H, Fautz R, Gerber E, et al. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104.
32. Rothe H. *Special Aspects of Cosmetic Spray Evalulation*. Unpublished data presented at the 26 September CIR Expert Panel meeting. Washington, DC; 9-26-2011.
33. Johnsen MA. The Influence of Particle Size. *Spray Technology Marketing*. 2004;14(11):24-27.
34. European Union. Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. 2009. Internet site Accessed September 13, 2013. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF
35. Scientific Committee on Consumer Products (SCCP). Opinion on amino acids obtained by hydrolysis of human hair. 2005. Accessed 2-4-2016. Report No. SCCP/0894/05; http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_005.pdf
36. Singaravelu S, Ramanathan G Raja MD, Barge S, Sivagnanam UT. Preparation and characterization of keratin-based biosheet from bovine horn waste as wound dressing material. *Materi Lett*. 2015;152:90-93.
37. Noda J, Imai T, Kida K, Otagiri M. The physicochemical and biopharmaceutical properties of fragmented keratin as a new drug carrier. *Biol Pham Bull*. 1996;19(3):466-473.
38. Barba C, Mendez S, Roddick-Lanzilotta A, Kelly R, Parra JL, COderech I. Wool peptide derivatives for hand care. *J Cosmet Sci*. 2007;58(2):99-107.
39. Consumer Product Testing Co. *Primary Dermal Irritation in Rabbits; Primary Ocular Irritation in Rabbits; Acute Oral Toxicity in Rats (25% Hydrolyzed Keratin from Hoof Meal)*. Unpublished data submitted by the Personal Care Products Council; 1983.
40. Food and Drug Research Laboratories, Inc. *Acute Oral Toxicity in Rats (20% Hydrolyzed Keratin from Hoof Meal)*. Unpublished data from the Personal Care Products Council; 1976.
41. Toxicol Laboratories Limited. *Single dose Oral Toxicity Study in the Rat (20% Hydrolyzed Keratin from Hoof Meal)*. Unpublished data submitted by the Personal Care Products Council; 1986.
42. Fernandes MM, Lima CF, Loureiro A, Gomes AC, Cavaco-Paulo A. Keratin-based peptide: Biological evaluation and strengthening properties on relaxed hair. *Int J Cosmet Sci*. 2012;34(4):338-346.
43. BioReliance. *Bacterial Reverse Mutation Assay Hydrolyzed Keratin (MW - 3,000 Da). BioReliance Study Number AA88DT.502.BTL*. Unpublished data submitted by Personal Care Products Council; 2004:15.
44. Active Concepts. *Bacterial Reverse Mutation Test (AC Cashmere Hydrolysate- Solution Hydrolyzed Keratin from Goat Wool)*. Unpublished data submitted by Personal Care Products Council; 2016.
45. Active Concepts. *Bacterial Reverse Mutation Test (AC Keratin Hydrolysate 30 - Solution Hydrolyzed Keratin from Sheep Wool)*. Unpublished data submitted by Personal Care Products Council; 2016.
46. Active Concepts. *Bacterial Reverse Mutation Test (AC Keratin Hydrolysate SD- Powder Hydrolyzed Keratin from Sheep Wool)*. Unpublished data submitted by Personal Care Products Council; 2016.
47. Anonymous. *Summary of Keratin Safety Studies*. Unpublished data submitted by Personal Care Products Council; 2016.
48. Toxicol Laboratories Limited. *Primary Skin Irritation Study: Hydrolyzed Keratin (MW - 125,000 Da) Study Ref. No. 63/8601*. Unpublished data submitted by Personal Care Products Council; 1986.
49. Active Concepts. *AC Cashmere Hydrolysate Irritation Analysis (AC Cashmere Hydrolysate - solution Hydrolyzed Keratin from Goat Wool)*. Unpublished data submitted by Personal Care Products Council; 2007.
50. Active Concepts. *Dermal and Ocular Irritation Tests (AC Keratin Hydrolysate 30 - Solution Hydrolyzed Keratin from Sheep Wool)*. Unpublished data submitted by Personal Care Products Council; 2015.
51. Active Concepts. *Dermal and Ocular Irritation Tests (AC Keratin Hydrolysate SD - Powder Hydrolyzed Keratin from Sheep Wool)*. Unpublished data submitted by Personal Care Products Council; 2012.
52. Consumer Product Testing Co. Primary dermal irritation in rabbits; primary ocular irritation in rabbits, acute oral toxicity in rats: Hydrolyzed Keratin (MW ~ 600 Da). Experiment Reference No: 81103-S. Unpublished data submitted by Personal Care Products Council; 1981.
53. Consumer Product Testing Co. Primary dermal irritation in rabbits; primary ocular irritation in rabbits; acute oral toxicity in rabbits: Hydrolyzed Keratin (MW ~ 500 Da) Experiment Reference No. 84229 -1. Unpublished data submitted by Personal Care Products Council; 1984.
54. Consumer Product Testing Co. The MatTek Corporation EpiDerm™ skin model *in vitro* toxicity testing system: Hydrolyzed Keratin (MW ~ 3,000 Da). Experiment Reference Number: V04-0032 -1. Unpublished data submitted by Personal Care Products Council; 2004.
55. Toxicol Laboratories Limited. *Primary Skin Irritation Study (20% Hydrolyzed Keratin from Hoof Meal)*. Unpublished data submitted by the Personal Care Products Council; 1986.

56. Food and Drug Research Laboratories, Inc. *Rabbit Eye Irritation Study (20% Hydrolyzed Keratin from Hoof Meal)*. Unpublished data submitted by the Personal Care Products Council; 1976.
57. Anonymous. *Summary: Study of Primary Cutaneous Irritation of a Pharmaceutical or Cosmetic Compound in the Male Rabbit*. Unpublished data submitted by Personal Care Products Council; 2001.
58. Food and Drug Research Laboratories, Inc. *Primary Skin Irritation study with Rabbits (20% Hydrolyzed Keratin from Hoof Meal)*. Unpublished data submitted by the Personal Care Products Council; 1976.
59. Consumer Product Testing Co. The Hen's egg test – utilizing the chorioallantoic membrane (HET-CAM): Hydrolyzed Keratin (MW - 3,000 Da) Experiment Reference Number: V04-0033 -1. Unpublished data submitted by Personal Care Products Council; 2004.
60. Toxicol Laboratories Limited. Eye irritation study: Hydrolyzed Keratin (MW - 125,000 Da) Study Ref. No. 64/8601. Unpublished data submitted by Personal Care Products Council; 1986.
61. Toxicol Laboratories Limited. Eye irritation study (20% Hydrolyzed Keratin from hoof meal). Unpublished data submitted by the Personal Care Products Council; 1986.
62. Anonymous. Study of ocular irritation of a pharmaceutical or cosmetic compound in the male rabbit. Unpublished data submitted by Personal Care Products Council; 2001.
63. Active Concepts. OECD TO 4420: In vitro skin sensitization (AC Cashmere Hydrolysate - solution Hydrolyzed Keratin from goat wool). Unpublished data submitted by Personal Care Products Council; 2015.
64. Active Concepts. OECD TO 442D: In vitro skin sensitization (AC Keratin Hydrolysate SD - powder Hydrolyzed Keratin from sheep wool). Unpublished data submitted by Personal Care Products Council; 2015.
65. Active Concepts. OECO TG 4420: In vitro skin sensitization (AC Keratin Hydrolysate 30 - solution Hydrolyzed Keratin from sheep wool). Unpublished data submitted by Personal Care Products Council; 2015.
66. Consumer Product Testing Co. Repeated insult patch test: Hydrolyzed Keratin (MW ~ 3,000 Da). Experiment Reference Number: C04-0181.01. Unpublished data submitted by Personal Care Products Council; 2004.
67. McFadden JP, Rycroft RJG, White IR, Wakelin SH, Basketter DA. Hydrolyzed protein shampoo additives are not a common contact allergen. *Contact Dermatitis*. 2000;43(4):243-243.
68. Niinimaki A, Niinimaki M, Makinen-Kiljunen S, Hannuksela M. Contact urticaria from protein hydrolysates in hair conditioners. *Allergy*. 1998;53(11):1078-1082.
69. Freeman S, Lee MS. Contact urticaria to hair conditioner. *Contact Dermatitis*. 1996;35(3):195-196.
70. Active Concepts. Product specification (AC Keratin Hydrolysate SD- powder Hydrolyzed Keratin from sheep wool). Unpublished data submitted by Personal Care Products Council; 2014.
71. Active Concepts. Product specification (AC Keratin Hydrolysate 30- solution Hydrolyzed Keratin from sheep wool). Unpublished data submitted by Personal Care Products Council; 2015.
72. Active Concepts. Product specification (AC Cashmere Hydrolysate- solution Hydrolyzed Keratin from goat wool). Unpublished data submitted by Personal Care Products Council; 2015.
73. Arnaud JC, Bore P. Ion-exchange chromatography for the analysis of protein derivatives and specific amino acids. *Cosmet Sci Tech*. 1985;4:223-244.
74. Personal Care Products Council. Concentration of Use by FDA Product Category: Hydrolyzed Keratin and other Keratin Ingredients. Unpublished data submitted by Personal Care Products Council; 4-9-2015.