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

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date to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a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

The 2015 Cosmetic Ingredient Review Expert Panel members are: Chairman, 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, DPA. This safety assessment was prepared by Christina L. Burnett, Scientific Analyst/Writer and Bart Heldreth, Ph.D., Chemist CIR.

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INTRODUCTION

The keratin and keratin-derived ingredients detailed in this report function mainly as skin and hair conditioning agents in personal care products. This report assesses the safety of the following 8 keratin ingredients:

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

This is the first CIR report on ingredients derived from the keratin family of proteins. However, the safety of several hydrolyzed proteins as used in cosmetics has previously been reviewed by the Panel. The Panel concluded that hydrolyzed collagen, hydrolyzed soy, hydrolyzed silk, hydrolyzed rice protein, and hydrolyzed corn protein are safe for use in cosmetics.[1-6] 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 MW of 3500 Da or less.[7]

Keratin occurs naturally in epithelial cells and is essential for normal tissue structure and function. Many of the reports found in the published literature presented efficacy studies of other cosmetic or pharmaceutical ingredients in which the effects of the ingredients on naturally-occurring keratin in skin, hair, or other tissues were evaluated. These studies were not relevant for assessing the safety of the keratin ingredients included in this assessment.

These ingredients may be derived from many different sources, such as human hair, bird feathers, and sheep's wool. These differing sources could potentially have unique compositions and impurities, which are currently not well-defined in this scientific literature review.

CHEMISTRY

Definition

The definition, structures, and functions of the keratin 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 and form the bulk of cytoplasmic epithelia and epidermal appendage structures (i.e., hair, wool, horn, nails and similar tissues in animals).[8] These keratins can be classified into two 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. Cosmetic ingredients utilize hard keratins (derived from hair, wool, horn, nails or other similar tissues in animals), which may be modified/extracted (i.e., hydrolyzed, oxidized, sulfonated, etc.) into other cosmetic ingredients. Such keratin proteins contain a much higher content of cysteine residues (than the "soft," cytoplasmic epithelial keratins) in their non-helical domains and thus form tougher and more durable structures via intra/intermolecular disulfide bond formation. The structural subunits of these keratins comprise two chains, which differ by molecular weight and sequence (designated types I and II), that each contain non-helical end terminal domains, and a highly-conserved, central α-helical domain.

Keratins are rather difficult to solubilize, even when compared to other proteins. Aside from mechanical means, such as grinding of materials such as animal horn, one of the first processes for extracting keratins involved rather harsh conditions using lime. Eventually, a number of oxidative and reductive methods were developed for extracting keratin. Many of the ingredients in this assessment involve extraction steps such as hydrolysis by acid or enzyme.[9]

Chemical and Physical Properties

Keratin is insoluble in water, and many keratin-based materials have molecular weights between 9-60 kDa.[10,11] Keratin proteins extracted from hair are classified into 3 broad groups: alpha-, beta-, and gamma-.[12] α-Keratin (found in hair fiber cortex) is low in sulfur content and has an average molecular weight of 60-80 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 molecular weight of ~15 kDa.

Molecular weights for hydrolyzed hair keratin are reported to be around 400 Da.[13] The molecular weights for hydrolyzed keratin have been reported to be as low as 150 Da, but may be around 1000 to 3000 Da.[13-15]

Method of Manufacturing

Keratin

Keratin may be produced by non-chemical and chemical methods. For the non-chemical methods, keratin may be dissolved and converted from wool via steam explosion or from feathers via superheated water.[10] Under conditions of steam explosion, wool is cooked at a high temperature (with steam ~220ºC) for several minutes (~10 min) and then followed by explosive decompression. The wool is disrupted into solid and liquid phases consisting of oligopeptides, water soluble peptides, and free amino acids. Under conditions of superheated water, feather barbs are treated with superheated water (liquid ~220ºC) for 2 h and then cooled. The result is oligopeptides with a molecular weight of 1.0 to 1.8 kDa.

For the chemical methods, wool keratin is extracted from reduction or oxidation of disulfide bonds. 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 the hydrogen bonds. Resultant keratins may have low or high sulfur content (low sulfur molecular weights between 45-60 kDa, high sulfur molecular weights between 11-28 kDa), or high glycine and tyrosine content (modular weight between 9-12 kDa).For example, it has been reported that keratin may be extracted from chicken feathers with reducing agents.[16]

Hydrolyzed Keratin

Hydrolyzed keratin may be prepared from sheep's wool.[17] 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 4-6 hours. When the target molecular weight is reached, the pH is adjusted to neutralize the enzyme. The resultant solution is a mixture of hydrolyzed keratin fractions with a molecular weight of ~ 1000 Da. The solution may be diluted to produce a 30% active material.

Hydrolyzed Keratin and Hydrolyzed Hair Keratin

A supplier reported that hydrolyzed hair keratin and hydrolyzed keratin are obtained through acid hydrolysis.[14] 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).[18] The resultant hydrolyzed proteins have molecular weights in the 2000-4000 Da range and all contain di- and tri-peptides.

Impurities/Composition

Keratins ubiquitously consist of central α-helical rod domains that are flanked by non-α-helical head and tail domains.[19] However, the amino sequences are not highly conserved across various source species (e.g., equine versus human), or even among tissue-specific function-types within one species (e.g., hair versus nail versus skin). Indeed, the differences in amino acid sequences between various keratins can be rather striking (e.g., many cysteine residues in hair keratins versus a very small number in epidermal keratins, to no cysteine residues in other types). Accordingly, the species and tissue-specific function-types of the keratin source could significantly impact the composition of a resulting cosmetic ingredient. While the sequences of some keratin proteins have been elucidated, with a definition like "the protein derived from hair, wool, horn, nails or other similar tissues in animals," the composition of a keratin-derived ingredient is virtually unknowable, without submission from raw material suppliers.

As with most non-synthetic raw materials, numerous environmental factors may profoundly affect the presence and concentration of impurities therein, dependent on the keratin source. Accordingly, manufactures should utilize best practices to ensure the lowest possible values of any impurities.

Keratin

Cysteine residues in keratin protein molecules make up 7% to 20% of the total amino acid residues.[10]

Hydrolyzed Keratin

Amino acid composition data on hydrolyzed keratin is described in Table 2.

USE

Cosmetic

The safety of the cosmetic ingredients included in this safety assessment is evaluated on the basis of the expected use in cosmetics. The data received from the FDA are those it collects from manufacturers on the use of individual ingredients in cosmetics by cosmetic product category in its Voluntary Cosmetic Registration Program (VCRP), and those from the cosmetic industry are submitted in response to a survey of the maximum reported use concentrations by category conducted by the Personal Care Products Council (Council).

According to the 2015 VCRP data, hydrolyzed keratin has the most reported uses of the ingredients listed in this safety assessment in cosmetic products, with a total of 573; more than half of the uses are in rinse-off non-coloring hair products (Table 3).[20] 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.[21-24] 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.[21,23]

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).[25] Hydrolyzed hair keratin is a substance prohibited in cosmetic products in the E.U. due to its human origin.

Non-Cosmetic

Non-cosmetic 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.[8,10-12,26,27]

TOXICOKINETICS

Absorption, Distribution, Metabolism, Excretion

Keratin

The tissue distribution of enzymatically and chemically fragmented keratin (MW ~ 8000 and 33,000, respectively) was studied in male ddY mice.[27] The radiolabeled test materials were 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 keratins were 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.[28] No further details regarding the penetration properties of the hydrolyzed keratin were provided.

TOXICOLOGICAL STUDIES

Acute Toxicity

Intravenous – Non-Human

Keratin

In an acute toxicity study, enzymatically and chemically fragmented keratin (MW ~ 8000 and 33,000, respectively) were administered intravenously to male ddY mice at doses of 300 mg/kg.[27] The test materials were dissolved in 1/15 M sodium phosphate buffer, which also was the control material. Body weight of the mice was measured every 2 d for 14 d. The mice were killed after 14 d, and their lungs, livers, kidneys, and spleens underwent histopathological examination. Body weight changes were similar to those of the control group. No significant changes were observed in the examined organs and tissues. The authors concluded that toxicity to fragmented keratins was significantly low.

Repeated 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

Keratin

The potential of keratin peptide (MW ~1600) to induce genetic toxicity was studied in a comet assay using human fibroblasts.[29] The keratin peptides were prepared in an aqueous formulation with 100% phosphate buffer or in an organic solvent formulation with 10% ethanol, 1.5% propylene glycol, 0.5% benzyl alcohol, and 88% phosphate buffer. Concentrations tested ranged from 0.025 g/L to 0.5 g/L. Positive and negative controls were utilized. The cells were incubated with the test materials and controls for either 1 h or 72 h. In the aqueous formulations, keratin peptide was not genotoxic at any concentration tested for either time of exposure. In the organic solvent formulations 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. The positive controls yielded expected results. The authors concluded that keratin peptide was not genotoxic in aqueous formulation.

Hydrolyzed Keratin

The potential of hydrolyzed keratin (MW = 3000; 14% peptide content) to induce gene mutation was studied in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 and in *Escherichia coli* strain WP2 uvrA using the reverse mutation assay.[30] The assay was performed with and without S9 metabolic activation at concentrations up to 5000 μg/plate. No positive mutagenic responses were observed with or without S9. It was concluded that hydrolyzed keratin was not mutagenic with or without metabolic activation.

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

Non-human and human dermal irritation studies are presented in Table 4.[16,18,28,31-34] Hydrolyzed keratin was non-irritating when tested at 100% in in vitro studies. Keratin (concentration not reported) and hydrolyzed keratin (tested neat) were not irritating in rabbit studies. No adverse effects were reported in an efficacy study of hydrolyzed keratin in a hand cream at 3%.

Ocular Irritation

Non-human ocular irritation studies are presented in Table 5.[18,31,32,35,36] No ocular irritation to hydrolyzed keratin was observed in in vitro studies at concentrations up to 5% and slight irritation potential was observed at 10%. Hydrolyzed keratin was minimally to non-irritating in Draize rabbit studies when tested neat.

Dermal Sensitization

Human dermal sensitization studies are presented in Table 6.[37-39] No sensitization to hydrolyzed keratin was observed when tested up to 5% in a patch studies.

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.[40] Prick testing elicited a strong positive (10 mm) wheal-and-flare response to the hair conditioner, which contained steartrimonium hydrolyzed animal protein. Further 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. Patch tests using the European standard series and a series of 15 common bases of medicines and cosmetics were negative.

SUMMARY

The keratin and 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 the 2015 VCRP data, hydrolyzed keratin has the most reported uses of the ingredients listed in this safety assessment in cosmetic formulations, with a total of 573; more than half of the uses are in rinse-off non-coloring hair formulations. 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 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.

Non-cosmetic 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 keratins were 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.

In an acute intravenous toxicity study of enzymatically and chemically fragmented keratin in mice, body weight changes were similar to those of the control group and no significant changes were observed in the examined organs and tissues.

Keratin peptide in aqueous formulation was not genotoxic in a comet assay. Hydrolyzed keratin was not mutagenic with or without metabolic activation at concentrations up to 5000 μg/plate.

Hydrolyzed keratin was non-irritating when tested at 100% in in vitro studies. Keratin (concentration not reported) and hydrolyzed keratin (tested neat) were not irritating in rabbit studies. No adverse effects were reported in an efficacy study of hydrolyzed keratin in a hand cream at 3%.

No ocular irritation to hydrolyzed keratin was observed in in vitro studies at concentrations up to 5% and slight irritation potential was observed at 10%. Hydrolyzed keratin was minimally to non-irritating in Draize rabbit studies when tested neat.

No sensitization to hydrolyzed keratin was observed when tested up to 5% in a patch studies.

An allergic reaction has been reported to a hair conditioner containing hydrolyzed keratin.

No relevant published 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.

DATA NEEDS

CIR is seeking types and concentrations of impurities and/or general composition of keratin and keratin-derived ingredients, particularly in relation to the different sources that these ingredients may be derived. Information on dermal penetration potential, and any additional toxicological data, especially for these ingredients in formulation at use concentration, would help the CIR Expert Panel assess the safety of the use of these ingredients in cosmetics and would improve the resulting safety assessment.

TABLES

Table 1. Definitions, Structures, and functions of the ingredients in this safety assessment.[41]

Ingredient/CAS No.	Definition & 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

Table 2. Amino acid composition of commercialized keratin hydrolysate (g/100 g protein).[42]

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

Table 3. Frequency and concentration of use according to duration and type of exposure for keratin ingredients.[20,43]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Keratin		**Hydrolyzed Hair Keratin**	
Totals†	**90**	**0.075**	**28**	**NR**
Duration of Use				
Leave-On	44	0.075	3	NR
Rinse Off	46	NR	25	NR
Diluted for (Bath) Use	NR	NR	NR	NR
Exposure Type				
Eye Area	6	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	2	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	81	0.075	28	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**	
Totals†	**573**	**0.000034-5**	**1**	**NR**
Duration of Use				
Leave-On	216	0.000034-5	NR	NR
Rinse Off	356	0.0001-0.88	1	NR
Diluted for (Bath) Use	1	<0.01	NR	NR
Exposure Type				
Eye Area	34	0.001-0.2	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation –Sprays	16; 117[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	37	0.001-0.21	NR	NR
Deodorant (underarm)	1[a]	NR	NR	NR
Hair - Non-Coloring	335	0.000034-5	1	NR
Hair-Coloring	164	0.0001-0.5	NR	NR
Nail	8	0.002-0.04	NR	NR
Mucous Membrane	9	0.01-0.028	NR	NR
Baby Products	NR	NR	NR	NR

NR = Not reported.

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

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

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

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

Table 4. Dermal irritation studies.

Ingredient	Concentration	Method	Results	Reference
		Non-Human – In Vitro		
Hydrolyzed Keratin	100% (MW = 3000)	EpiDerm skin model	Non-irritating	[33]
Hydrolyzed Keratin	2 forms tested (MW = 2000-4000), solution concentration not reported, powder undiluted	MatTek EpiDerm assay	Non-irritating	[18]
		Non-Human – In Vivo		
Keratin	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	[16]
Hydrolyzed Keratin	Neat (MW = 500)	Draize primary dermal irritation study in 6 New Zealand white rabbits; occluded for 24 h	PII = 2.15. Not a primary irritant.	[32]
Hydrolyzed Keratin	Neat (MW = 125,000)	Draize primary dermal irritation study in 6 female New Zealand White rabbits; occluded for 24 h	PII = 2.0. Not a primary irritant.	[34]
Hydrolyzed Keratin	Neat (MW = 600)	Draize primary dermal irritation study in 6 New Zealand white rabbits; occluded for 24 h	PII = 0.0. Not a primary irritant.	[31]
		Human – In Vivo		
Hydrolyzed Keratin in a hand cream, enzymatically hydrolyzed	3% (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	No adverse effects	[28]

Table 5. Ocular irritation studies.

Ingredient	Concentration	Method	Results	Reference
		Non-Human – In Vitro		
Hydrolyzed Keratin	1%, 5% and 10% (MW = 3000)	HET-CAM method	Practically no irritation potential at 1% and 5%. Slight irritation potential at 10%.	[35]
Hydrolyzed Keratin	2 forms tested (MW = 2000-4000), solution concentration not reported, powder undiluted	MatTek EpiOcular assay	Non-irritating	[18]
		Non-Human – In-Vivo		
Hydrolyzed Keratin	Neat (MW = 500)	Draize ocular irritation study in 6 New Zealand white rabbits; unrinsed eyes	Minimal ocular irritant	[32]
Hydrolyzed Keratin	Neat (MW = 125,000)	Draize ocular irritation study in 6 female New Zealand White rabbits; ;unrinsed eyes	Non-irritating	[36]
Hydrolyzed Keratin	Neat (MW = 600)	Draize ocular irritation study in 6 New Zealand white rabbits; unrinsed eyes	Non-irritating	[31]

Table 6. Dermal sensitization studies.

Ingredient	Concentration	Method	Results	Reference
		Human		
Hydrolyzed Keratin	Not reported (MW = 3000)	HRIPT with 51 subjects; semi-occlusive	No dermal irritation or sensitization	[37]
Hydrolyzed Keratin	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	[38]
Multiple Hydrolyzed Proteins including Hydrolyzed Keratin	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, group2 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.	[39]

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