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Final Report on the Safety Assessment of Hydrolyzed Collagen

Hydrolyzed Collagen is a hydrolysate derived from animal byproducts. It is used in cosmetic products as a conditioner or moisturizer at concentrations less than 5 percent.

Hydrolyzed Collagen was practically nontoxic when administered orally or dermally in acute animal toxicity studies. This ingredient was minimally irritating to rabbit eyes when tested full-strength. Primary skin irritation tests in rabbits indicated that Hydrolyzed Collagen was nonirritating or minimally irritating when tested full-strength. Subchronic dermal studies on 2 cosmetic formulations containing 2 percent Hydrolyzed Collagen were negative for systemic toxicity. Hydrolyzed Collagen was nonsensitizing in guinea pigs.

In clinical studies, Hydrolyzed Collagen produced no skin irritation, sensitization, or indication of phototoxicity.

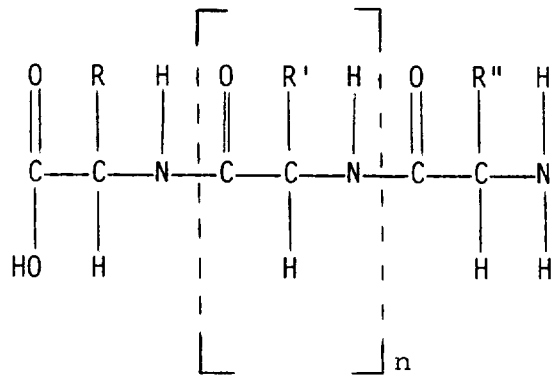
On the basis of the available animal and clinical data, it is concluded that Hydrolyzed Collagen is safe as a cosmetic ingredient in the present practices of use and concentration.

INTRODUCTION

This report presents the available information published between 1940 and the present and the unpublished cosmetic industry data on Hydrolyzed Collagen (formerly Hydrolyzed Animal Protein).

CHEMICAL AND PHYSICAL PROPERTIES

Hydrolyzed Collagen is defined as a collagen hydrolysate derived by acid, enzyme, or other method of hydrolysis.[1] It is a polypeptide of molecular weight 1000 to 10,000 with the following generalized structure:[2]



The typical amino acid composition is given in Table 1 along with the corresponding structures of R, R′, and R″.

Hydrolyzed Collagen is an off-white to white hygroscopic powder. It is also available as a viscous, amber aqueous solution.[2] The physicochemical properties of Hydrolyzed Collagen and its solution are given in Table 2.

Hydrolyzed Collagen can be prepared by any 1 of 3 methods: alkaline hydrolysis of bovine skin products followed by enzymatic hydrolysis to the desired molecular weight, enzymatic hydrolysis of fresh animal byproducts or bovine-derived leather, or acid or alkaline hydrolysis of chrome leather fold splinters with inorganic acids or lyes to a defined molecular weight. The hydrolysate produced by the latter method is purified in an aqueous solution and then by precipitation and filtration to effectively remove the heavy ions.[2] Acid hydrolysis tends to split the polypeptide bond between proline (or hydroxyproline) and other amino acids, with very little specificity for which amino acid donates its amino group to the peptide bond.[3]

The spectrum of amino acids resulting from the hydrolysis of collagen differs substantially from that of other proteins by its high content of glycine and proline and low content of histidine, tryptophan, and cystine (Table 1). Collagen also contains 2 amino acids, hydroxyproline and hydroxylysine, not found in other proteins.[4]

Hydrolyzed Collagen is analyzed primarily by column chromatography. The literature cites numerous chromatographic methods.[5-9] Its solution can be positively identified by comparison to a standard infrared spectrum.[10] The 2 amino acids found only in collagen, hydroxyproline and hydroxylysine, allow for differentiation between collagen hydrolysates and other protein hydrolysates.[4]

Hydrolyzed Collagen is stable under the conditions of normal cosmetic use. However, the addition of perfumes high in aldehyde content may cause color reactions and odor changes due to the reaction of active carbonyl groups with the amino group of the hydrolysate.[2,11] Burnett[11] has found that cosmetics containing proteins are better preserved at successively lower pH values, whereas Frech et al.[12] found that sodium acetate is an effective preservative in protein hydrolysate solutions. Hydrolyzed Collagen has shown a 15 to 45 percent reduction in viscosity after storage for 1 month at room temperature.[13]

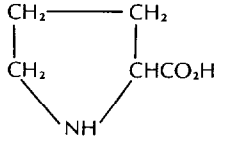
TABLE 1. Typical Amino Acid Composition of Hydrolyzed Collagen[2]

Amino Acid	*R, R', or R''*	*Typical Composition* (%)*
Glycine	$-H$	20.0–30.5
Alanine	$-CH_3$	8.0–11.0
Serine	$-CH_2OH$	2.9–4.1
Threonine	$-CHOHCH_3$	1.8–2.6
Proline	CYCLIC†	13.7–18.0
Hydroxyproline	CYCLIC†	12.1–14.5
Valine	$-CHCH_3CH_3$	2.1–3.4
Isoleucine	$-CHCH_3CH_2CH_3$	1.3–1.8
Leucine	$-CH_2CHCH_3CH_3$	2.8–3.5
Phenylalanine	$-CH_2C_6H_5$	1.1–2.6
Tyrosine	$-CH_2C_6H_4OH$	0.2–1.0
Cystine/cysteine	$-$†$/-CH_2-SH$	0.0–0.9
Methionine	$-CH_2CH_2SCH_3$	0.7–0.9
Aspartic acid	$-CH_2CO_2H$	5.7–9.0
Glutamic acid	$-CH_2CH_2CO_2H$	10.0–11.7
Arginine	$-CH_2CH_2CH_2C(NH)_2NH_2$	7.8–9.0
Histidine	$NHCHNCHCCH_2-$	0.7–1.0
Lysine	$-CH_2CH_2CH_2CH_2NH_2$	3.9–5.2
Hydroxylysine	$-(CH_2)_2CHOHCH_2NH_2$	0.7–1.2

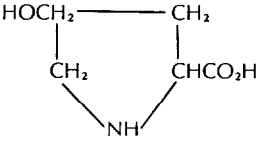
*Prepared by alkaline hydrolysis of bovine skin products to form a food-grade gelatin, followed by enzyme hydrolysis to the desired molecular weight.

†These do not conform to the generalized formula.

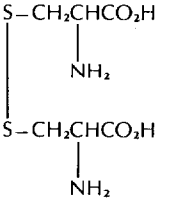
Proline:



Hydroxyproline:



Cystine:



USE

Cosmetic Use

Hydrolyzed Collagen is used in cosmetics, primarily hair and skin care products, because of its conditioning and moisturizing properties. It is generally used

TABLE 2. Physicochemical Properties of Hydrolyzed Collagen[2]

Property	*Value*	
	Powder	*Solution*
Molecular weight	1000 to 10,000	–
Solids content (105°C/16 hours)	–	35% min.
Moisture content (vacuum oven 90°C/6 hours)	8.0% max.	–
pH	–	4.0–6.5 (10% aqueous solution)
Nitrogen	12.0% min.	8.0% min.
Ash content	12.0% max.	5.0% max.
Iron	–	3 ppm max.
Heavy metals	–	25 ppm max.

at concentrations <5 percent in the following product categories: baby shampoos, bath, eye makeup, hair, hair coloring, makeup, manicuring, personal cleanliness, shaving, skin care, and tanning preparations.[2,13–16]

Table 3 presents the FDA product formulation data for Hydrolyzed Collagen.[15] The cosmetic product formulation computer printout that is made available by the Food and Drug Administration (FDA) is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations. Ingredients are listed in prescribed concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at <100 percent concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a 2- to 10-fold error in the assumed ingredient concentration.

In 1981, approximately 95 percent of the 923 formulations containing Hydrolyzed Collagen incorporated the hydrolysate at concentrations of <5 percent: 23 percent of these at concentrations ≤0.1 percent, 50 percent at concentrations >0.1 to 1 percent, and 22 percent at concentrations >1 to 5 percent. Hair preparations accounted for 66 percent of the total product listings of Hydrolyzed Collagen, with the second highest listing (16 percent) found in skin care products.[15]

The formulation data presented in Table 3 indicate that cosmetic products containing Hydrolyzed Collagen may contact all external body surfaces and hair, as well as the eyes. These products may be used daily or occasionally over a period of up to several years. The frequency and length of application could result in continuous exposure.

Noncosmetic Use

Hydrolyzed Collagen is used in soaps to impart a soft texture to skin.[11] It is also used in a treatment for duodenal and gastric ulcers and gastritis.[17] A protective film-forming composition containing Hydrolyzed Collagen is also applied to cow teats.[18]

GENERAL BIOLOGY

Substantivity

The literature contains many studies documenting the substantivity of Hydrolyzed Collagen to human hair and skin. Hydrolyzed Collagen generally is adsorbed on hair; the amount of protein adsorbed is measured by hydroxyproline analysis.[13,19] Kim et al. found that adsorbed Hydrolyzed Collagen increased the tensile strength and elongation of hair. Increasing concentrations of Hydrolyzed Collagen increased adsorption.[19,20] Hydrolyzed Collagens of average molecular weight 1000 and 2000 have greater substantivity to hair and skin, respectively, than those of average molecular weight 10,000.[13,21] Brooks[22] stated that Hydrolyzed Collagen substantivity is due to the distribution of terminal amino groups along (primarily at the lysine and arginine residues) and at the ends of the polypeptide.

Cooperman and Johnsen[23] studied the penetration of Hydrolyzed Collagen into both unbleached and bleached hair strands. In the hair strands treated with the lower molecular weight Hydrolyzed Collagens, the cuticle contained the highest percentage of protein. However, the cortex, by virtue of its greater bulk, contained the greater amount of protein. Hair strands treated with the higher molecular weight Hydrolyzed Collagens had equal quantities of protein in the cuticle and cortex. Protein penetration increased with increasing damage to hair.

As a hygroscopic compound, Hydrolyzed Collagen helps bind water to the hair and skin surfaces.[11,13,24,25] Brooks[22] found that Hydrolyzed Collagen binds water better at higher relative humidities and at pH 5 rather than pH 7 or 9. Hydrolyzed Collagen potentiates epidermal metabolism by providing a suitable, moist environment on the surface of the epidermis for healthy skin and hair.[11]

The amphoteric nature of Hydrolyzed Collagen makes it an acceptable buffering agent for alkali in permanent waving products. It is incorporated in waving formulations to avoid or minimize damage to hair. A protective application of Hydrolyzed Collagen is sometimes made prior to waving. Hydrolyzed Collagen is also used in hair dyes to insure uniformity in dyeing.[11]

General Effects

Various enzymes will hydrolyze collagen: trypsin,[26] intracellular proteolytic enzymes of *Oidiodendron kalari*,[27] *Streptomyces griseus* protease,[28,29] collagenases of genera *Bacteroides, Clostridium,* and *Peptostreptococcus*,[30,31] rat hepatic lysosomal extracts,[32] collagenases from rabbit synovial fibroblasts,[33] and cathepsin B and collagenolytic cathepsin from human placenta.[34]

TABLE 3. Product Formulation Data[15]

Product Category	Total No. of Formulations in Category	Total No. Containing Ingredient	No. of Product Formulations Within Each Concentration Range (%)						
			>50	>25–50	>10–25	>5–10	>1–5	>0.1–1	≤0.1
Hydrolyzed Collagen									
Baby shampoos	35	1	–	–	–	–	–	–	1
Bath oils, tablets, and salts	237	2	–	–	–	–	2	–	–
Bubble baths	475	2	–	–	–	–	–	2	–
Other bath preparations	132	2	–	–	–	–	–	2	–
Eyeliner	396	1	–	–	–	–	–	–	1
Eye shadow	2582	6	–	–	–	–	–	5	1
Mascara	397	28	–	–	–	–	–	15	13
Other eye makeup preparations	230	5	–	–	–	–	1	1	2
Hair conditioners	478	174	2	1	2	13	60	79	17
Hair sprays (aerosol fixatives)	265	7	–	–	–	–	–	1	6
Hair straighteners	64	7	–	–	–	–	–	7	–
Permanent waves	474	70	–	–	1	8	24	21	16
Hair rinses (noncoloring)	158	34	–	–	–	1	10	17	6
Hair shampoos (noncoloring)	909	224	–	–	–	3	36	133	52
Tonics, dressings, and other hair grooming aids	290	35	1	–	1	–	11	17	5
Wave sets	180	39	–	–	2	–	4	19	14
Other hair preparations (noncoloring)	177	18	–	–	–	1	6	7	4
Hair tints	15	14	–	–	–	–	13	1	–
Hair rinses (coloring)	76	24	–	–	–	–	–	–	24

Hair bleaches	111	7	–	–	–	–	3	3	1
Other hair coloring preparations	49	1	–	–	–	–	–	1	–
Blushers (all types)	819	5	–	–	–	–	–	5	–
Face powders	555	5	–	–	–	–	–	2	3
Makeup foundations	740	10	–	–	–	–	–	8	2
Lipstick	3319	15	–	–	–	–	–	9	6
Makeup bases	831	15	–	–	–	–	–	10	5
Cuticle softeners	32	3	–	–	–	–	–	1	2
Nail creams and lotions	25	6	–	1	–	1	2	1	1
Nail polish and enamel	767	1	–	–	–	–	1	–	–
Nail polish and enamel remover	41	2	–	–	–	–	–	–	2
Other manicuring preparations	50	6	–	–	–	–	3	1	2
Bath soaps and detergents	148	3	–	–	–	–	1	2	–
Aftershave lotions	282	3	–	–	–	–	–	3	–
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	27	–	–	–	–	4	14	9
Face, body, and hand skin care preparations (excluding shaving preparations)	832	46	–	–	–	1	4	37	4
Moisturizing skin care preparations	747	43	–	–	1	1	11	23	7
Night skin care preparations	219	11	–	–	–	1	5	5	–
Paste masks (mudpacks)	171	6	–	–	–	–	3	1	2
Skin fresheners	260	7	–	–	–	–	1	5	1
Wrinkle smoothers (removers)	38	1	–	–	–	–	1	–	–
Other skin care preparations	349	7	–	1	–	–	2	2	2
1981 TOTALS		923	3	3	7	30	208	461	211

In other studies, a proteinase from *Pseudomonas aeruginosa* catalyzed the hydrolysis of collagen,[35] whereas anthocyanosides isolated from *Vaccinium myrtillis* decreased collagen hydrolysis.[36] Hydrolyzed Collagen induced the activity of an extracellular collagenase produced by a marine *Vibrio*,[37] and a secondary vitamin K deficiency in rats increased the hydrolysis of collagen.[38] The epimerization of trans-4-hydroxy-L-proline to cis-4-hydroxy-D-proline during acid hydrolysis of collagen has also been documented.[39]

Collagen hydrolysates have been studied for their stimulatory effect on the healing of open wounds.[40] The ears of 1 rabbit were incised: 1 was kept as a control and 1 was treated with Hydrolyzed Collagen. The same rate of healing was observed for the first few days; thereafter, the treated ear healed more rapidly.[16]

Animal Toxicology

Acute Toxicity

Oral

Hydrolyzed Collagen (100 percent) was analyzed for acute oral toxicity in rats in 2 tests. LD_{50}s were >10 g/kg and >15 g/kg, respectively. In each study, investigators concluded that Hydrolyzed Collagen was nontoxic by ingestion[41,42] (Table 4).

Two shampoo formulations, each containing 2 percent Hydrolyzed Collagen, were tested for acute oral toxicity in mice and rats. LD_{50}s were >15 ml/kg in both mice and rats. The investigators concluded that each formulation was practically nontoxic[43,44] (Table 4).

A hair conditioner containing 0.5 percent Hydrolyzed Collagen had an oral LD_{50} of >40.0 ml/kg in rats, estimated by interpolation from the probit response curve. The investigators' observations included decreased activity, rales, diarrhea, salivation, and an increase in body weight[46] (Table 4).

Ocular Irritation

Four lots of Hydrolyzed Collagen (100 percent) were evaluated for ocular irritation by a modified Draize eye test. A 0.1 ml sample of Hydrolyzed Collagen

TABLE 4. Acute Oral Toxicity of Hydrolyzed Collagen

Compound	*Species*	*LD_{50}*	*Results/Comments*	*Reference*
Hydrolyzed Collagen, 100%	Rat	>10 g/kg	Nontoxic	41
Hydrolyzed Collagen, 100%	Rat	>15 g/kg	Nontoxic	42
Hydrolyzed Collagen, 2% in a shampoo	Mouse	>15 g/kg	Practically nontoxic	43
Hydrolyzed Collagen, 2% in a shampoo	Rat	>15 g/kg	Practically nontoxic	44
Hydrolyzed Collagen 0.5% in a hair conditioner	Rat	>40.0 ml/kg	–	45

was instilled into the conjunctival sac of 1 eye of each of 6 albino rabbits. Eyes were scored by Draize classification (max, 110) at 1, 2, 3, 4, and 7 days or until negative. The 4 lots had average irritation scores of 5, 4, 3, 0; 6, 3, 1, 0; 5, 3, 3, 0; and 6, 3, 3, 0 on Days 1, 2, 3, and 4, respectively. As judged by the Draize classification of eye irritation, Hydrolyzed Collagen was mildly irritating[47] (Table 5).

Hydrolyzed Collagen was evaluated as a 50 percent aqueous solution in a modified Draize eye irritation test. A 0.1 ml sample of the solution was instilled into the conjunctival sac of 1 eye of each of 6 albino rabbits; the other eye served as a control. The eyes were examined for 7 days or until negative. Average irritation scores were 1 and 0 (max, 110) on Days 1 and 2, respectively. As judged by the Draize classification of eye irritation, the test solution was minimally irritating[48] (Table 5).

A shampoo formulation containing 2 percent Hydrolyzed Collagen was tested for ocular irritation in 9 albino rabbits. The shampoo was diluted to a concentration of 20 percent (w/v) with distilled water, and a 0.1 ml sample was instilled into the conjunctival sac of the left eye. Each right eye served as a control.

TABLE 5. Ocular Irritation of Hydrolyzed Collagen

Compound	*Number of Rabbits/Test Groups*	*Results/Comments*	*Reference*
Hydrolyzed Collagen, 100% (4 lots)	6	Average irritation scores (max, 110) on Days 1, 2, 3, and 4, respectively were: Lot 1–5, 4, 3, 0 Lot 2–6, 3, 1, 0 Lot 3–5, 3, 3, 0 Lot 4–6, 3, 3, 0; Hydrolyzed Collagen was concluded to be mildly irritating	47
Hydrolyzed Collagen, 50% aqueous solution	6	Average irritation scores (max, 110) were 1 and 0 on Days 1 and 2, respectively; test solution concluded to be minimally irritating	48
Hydrolyzed Collagen, 0.2% in a shampoo, tested as a 20% (w/v) aqueous dilution	9/ 3 rinsed 6 unrinsed	No irritation observed in rinsed eyes; unrinsed eyes had mean total scores (max, 130) of 3.33, 0.67, 0.0, and 0.0 at 24, 48, and 72 hours and 7 days, respectively; concluded that shampoo was a nonirritant but could cause minimal irritation under test conditions	49
Hydrolyzed Collagen, 0.5% in a hair conditioner	9/ 3 unrinsed 3 rinsed 3 received 1:9 dilution with water	Conjunctival scores of 2 or less (max, 110) seen in all unrinsed eyes, 1 rinsed, and 1 dilution eye; effects cleared by 72, 72, and 48 hours, respectively; concluded that hair conditioner was a mild, transient irritant under all test conditions	50

The eyes of 3 rabbits were rinsed with water 2 seconds after application; the other 6 were not rinsed. Eyes were scored at 24, 48, and 72 hours and 7 days. The unrinsed treated eyes had conjunctival irritation at 24 hours consisting of slight erythema (6 rabbits), very slight edema (3 rabbits), and slight discharge (1 rabbit). Irritation decreased progressively, and all unrinsed eyes were normal at 72 hours. No irritation was observed in the rinsed eyes throughout the 7-day period. Mean total scores for the unrinsed eyes were 3.33, 0.67, 0.0, and 0.0 (max, 130) at 24, 48, and 72 hours and 7 days, respectively. The shampoo was found to be a nonirritant. However, under test conditions, it could cause minimal eye irritation[49] (Table 5).

A hair conditioner containing 0.5 percent Hydrolyzed Collagen was tested for ocular irritation in 9 albino rabbits. A 0.1 ml sample was applied to the right eye of each rabbit. The rabbits were divided into groups of 3: Group I received the product full-strength, Group II received a full-strength application followed by rinsing 4 seconds later, and Group III received a 1:9 dilution of the conditioner with water. Ocular reactions were recorded 24, 48, and 72 hours and 7 days after instillation of the test material. Conjunctival effects (score of 2 or less, max, 110) were seen in all 3 rabbits of Group I and in 1 rabbit of Groups II and III. These effects had disappeared by 72 hours in Groups I and II and by 48 hours in Group III. The investigators concluded that the hair conditioner was a mild, transient irritant when applied full-strength with or without rinsing, or diluted 1:9 with water[50] (Table 5).

Dermal/Primary Skin Irritation

Hydrolyzed Collagen (100 percent) was tested for primary skin irritation using 6 rabbits. The backs of the rabbits were clipped and divided into 2 sections, of which 1 was abraded. Hydrolyzed Collagen was applied to both sections under gauze pads for 24 hours. Sites were scored upon patch removal and 48 hours later. No reactions were noted. The investigators concluded that Hydrolyzed Collagen was nonirritating[41] (Table 6).

Hydrolyzed Collagen was applied full-strength to the shaved backs of 9 rabbits in a modified Draize primary skin irritation test. A 0.1 ml sample was applied to each rabbit by occlusive filter disc for 24 hours. Four lots of Hydrolyzed Collagen were evaluated. Sites were graded upon disc removal and 48 hours later. Average irritation socres (max, 4) for the 4 lots were 0.25, 0.20, 0.16, and 0.26. The investigators concluded the Hydrolyzed Collagen was minimally irritating[51] (Table 6).

Hydrolyzed Collagen was evaluated as a 50 percent aqueous solution for primary skin irritation using 9 albino rabbits. Samples of 0.1 ml were applied by occlusive filter disc to the shaved skin of the rabbits for 24 hours. Applications were made for 3 consecutive days. Sites were scored for irritation 24 hours after each application. The maximum average irritation response was 1.33 (max, 4); the test solution was considered mildly irritating[52] (Table 6).

Various concentrated solutions of a soap containing 26 to 28 percent Hydrolyzed Collagen were brushed onto the skin of guinea pigs (number unspecified). Skin changes were monitored clinically and microscopically. Body weights were recorded and necropsies performed on a number of the animals. No internal in-

juries were noted. The soap was considered significantly less irritating than a lauryl sulfate solution of the equivalent concentration[53] (Table 6).

A shampoo formulation containing 2 percent Hydrolyzed Collagen was evaluated for skin irritation and acute dermal toxicity. Three groups of 4 rabbits each received doses of 1.5, 3, and 6 ml/kg of the test shampoo, and a fourth group received 6 ml/kg of a regular shampoo as a comparative control. Backs of all rabbits were clipped; 2 in each group also were abraded. The shampoos were applied full-strength under an occlusive wrap for 24 hours. Sites were evaluated according to Draize at 24, 48, and 72 hours and 7 and 14 days. At 24 and 48 hours, moderate erythema (score of 2 on 0 to 4 scale) was seen in all control rabbits and 1 high-dose nonabraded rabbit. Slight erythema (score of 1) was noted in all others, and no edema was noted. The skin appeared darkened at 48 hours in 1 control rabbit and in the high-dose rabbit with moderate erythema. At 72 hours, no erythema or edema was noted. However, the same 2 rabbits still had darkened skin, whereas peeling and scaling of the skin was observed in the others. At 7 and 14 days, treated skin sites appeared normal except for areas of pustular dermatitis due to a secondary infection; 1 high-dose rabbit had darkened, thickened skin (Day 7), and 1 had dry, cracked, and peeling skin (Day 14). The investigators reported that application of the shampoo caused erythema and burning of the skin of the rabbit. Its application, under exaggerated conditions, was responsible for the degeneration and necrosis of the skin of some rabbits and was followed by secondary bacterial infection in others as manifested by a pustular dermatitis. There were no significant differences in hematological and urinalysis values. A dose-related decrease in feed consumption and body weight gain was observed. However, there were no other signs of systemic toxicity. Necropsy of the 3 rabbits that died during the study (2 control, 1 high dose) indicated that acute pneumonia was the prime contributing factor[54] (Table 6).

Two other shampoo formulations, each containing 2 percent Hydrolyzed Collagen, were evaluated for skin irritation using identical tests. The first shampoo was administered full-strength, whereas the second was diluted to 20 percent (w/v) in distilled water. Samples of 0.5 ml of the shampoo or dilution were administered to the intact and abraded skin of 6 rabbits and occluded for 24 hours. Sites were scored according to Draize at 24, 48 (shampoo only), and 72 hours. Moderate to severe irritation was observed in the rabbits treated with the full-strength shampoo; very slight to mild irritation was seen in the rabbits receiving the dilution treatments (Table 6). The shampoo and dilution had primary irritation scores (PIS) of 4.67 and 1.46, respectively, where a score of 5 (max, 8) is considered to be a primary skin irritant. The investigators concluded that the shampoo was a dermal irritant to the intact and abraded skin of rabbits, whereas the dilution was classified as a nonirritant. However, it was noted that mild irritation can occur under these test conditions.[55,56]

A hair conditioner containing 0.5 percent Hydrolyzed Collagen was evaluated for dermal irritation in a combined irritation and phototoxicity test. Occlusive patches containing 0.20 ml of the conditioner were applied to the backs of 6 clipped rabbits for 48 hours. Sites were scored for erythema and edema at 49, 72, and 96 hours; all rabbits had a score of 0 (max, 8). It was concluded that the hair conditioner was not a primary dermal irritant[57] (Table 6).

TABLE 6. Primary Skin Irritation/Dermal Toxicity of Hydrolyzed Collagen

Compound	*Test Method*	*Number and Species of Animals*	*Results/Comments*	*Reference*
Hydrolyzed Collagen, 100%	Applied under gauze pads for 24 hours. I, A*	6 rabbits	No reactions PII† of 0; nonirritating	41
Hydrolyzed Collagen, 100% (4 lots)	Occlusive disc for 24 hours–I	9 rabbits	Average irritation scores (max, 4) for 4 lots were 0.25, 0.20, 0.16, and 0.26; concluded to be minimally irritating	51
Hydrolyzed Collagen, 50% aqueous solution	Occlusive disc for 24 hours, 3 consecutive applications–I	9 rabbits	Maximum average irritation response was 1.33 (max, 4); test solution considered mildly irritating	52
Hydrolyzed Collagen, 26–28% in a soap	Various concentrations brushed on–I	Guinea pigs, unspecified number	Considered significantly less irritating than a lauryl sulfate solution of the equivalent concentration; no internal injuries noted	53
Hydrolyzed Collagen, 2% in a shampoo	Occlusive patch for 24 hours–I, A–doses of 1.5, 3, and 6 ml/kg	12 rabbits	Moderate erythema observed in all control rabbits and 1 high-dose rabbit (score of 2, max, 4), slight erythema (score of 1) in all others, clearing by 72 hours; no edema noted; several rabbits had darkened or cracked and peeling skin, pustular dermatitis due to secondary infection in others; dose-related decrease in feed con-	54

			sumption and body weight gain; no other signs of systemic toxicity; conclusion: under exaggerated conditions, caused erythema and burning of rabbit skin and was responsible for the degeneration and necrosis of skin, allowing secondary infection	
Hydrolyzed Collagen, 2% in a shampoo	Occlusive patch for 24 hours–I, A	6 rabbits	Moderate to severe and well-defined erythema was observed in 5 and 1 rabbits, respectively, at 24 hours, with similar results at 72 hours; very slight, slight, and moderate edema was observed in 1, 3, and 2 rabbits, respectively, at 24 hours, decreasing minimally by 72 hours; PIS‡ of 4.67 (max, 8); concluded to be dermal irritant on I and A skin	55
Hydrolyzed Collagen, 2% in a shampoo–diluted to 20% (w/v) in distilled water	Occlusive patch for 24 hours–I, A	6 rabbits	Well-defined and very slight erythema was observed in 3 and 3 rabbits, respectively, at 24 hours, diminishing to only very slight scores in 5 rabbits at 72 hours; slight and very slight edema was observed in 1 and 3 rabbits, respectively, at 24 hours, clearing totally by 72 hours; PIS of 1.46 (max, 8); concluded to be a nonirritant on I and A skin but can cause mild irritation under study conditions	56
Hydrolyzed Collagen, 0.5% in a hair conditioner	Occlusive patch for 48 hours–I	6 rabbits	No erythema or edema; all rabbits had individual score of 0 (max, 8); concluded to be not a primary dermal irritant	57

*I, intact; A, abraded.
†PII, primary irritation index.
‡PIS, primary irritation scores.

Subchronic Toxicity

Dermal

A hair preparation containing 2 percent Hydrolyzed Collagen was tested for subchronic dermal toxicity. Three groups of 2 male and 2 female rabbits received 100, 1000, or 3200 mg/kg of the test shampoo. Control groups received 2000 mg/kg of a marketed antidandruff shampoo, 3220 mg/kg of the test shampoo without the active drug ingredient, and 1 ml water/kg. All test and control applications were made daily for 30 days, were left on the skin for 15 minutes, and were then removed with water. The skin of 1 male and 1 female in each group was abraded weekly. The only treatment-related finding was local skin irritation. No deaths, abnormal behavior, gross or microscopic lesions were associated with treatment.[58]

Another shampoo containing 2 percent Hydrolyzed Collagen was tested for dermal toxicity using Yorkshire pigs (white). Three groups of 2 male and 2 female pigs (1 of each sex abraded twice weekly) received applications of 0.5, 1.0, and 2.0 ml/kg. Control groups received 2.0 ml/kg saline and 2.0 ml/kg of another marketed shampoo. Applications were made to the clipped back of each pig twice daily for 4 weeks. Treated sites were rinsed with warm water 1 hour after each application. All pigs were given a general physical examination before and at 4 weeks observation. Body weights were recorded weekly, and blood samples were obtained for routine hematological and serum chemistry evaluations. All animals were necropsied. The abraded skin of 1 high-dose female was slightly irritated; the skin of all others appeared unremarkable. No dose-related effects were determined by physical examination, hematological and serum chemistry evaluations, necropsy examination, and histopathological evaluation. A statistically significant dose-regression relationship for male gonad weights was not considered indicative of systemic toxicity due to the immaturity and variation in size of the testes of these young pigs. Minimal focal inflammatory cellular infiltration was noted in the treated dermis of 1 middle-dose and 1 high-dose pig. However, this same condition was observed in the untreated skin of 1 saline control and 1 high-dose pig.[59]

Sensitization

Hydrolyzed Collagen was tested for sensitization using 2 male white guinea pigs. A 0.1 percent solution of Hydrolyzed Collagen in physiological saline was injected intracutaneously into the clipped back or flank of each guinea pig every other day or 3 times weekly for a total of 10 injections. The first injection consisted of 0.05 ml of the test solution; each succeeding injection consisted of 0.1 ml. Sites were scored for diameter, height, and color 24 hours after each injection. After a 2-week rest period, a challenge injection of 0.05 ml was administered into a different site. Induction injections gave average diameter scores of 7.6 and 8.9 mm, average heights of 0.3 and 0.2 mm, and an average color of pink for the 2 guinea pigs. On challenge, 1 animal had no reaction, whereas the second had a reaction of diameter 5 mm, no height, and a pink color. Both animals had a sensitization score of 0 (9 or above is severely sensitizing), classifying Hydrolyzed Collagen as a nonsensitizer.[41]

A shampoo formulation containing 2 percent Hydrolyzed Collagen was

tested for sensitization using a modification of the Buehler and Griffith[60] method. A total of 3 inductive applications, 1 per week, were made to the clipped back of 10 guinea pigs. The first inductive application consisted of 0.5 ml of a 1, 5, and 10 percent dilution (distilled water v/v) occlusively patched on 3 separate sites on the right side of the animal for 24 hours. The second and third inductions consisted of 0.5 ml of a 10 percent aqueous dilution occlusively patched on the left side of the animal for 6 hours. A 24-hour challenge patch was applied 2 weeks later to an untreated site on the animal's right side. Sites were scored for erythema 24 hours after each application. Dinitrochlorobenzene (DNCB) was tested as a positive control. The first and third inductive patches produced no erythema, and the second produced very slight erythema in 2 guinea pigs. No erythema was observed at challenge. The investigators concluded that the shampoo formulation did not cause sensitization.[45]

Phototoxicity

A shampoo formulation containing 2 percent Hydrolyzed Collagen was tested for phototoxicity using 2 guinea pigs. The shampoo was tested as a 20 percent (w/v) mixture in distilled water, and 8-methoxypsoralen was tested undiluted as a positive control. The back of each animal was clipped and divided into 4 sites; 2 received 0.1 ml of the shampoo and 2 received 0.05 ml of 8-methoxypsoralen. Fifteen to twenty minutes later, the right side of each animal was shielded with cardboard while the animals were irradiated for 1 hour with UVA light (320 to 400 nm) using a No. F40 BL 40W Westinghouse Blacklight. Sites were graded for erythema (max, 4) 24 hours after exposure. All exposed and unexposed sites treated with the shampoo dilution had scores of 0. 8-Methoxypsoralen gave a mean score of 3.5 for the irradiated sites and a score of 0 for the nonirradiated sites. The investigators concluded that the shampoo formulation was not phototoxic in guinea pigs.[61]

A hair conditioner containing 0.5 percent Hydrolyzed Collagen was also analyzed for phototoxicity using 6 rabbits. One rabbit received 8-methoxypsoralen as a positive control. A 0.20 ml sample of the conditioner was applied to a gauze patch, evaporated for 5 minutes, and then placed on the clipped back of the rabbit and occluded. Two patches were applied to each animal. Two hours later, 1 patch was removed and the other protected with aluminum foil while the animals were irradiated for 15 minutes with Sylvania lights No. F-40-BLB. The patches were then replaced until 48 hours posttreatment, at which time all patches were removed. Sites were scored for erythema and edema (max, 8) 1 hour later and at 72 and 96 hours. Each rabbit had an individual score of 0 for both irradiated and nonirradiated sites. The hair conditioner was neither a primary dermal irritant nor a phototoxic irritant to rabbit skin.[57]

CLINICAL ASSESSMENT OF SAFETY

Irritation and Sensitization

Hydrolyzed Collagen (100 percent) was tested for skin irritation on 20 humans. A single patch containing 0.1 ml of Hydrolyzed Collagen was applied to the volar forearm or the inner aspect of the arm of each subject. A standard con-

trol was also tested. Reactions were recorded 2 and 24 hours after patch removal. The irritation score for Hydrolyzed Collagen and the controls in all 20 subjects was 0 (max, 4). No significant difference in irritancy potential existed between Hydrolyzed Collagen and the control[62] (Table 7).

Patch tests were performed on 33 subjects (18 men and 15 women) using Hydrolyzed Collagen at concentrations of 2 and 20 percent. Occlusive patches containing Hydrolyzed Collagen at each concentration were applied to the breast or arm for 24 hours. Sites were scored at 24, 48, and 72 hours; no reactions were observed[63] (Table 7).

A 21-day cumulative irritation test was conducted on a hair conditioner containing 0.5 percent Hydrolyzed Collagen. Semiocclusive patches with 0.5 ml of the conditioner were applied to the upper part of the back of 20 subjects for 24 hours. Patches were then removed, evaluated 30 minutes later, and a new patch was applied. These procedures were repeated for 15 applications, allowing for 21-day continuous exposure. Mineral oil was used as the standard control. Of the 17 subjects who completed the study, only 1 had any reaction, giving a cumulative irritation score of 0.5 (max, 84). The mean cumulative irritation score of 0.03 was exactly comparable to that of mineral oil (0.03). The investigators concluded that the product as used by label directions would not present any medical hazard to the consumer[64] (Table 7).

Various compositions of a soap containing 26 to 28 percent Hydrolyzed Collagen were applied daily to the skin as a 5 percent solution for 10 to 48 days. A large number of healthy subjects and people with dermatitis were used. A low degree of irritation was seen even at high concentrations of the least irritating composition. No sensitization was observed. The treated skin area was examined microscopically; those with acute dermatitis had moderate irritation[53] (Table 7).

Five cosmetic formulations were evaluated for irritation and sensitization in repeated insult patch tests (RIPT). Three of these, a morning cream, a suntan lotion, and a night cream, containing 3.0, 2.2, and 3.0 percent Hydrolyzed Collagen, respectively, were tested in the same manner. A series of 10 48-hour occlusive patches containing the undiluted formulation was applied to the back of each subject. Sites were graded after each removal and 24 hours after removal of the tenth patch (morning cream and suntan lotion only). After approximately an 11-day rest period, challenge patches were applied, occluded for 48 hours, and scored upon removal and 24 hours later. Scattered irritant responses after the third application were seen in the 103 subjects who completed the induction phase for the morning cream and suntan lotion. The maximum number of responses seen at any 1 reading for the suntan lotion was 11 with erythema and 8 with very mild erythema. Maximum response to the morning cream was 5 with erythema and 10 with very mild erythema. Of the 96 subjects who completed the challenge phase, 1 had erythema and 1 had very mild erythema to the suntan lotion at 24 hours. However, the panelist with erythema had a negative reaction on rechallenge. No reactions were observed on challenge with the morning cream. Two of the 113 panelists completing the induction phase testing of the night cream had irritant responses: 1 had very mild erythema and 1 had erythema. One of the 103 subjects completing the challenge phase had erythema. The investigators concluded that the morning cream, suntan lotion, and night cream were mildly irritating, definitely irritating, and nonirritating, respectively, whereas none of the formulations gave significant evidence of sensitization[65,66] (Table 7).

TABLE 7. Clinical Irritation and Sensitization

Ingredient	*Type of Test*	*Number of Humans*	*Results/Comments*	*Reference*
Hydrolyzed Collagen, 100%	Single patch-type and duration not specified	20	Average irritation score of 0 for all 20 subjects (max, 4); no significant difference between test material and control	62
Hydrolyzed Collagen, 2 and 20%	Single, 24-hour occlusive patch	33	No reactions were observed	63
Hydrolyzed Collagen, 0.5% in a hair conditioner	21-day cumulative irritation test	17	One subject had a reaction, giving a cumulative irritation score of 0.5 (max, 84); mean cumulative irritation score of 0.03 was exactly comparable to control; product should not present any medical hazard; nonirritant	64
Hydrolyzed Collagen, 26–28% in a soap—tested as 5% solution of the soap	Applied daily to skin for 10–48 days	Large number (unspecified), both healthy subjects and some with dermatitis	Low degree of irritation, no sensitization was observed; those with acute dermatitis showed moderate irritation	53
Hydrolyzed Collagen, 3.0% in a morning cream	RIPT*	103–I† 96–C	Scattered irritant responses, maximum response at any one reading was 10 very mild erythemas and 5 erythemas; no reactions on challenge; mildly irritating and nonsensitizing	65
Hydrolyzed Collagen, 2.2% in a suntan lotion	RIPT	103–I 96–C	Scattered irritant responses, maximum response at any one reading was 8 very mild erythemas and 11 erythemas; 2 reactions to the challenge, 1 very mild erythema and 1 erythema; negative reaction on rechallenge; definitely irritating and nonsensitizing	65
Hydrolyzed Collagen, 3.0% in a night cream	RIPT	113–I 103–C	Two irritant responses: 1 very mild erythema and 1 erythema; 1 erythema reaction on challenge; nonirritating and nonsensitizing	66
Hydrolyzed Collagen, 0.5% in a mascara	RIPT	205	One subject exhibited faint erythema during the induction phase; no reactions at challenge; nonirritating and nonsensitizing	67

TABLE 7. (Continued)

Ingredient	*Type of Test*	*Number of Humans*	*Results/Comments*	*Reference*
Hydrolyzed Collagen, 0.5% in a hair conditioner tested as a 0.1% dilution	RIPT	207–I 201–C	Mean cumulative irritation scores (max, 50) as follows: 167 subjects had score of 0 22 subjects had score of 1 12 subjects had score of 2 1 subject had score of 3 1 subject had score of 4 3 subjects had score of 5 1 subject had score of 6; one subject exhibited erythema at challenge; however, this panelist reacted to 10 of the other 13 products and had no reaction on rechallenge	68
Hydrolyzed Collagen, 0.5% in a mascara	Controlled use test, 4 weeks of daily use	27	No reactions were observed	69

*Repeated insult patch test.
†I, induction; C, challenge.

The fourth formulation tested by RIPT was a mascara containing 0.5 percent Hydrolyzed Collagen. Occlusive patches containing the undiluted mascara were applied to the upper backs of 205 subjects on Monday, Wednesday, and Friday for 3 consecutive weeks. Patches were removed and sites graded just prior to the next scheduled patch application. Following a 2-week rest, 2 consecutive 48-hour challenge patches were applied to adjacent sites on the back. Sites were graded at 48 and 96 hours. One subject had faint erythema during the induction phase; no reactions were observed at challenge. The mascara was found to be neither an irritant nor a sensitizer[67] (Table 7).

The fifth formulation evaluated by RIPT was a hair conditioner containing 0.5 percent Hydrolyzed Collagen. A 0.1 percent dilution of the conditioner was applied using semioccluded patches to the upper backs of the subjects for 48 hours. Patches were then removed, sites evaluated, and new patches applied for a total of 10 applications. Following a 2-week rest period, challenge patches were applied to the subjects' thighs. Mean cumulative irritation scores (max, 50) were as follows: 167 subjects had a score of 0, 22 subjects had a score of 1, 12 subjects had a score of 2, 3 subjects had scores of 3, 4, and 6 (each), and 3 subjects had a score of 5. Of the 201 subjects completing the challenge phase of the study, 1 had erythema. However, this panelist also reacted to 10 of the other 13 substances being tested. A rechallenge on the other thigh produced no reaction[68] (Table 7).

A mascara formulation containing 0.5 percent Hydrolyzed Collagen was evaluated by a 4-week controlled use test. Twenty-seven women used the product daily as per normal instructions. No reactions were observed[69] (Table 7).

A prospective study of cosmetic-induced dermatitis by 11 dermatologists of the North American Contact Dermatitis Group (NACDG) identified 1 case of dermatitis associated with use of Hydrolyzed Collagen from among a total of 487 cases.[70]

Phototoxicity/Photosensitization

A mascara formulation containing 0.5 percent Hydrolyzed Collagen was tested for phototoxicity/photosensitization on a panel of 23 humans. Occlusive patches containing 0.1 g/cm^2 of the mascara were applied to the backs of the subjects for 24 hours. Patches were then removed, evaluated, and irradiated with 3 times the individual's MED using a xenon arc solar simulator (150 W) filtered to produce a continuous UVA-UVB emission spectrum (290 to 400 nm). Sites were evaluated 48 hours later, and the procedures of application, patching, and irradiation were repeated for a total of 7 applications. No reactions were observed. The investigators concluded that the mascara was neither phototoxic nor a photosensitizer.[71]

Domsch et al.[72] have found that UV-induced erythema was decreased by rubbing Hydrolyzed Collagen (mean molecular weight of 1500) into the skin. A 10 percent solution of Hydrolyzed Collagen applied immediately and 24 hours after irradiation decreased erythema by 20 percent at 24 hours and 25 percent at 48 hours.

SUMMARY

Hydrolyzed Collagen is a collagen hydrolysate derived by acid, enzyme, or other method of hydrolysis. It is a white to off-white hygroscopic powder of molecular weight 1000 to 10,000 and is also available as a viscous, amber aqueous solution.

Hydrolyzed Collagen can be prepared by any 1 of 3 methods: alkaline hydrolysis of bovine skin products followed by enzymatic hydrolysis to the desired molecular weight, enzymatic hydrolysis of fresh animal byproducts or bovine-derived leather, or acid or alkaline hydrolysis of chrome leather fold splinters with inorganic acids or lyes to a defined molecular weight. The hydrolysis of collagen yields a high content of glycine and proline compared to other proteins, as well as 2 unique amino acids, hydroxyproline and hydroxylysine. Hydrolyzed Collagen is usually analyzed by column chromatography.

Hydrolyzed Collagen is used in cosmetics, primarily hair and skin care products, because of its conditioning and moisturizing properties. It is usually incorporated at concentrations <5 percent and was in 923 formulations reported in 1981. Cosmetic products containing Hydrolyzed Collagen may contact all external body surfaces and hair, as well as the eyes. Frequency and length of application could result in continuous exposure.

Many studies have documented the substantivity of Hydrolyzed Collagen to human hair and skin. Hydrolyzed Collagen generally is adsorbed on hair, although it has been shown to penetrate the cuticle and cortex. Increasing concentrations of Hydrolyzed Collagen increased adsorption, as did increasing damage to hair. Hydrolyzed Collagen also binds water to the hair and skin surfaces and is used as a buffering agent for alkali in permanent waving preparations.

Acute toxicity studies found Hydrolyzed Collagen and formulations containing Hydrolyzed Collagen practically nontoxic when administered orally to mice and rats. Dermal studies gave no indication of systemic toxicity when formulations containing Hydrolyzed Collagen were applied to rabbits and guinea pigs. However, a shampoo formulation (2 percent Hydrolyzed Collagen) administered to rabbits under exaggerated conditions did cause erythema and burning, leading to degeneration and necrosis of the skin.

Hydrolyzed Collagen was minimally irritating to rabbit eyes when tested full-strength and in formulation. Primary skin irritation tests in rabbits indicated that Hydrolyzed Collagen was nonirritating or minimally irritating when tested full-strength, whereas a 50 percent aqueous solution of Hydrolyzed Collagen was mildly irritating. Shampoo formulations containing Hydrolyzed Collagen (2 percent) were generally nonirritating when tested as dilutions. However, these were irritating under the exaggerated conditions of a full-strength application. A soap (26 to 28 percent Hydrolyzed Collagen) and hair conditioner (0.5 percent Hydrolyzed Collagen) produced no dermal irritation in guinea pigs and rabbits, respectively.

Subchronic dermal studies on 2 cosmetic formulations containing 2 percent Hydrolyzed Collagen were negative for systemic toxicity in rabbits and Yorkshire pigs.

Hydrolyzed Collagen was nonsensitizing in guinea pigs. Cosmetic formulations containing Hydrolyzed Collagen (2, 2, and 0.5 percent) were also nonsensitizing and nonphototoxic in guinea pigs and rabbits.

In clinical studies, Hydrolyzed Collagen produced no skin irritation. Formulations containing Hydrolyzed Collagen at concentrations ranging from 0.5 to 28 percent produced some irritation. However, no significant evidence of sensitization was observed in any study. No phototoxicity or photosensitization was evident in a study of a mascara containing 0.5 percent Hydrolyzed Collagen. It has been reported that UV-induced erythema was decreased by rubbing Hydrolyzed Collagen into the skin after irradiation.

CONCLUSION

On the basis of the available animal and clinical data presented in this report, the Panel concludes that Hydrolyzed Collagen is safe as a cosmetic ingredient in the present practices of use and concentration.

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