Safety Assessment of Tissue-Derived Proteins and Peptides as Used in Cosmetics

Status:	Scientific Literature Review for Public Comment
Release Date:	April 13, 2017
Panel Meeting Date:	June 12-13, 2017

All interested persons are provided 60 days from the above date to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a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 J. Gill.

The 2017 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.

1620 L St NW, Suite 1200◊ Washington, DC 20036-4702 ◊ ph 202.331.0651 ◊fax 202.331.0088
◊ cirinfo@cir-safety.org

INTRODUCTION

The tissue-derived proteins and peptides detailed in this report are described by the *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*) to function mainly as skin and hair conditioning agents in personal care products.[1] This report assesses the safety of the following 18 tissue-derived ingredients:

Ammonium Hydrolyzed Collagen
Calcium Hydrolyzed Collagen
Collagen
Elastin
Fibronectin
Gelatin
Hydrolyzed Actin
Hydrolyzed Collagen
Hydrolyzed Collagen Extract
Hydrolyzed Elastin
Hydrolyzed Fibronectin
Hydrolyzed Gelatin
Hydrolyzed Reticulin
Hydrolyzed Spongin
MEA -Hydrolyzed Collagen
Soluble Collagen
Soluble Elastin
Zinc Hydrolyzed Collagen

The safety of several hydrolyzed proteins as used in cosmetics has been reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel (Panel) in several previous assessments. The Panel concluded that Hydrolyzed Keratin (finalized in 2016), Hydrolyzed Collagen (published in 1985, re-review with reaffirmed conclusion published in 2006) Hydrolyzed Soy Protein (finalized in 2015), Hydrolyzed Silk (finalized in 2015), Hydrolyzed Rice Protein (published in 2006), and Hydrolyzed Corn Protein (published in 2011) are safe for use in cosmetics.[2-8] 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.[9] The CIR is concurrently reviewing the safety of plant-derived and milk-derived proteins in separate reports.

Actin, collagen, elastin, fibronectin, gelatin, and reticulin all occur naturally and are essential components in mammalian tissues. Much of the available published literature evaluated the effects of pharmaceutical or other agents on these proteins in their naturally occurring tissues. These studies were not considered relevant for assessing the safety of the tissue-derived ingredients as used in cosmetics and are not included in this assessment.

The sources for these cosmetic ingredients may be from many different land or marine animals. These differing sources could potentially produce or result in tissue-derived proteins with unique properties, which may result in varying compositions and impurities within a single ingredient (e.g., Hydrolyzed Collagen from animals such as cows may have some impurities that are different from Hydrolyzed Collagen obtained from fish). When the specific cosmetic ingredient names are discussed in this report, capitalization as presented in the *Dictionary* (see list above) will be used (i.e. Collagen). When referring generally to the naturally-occurring protein from which these ingredients are derived, lower-case lettering will be used (i.e., collagen).

CHEMISTRY

Definition

The definitions and functions of the tissue-derived proteins and peptides are described in Table 1. General and more specific descriptions of these ingredients are found below and in sub-sections, respectively.

Tissue protein derivatives form a broad category of materials that are prepared by extraction from animal tissue and partial hydrolysis to yield cosmetic ingredients. Proteins and protein hydrolysates, including those of animal tissue, are used as conditioning agents in hair and skin products. These proteins are present in many types of tissue, including skin.

The most common protein in mammals is collagen, making up approximately 30% of all proteins by mass.[10,11] The collagen family is comprised of 28 members (named collagen I to collagen XXVIII) that all have at least one triple helix in their structure at varying degrees (see further description below).[11] The most common are mainly the fibril-forming collagens (types I, II, III, and V) that are found in skin, cartilage, reticulate, and cell surfaces. Most of the other proteins addressed in this report are derivatives of collagen, are co-located with collagen in tissues, or are both. Gelatin, for example, is a product obtained by the partial hydrolysis of collagen derived from the skin, white connective tissue, and bones of animals.[10] Reticulin is a type of fiber in connective tissue composed of type III collagen secreted by reticular cells. Actin, elastin, and fibronectin are discrete in structure from collagens, but are commonly co-located with collagen in tissue (e.g., fibronectin commonly provides rigidity on the edges of primarily collagen-based tissues). Spongin, however, is a collagen-like protein found only in marine sponges (constituting the small skeletal elements, or spicules, in the animal).

The preparation of protein hydrolysates can be afforded via acid, enzyme or other methodologies. These methodologies, and the degree to which they are utilized, may profoundly affect the size and reactivity of such hydrolysates. In most ingredients in this report, even in ingredients without "hydrolyzed" in the name, the proteins are at least hydrolyzed to some degree as a necessary part of extraction or solubilization. Further steps towards solubilization of these macromolecules are commonly achieved via reaction with an alkaline substance to generate a protein salt (e.g., Calcium Hydrolyzed Collagen).

Actin

Actin is a major protein of muscle and an important component of all eukaryotic cells.[10] α-Actin is found in differentiated muscle cells, while β-actin and γ-actin are in all non-muscle cell types.

Collagen

Collagen is the main constituent of skin (comprising 70% to 80% dry weight of the dermis) and connective tissue, and is the organic substance of bones and teeth.[10,12] Collagen is primarily responsible for the skin's tensile strength. One collagen molecule consists of 3 polypeptide chains, each containing approximately 1000 amino acids in a primary sequence that is rich in proline, hydroxyproline, and hydroxylysine. Collagen is not just one discrete, ubiquitous protein sequence, but is a protein superfamily that is diversified across different tissue/function types and source species, including cattle, chicken, and fish.[11,13] The common structural feature of collagen proteins is the presence of a triple helix. However, the percentage of each protein that this helix makes up can vary across different members of the collagen superfamily from as little as 10% to nearly 100%. The diversity of the collagen superfamily is further increased by the presence or absence of several α-chains, the existence of several molecular isoforms and supramolecular structures of specific collagen types, and the use of different methods of extraction/hydrolysis.

Elastin

Elastin is the primary component of the elastic, load-bearing fibers of animal connective tissue.[10] It is an insoluble, highly cross-linked hydrophobic protein that is rich in nonpolar amino acid residues, such as valine, leucine, isoleucine, and phenylalanine. There are two types of elastin: Type 1 is derived from bovine neck ligaments, aorta, skin, and related tissues; Type 2 is derived from cartilage and its derivatives.[14] In skin, elastin is the intact elastic fiber network that comprises approximately 2% to 4% of the dermis by volume.[12]

Fibronectin

Fibronectin is a multifunctional glycoprotein found on cell surfaces, in body fluids (especially plasma), in soft connective tissue matrices, and in most basement membranes.[10]

Gelatin

Gelatin is a heterogeneous mixture of water-soluble proteins of high average molecular weight that are derived from the denaturation and hydrolysis of collagen.[10] Glycine or alanine accounts for one third to one half of the amino acid residues, while another quarter is composed of proline or hydroxyproline.

Reticulin

Reticulin is a connective tissue protein that occurs wherever connective tissue forms a boundary[10]

Physical and Chemical Properties

The molecular weight ranges for some of the tissue-derived proteins and peptides are presented in Table 2.

Collagen

Collagen has a pH range of 3.8 to 4.7[13]

Hydrolyzed Collagen

Hydrolyzed Collagen may be a powder or solution.[3] A 10% aqueous solution has a pH of 4.0-6.5.

Elastin

Purified elastin is a pale yellow color and is a bluish fluorescence in UV light.[10] It resists acid and alkaline hydrolysis. It is practically insoluble in hydrogen-bond-breaking solvents at temperatures up to 100 ºC. It is nearly impossible to bring into solution except by using hydrolytic reagents capable of rupturing peptide bonds.

Fibronectin

Fibronectin can be a solution or a lyophilized powder.[15]

Gelatin

Gelatin is a vitreous, brittle solid that is colorless to faintly yellow.[10,16] It is practically odorless and tasteless. When gelatin granules are immersed in cold water, they hydrate into discrete, swollen particles. When warmed, gelatin disperses into water. Warm-blooded animal sourced gelatin has a gel point of 30 to 35 ºC while cold-water ocean fish sourced gelatin has a gel point between 5 and 10 ºC. Gelatin is soluble in aqueous solution of polyhydric alcohols like glycerin and acetic acid and is insoluble in alcohol, chloroform, ether, and most other organic solvents.

Soluble Elastin

Soluble Elastin is reported to be a cream-colored powder that is soluble in water and ethanol.[14]

Method of Manufacturing

Methods used to manufacture protein hydrolysates typically yield broad molecular weight distributions of peptides, ranging from 500 to 30,000 daltons (Da).[17] However, certain enzymes, such as papain, can routinely yield narrower distributions of 500 to 10,000 Da. For example, if the average molecular weight of an amino acid is 135 Da, then, under the broader distribution figures (i.e., 500 to 30,000 Da), these ingredients are approximately 4 to 220 amino acids in length (and approximately 4 to 74 amino acids in length under the narrower distribution, i.e., 500 to 10,000 da).[18]

Collagen

The *Merck Index* reports that collagen may be prepared by dissolving the mineral part of bones with phosphoric acid.[10]

Hydrolyzed Collagen

As given in the CIR safety assessment and re-review of this ingredient, Hydrolyzed Collagen may be prepared by alkaline hydrolysis of bovine or fish collagen, followed by enzymatic hydrolysis to the desired molecular weight.[3,4]

Elastin

Elastin may be sourced from numerous farm animals, such as cattle or goats.[14] It has been reported that pure elastin can be prepared from cattle aortas through extraction with sodium hydroxide at 100º C and filtration (which both may be repeated several times), precipitation, neutralization with hydrochloric acid, and washing to remove residual salt. The resultant extract may then be purified by autoclaving or by amylase pretreatment. Elastin may also be produced as a byproduct of the purification of elastin. Native elastin is reported to be too insoluble for use in cosmetic formulations.

Hydrolyzed Elastin

Hydrolyzed elastin is reported to be prepared from the skin of codfish or from bovine neck tendons.[19,20] The fibrous tissue is washed and purified to remove soil and other residual materials and then dried. The dried elastin fibers are then hydrolyzed for several hours until the target molecular weight is reached. The final product is a solution, with the bovine source material being concentrated to a 30% active content.

Hydrolyzed Elastin (MW = 3000-4000 Da) may be obtained by hydrolyzing animal ligaments or hides by enzymes such as pancreatic elastase, ficin, pepsin or trypsin. Hydrolyzed Elastin may also be obtained through acid hydrolysis at high temperatures (70-100º C, depending on acid) at several 1 hour intervals.

A supplier reported that Hydrolyzed Elastin is manufactured by enzymatic hydrolysis for a specific duration of time and at an elevated temperature (details not provided).[21] The resultant hydrolyzed protein has molecular weights in the 2000 to 4000 Da range and all contain di- and tri-peptides.

Gelatin

According to the *Food Chemicals Codex*, gelatin is the product obtained from the acid, alkaline, or enzymatic hydrolysis of collagen.[16] Type A gelatin is produced by the acid processing of collagenous raw materials and exhibits an isoelectric point between pH 7 and pH 9. Type B gelatin is produced by the alkaline or lime processing of collagenous raw materials and exhibits an isoelectric point between pH 4.6 and pH 5.2.

Soluble Collagen

Soluble Collagen is reported to be extracted from bovine dermal tissues, bony fish skins, or tropical fish' swim bladders using neutral salt solutions.[22]

Soluble Elastin

Water-soluble elastin hydrolysates may be obtained by acid treatment of cattle ligaments at 80º C and a pH less than 4, followed by filtration, grinding, enzymatic treatment at pH 9/13(alkaline proteases in the presences of urea), and finally neutralizing enzymes at 90º C.[14]

Composition

The amino acid composition for Collagen, Soluble Collagen, and Elastin is presented in Table 3.

Impurities

Several of the ingredients in this safety assessment, including Hydrolyzed Collagen, Hydrolyzed Elastin, and Gelatin, may be bovine sourced; however, these ingredients are highly processed and, as such, the U. S. Food and Drug Administration (FDA) does not consider them risk materials for transmission of infectious agents (i.e. bovine spongiform encephalopathy) in cosmetic products (21 CFR §700.27).

Collagen

An analysis for 3 different Collagen products found the level of arsenic to be less than 1 ppm. [13]

Hydrolyzed Collagen

The maximum concentrations of iron and heavy metals reported in Hydrolyzed Collagen were 3 ppm and 25 ppm, respectively. [3]

Elastin and Hydrolyzed Elastin

Impurities in commercial elastin-based preparations include contamination by lipoid substances from the raw materials and products of collagen degradations.[23]

Gelatin

According to the *Food Chemicals Codex*, gelatin must contain no more than 0.0005% sulfur dioxide, 10 mg/kg chromium, 1.5 mg/kg lead, and 0.3 mg/kg pentachlorophenol.[16]

USE

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the 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 the FDA 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 2017 VCRP data, Soluble Collagen is used in 425 formulations; the majority of uses are in leave-on skin care products (Table 4).[24] Gelatin has the second greatest number of overall uses reported, with a total of 334; the majority of the uses are in rinse-off bath soaps and detergents. The results of the concentration of use

survey conducted in 2016 by the Council indicate Collagen has the highest reported maximum concentration of use; it is used at up to 96% in face and neck skin care products.[25] Gelatin is used at up to 66% in bath oils, tablets, and salts.

Historic and current use data for Hydrolyzed Collagen is reported in Table 5. In 2017, Hydrolyzed Collagen was reported to be used in 543 formulations; the majority of uses are in leave-on skin care products. The number of uses of Hydrolyzed Collagen have declined since the initial safety assessment in 1981 and the re-review in 2002 (923 and 570 uses, respectively.[3,4] The maximum use concentration of Hydrolyzed Collagen was reported to be 16.5% in hair tonics and dressings in 2017; it was previously reported to be used at concentrations greater than 50%.[3,25]

Ingredients with no reported uses in the VCRP or by Council are listed in Table 6.

In some cases, reports of uses were received from the VCRP, but no concentration of use data were provided. For example, Elastin is reported to be used in 46 formulations, but no use concentration data were provided. In other cases, no uses were reported to the VCRP, but a maximum use concentration was provided in the industry survey. For example, Ammonium Hydrolyzed Collagen was not reported in the VCRP database to be in use, but the industry survey indicated that it is used in several formulations at concentrations up to 0.12%.

Some of these ingredients may be used in products that can come into contact with mucous membranes and the eyes. For example, Collagen is used in bath soaps and detergents at up to 0.67% and in eye makeup preparations at up to 0.2%.[25] Additionally, some of these ingredients were reported to be used in hair care products, skin care preparations, face powders, and fragrances and could possibly be inhaled. For example, Hydrolyzed Collagen was reported to be used in hair spray at a maximum concentration of 0.28% and Soluble Collagen was reported to be used in face powders at a maximum concentration of 0.0035%. 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.[26-29] 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.[26,27] Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[30-32]

The tissue-derived protein and peptide ingredients described in this safety assessment are not restricted from use in any way under the rules governing cosmetic products in the European Union; however, monoalkanolamine ingredients must not have a secondary amine content that exceeds 0.5%, and water-soluble zinc salt ingredients must not have more than 1% zinc in ready for use preparations.[33]

Non-Cosmetic

The FDA determined that the use of peptones as direct food substances is generally recognized as safe (GRAS). These GRAS peptones are defined as "the variable mixture of polypeptides, oligopeptides, and amino acids that are produced by partial hydrolysis of …animal tissue or gelatin,…" (21 CFR §184.1553). The FDA requires allergen labeling when one or more of the eight major food allergens, which includes fish, are included in food.[34]

Collagen

Non-cosmetic uses of collagen include fibers in sutures, leather substitutes, coatings as a gel in photographic emulsions, and food casings.[10]

Gelatin

Non-cosmetic uses of gelatin include uses in food as a stabilizer, thickener, texturizer, firming agent, surface-active agent, or surface-finishing agent.[10,16] Gelatin is also used in the manufacturing of rubber substitute, adhesives, cements, lithographic and printing inks, plastic compounds, artificial silk, photographic plates and films, matches, and light filters for mercury lamps.[10] It is also used as a clarifying agent, in hectographic masters, sizing paper and textiles, and for inhibiting crystallization in culture preparations in bacteriology. In pharmaceuticals, gelatin is a suspending agent, an encapsulating agent, a tablet binder, and a tablet and coating agent.

Gelatin is a category I active ingredient in ophthalmic demulcent over-the-counter (OTC) drug products at up to 0.01% (21CFR §349.12).

TOXICOKINETICS

Gelatin

The bioavailability of gelatin derived from Nile tilapia scales was determined in an oral pharmacokinetic study in rats.[35] Five groups of six female Sprague-Dawley rats received 4000 mg/kg body weight gelatin intragastrically (i.g.), 400 mg/kg hydroxyproline i.g., 400 mg/kg hydroxyproline intravenously (i.v.), normal saline i.g., or normal saline i.v. Blood plasma was then drawn from the rats at different times over 24 h to determine the hydroxyproline concentration. The bioavailability of the gelatin was indirectly measured by the bioavailability of hydroxyproline in gelatin. The relative and absolute bioavailability of gelatin was 74.12% and 85.97%, respectively. The amino acid profile of plasma showed 41.91% of the digested gelatin was absorbed from the intestine in peptide form. The authors of this study concluded that gelatin had high oral bioavailability.

TOXICOLOGICAL STUDIES

Acute

Animal –Dermal

Hydrolyzed Collagen

Hydrolyzed Collagen at up to 2% in formulation was practically nontoxic when administered dermally in acute toxicity studies.[3]

Animal - Oral

Collagen

The safety of a product containing approximately 60% collagen type II (from chicken sternal cartilage), 20% chondroitin sulfate, and 10% hyaluronic acid was investigated in 5 male and 5 female Sprague-Dawley rats.[36] The rats received a single oral dose of 5000 mg/kg body weight and were observed for clinical signs of toxicity for 14 days. All rats survived the observation period and had normal body weight gains. On the 15th day of the study, the rats were killed and underwent macroscopic necropsy: no gross pathological lesions were observed in any of the animals.

Hydrolyzed Collagen

Hydrolyzed Collagen was practically nontoxic when administered orally (up to 100%) in acute toxicity studies.[3]

Short-Term Toxicity Studies

Animal – Oral

Gelatin

In a rat study of the ability of shark skin gelatin to increase bone mineral density, no adverse effects were reported.[37] The female Wistar rats (n=40) were ovariectomized approximately a week after the start of receiving a low-protein diet and then received shark gelatin as oral doses of 10, 20, or 40 mg/100 g body weight/day for 2 weeks. Control animals were given ovalbumin at 20 mg/100 g body weight/day. No significant differences between experimental groups and the controls were observed in final body weight, feed intake, femoral bone weight, or femoral bone length.

Subchronic Toxicity Studies

Animal – Dermal

Hydrolyzed Collagen

Subchronic dermal studies on 2 cosmetic formulations containing 2% Hydrolyzed Collagen were negative for systemic toxicity.[3]

Animal - Oral

Collagen

The safety of a product containing approximately 60% collagen type II, (from chicken sternal cartilage), 20% chondroitin sulfate, and 10% hyaluronic acid was investigated in 40 male and 40 female Sprague-Dawley rats.[36] The rats were divided into groups of 10 animals/sex and received the test material in distilled water at 0, 30,

300, or 1000 mg/kg body weight once daily via gavage for 90 days. Animals were observed twice daily for mortality and detailed observations for clinical signs of toxicity were performed once weekly. Body weight and feed consumption were measured weekly. Hematology samples were collected a week before the end of dosing and the animals were killed at the end of the dosing period. A gross necropsy was performed on all animals and tissues were preserved for histopathological examination.

All animals survived until the end of the dosing period and no adverse effects or clinical signs of toxicity were observed during treatment. No significant findings were observed in changes in average body weights, average body weight gain, or hematology parameters. A statistically significant but small decrease in alkaline phosphatase activity in the 1000 mg/kg/day males was observed, but was not considered adverse. Minimal but statistically significant increases in albumin in 300 mg/kg/day males and in globulin in 1000 mg/kg/day females were not considered to be toxicologically significant since these were not dose-related. Statistically significant, but minimal, changes in average brain weight in the low dose females (higher than controls) and spleen to brain weight ratios in the intermediate dose group males (lower than controls) were also not considered to be toxicologically significant. No treatment-related histopathologic changes or gross abnormalities were observed. The researchers concluded that the test material containing collagen type II was tolerated well in this rat study.[36]

Human - Oral

Hydrolyzed Collagen/Gelatin

In a 4-month dietary intake study of hydrolyzed collagen (interchangeably reported as gelatin) for the potential role in enhancing bone remodeling in children, no adverse effects were observed.[38] The randomized double-blind study divided the children (ages 6-11) in to 3 groups that received placebo (n=18), hydrolyzed collagen (n=20), or hydrolyzed collagen+calcium (n=22) daily 250 ml dose.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

No published DART studies on tissue-derived proteins and peptides were discovered and no unpublished data were submitted.

GENOTOXICITY

No published genotoxicity studies on tissue-derived proteins and peptides were discovered and no unpublished data were submitted.

CARCINOGENICITY

No published carcinogenicity studies on tissue-derived proteins and peptides were discovered and no unpublished data were submitted.

OTHER RELEVANT STUDIES

Type 1 Hypersensitivity

Skin prick tests and histamine release tests of fish gelatin and codfish were completed in 30 fish-allergic patients (diagnosed in accordance with European Academy of Allergy and Clinical Immunology Guidelines).[39] Codfish-specific IgE was also measured in the patients and they underwent double-blinded, placebo-controlled food challenges with fish gelatin. All 30 patients had positive skin prick tests, histamine release tests, and specific IgE to codfish. Skin prick tests and histamine release tests with fish gelatin were positive in 3/30 and 7/30 patients, respectively. Oral challenge resulted in two patients reporting mild subjective reactions. One patient had a mild reaction to the placebo but not the fish gelatin. The proportion of truly sensitive patients was estimated to be 0.03. The study authors concluded that the fish gelatin in the study presented no risk to fish-allergic patients at doses typically used in foods (3.61 g).

The potential for tuna skin-derived gelatin to induce allergic reaction in patients with fish allergy or sensitization was investigated using the serum samples of 100 consecutive allergic patients. [40] Serum IgE antibodies were tested against hydrolyzed or non-hydrolyzed gelatin extracted from yellowfin tuna skin and compared to extracts of yellowfin tuna flesh and skin and bovine or porcine gelatins. Of the 100 samples tested, only 3 exhibited reactivity to tuna skin-derived gelatin (1 hydrolyzed, 2 non-hydrolyzed). No cross-reactivity was observed between bovine/porcine gelatin and fish gelatin.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

In Vitro

Hydrolyzed Elastin

In a MatTek™ EpiDerm assay, solutions of Hydrolyzed Elastin (concentration not reported; MW = 2000-4000 Da) were predicted to be non-irritating.[21] Two forms of the ingredient were tested, with one sourced from fish. The source of the form was not reported.

Animal

Hydrolyzed Elastin

Hydrolyzed Elastin (MW = 3000 Da) was not a primary irritant when tested neat in a Draize primary dermal irritation study in 6 New Zealand white rabbits.[41] The test sites were occluded for 24 h. The primary irritation index score was 0.38.

Hydrolyzed Collagen

Primary skin irritation tests in rabbits indicated that Hydrolyzed Collagen was nonirritating or minimally irritating when tested at up to 100%.[3]

Human

Hydrolyzed Collagen

Irritation was not observed in human volunteers with healthy skin at concentrations up to 28%, but moderate irritation was observed in volunteers with dermatitis.[3]

Sensitization

Animal

Hydrolyzed Collagen

Hydrolyzed Collagen was nonsensitizing in guinea pig studies at up to 2%.[3]

Human

Hydrolyzed Collagen

Formulations containing 0.5% to 28% Hydrolyzed Collagen produced some irritation but no sensitization in human repeated insult patch tests (HRIPTs).[3]

Hydrolyzed Elastin

In a HRIPT with 52 subjects, Hydrolyzed Elastin (25% w/v in corn oil; MW = 3000 Da) did not produce dermal irritation or dermal sensitization.[42] The test patches were occlusive.

Phototoxicity

Hydrolyzed Collagen

Hydrolyzed Collagen at up to 2% was not phototoxic to animals, nor was it phototoxic or photosensitizing to humans at up to 0.2%.[3] *UV-induced erythema was decreased after application of 10% solution of Hydrolyzed Collagen (MW = 1500 Da) onto the skin after irradiation.*

OCULAR IRRITATION STUDIES

In Vitro

Hydrolyzed Elastin

In a MatTek™ EpiOcular assay, solutions of Hydrolyzed Elastin (concentration not reported; MW = 2000-4000 Da) were predicted to be non-irritating.[21] Two forms of the ingredient were tested, with one sourced from fish. The source of the other form was not reported.

Animal

Hydrolyzed Collagen

Hydrolyzed Collagen was minimally irritating to rabbit eyes when tested full-strength.[3]

Hydrolyzed Elastin

Hydrolyzed Elastin (MW = 3000 Da) was not a primary irritant when tested neat in a Draize ocular irritation study in 6 New Zealand white rabbits.[41] The treated eyes of the animals were not rinsed.

CLINICAL STUDIES

Case Reports

Elastin

A 26-year-old woman with a history of fish allergy experienced urticarial eruptions following use of a cosmetic cream containing codfish-derived elastin.[43] The patient's serum total IgE level was 442 kU/L, and strong radioallergosorbent test scores for specific IgE were observed for tuna, salmon, mackerel, flatfish, codfish, horsemackerel, sardine, and salmon roe. No prick-tests were performed because of the patient's history of severe symptoms. Immunoblot analysis revealed that the patient had IgE antibodies against codfish elastin, parvalbumin, collagen, and transferrin. The molecular weight range of the proteins that the patient's serum reacted with was 10,000 to 20,000 Da, which corresponded to the range of codfish elastin. The company that produced the cosmetic cream reported that the elastin in the cosmetic cream was derived from the skin and soft tissue of codfish.

Collagen, Hydrolyzed Collagen, Soluble Collagen

A 30-year-old woman with a history of atopic dermatitis experienced anaphylaxis twice on separate occasions, once after consuming a fortified yogurt containing hydrolyzed fish collagen and once after consuming a gummy candy containing hydrolyzed fish collagen.[44] Fifteen months prior to the anaphylactic episodes, the patient had been applying a moisturizer containing fish atelocollagen (a water-soluble collagen) to her impaired facial skin. The atelocollagen in the product has a molecular weight of 350,000 Da. Skin prick tests on the patient were positive for hydrolyzed fish collagen in the food products, the moisturizer, fish atelocollagen, and fish gelatin. The tests were negative for gelatin derived from porcine skin or bovine bone. The patient denied anaphylactic reactions following ingestion of raw or cooked fish. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and IgE western blot analyses showed that the patient's serum reacted with an approximately 140,000 Da protein of fish atelocollagen and a 120,000 Da protein of gelatin from fish collagen. Weak reactions were observed with bovine bone gelatin protein and no reactions were observed to porcine skin gelatin protein or hydrolyzed fish collagen protein. The researchers of this case study speculated that the fish atelocollagen (350,000 Da) was degraded on the skin surface by proteases into smaller peptides and induced sensitization, but did not rule out the possibility that intact collagen or degradation products with greater than 4500 Da were antigens because of the patient's impaired skin.

A 22-year-old female reported contact urticaria following use of a hair conditioner that contained steartrimonium hydrolyzed animal protein.[45] She had a similar, less severe reaction the year before to another hair conditioner that also contained this ingredient. The patient also had a history of hay fever and recurrent hand dermatitis. Prick testing elicited strongly positive wheal and flare response to both hair conditioners, steartrimonium hydrolyzed animal protein, and other hair conditioners that contained protein, including hydrolyzed collagen in some products. Negative reactions were observed when the patient was tested with protein-free hair products. Prick tests with the standard series of allergens yielded positive results for grass mix, rye, English plantain, dust mite, cow's milk, soybean, baker's yeast, and wholegrain wheat. Tests with raw meat were negative. The patient's total IgE was 221. Radioallergosorbent tests (RAST) were negative to pork, beef, chicken, and mutton.

Hydrolyzed Collagen

A study of sensitization to protein hydrolysates in hair care products was performed in 3 groups of patients.[46] The first group, which comprised 11 hairdressers with hand dermatitis, submitted to scratch and prick tests with 22 trademarked protein hydrolysates, including Soluble Collagen and Hydrolyzed Collagen, as well as quaternized hydrolyzed proteins. The second test group comprised 1260 consecutive adults with suspected allergic respiratory disease: they were subjected to skin prick tests with 1 to 3 of the protein hydrolysates. The third group of patients comprised 28 adults with atopic dermatitis and was also tested with a protein hydrolysate via a skin prick test.

Positive reactions were seen in a total of 12 patients (all female with atopic dermatitis) from 3 of the 22 protein hydrolysates. All 12 had reactions to hydroxypropyl trimonium hydrolyzed collagen. Three of the 12 also had a reaction to one trademarked version of Hydrolyzed Collagen (1% solution), while 1 other had a reaction to hydroxypropyl trimonium hydrolyzed milk protein.[46]

SUMMARY

This report assesses the safety of 18 tissue-derived ingredients, including Hydrolyzed Collagen, which has been previously reviewed by the Panel. Summary information presented in this safety assessment from the previous report will not be summarized below.

Soluble Collagen is used in 425 formulations; the majority of uses are in leave-on skin care products. Gelatin has the second greatest number of overall uses reported, with a total of 334; the majority of the uses are in rinse-off bath soaps and detergents. The results of the concentration of use survey conducted in 2016 by the Council indicate Collagen has the highest reported maximum concentration of use; it is used at up to 96% in face and neck skin care products. Gelatin is used at up to 66% in bath oils, tablets, and salts.

A toxicokinetics study of fish-derived gelatin (4000 mg/kg) in rats found that gelatin has a high oral bioavailability.

A product containing 60% chicken-derived collagen did not produce acute toxic effects in rats that were given a single oral dose of 5000 mg/kg.

No adverse effects were reported in a 2 week oral study of shark skin-derived gelatin in ovariectomized rats that received the test material at up to 40 mg/100 g daily.

In subchronic toxicity studies, rats tolerated daily oral dosing of a test material containing 60% collagen. No adverse effects were reported in a 4 month study of a dietary supplement containing a 250 ml dose of hydrolyzed collagen in human children

Gelatin and other tissue-derived proteins may be sourced from fish, which is a major food allergen that can produce Type 1 (immediate) reactions in sensitized individuals. Researchers have reported a low risk of IgE-mediated reactions to fish gelatin in individuals with fish allergies.

In vitro dermal irritation studies have predicted solutions of Hydrolyzed Elastin (MW = 2000-4000 Da) to be non-irritating. The ingredient (MW = 3000 Da) was not a primary dermal irritant when tested neat in rabbits.

In a HRIPT, Hydrolyzed Elastin (25% w/v in corn oil; MW = 3000 Da) did not produce dermal irritation or dermal sensitization.

Hydrolyzed Elastin was not predicted to be an ocular irritant in in vitro assays and it was not an ocular irritant in rabbit eyes when tested neat.

Case reports of dermal sensitization to cosmetics containing Elastin and Collagen derived from fish have been described in the published literature. Reactions to Hydrolyzed Collagen have been reported as well.

No relevant published DART, genotoxicity, or carcinogenicity studies on tissue-derived proteins and peptides 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 tissue-derived ingredients, particularly in relation to the different sources from which these ingredients may be derived. Information on dermal penetration potential and any additional toxicological data (especially at use concentration) would help the CIR Expert Panel assess the safety these ingredients as used in cosmetics and would improve the resulting safety assessment.

TABLES

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

Ingredient CAS No.	Definition	Function
Ammonium Hydrolyzed Collagen 68951-88-2 [generic to ammonium hydrolyzed proteins]	Ammonium Hydrolyzed Collagen is the ammonium salt of Hydrolyzed Collagen.	hair conditioning agents; skin-conditioning agents-misc.
Calcium Hydrolyzed Collagen	Calcium Hydrolyzed Collagen is the calcium salt of Hydrolyzed Collagen.	nail conditioning agents; skin-conditioning agents-misc.
MEA-Hydrolyzed Collagen	MEA-Hydrolyzed Collagen is the monoethanolamine salt of Hydrolyzed Collagen.	hair conditioning agents; skin-conditioning agents-misc.
Zinc Hydrolyzed Collagen	Zinc Hydrolyzed Collagen is the zinc salt of Hydrolyzed Collagen.	hair conditioning agents; skin-conditioning agents-misc.
Hydrolyzed Collagen 73049-73-7 [generic to animal peptones] 92113-31-0	Hydrolyzed Collagen is the hydrolysate of animal or fish collagen derived by acid, enzyme or other method of hydrolysis. It is characterized by a significant level of hydroxyproline residues.	hair conditioning agents; nail conditioning agents; skin-conditioning agents-misc.
Hydrolyzed Collagen Extract	Hydrolyzed Collagen Extract is the extract of Hydrolyzed Collagen.	skin protectants
Soluble Collagen	Soluble Collagen is a non-hydrolyzed, native protein derived from the connective tissue of animals. It consists essentially of a mixture of the precursors of mature collagen. It has a triple helical structure and is predominantly not cross-linked.	hair conditioning agents; skin-conditioning agents-misc.
Collagen 9007-34-5	Collagen is the protein found in cartilage and other connective tissues in animals.	hair conditioning agents; skin-conditioning agents-misc.
Gelatin 9000-70-8	Gelatin is a product obtained by the partial hydrolysis of collagen derived from the skin, white connective tissue and bones of animals.	binders; hair conditioning agents; lytic agents; oral health care drugs; skin-conditioning agents-misc.; viscosity increasing agents-aqueous
Hydrolyzed Gelatin 68410-45-7 [specific to enzymatic digest product]	Hydrolyzed Gelatin is the hydrolysate of Gelatin derived by acid, enzyme or other method of hydrolysis.	skin-conditioning agents-misc.
Hydrolyzed Reticulin 73049-73-7 [generic to animal peptones] 99924-37-5	Hydrolyzed Reticulin is the hydrolysate of the reticulin portion of animal connective tissue derived by acid, enzyme or other method of hydrolysis. [Reticulin is a type of fiber in connective tissue composed of type III collagen secreted by reticular cells]	hair conditioning agents; skin-conditioning agents-misc.
Hydrolyzed Actin 73049-73-7 [generic to animal peptones]	Hydrolyzed Actin is the hydrolysate of actin derived by acid, enzyme or other method of hydrolysis.	hair conditioning agents; skin-conditioning agents-misc.
Elastin 9007-58-3	Elastin is a fibrous protein found in the connective tissue of animals.	hair conditioning agents; skin-conditioning agents-misc.
Soluble Elastin	Soluble Elastin a water soluble non-hydrolyzed, native protein derived from Elastin.	skin-conditioning agents-misc.
Hydrolyzed Elastin 100085-10-7 73049-73-7 [generic to animal peptones] 91080-18-1	Hydrolyzed Elastin is the hydrolysate of elastin derived by acid, enzyme or other method of hydrolysis.	hair conditioning agents; skin-conditioning agents-emollient; skin-conditioning agents-misc.
Fibronectin 98725-78-1	Fibronectin is a glycoprotein found in connective tissues, basement membranes, in plasma and other body fluids.	hair conditioning agents; skin-conditioning agents-misc.
Hydrolyzed Fibronectin 100085-35-6 73049-73-7 [generic to animal peptones]	Hydrolyzed Fibronectin is the hydrolysate of Fibronectin derived by acid, enzyme or other method of hydrolysis.	hair conditioning agents; skin-conditioning agents-misc.
Hydrolyzed Spongin	Hydrolyzed Spongin is the hydrolysate of spongin derived by acid, enzyme or other method of hydrolysis. [Spongin is a collagen-type protein, common to marine sponges]	skin-conditioning agents-misc.

Table 2. Reported molecular weights of tissue-derived proteins[3,10,14,23,47,48]

Ingredient	Molecular Weight (Da) Range
Collagen (native)	130,000 to > 1,000,000
Soluble Collagen	30,000-40,000, but may be up to an average of 300,000
Hydrolyzed Collagen	1000 to 25,000
Hydrolyzed Actin	58.4% < 5000; 41.4% > 5000 and < 30,000
Hydrolyzed Elastin	500 to 150,000
Fibronectin	>200,000

Table 3. Amino acid residue profile of collagen, soluble collagen, and elastin (residues per 1000).[13,14,49]

Amino Acid	Collagen	Soluble Collagen	Elastin
Hydroxyproline	73-98	95.9-105.8	7.1
Aspartic acid	42-48	43.9-48.3	7.3
Threonine	17-19	15.2-21.1	10.1
Serine	22-31	28.3-44.1	9.0
Glutamic acid	73-80	68.3-86.1	17.4
Proline	121-125	115.6-144.8	125.4
Glycine	325-347	310.3-324.0	316.2
Alanine	112-114	88.2-107.3	223.3
Cysteine	not determined	not determined	not determined
Valine	19-26	not determined	134.0
Methionine	not determined	3.9-8.3	not detected
Isoleucine	11-14	11.8-13.0	26.6
Leucine	24-31	25.5-29.3	64.7
Tyrosine	1-7	1.8-3.3	6.1
Phenylalanine	13-16	10.7-16.3	33.6
Histidine	4-6	4.8-6.5	0.5
Hydroxylysine	not determined	8.3-9.8	not detected
Lysine	26-31	25.8-27.7	3.6
Arginine	50-55	46.5-52.0	6.0
Tryptophan	not determined	not determined	not determined

Table 4. Frequency (2016) and concentration of use (2017) according to duration and type of exposure for tissue-derived proteins and peptides.[24,25]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Ammonium Hydrolyzed Collagen		**Collagen**		**Elastin**		**Fibronectin**	
Totals†	**NR**	**0.03-0.12**	**299**	**0.00005-96**	**46**	NR	**2**	**NR**
Duration of Use								
Leave-On	NR	0.1-0.12	241	0.0001-96	40	NR	2	NR
Rinse Off	NR	0.03-0.05	58	0.00005-1.3	6	NR	NR	NR
Diluted for (Bath) Use	NR	NR	NR	NR	NR	NR	NR	NR
Exposure Type								
Eye Area	NR	NR	31	0.00012-0.2	2	NR	NR	NR
Incidental Ingestion	NR	NR	5	0.0035-0.05	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	55[b]; 94[c]	0.00095[b]	11[b]; 11[c]	NR	2[c]	NR
Incidental Inhalation-Powder	NR	0.1[a]	94[c]	0.0018-96[a]	11[c]	NR	2[c]	NR
Dermal Contact	NR	0.03-0.12	240	0.00005-96	42	NR	2	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	0.05	39	0.00095-1.3	4	NR	NR	NR
Hair-Coloring	NR	NR	1	NR	NR	NR	NR	NR
Nail	NR	NR	4	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	8	0.0035-0.67	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

	Gelatin		**Hydrolyzed Actin**		**Hydrolyzed Elastin**		**Hydrolyzed Fibronectin**	
Totals†	**334**	**0.000052-66**	**20**	**0.00075-1.6**	**278**	**0.00000035-5**	**10**	**0.025-0.05**
Duration of Use								
Leave-On	33	0.0003-37.8	18	1.6	211	0.00000035-5	9	0.05
Rinse Off	301	0.000052-9.5	2	0.00075	67	0.0000035-2.5	1	0.025
Diluted for (Bath) Use	NR	0.21-66	NR	NR	NR	NR	NR	NR
Exposure Type								
Eye Area	NR	0.5-1	5	1.6	10	0.00035-0.5	1	NR
Incidental Ingestion	3	NR	NR	NR	2	0.00035-0.15	NR	NR
Incidental Inhalation-Spray	19[b]; 6[c]	0.03; 0.009-0.095[b]; 0.016[c]	7[b]; 6[c]	NR	57[b]; 88[c]	0.00031-0.42; 0.002-0.24[b]	4[b]; 3[c]	NR
Incidental Inhalation-Powder	1; 6[c]	0.016[c]; 0.03-37.8[a]	6[c]	NR	88[c]; 2[a]	0.0000035; 0.00025-5[a]	3[c]	NR
Dermal Contact	286	0.0003-66	10	NR	227	0.00000035-5	10	0.025-0.05
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	12	0.000052-0.5	7	0.00075	48	0.00031-2.5	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	33	0.001-0.02	NR	NR	NR	NR	NR	NR
Mucous Membrane	257	0.0011-66	NR	NR	5	0.00035-0.15	NR	NR
Baby Products	NR	NR	NR	NR	4	NR	NR	NR

Table 4. Frequency (2016) and concentration of use (2017) according to duration and type of exposure for tissue-derived proteins and peptides.[24,25]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Hydrolyzed Reticulin		**MEA-Hydrolyzed Collagen**		**Soluble Collagen***		**Soluble Collagen Extract‡**	
Totals†	NR	**0.025-0.05**	NR	**0.03-0.12**	**425**	**0.0000005-0.7**	**2**	NR
Duration of Use								
Leave-On	NR	0.05	NR	0.1-0.12	368	0.0000005-0.7	2	NR
Rinse Off	NR	0.025	NR	0.03-0.06	57	0.000025-0.014	NR	NR
Diluted for (Bath) Use	NR	NR	NR	NR	NR	0.000035-0.005	NR	NR
Exposure Type								
Eye Area	NR	NR	NR	NR	51	0.00003-0.05	NR	NR
Incidental Ingestion	NR	NR	NR	NR	6	0.00035-0.01	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR	104[b]; 141[c]	0.000005-0.0035; 0.00035-0.0035[b]	1[b]; 1[c]	NR
Incidental Inhalation-Powder	NR	NR	NR	0.1[a]	141[c]	0.0035; 0.0001-0.7[a]	1[c]	NR
Dermal Contact	NR	0.025-0.5	NR	0.03-0.12	379	0.000005-0.7	2	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	0.06	15	0.00001-0.014	NR	NR
Hair-Coloring	NR	NR	NR	NR	1	0.000025-0.0005	NR	NR
Nail	NR	NR	NR	NR	NR	0.0000005-0.01	NR	NR
Mucous Membrane	NR	NR	NR	NR	9	0.000035-0.01	NR	NR
Baby Products	NR	NR	NR	NR	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 powders, but it is not specified whether the reported uses are powders.

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

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

* Includes 25 uses listed in the VCRP as “soluble animal collagen”.

‡ Not listed in the INCI Dictionary, possibly the same as Collagen Extract.

Table 5. Historic and current frequency and concentration of use according to duration and type of exposure for Hydrolyzed Collagen.[24,25]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	1981 uses/concentrations		**2002 uses/2004 concentrations**		**2017 uses/2016 concentrations**	
Totals†	**923**	**≤0.1 - >50**	**570[d]**	**0.000004-6**	**543**	**0.00003-16.5**
Duration of Use						
Leave-On	284	≤0.1 - ≤50	245	0.000004-6	365	0.00003-16.5
Rinse Off	633	≤0.1 - >50	321	0.007-0.2	177	0.00003-3
Diluted for (Bath) Use	6	>0.1-5	4	NR	1	NR
Exposure Type						
Eye Area	40	≤0.1 - ≤5	21	0.000004-3	23	0.001-3.2
Incidental Ingestion	15	≤1	7	1	5	0.01-0.1
Incidental Inhalation-Spray	7; 96[a]; 46[b]	<1; >0.1 - >50[a]; ≤10[b]	3; 108[a]; 38[b]	0.000004-1[a]; 0.06-6[b]	116[a]; 139[b]	0.0017-0.28; 0.0092-16.5[a]
Incidental Inhalation-Powder	5; 46[b]	≤1; ≤10[b]	4; 38[b]	0.5; 0.06-6[b]	3; 139[b]; 2[c]	0.0015-5[c]
Dermal Contact	207	≤0.1 - ≤25	210	0.000004-6	401	0.0003-5
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	609	>0.1 - >50	331	0.02-0.2	121	0.0003-16.5
Hair-Coloring	46	≤0.1 - ≤5	4	NR	3	0.15-1.2
Nail	18	≤0.1 - ≤50	9	NR	7	0.00003-0.01
Mucous Membrane	24	>0.1-5	28	0.1-1	22	0.0024-0.1
Baby Products	1	≤0.1	NR	NR	4	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
d. Majority of the uses were categorized as "Hydrolyzed Animal Protein" in the VCRP database.

Table 6. Ingredients not reported in use.
Calcium Hydrolyzed Collagen
Zinc Hydrolyzed Collagen
Hydrolyzed Collagen Extract
Hydrolyzed Gelatin
Soluble Elastin
Hydrolyzed Spongin

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