Toxicological profile for

Gelatin

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

Not applicable.

1.2. Synonyms

Gelatin; Gelfoam; Gelatin, unspecified; Gelatin foam; Gelatina; Gelofusine; Pharmagel A; Pharmagel Adb; Pharmagel B; Puragel; Spongiofort; CHEBI:5291; Collagens, gelatins; EINECS 232-554-6; Emagel; FreAlagin AD; FreAlagin M; gelatin (ep monograph); gelatin (ii); gelatin (mart.); gelatin limed bone; gelatin,absorbable; gelatin,absorbable film; gelatin,absorbable powder; gelatin,absorbable sponge; Gelatin velvatex; Gelofusin; UNII-1T8387508X; UNII-2G86QN327L; UNII-6EG4DCS5TJ; UNII-7Z075S9991; UNII-A7JR5F8DLH; UNII-AHQ60JKI5D; UNII-F5AJW0ONK4; UNII-JSM64OJO9B; UNII-WIL1404U79; (PubChem)

1.3. Molecular formula

No data available to us at this time.*1.4. Structural Formula*

1.4. Structural Formula

1.4. Structural Formula

Not applicable.

1.5. Molecular weight (g/mol)

Not applicable.

1.6. CAS registration number

9000-70-8

1.7. Properties

1.7.1. Melting point

(°C): No data available to us at this time.

1.7.2. Boiling point

(°C): No data available to us at this time.

1.7.3. Solubility

Soluble in hot water, glycerol, acetic acid; insoluble in organic solvents, insoluble in alcohol, chloroform, ether, fixed & volatile oils (HSDB, 2002)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

No data available to us at this time.

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): No data available to us at this time.

1.7.8. Decomposition temperature

(°C): No data available to us at this time.

1.7.9. Stability

Stable in air when dry, subject to microbic decomp when moist or in soln (HSDB, 2002)

1.7.10. Vapor pressure

No data available to us at this time.

1.7.11. log Kow

No data available to us at this time.

2. General information

2.1. Exposure

Gelatin (CAS RN 9000-70-8) is listed (at concentrations where specified) as an ingredient in personal care (15-30%) and pesticide (12%) products by the CPID.

Gelatin (CAS RN 9000-70-8) is used as a film forming and viscosity controlling agent in cosmetics in the EU. As taken from CosIng (Cosmetic substances and ingredients database).

“Used to make lithographic and printing inks, plastic compounds, artificial silk, matches, pharmaceutical capsules, and light filters for mercury lamps; Used as clarifying agent, pharmaceutical aid (suspending agent), gelling component and additive for foods, carrier in photographic films and papers, and microencapsulating agent; Also used in electroplating, hectographic masters, paper and textile sizing, blood substitutes and extenders, cosmetics, ointments, bacteriology, and other medical and veterinary applications.”

“Industrial Processes with risk of exposure: Electroplating, Photographic Processing, Pulp and Paper Processing, Textiles (Fiber & Fabric Manufacturing)”

As taken from Haz-Map, 2021.

Occupational exposure limit in Russia – 10 mg/m3, JUN2003.

As taken from RTECS, 2019.

Gelatine (CAS RN 9000-70-8) is listed as a fragrance ingredient by IFRA.

Gelatin (CAS RN 9000-70-8) is used as a binder, coating agent, emulsifying agent, encapsulating agent, film former, gelling agent, stabilizing agent, thickening agent and viscosity increasing agent in non-medicinal natural health products (Health Canada, 2021).

“Gelatin (CAS RN 9000-70-8) is used in: binders, hair conditioning agents, lytic agents, oral health care drugs, skin-conditioning agents-misc., viscosity increasing agents – aqeous. (…)
“Gelatin is used in a total of 334 formulations; 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.43 Gelatin is used at up to 66% in bath oils, tablets, and salts. (…)
Non-cosmetic uses of Gelatin include uses in food as a stabilizer, thickener, texturizer, firming agent, surfaceactive agent, or surface-finishing agent.11,20 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.11 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).”

CIR (2022)

2.2. Combustion products

No data available to us at this time.

2.3. Ingredient(s) from which it originates

“Gelatin is the name given to the proteins formed when the connective tissues of animals are boiled. They have the property of dissolving in hot water and forming a jelly when cooled. Gelatin is thus a large molecular weight protein formed from hydrolysis of collagen. Gelatin solutions were first used as colloids in man in 1915. The early solutions had a high molecular weight (about 100,000). This had the advantage of a significant oncotic effect but the disadvantages of a high viscosity and a tendency to gel and solidify if stored at low temperatures.”

As taken from Mitra S and Khandelwal P (2009). Are All Colloids Same? How to Select the Right Colloid? Indian J Anaesth. 53(5): 592–607. Pubmed, 2011 available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2900092/

Common/vernacular names: Agar-agar, gelosa, gelose, layor carang, and vegetable gelatin; also, Chinese gelatin, colle du Japon, Japanese gelatin, and Japanese isinglass.

Source: Red algae, including Gelidium cartilagineum (L.) Gaill., Gelidium amansii Lamour., Gracilaria confervoides (L.) Grev., other Gelidium and Gracilaria species as well as species of the genera Pterocladia, Ahnfeltia, Acanthopeltis, and Suhria.

As taken from Khan IA and Abourashed EA, 2010.

“A complex combination of proteins obtained by hydrolysis of collagen by boiling skin, tendons, ligaments, bones, etc.”

As taken from CosIng (Cosmetic substances and ingredients database).

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) reviewed the safety of 19 ectodermal-derived proteins and peptides, which function mainly as skin and/or hair conditioning agents in

cosmetics, including gelatin. It concluded that “these ingredients are safe in the present practices of use and concentration” (CIR, 2017).

“A mixture of proteins made from denatured collagen”

As taken from Haz-Map, 2021

“In this study, we aimed to obtain gelatin from the marine snail Rapana venosa using acidic and enzymatic extraction methods and to characterize these natural products for cosmetic and pharmaceutical applications. Marine gelatins presented protein values and hydroxyproline content similar to those of commercial mammalian gelatin, but with higher melting temperatures. Their electrophoretic profile and Fourier transform infrared (FTIR) spectra revealed protein and absorption bands situated in the amide region, specific for gelatin molecule. Scanning electron microscopy (SEM) analysis showed significant differences in the structure of the lyophilized samples, depending on the type of gelatin. In vitro studies performed on human keratinocytes showed no cytotoxic effect of acid-extracted gelatin at all tested concentrations and moderate cytotoxicity of enzymatic extracted gelatin at concentrations higher than 0.5 mg/mL. Also, both marine gelatins favored keratinocyte cell adhesion. No irritant potential was recorded as the level of IL-1α and IL-6 proinflammatory cytokines released by HaCaT cells cultivated in the presence of marine gelatins was significantly reduced. Together, these data suggest that marine snails are an alternative source of gelatins with potential use in pharmaceutical and skincare products.” As taken from Gaspar-Pintiliescu A et al. 2019. Mar. Drugs 17(10), 589. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31627413/

“Gelatin is a heterogeneous mixture of water-soluble proteins of high average molecular weight that are derived from the denaturation and hydrolysis of Collagen.11 Glycine or alanine accounts for 1 third to 1 half of the amino acid residues, while another quarter is composed of proline or hydroxyproline. (…)

According to 21 CFR§700.27, Gelatin is `… a product that has been obtained by the partial hydrolysis of collagen derived from hides, connective tissue, and/or bones of cattle and swine. Gelatin may be either Type A (derived from an acid-treated precursor) or Type B (derived from an alkali-treated precursor) that has gone through processing steps that include filtration and sterilization or an equivalent process in terms of infectivity reduction.`”

CIR (2022)

3. Status in legislation and other official guidance

FDA Requirements:

REGULATION: FDA - GRAS. [Furia, T.E. (ed.). CRC Handbook of Food Additives. 2nd ed. Cleveland: The Chemical Rubber Co., 1972., p. 857] **PEER REVIEWED**

As taken from HSDB, 2002.

Included on the US FDA’s list of Substances Added to Food (formerly EAFUS) as an anticaking or free-flow agent, drying agent, flavoring agent or adjuvant, formulation aid, humectant, processing aid, solvent or vehicle, stabilizer or thickener, surface-finishing agent, and texturizer, and included under 21 CFR sections 133.178 (Pasteurized neufchatel cheese spread with other foods), 133.179 (Pasteurized process cheese spread), 172.230 (Microcapsules for flavoring substances), 172.255 (Polyacrylamide), 172.280 (Terpene resin) and 182.70 (Substances migrating from cotton and cotton fabrics used in dry food packaging).

As taken from FDA, 2024

Included on the US FDA’s database of Select Committee on GRAS Substances (SCOGS). The SCOGS Select Committee concluded that “there is no evidence in the available information on

gelatin that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current or might reasonably be expected in the future." (FDA, 2015).

"**Guidance on Gelatin** - In 1994, representatives of the gelatin industry presented preliminary data to FDA staff concerning an experimental study of the infectivity of TSE-infected tissue that had undergone one of two processes (lime or acid) used to make gelatin. Based on these data, FDA decided not to include gelatin as part of its recommendations concerning other bovine ingredients in FDA-regulated products. A notice in the Federal Register of August 29, 1994, summarized FDA's recommendations to reduce any potential BSE risk and clarified that FDA's recommendations at that time did not extend to gelatin for human use produced from bovine materials from BSE countries.

Recent Review of Gelatin Guidance - In 1996, FDA decided to review its previous guidance on the use of gelatin because of new information suggesting that BSE may be transmissible to humans and because of updated data from the study on the effect of gelatin processing on infectivity.

During the April 1997 meeting of the TSE advisory committee, information on industry practices and the results of the research study were presented. The study involved mouse brain tissue that had been infected with scrapie (as a BSE model).1 The tissue was treated with lime or with acid according to gelatin manufacturing conditions. Neither the acid nor the lime treatment completely inactivated the infectious agent. A second infectivity study is due to be completed in late 1997 or early 1998.

The advisory committee members stated opinions on questions raised by FDA and were polled on their answers to the final question, "Does current scientific evidence justify continuing to exempt gelatin from restrictions recommended by FDA for other bovine-derived materials from BSE countries?" Ten of the 14 members responded "no"or a "qualified no"to this question (see Appendix B for a summary of the advisory committee meeting).

Recommendations - FDA has been reviewing the currently available scientific information, including information provided on behalf of the Gelatin Manufacturers of Europe and the Gelatin Manufacturers Institute of America. FDA also considered the advisory committee's recommendations and other available information. Based on this review, FDA proposes the following recommendations concerning the acceptability of gelatin for use in FDA-regulated products intended for human use:

- In order to ensure that all parties in the distribution chain take appropriate responsibility, importers, manufacturers, and suppliers should determine the tissue, species, and country source of all materials to be used in processing gelatin for human use.
- Bones and hides from cattle that shows signs of neurological disease, from any source country, should not be used as raw material for the manufacture of gelatin.
- Gelatin produced from bones and hides obtained from cattle residing in, or originating from, countries reporting BSE or from countries that do not meet the latest BSE-related standards of the Office International des Epizooties (OIE)2 (see Appendix C) should not be used either in injectable, ophthalmic, or implanted FDA-regulated products, or in their manufacture.
- At this time, there does not appear to be a basis for objection to the use of gelatin in FDA-regulated products for oral consumption and cosmetic use by humans when the gelatin is produced from bones obtained from cattle residing in, or originating from, BSE countries, if the cattle come from BSE-free herds and if the slaughterhouse removes the heads, spines, and spinal cords directly after slaughter. Nor does there appear to be a basis for objection to gelatin for oral consumption and cosmetic use which is produced from bones from countries which have not reported BSE but which fail to meet OIE standards if the slaughterhouse removes the heads, spine, and spinal cords after slaughter. Gelatin

processors should ensure that slaughterhouses that supply bovine bones for gelatin production remove heads, spines, and spinal cords as the first procedure following slaughter.

- At this time, there does not appear to be a basis for objection to the use of gelatin produced from bovine hides, from any source country, in FDA-regulated products for oral consumption and cosmetic use by humans use if processors ensure that the bovine hides have not been contaminated with brain, spinal cord, or ocular tissues of cattle residing in, or originating from, BSE countries and if they exclude hides from cattle that have signs of neurological disease (see #2).
- At this time, there does not appear to be a basis for objection to the use of gelatin produced from bovine hides and bones in FDA-regulated products for human use if the gelatin is produced from U.S.-derived raw materials or from cattle born, raised, and slaughtered in other countries that have no reported BSE cases and that meet OIE BSE standards.
- At this time, there does not appear to be a basis for objection to the use of gelatin produced from porcine skins, from any source country, in FDA-regulated products for human use. Processors should ensure that gelatin made from porcine skins is not cross-contaminated with bovine materials originating from BSE countries or from countries that do not meet OIE standards."

As taken from FDA, 1997.

Gelatins (CAS RN 9000-70-8) are listed in the US EPA InertFinder Database as approved for food and non-food use pesticide products.

Gelatine (CAS RN 9000-70-8) is listed as a fragrance ingredient by the International Fragrance Association (IFRA).

Gelatins (CAS RN 9000-70-8) are not registered under REACH (ECHA).

Gelatins (CAS RN 9000-7-8) are not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2024).

Gelatins (CAS RN 9000-70-8) are listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR and 2020 CDR Full Exempt lists (Chemical Data Reporting Rule).

US EPA TSCA (Toxic Substances Control Act) inventory.

Permitted for use in fruit juices and nectars as an anti-foaming agent (Codex, 2015).

Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) – Edible Gelatin

Synonyms:	GELATIN EDIBLE
Chemical Names:	GELATIN
CAS number:	9000-70-8
Functional Class:	Food Additives EMULSIFIER FOOD_ADDITIVE GELLING_AGENT

	STABILIZER
Evaluation year:	1970
ADI:	NOT LIMITED
Meeting:	14
Specs Code:	N
Report:	NMRS 48/TRS 462-JECFA 14/12
Tox Monograph:	NOT PREPARED
Specification:	COMPENDIUM ADDENDUM 12/FNP 52 Add. 12/67 (METALS LIMITS) (2004). R; FAO JECFA Monographs 1 vol.2/1
Previous Years:	1970, FAS 70.40/NMRS 48B-JECFA 14/17; COMPENDIUM/563. N

As taken from JECFA, 2021

Gelatins (CAS RN 9000-70-8) have been identified as being of “low concern to human health by application of expert validated rules under the NICNAS targeted tier I approach” and pose “no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework” (AICIS, 2017).

Gelatin (CAS RN 9000-70-8) is included on the US FDA’s list of inactive ingredients for approved drug products. It is permitted for use as an ingredient in various products, at the following maximum potencies per unit doses and maximum daily exposures (MDEs):

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose	Maximum Daily Exposure (MDE)
GELATIN	BUCCAL	GUM, CHEWING	9000708	2G86QN327L		102 mg
GELATIN	DENTAL	PASTE	9000708	2G86QN327L	16.8 %w/w	
GELATIN	INTRACAVITARY	INJECTION	9000708	2G86QN327L	0.05 ml	
GELATIN	INTRAMUSCULAR	INJECTION	9000708	2G86QN327L	16 %w/v	
GELATIN	INTRAMUSCULAR	INJECTION, POWDER, FOR SOLUTION	9000708	2G86QN327L	14 mg	
GELATIN	INTRAMUSCULAR	INJECTION, POWDER, FOR SUSPENSION	9000708	2G86QN327L		2 mg
GELATIN	INTRAMUSCULAR	INJECTION, POWDER, LYOPHILIZED, FOR SOLUTION	9000708	2G86QN327L		1 mg
GELATIN	INTRAMUSCULAR	INJECTION,	9000708	2G86QN327L	1.3 mg	

		SUSPENSION				
GELATIN	INTRAVENOUS	INJECTION	9000708	2G86QN327L	20 mg	
GELATIN	INTRAVENOUS	INJECTION, POWDER, FOR SOLUTION	9000708	2G86QN327L	14 mg	
GELATIN	INTRAVENOUS	SOLUTION	9000708	2G86QN327L	34.8 mg	
GELATIN	NASAL	DROPS	9000708	2G86QN327L	50 mg/1ml	
GELATIN	ORAL	CAPSULE	9000708	2G86QN327L		6436 mg
GELATIN	ORAL	CAPSULE, COATED	9000708	2G86QN327L		288 mg
GELATIN	ORAL	CAPSULE, COATED PELLETS	9000708	2G86QN327L	65 mg	
GELATIN	ORAL	CAPSULE, COATED, EXTENDED RELEASE	9000708	2G86QN327L	NA	
GELATIN	ORAL	CAPSULE, DELAYED RELEASE	9000708	2G86QN327L		967 mg
GELATIN	ORAL	CAPSULE, EXTENDED RELEASE	9000708	2G86QN327L		1229 mg
GELATIN	ORAL	CAPSULE, LIQUID FILLED	9000708	2G86QN327L		1042 mg
GELATIN	ORAL	DROPS	9000708	2G86QN327L	NA	
GELATIN	ORAL	ELIXIR	9000708	2G86QN327L	NA	
GELATIN	ORAL	PASTILLE	9000708	2G86QN327L	143 mg	
GELATIN	ORAL	POWDER	9000708	2G86QN327L	100 mg	
GELATIN	ORAL	POWDER, FOR SUSPENSION	9000708	2G86QN327L	NA	
GELATIN	ORAL	SOLUTION	9000708	2G86QN327L	34.8 mg	
GELATIN	ORAL	SYRUP	9000708	2G86QN327L	NA	
GELATIN	ORAL	TABLET	9000708	2G86QN327L		46 mg
GELATIN	ORAL	TABLET, CHEWABLE	9000708	2G86QN327L		24 mg
GELATIN	ORAL	TABLET, COATED	9000708	2G86QN327L	42.12 mg	
GELATIN	ORAL	TABLET, DELAYED RELEASE	9000708	2G86QN327L	19 mg	
GELATIN	ORAL	TABLET, EXTENDED RELEASE	9000708	2G86QN327L		239 mg
GELATIN	ORAL	TABLET, FILM COATED	9000708	2G86QN327L		40 mg
GELATIN	ORAL	TABLET, FILM COATED,	9000708	2G86QN327L	NA	

		EXTENDED RELEASE				
GELATIN	ORAL	TABLET, ORALLY DISINTEGRATING	9000708	2G86QN327L		120 mg
GELATIN	ORAL	TABLET, SUGAR COATED	9000708	2G86QN327L	NA	
GELATIN	ORAL	WAFER	9000708	2G86QN327L	NA	
GELATIN	RESPIRATORY (INHALATION)	CAPSULE	9000708	2G86QN327L	NA	
GELATIN	RESPIRATORY (INHALATION)	POWDER	9000708	2G86QN327L	100 mg	
GELATIN	SUBCUTANEOUS	INJECTION	9000708	2G86QN327L	16 %w/v	
GELATIN	SUBCUTANEOUS	INJECTION, POWDER, FOR SOLUTION	9000708	2G86QN327L	14 mg	
GELATIN	SUBLINGUAL	TABLET	9000708	2G86QN327L		19 mg
GELATIN	SUBLINGUAL	TABLET, ORALLY DISINTEGRATING	9000708	2G86QN327L		13 mg
GELATIN	TOPICAL	SYSTEM	9000708	2G86QN327L		1050 mg
GELATIN	VAGINAL	SUPPOSITORY	9000708	2G86QN327L	NA	

As taken from FDA, 2024b

Gelatin (CAS RN 9000-70-8) is classified as an NHP [Natural Health Product] under Schedule 1, item 2 (an isolate) of the Natural Health Products Regulations (Health Canada, 2021).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

“The amount metabolized is low: perhaps 3%.”

As taken from Mitra S and Khandelwal P (2009). Are All Colloids Same? How to Select the Right Colloid? Indian J Anaesth. 53(5): 592–607. Pubmed, 2011.

4.2. Absorption, distribution and excretion

“.../Elimination of gelatine used as plasma substitute/ is rapid & constitutes no drawback to using these soln.” [National Research Council. Drinking Water & Health Volume 1. Washington, DC: National Academy Press, 1977., p. 285] **PEER REVIEWED**

“...It is completely absorbed in 4 to 6 wk, it may be left in place after closure of operative wound.” [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 955] **PEER REVIEWED**

“Intravascular retention, dispersal, excretion, & break-down of gelatin plasma substitutes are discussed.” [zekorn d; intravascular retention, dispersal, excretion, and break-down of gelatin plasma substitutes; bibl haematol (BASEL) 33: 131 (1969)] **PEER REVIEWED**

As taken from HSDB, 2002.

“It is rapidly excreted by the kidney. Following infusion, its peak plasma concentration falls by half in 2.5 hours. Distribution (as a percent of total dose administered) by 24 hours is 71% in the urine, 16% extravascular and 13% in plasma.”

As taken from Mitra S and Khandelwal P (2009). Are All Colloids Same? How to Select the Right Colloid? Indian J Anaesth. 53(5): 592–607. Pubmed, 2011 available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2900092/

High levels of protein have been reported in the urine following treatment with intravenous succinylatred gelatine solution, probably due to the urinary excretion of gelatin-derived protein (Raghunath et al. 2013).

“The bioavailability of Gelatin derived from Nile tilapia scales was determined in an oral pharmacokinetic study in rats. 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 di- and tripeptide form. The authors of this study concluded that Gelatin had high oral bioavailability”

As taken from CIR, 2017

4.3. Interactions

“Topical application of antifungals does not have predictable or well-controlled release characteristics and requires reapplication to achieve therapeutic local concentration in a reasonable time period. In this article, the efficacy of five different US Food and Drug Administration-approved antifungal-loaded (amphotericin B, natamycin, terbinafine, fluconazole, and itraconazole) electrospun gelatin fiber mats were compared. Morphological studies show that incorporation of polyenes resulted in a two-fold increase in fiber diameter and the mats inhibit the growth of yeasts and filamentous fungal pathogens. Terbinafine-loaded mats were effective against three filamentous fungal species. Among the two azole antifungals compared, the itraconazole-loaded mat was potent against Aspergillus strains. However, activity loss was observed for fluconazole-loaded mats against all of the test organisms. The polyene-loaded mats displayed rapid candidacidal activities as well. Biophysical and rheological measurements indicate strong interactions between polyene antifungals and gelatin matrix. As a result, the polyenes stabilized the triple helical conformation of gelatin and the presence of gelatin decreased the hemolytic activity of polyenes. The polyene-loaded fiber mats were noncytotoxic to primary human corneal and sclera fibroblasts. The reduction of toxicity with complete retention of activity of the polyene antifungal-loaded gelatin fiber mats can provide new opportunities in the management of superficial skin infections.” As taken from Lakshminarayanan R et al. 2014. Int. J. Nanomedicine 9, 2439-58. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24920895

“We previously reported that the EGFR2R-lytic hybrid peptide has cytotoxic and anti-tumor activities both in vitro and in vivo. In this study, to improve the peptide pharmacokinetics and its anti-tumor activity after intravenous injection, we prepared biodegradable gelatin hydrogel nanoparticles as the delivery system of peptide. The complex is formed through the electrostatic interaction between the cationic peptide and anionic gelatin. In vitro release studies confirmed that the peptide was released from the complex in phosphate-buffered saline (PBS) solution containing fetal bovine serum at 37C within 48h, whereas little release was observed in PBS solution. In vivo release studies indicated that the anti-tumor activity of the complex was more effective than that of peptide treatment alone, and high tumor accumulation of the peptide was observed in the mice treated with the complex. Furthermore, the plasma area under the concentration curve (AUC) and half-life (T1/2) values of the complex were higher than those of the peptide treatment alone,

respectively. These results demonstrate that the rate of peptide release was controlled by the gelatin, and that the complex had a longer circulation time and enhanced its anti-tumor activity in vivo." As taken from Gaowa A et al. 2014. J. Control. Release 176, 1-7. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24378440

"Recent studies suggest that dihydroartemisinin (DHA), a derivative of artemisinin isolated from the traditional Chinese herb Artemisia annua L., has anticancer properties. Due to poor water solubility, poor oral activity, and a short plasma half-life, large doses of DHA have to be injected to achieve the necessary bioavailability. This study examined increasing DHA bioavailability by encapsulating DHA within gelatin (GEL) or hyaluronan (HA) nanoparticles via an electrostatic field system. Observations from transmission electron microscopy show that DHA in GEL and HA nanoparticles formed GEL/DHA and HA/DHA aggregates that were approximately 30-40 nm in diameter. The entrapment efficiencies for DHA were approximately 13 and 35% for the GEL/DHA and HA/DHA aggregates, respectively. The proliferation of A549 cells was inhibited by the GEL/DHA and HA/DHA aggregates. Fluorescent annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining displayed low background staining with annexin V-FITC or PI on DHA-untreated cells. In contrast, annexin V-FITC and PI stains dramatically increased when the cells were incubated with GEL/DHA and HA/DHA aggregates. These results suggest that DHA-aggregated GEL and HA nanoparticles exhibit higher anticancer proliferation activities than DHA alone in A549 cells most likely due to the greater aqueous dispersion after hydrophilic GEL or HA nanoparticles aggregation. These results demonstrate that DHA can aggregate with nanoparticles in an electrostatic field environment to form DHA nanosized aggregates." As taken from Sun Q et al. 2014. J. Biomed. Mater. Res. B Appl. Biomater. 102(3), 455-62. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24039154?dopt=AbstractPlus

"Purpose: Solubilizers play an important role in dissolution of pharmacological ingredients and should properly dissolve the active principle(s) while preserving its activities. This study investigated the effect of starch, gelatin, methylcellulose and polyvinylpyrrolidone 10000 in the preservation of the androgenic activity of the methanol extract Basella alba (MEBa). Methods: Different groups of male albino rats were orally given the MEBa (1 mg/kg) dissolved into either 1% gelatin (1% gel), %1 methylcellulose (1% MC) and 1% polyvinylpyrrolidone 10000 (1% PVP 10000) or 2% starch solution (2% SS) for 30 days. Thereafter, animals were sacrificed and serum testosterone and creatinine levels as well as alanine aminotransferase (ALT) activity determined. Vital and reproductive organs were dissected out and weighed, while liver thiobarbituric acid reactive substances (TBARS) and glutathione levels were determined. Results: Different treatments did not affect the animal body and organ weights. The MEBa stimulatory effect on testosterone production was preserved with 2% SS and 1% PVP 10000 as vehicles. Increased liver glutathione and TBARS levels were also observed in the animals fed with the MEBa dissolved in 2% SS and 1% Gel, respectively, while other biochemical parameters remained unchanged. Conclusion: Starch and polyvinylpyrrolidone 10000 stand as good preservation agents for MEBa androgenic activity, with starch exhibiting additional antioxidant activity through increase of glutathione levels." As taken from Nantia E et al. 2017. Adv. Pharm. Bull. 7(1), 103-108. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28507943

5. Toxicity

5.1. Single dose toxicity

"There is no documented evidence of a deleterious nature to humans from the ingestion of gelatin, other than a rare allergenic response, when the diet has provided an adequate amount of the amino acid tryptophan and is deficient in several others, and thus is of low nutritive value.".

"No significant adverse findings other than rare hypersensitivity have been found in the examination of data from feeding and biochemical experiments. Thus, there is no evidence to demonstrate a hazard to the public at the level gelatin is consumed as a food or a food ingredient."

As taken from FDA, 2015.

5.2. Repeated dose toxicity

“There is no documented evidence of a deleterious nature to humans from the ingestion of gelatin, other than a rare allergenic response, when the diet has provided an adequate amount of the amino acid tryptophan and is deficient in several others, and thus is of low nutritive value.”.

”No significant adverse findings other than rare hypersensitivity have been found in the examination of data from feeding and biochemical experiments. Thus, there is no evidence to demonstrate a hazard to the public at the level gelatin is consumed as a food or a food ingredient.”

As taken from FDA, 2015.

“In a rat study of the ability of shark skin Gelatin to increase bone mineral density, no adverse effects were reported. 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.

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. 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”

As taken from CIR, 2017

5.3. Reproduction toxicity

Type of Test	**Route of Exposure**	**Species Observed**	**Dose Data**	**Sex/ Duration**	**Toxic Effects**	**Reference**
TDLo - Lowest published toxic dose	Intraperitoneal	Rodent - mouse	700 mg/kg	female 7-13 day(s) after conception	Reproductive - Specific Developmental Abnormalities - urogenital system Reproductive - Effects on Newborn - growth statistics (e.g.%, reduced weight gain)	OYYAA2 Oyo Yakuri. Pharmacometrics. (Oyo Yakuri Kenkyukai, CPO Box 180, Sendai 980-91, Japan) V.1- 1967- Volume(issue)/page/year: 8,981,1974

As taken from RTECS, 2019.

5.4. Mutagenicity

No data available to us at this time.

5.5. Cytotoxicity

Gelatin nanoparticles as a new and simple gene delivery system (Abstract).

PURPOSE: The aim of this study was to evaluate cationized gelatin nanoparticles as biodegradable and low cell toxic alternative carrier to existing DNA delivery systems.

METHODS: Native gelatin nanoparticles were produced using a two-step desolvation method. In order to bind DNA by electrostatic interactions onto the surface of the particles, the quaternary amine cholamine was covalently coupled to the particles. The modified nanoparticles were loaded with different amounts of plasmid in varying buffers and compared to polyethyleneimine-DNA complexes (PEI polyplexes) as gold standard. Transfection ability of the loaded nanoparticles was tested on B16 F10 cells. Additionally, the cell toxicity of the formulations was monitored.

RESULTS: Different setups resulted in efficient gene delivery displayed by exponential increase of gene expression. The gene expression itself occurred with a certain delay after transfection. In contrast to PEI polyplexes, cationized gelatin nanoparticles almost did not show any significant cytotoxic effects.

CONCLUSIONS: Cationized gelatin nanoparticles have shown the potential of being a new effective carrier for nonviral gene delivery. The major benefit of gelatin nanoparticles is not only the very low cell toxicity, but also their simple production combined with low costs and multiple modification opportunities offered by the matrix molecule.

As taken from Zwiorek K et al. J Pharm Pharm Sci. 2005 Feb 3; 7(4):22-8. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/15850545

“Topical application of antifungals does not have predictable or well-controlled release characteristics and requires reapplication to achieve therapeutic local concentration in a reasonable time period. In this article, the efficacy of five different US Food and Drug Administration-approved antifungal-loaded (amphotericin B, natamycin, terbinafine, fluconazole, and itraconazole) electrospun gelatin fiber mats were compared. Morphological studies show that incorporation of polyenes resulted in a two-fold increase in fiber diameter and the mats inhibit the growth of yeasts and filamentous fungal pathogens. Terbinafine-loaded mats were effective against three filamentous fungal species. Among the two azole antifungals compared, the itraconazole-loaded mat was potent against Aspergillus strains. However, activity loss was observed for fluconazole-loaded mats against all of the test organisms. The polyene-loaded mats displayed rapid candidacidal activities as well. Biophysical and rheological measurements indicate strong interactions between polyene antifungals and gelatin matrix. As a result, the polyenes stabilized the triple helical conformation of gelatin and the presence of gelatin decreased the hemolytic activity of polyenes. The polyene-loaded fiber mats were noncytotoxic to primary human corneal and sclera fibroblasts. The reduction of toxicity with complete retention of activity of the polyene antifungal-loaded gelatin fiber mats can provide new opportunities in the management of superficial skin infections.” As taken from Lakshminarayanan R et al. 2014. Int. J. Nanomedicine 9, 2439-58. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24920895

“In this study, we investigated the effect of (131)I gelatin microspheres ((131)I-GMSs) on human hepatocellular carcinoma cells (HepG2) in nude mice (Balb/c) and the biodistribution of (131)I-GMSs after intratumoral injection. The treatment group and control group animals received intratumoral injections of 1 mCi (131)I-GMSs and GMSs unlabeled (131)I, respectively. The size of the implanted tumor was measured once a week for 8 weeks, and the survival time was calculated from the day of injection to 64 days post-injection. Another 35 animals received intratumoral injections of 0.2 mCi (131)I-GMSs and were subject to single-photon emission computed tomography (SPECT) on days 1, 8, 16, 24 and 32 post-injection. Samples of various organs were collected and used to calculate tissue concentrations on days 1, 4, 8, 16 and 24. Free thyroxine (FT4) in fetal bovine serum was tested to evaluate thyroid function. The tumors were collected for

histological examination. (131)I-GMSs produced a pronounced reduction in HepG2 tumor volume, and the overall survival was 73.3% in the treatment group and only 13.3% in the control group ($P < 0.001$). Tissue radioactivity concentration measurements and SPECT demonstrated that the injected (131)I-GMSs mainly accumulated within the tumors. The concentration of FT4 was stable during the observation period. The microspheres could be observed by histological methods on day 32. (131)I-GMSs suppressed the growth of HepG2 in the nude mice and were retained in the tumor for a long period of time after injection. Direct intratumoral injection of (131)I-GMSs offers a promising modality for the treatment of hepatocellular carcinoma." As taken from Chi JL et al. 2014. Radiat. Res. 181(4), 416-24. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24720750

"INTRODUCTION: The aim of this study was to investigate the effects of 131I gelatin microspheres (131I-GMS) on human breast cancer cells (MCF-7) in nude mice and the biodistribution of 131I-GMSs following intratumoral injections. METHODS: A total of 20 tumor-bearing mice were divided into a treatment group and control group and received intratumoral injections of 2.5 mci 131I-GMSs and nonradioactive GMSs, respectively. Tumor size was measured once per week. Another 16 mice received intratumoral injections of 0.4 mci 131I-GMSs and were subjected to single photon emission computed tomography (SPECT) scans and tissue radioactivity concentration measurements on day 1, 4, 8 and 16 postinjection. The 20 tumor-bearing mice received intratumoral injections of 0.4 mci [131I] sodium iodide solution and were subjected to SPECT scans and intratumoral radioactivity measurements at 1, 6, 24, 48 and 72 h postinjection. The tumors were collected for histological examination. RESULTS: The average tumor volume in the 131I-GMSs group on post-treatment day 21 decreased to $86.82 \pm 63.6\%$, while it increased to $893.37 \pm 158.12\%$ in the control group ($P < 0.01$ vs. the 131I-GMSs group). 131I-GMSs provided much higher intratumoral retention of radioactivity, resulting in $19.93 \pm 5.24\%$ of the injected radioactivity after 16 days, whereas the control group retained only $1.83 \pm 0.46\%$ of the injected radioactivity within the tumors at 1 h postinjection. CONCLUSIONS: 131I-GMSs suppressed the growth of MCF-7 in nude mice and provided sustained intratumoral radioactivity retention. The results suggest the potential of 131I-GMSs for clinical applications in radiotherapy for breast cancer." As taken from Li CC et al. 2014. Radiat. Oncol. 9, 144. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24958442

"Local and rapid heating by microwave (MW) irradiation is important in the clinical treatment of tumors using hyperthermia. We report here a new thermo-seed technique for the highly efficient MW irradiation ablation of tumors in vivo based on gelatin microcapsules. We achieved 100% tumor elimination in a mouse model at an ultralow power of 1.8 W without any side-effects. The results of MTT assays, a hemolysis test and the histological staining of organs indicated that the gelatin microcapsules showed excellent compatibility with the physiological environment. A possible mechanism is proposed for MW hyperthermia using gelatin microcapsules. We also used gelatin microcapsules capped with CdTe quantum dots for in vivo optical imaging. Our study suggests that these microcapsules may have potential applications in imaging-guided cancer treatment." As taken from Du Q et al. 2015. Nanoscale 7(7), 3147-54. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25613756

"As a biomaterial, it is well established that gelatin exhibits low cytotoxicity and can promote cellular growth. However, to circumvent the potential toxicity of chemical crosslinkers, reagent-free crosslinking methods such as electron irradiation are highly desirable. While high energy irradiation has been shown to exhibit precise control over the degree of crosslinking, these hydrogels have not been thoroughly investigated for biocompatibility and degradability. Here, NIH 3T3 murine fibroblasts are seeded onto irradiated gelatin hydrogels to examine the hydrogel's influence on cellular viability and morphology. The average projected area of cells seeded onto the hydrogels increases with irradiation dose, which correlates with an increase in the hydrogel's shear modulus up to 10 kPa. Cells on these hydrogels are highly viable and exhibits normal cell cycles, particularly when compared to those grown on glutaraldehyde crosslinked gelatin hydrogels. However,

proliferation is reduced on both types of crosslinked samples. To mimic the response of the hydrogels in physiological conditions, degradability is monitored in simulated body fluid to reveal strongly dose-dependent degradation times. Overall, given the low cytotoxicity, influence on cellular morphology and variability in degradation times of the electron irradiated gelatin hydrogels, there is significant potential for application in areas ranging from regenerative medicine to mechanobiology." As taken from Wisotzki EI et al. 2016. Macromol. Biosci. 16(6), 914-24. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26937853

5.6. Carcinogenicity

"In this study, we investigated the effect of (131)I gelatin microspheres ((131)I-GMSs) on human hepatocellular carcinoma cells (HepG2) in nude mice (Balb/c) and the biodistribution of (131)I-GMSs after intratumoral injection. The treatment group and control group animals received intratumoral injections of 1 mCi (131)I-GMSs and GMSs unlabeled (131)I, respectively. The size of the implanted tumor was measured once a week for 8 weeks, and the survival time was calculated from the day of injection to 64 days post-injection. Another 35 animals received intratumoral injections of 0.2 mCi (131)I-GMSs and were subject to single-photon emission computed tomography (SPECT) on days 1, 8, 16, 24 and 32 post-injection. Samples of various organs were collected and used to calculate tissue concentrations on days 1, 4, 8, 16 and 24. Free thyroxine (FT4) in fetal bovine serum was tested to evaluate thyroid function. The tumors were collected for histological examination. (131)I-GMSs produced a pronounced reduction in HepG2 tumor volume, and the overall survival was 73.3% in the treatment group and only 13.3% in the control group ($P < 0.001$). Tissue radioactivity concentration measurements and SPECT demonstrated that the injected (131)I-GMSs mainly accumulated within the tumors. The concentration of FT4 was stable during the observation period. The microspheres could be observed by histological methods on day 32. (131)I-GMSs suppressed the growth of HepG2 in the nude mice and were retained in the tumor for a long period of time after injection. Direct intratumoral injection of (131)I-GMSs offers a promising modality for the treatment of hepatocellular carcinoma." As taken from Chi JL et al. 2014. Radiat. Res. 181(4), 416-24. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24720750

"INTRODUCTION: The aim of this study was to investigate the effects of 131I gelatinmicrospheres (131I-GMS) on human breastcancer cells (MCF-7) in nude mice and the biodistribution of 131I-GMSs following intratumoral injections. METHODS: A total of 20 tumor-bearing mice were divided into a treatment group and control group and received intratumoral injections of 2.5 mci 131I-GMSs and nonradioactive GMSs, respectively. Tumor size was measured once per week. Another 16 mice received intratumoral injections of 0.4 mci 131I-GMSs and were subjected to single photon emission computed tomography (SPECT) scans and tissueradioactivity concentration measurements on day 1, 4, 8 and 16 postinjection. The 20 tumor-bearing mice received intratumoral injections of 0.4 mci [131I] sodium iodide solution and were subjected to SPECT scans and intratumoral radioactivity measurements at 1, 6, 24, 48 and 72 h postinjection. The tumors were collected for histological examination. RESULTS: The average tumor volume in the 131I-GMSs group on post-treatment day 21 decreased to $86.82 \pm 63.6\%$, while it increased to $893.37 \pm 158.12\%$ in the control group ($P < 0.01$ vs. the 131I-GMSs group). 131I-GMSs provided much higher intratumoral retention of radioactivity, resulting in $19.93 \pm 5.24\%$ of the injected radioactivity after 16 days, whereas the control group retained only $1.83 \pm 0.46\%$ of the injected radioactivity within the tumors at 1 h postinjection. CONCLUSIONS: 131I-GMSs suppressed the growth of MCF-7 in nude mice and provided sustained intratumoral radioactivity retention. The results suggest the potential of 131I-GMSs for clinical applications in radiotherapy for breastcancer." As taken from Li CC et al. 2014. Radiat. Oncol. 9, 144. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24958442

“Local and rapid heating by microwave (MW) irradiation is important in the clinical treatment of tumors using hyperthermia. We report here a new thermo-seed technique for the highly efficient MW irradiation ablation of tumors in vivo based on gelatin microcapsules. We achieved 100% tumor elimination in a mouse model at an ultralow power of 1.8 W without any side-effects. The results of MTT assays, a hemolysis test and the histological staining of organs indicated that the gelatin microcapsules showed excellent compatibility with the physiological environment. A possible mechanism is proposed for MW hyperthermia using gelatin microcapsules. We also used gelatin microcapsules capped with CdTe quantum dots for in vivo optical imaging. Our study suggests that these microcapsules may have potential applications in imaging-guided cancer treatment.” As taken from Du Q et al. 2015. Nanoscale 7(7), 3147-54. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25613756

“Three-dimensional (3-D) tissue engineered constructs provide a platform for examining how the local extracellular matrix contributes to the malignancy of various cancers, including human glioblastoma multiforme. Here, we describe a simple and innovative 3-D culture environment and assess its potential for use with glioblastoma stem cells (GSCs) to examine the diversification inside the cell mass in the 3-D culture system. The dissociated human GSCs were cultured using gelatin foam. These cells were subsequently identified by immunohistochemical staining, reverse transcriptase-polymerase chain reaction, and Western blot assay. We demonstrate that the gelatin foam provides a suitable microenvironment, as a 3-D culture system, for GSCs to maintain their stemness. The gelatin foam culture system contributes a simplified assessment of cell blocks for immunohistochemistry assay. We show that the significant transcription activity of hypoxia and the protein expression of inflammatory responses are detected at the inside of the cell mass in vitro, while robust expression of PROM1/CD133 and hypoxia-induced factor-1 alpha are detected at the xenografted tumor in vivo. We also examine the common clinical trials under this culture platform and characterized a significant difference of drug resistance. The 3-D gelatin foam culture system can provide a more realistic microenvironment through which to study the in vivo behavior of GSCs to evaluate the role that biophysical factors play in the hypoxia, inflammatory responses and subsequent drug resistance.” As taken from Yang MY et al. 2015. J. Biomed. Mater. Res. B Appl. Biomater. 103(3), 618-28. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/24966152

“The incidence of tumors in experimental animals (mice) injected subcutaneously with gelatin in various strength solutions, did not differ from that in untreated control animals.”

As taken from FDA, 2015.

5.7. Irritation/immunotoxicity

“450 stationary pt covering 5 age groups were randomly allocated 3 batches of modified fluid gelatin (neo-plasmagel). the incidence of allergoid & anaphylactoid reactions depended on the pre-medication (p less than 0.025).” [SCHONING B, KOCH H; ANAESTHESIST 30(1) 34 (1981)] **PEER REVIEWED**

“A case of anaphylactic reaction to an infusion of modified fluid gelatin (plasmagel) which occurred just before the induction of anaesthesia is reported.” [SAISSY JM ET AL; ANAPHYLACTIC REACTIONS TO MODIFIED FLUID GELATINS. (A CASE OF ALLERGY TO PLASMAGEL); ANN ANESTHESIOL FR 21(2) 148 (1980)] **PEER REVIEWED**

“Allergic reactions can occur.” [Rossoff, I.S. Handbook of Veterinary Drugs. New York: Springer Publishing Company, 1974., p. 237] **PEER REVIEWED**

As taken from HSDB, 2002.

Gelatin-induced T-cell activation in children with nonanaphylactic-type reactions to vaccines containing gelatin (Abstract).

BACKGROUND: Many cases of anaphylactic or nonanaphylactic reactions have been reported to measles-mumps-rubella vaccine or its component vaccines that contain gelatin as a stabilizer. Increased levels of specific IgE antibodies to gelatin have been reported in children with anaphylactic reactions. However, IgE is not increased in cases of nonanaphylactic reaction, and the mechanisms of the reaction are still controversial.

OBJECTIVE: The study was aimed to elucidate the relationship between nonanaphylactic reaction and gelatin.

METHODS: We investigated in vitro induction of activated memory helper T cells (CD4(+)CD25(+)CD45RO+ cells) in response to gelatin in children with nonanaphylactic reactions to vaccines containing gelatin.

RESULTS: In patients with delayed-type sensitivity to gelatin confirmed with a positive skin test response, CD4(+)CD25(+)CD45RO+ cells were significantly more strongly induced in culture containing gelatin than in control cultures. However, there was no significant difference between cultures with gelatin and those with control solvent in patients without reactions after vaccination. Of 76 patients with nonanaphylactic reactions after immunization with vaccine containing gelatin, 61 had an increased lymphocyte stimulation index to gelatin versus control children.

CONCLUSION: These results suggest the possibility that nonanaphylactic reactions to gelatin-containing vaccine in Japan might be mediated by delayed hypersensitivity reactions against gelatin.

As taken from Taniguchi K et al. J Allergy Clin Immunol. 1998 Dec;102(6 Pt 1):1028-32. PubMed, 2011.

A clinical analysis of gelatin allergy and determination of its causal relationship to the previous administration of gelatin-containing acellular pertussis vaccine combined with diphtheria and tetanus toxoids (Abstract).

BACKGROUND: The number of patients with allergic reactions after administration of gelatin-containing live vaccines is increasingly reported in Japan. These allergic reactions appear to be caused by gelatin allergy. It is still unknown how the patients were sensitized to gelatin.

OBJECTIVE: To determine the incidence of gelatin allergy and to identify contributing factors to gelatin allergy, we investigated the following clinical aspects: the development of IgE antibodies to gelatin and the relationship of the patients' past history of acellular pertussis vaccine combined with diphtheria and tetanus toxoid (DTaP) to the development of gelatin allergy.

METHODS: We evaluated 366 patient reports, submitted from 1994 to 1997, of adverse reactions after immunization with monovalent measles, mumps, and rubella vaccines containing 0.2% gelatin as stabilizer. On the basis of physician reports, the patients were categorized as to the nature of the adverse reaction. We determined the presence of IgE antibodies to gelatin and obtained past immunization history.

RESULTS: The 366 reported patients were categorized as follows: 34 with anaphylaxis, 76 with urticaria, 215 with nonurticarial generalized eruption, and 41 with local reactions only. In 206 patients from whom serum was available, IgE antibodies to gelatin were detected in 25 of 27 (93%) with anaphylaxis, 27 of 48 (56%) with urticaria, and 8 of 90 (9%) with a generalized eruption. None of a group of 41 patients with only local reactions at the injected site and none of a control group of 29 subjects with no adverse reaction had such antibodies. Among 202 patients for whom prior vaccine information was available, all had received DTaP vaccines. Among those for whom the prior DTaP vaccine could be determined to contain gelatin or be free of gelatin, 155 of 158 (98%) subjects had received gelatin-containing DTaP vaccines. This rate is higher than would be expected on the basis of the market share of gelatin-containing (vs gelatin-free) DTaP vaccines

(75%). Furthermore, before 1993, when a trivalent measles, mumps, and rubella vaccine (with the same 0.2% gelatin content as the monovalent vaccines) was used and administered before DTaP vaccination, no reports of anaphylaxis to the measles, mumps, and rubella vaccine were received.

CONCLUSION: Most anaphylactic reactions and some urticarial reactions to gelatin-containing measles, mumps, and rubella monovalent vaccines are associated with IgE-mediated gelatin allergy. DTaP immunization histories suggest that the gelatin-containing DTaP vaccine may have a causal relationship to the development of this gelatin allergy.

As taken from Nakayama T et al.J Allergy Clin Immunol. 1999, Feb; 103(2 Pt 1):321-5. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/9949325?dopt=AbstractPlus

IgE sensitization to gelatin: the probable role of gelatin-containing diphtheria-tetanus-acellular pertussis (DTaP) vaccines (Abstract).

We recently found that most events of anaphylaxis to live attenuated viral vaccines containing gelatin as a stabilizer might be caused by the gelatin. However, the mechanism that the children were sensitized to gelatin was unclear. In Japan, both diphtheria-tetanus-acellular pertussis (DTaP) vaccines with and without gelatin are available. We explored the possibility that gelatin-containing DTaP vaccines before live viral vaccines sensitize children to gelatin. We received the serum samples of 87 children who had systemic immediate-type reactions including anaphylaxis to the vaccines from both physicians and vaccine manufacturers throughout Japan. We then surveyed the DTaP vaccination histories of the children who demonstrated anti-gelatin IgE. Of the above 87 children, 79 (91%) had anti-gelatin IgE. We successfully collected DTaP vaccination histories including the manufacturers' names and numbers of doses on 55 children. Only one child had not received any DTaP vaccine, the other 54 had received gelatin-containing DTaP vaccines and none received gelatin-free DTaP vaccines. We concluded that there was a strong causal relationship between gelatin-containing DTaP vaccination, anti-gelatin IgE production, and risk of anaphylaxis following subsequent immunization with live viral vaccines which contain a larger amount of gelatin.

As taken from Sakaguchi M, Inouye S. Vaccine. 2000 Apr 3;18(19):2055-8. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/10706969?dopt=AbstractPlus

“In a double-blind placebo-controlled food challenge (DBPCFC) study of 30 patients with clinical allergy to fish, no patient reacted to a cumulative dose of 3.6 g of fish gelatin, although one patient showed a mild, confirmed subjective response to 7.61 g, but not 14.61 g” (Hansen et al 2004; cited in EFSA, 2007).

In a selected population of 141 beef and/or pork meat–sensitized children, 93% had beef meat–specific, 84% pork meat–specific and 79% both beef and pork meat–specific IgE antibodies. Porcine-gelatin specific IgE antibodies were detected in 16% and 38% of beef and pork meat–sensitized children, respectively, that were cross-reactive. The authors suggested that the presence of IgE anti-gelatin may place them at risk for potential allergic reactions after exposure to gelatin-containing foods, vaccines, or other medical products (Bogdanovich et al. 2009).

“BACKGROUND: We have observed patients clinically allergic to red meat and meat-derived gelatin. OBJECTIVE: We describe a prospective evaluation of the clinical significance of gelatin sensitization, the predictive value of a positive test result, and an examination of the relationship between allergic reactions to red meat and sensitization to gelatin and galactose-α-1,3-galactose (α-Gal). METHODS: Adult patients evaluated in the 1997-2011 period for suspected allergy/anaphylaxis to medication, insect venom, or food were skin tested with gelatin colloid. In vitro (ImmunoCAP) testing was undertaken where possible. RESULTS: Positive gelatin test results were observed in 40 of 1335 subjects: 30 of 40 patients with red meat allergy (12 also clinically allergic to gelatin), 2 of 2 patients with gelatin colloid-induced anaphylaxis, 4 of 172 patients with idiopathic anaphylaxis (all responded to intravenous gelatin challenge of 0.02-0.4 g), and 4 of 368 patients with drug allergy. Test results were negative in all patients with venom allergy (n = 241),

nonmeat food allergy (n = 222), and miscellaneous disorders (n = 290). ImmunoCAP results were positive to α-Gal in 20 of 24 patients with meat allergy and in 20 of 22 patients with positive gelatin skin test results. The results of gelatin skin testing and anti-α-Gal IgE measurements were strongly correlated (r = 0.46, P < .01). α-Gal was detected in bovine gelatin colloids at concentrations of approximately 0.44 to 0.52 μg/g gelatin by means of inhibition RIA. CONCLUSION: Most patients allergic to red meat were sensitized to gelatin, and a subset was clinically allergic to both. The detection of α-Gal in gelatin and correlation between the results of α-Gal and gelatin testing raise the possibility that α-Gal IgE might be the target of reactivity to gelatin. The pathogenic relationship between tick bites and sensitization to red meat, α-Gal, and gelatin (with or without clinical reactivity) remains uncertain". As taken from Mullins RJ et al. 2012. J. Allergy Clin. Immunol. 129, 1334-1342. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22480538.

The IgE profile of a patient who experienced a severe anaphylactic reaction on ingestion of marshmallows containing fish gelatin was analyzed in detail. The 12-year-old boy had a severe anaphylactic reaction, with urticaria, angioedema, and asthma starting 20 minutes after eating beef and several kosher marshmallows from a barbecue grill (no fish was prepared or consumed). Specific IgE binding to fish gelatin (ELISA, immunoblot, and CAP inhibition), and a positive skin test responses with fish gelatin are strong evidence for an anaphylactic reaction provoked by the consumption of fish gelatin contained in kosher marshmallows (Kuehn et al. 2009).

"The use of topical hemostatic agents is widespread and has been shown to reduce bleeding during a wide variety of surgical procedures. Nonetheless, as biologically active agents, there is potential for allergic reactions to these products. PURPOSE: This is a report of intraoperative anaphylaxis to gelatin associated with the use of two topical hemostatic agents. STUDY DESIGN: Case report. PATIENT SAMPLE: A patient with anaphylaxis during anterior spinal fusion. OUTCOME MEASURES: Laboratory assays for tryptase, gelatin-specific immunoglobulin E (IgE), and total IgE. METHODS: A 14-year-old male with myelomeningocele and scoliosis was treated with anterior spinal fusion from T12 to L3. Gelfoam sponges were applied during the preparation of the disc spaces. Approximately 1 hour later, Floseal hemostatic matrix was applied to a briskly bleeding screw hole in the L3 vertebral body, and the patient experienced an abrupt onset of hypotension and ventilatory difficulty. Epinephrine, dexamethasone, and blood products were administered for hemodynamic support while the surgical site was closed. Removal of the drapes revealed a widespread erythematous rash, and the patient was then transferred to the intensive care unit. When stable 3 days later, he returned to the operating room for completion of the spinal fusion. RESULTS: Postoperative laboratory assays were sent that revealed elevated levels of tryptase, total IgE, porcine, and bovine gelatin-specific IgE. The patient was counseled to avoid gelatin-containing products. At 6-month follow-up, his instrumented spine was radiographically fused and he reported no further allergic issues.CONCLUSIONS: Anaphylaxis may occur because of animal gelatin components of topical hemostatic agents. Previous reports have focused on the thrombin components. Care should be taken in the administration of these products, particularly in the atopic individual" As taken from Spencer HT et al. 2012. Spine J. 12, e1-e6. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23021035.

Intraoperative anaphylaxis induced by the gelatin component of thrombin-soaked gelfoam in a pediatric patient has also been reported (Khoriaty et al. 2012)

A case of anaphylactic shock to gelatin was seen in a 64-yr-old woman given a gelatin infusion during general anaesthesia 60 days after a previous uneventful administration (Marrel et al. 2011).

“The Measles-Mumps-Rubella (MMR) vaccine is often postponed in egg-allergic patients due to fear of anaphylactic reaction at the time of injection of this vaccin produced on egg derivates. However, this vaccine is recommended by health authorities, especially in case of increased measles incidence, and international recommendations indicate that there is no need for predictive allergological work-up and that the MMR vaccine is well tolerated in egg-allergic patients. We report on the case of a 12-year-old child with severe immediate-type egg allergy. Immediate-reading intradermal skin tests performed prior to the MMR vaccine were positive. Subsequent allergological work-up revealed a gelatin sensitization, and the child tolerated injections of the vaccine given according to a tolerance induction protocol. Gelatin is used as a stabilizer in numerous vaccines and may be responsible for immediate-type hypersentivity reactions to gelatin-containing vaccines. In case of reaction induced by the MMR vaccine, one needs to explore a potential gelatin sensitization/allergy. The MMR vaccine should be given and is well tolerated in patients with immediate-type egg hypersensitivity, even when gelatin sensitization is combined.” As taken from Dumortier B et al. 2013. Arch. Pediatr. 20(8), 867-70. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23850052

“BACKGROUND: Recent observations have disclosed that the galactose-alpha (1,3)-galactose (alpha-gal) moiety of non-primate glycoproteins can constitute a target for meat allergy. OBJECTIVE: To describe adults with allergic reactions to mammalian meat, dairy products and gelatin. To investigate whether patients could demonstrate sensitization to activated recombinant human coagulation factor VII ectapog alpha that is produced in baby hamster kidney cells. METHODS: Ten adults with mammalian meat, dairy products and gelatin allergies were examined using quantification of specific IgE and/or skin prick test for red meat, milk, milk components, gelatin, cetuximab and eptacog alpha. RESULTS: Most patients demonstrate quite typical clinical histories and serological profiles, with anti-alpha-gal titers varying from less than 1% to over 25% of total serum IgE. All patients demonstrate negative sIgE for gelatin, except the patient with a genuine gelatin allergy. All patients also demonstrated a negative sIgE to recombinant milk components casein, lactalbumin and lactoglobulin. Specific IgE to eptacog was positive in 5 out of the 9 patients sensitized to alpha-gal and none of the 10 control individuals. CONCLUSION: This series confirms the importance of the alpha-gal carbohydrate moiety as a potential target for allergy to mammalian meat, dairy products and gelatin (oral, topical or parenteral) in a Flemish population of meat allergic adults. It also confirms in vitro tests to mammalian meat generally to be more reliable than mammalian meat skin tests, but that diagnosis can benefit from skin testing with cetuximab. Specific IgE to gelatin is far too insensitive to diagnose alphaa-gal related gelatin allergy. IgE binding studies indicate a potential risk of alpha-gal-containing human recombinant proteins produced in mammalians.” As taken from Ebo DG et al. 2013. Acta Clin. Belg. 68(3), 206-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24156221

The patient is a 6-year 11-month-old girl who has a known history of peanut, tree nut, and shellfish allergy as well as persistent asthma that has been suboptimally controlled. She had pollen-food allergy syndrome from melons and bananas. She developed a febrile illness approximately 24 hours before the reaction occurred, which prompted administration of antipyretics. Her mother noted that ibuprofen had been given numerous times throughout her life (at least 40 times) in tablet form, and she had no history of reactions to medications. To our knowledge, this report is the first case of anaphylaxis from ingestion of an oral medication containing gelatin. Allergic reactions to gelatin in foods, cosmetics, pharmaceutical products, and medications are rarely reported complications, given the relatively widespread use of gelatin-containing products and possibly from the low prevalence of true gelatin allergy. Other factors that may influence the incidence of clinical reactions may include differences in allergenicity of the protein due to dose, processing of the

allergen, genetic background of the patient, gelatin type (bovine, porcine, or fish origin), and differences in reporting of adverse events (especially from vaccines). The previous reactions to the influenza vaccine could have represented mild reactions, because some forms of the influenza vaccine can contain as much as 250 μg of gelatin. 3 Her underlying illness with fever, fluid hydration status, and history of asthma could have contributed to the severity of her reaction and/or lowered her reaction threshold. Regardless of the cause, the patient's mother correctly identified an allergic reaction and administered epinephrine when the patient became pulseless. We advised the patient to continue avoidance of all types of bovine- and porcine-derived gelatin because of cross-reactivity. Gelatin allergy is an uncommon but real type of food allergy and may present at any time. When considering allergic reactions to medications, it is crucial to also consider food components of the medication that may be the cause of the reactions. With the current rise in the prevalence of atopic disorders and specifically food allergies, we may begin to see more patients with gelatin allergy. Land MH et al. (2013). Near fatal anaphylaxis from orally administered gelatin capsule. J. Allergy Clin. Immunol. Pract. 1(1), 99-100. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24229830

"There is no documented evidence of a deleterious nature to humans from the ingestion of gelatin, other than a rare allergenic response, when the diet has provided an adequate amount of the amino acid tryptophan and is deficient in several others, and thus is of low nutritive value.".

"No significant adverse findings other than rare hypersensitivity have been found in the examination of data from feeding and biochemical experiments. Thus, there is no evidence to demonstrate a hazard to the public at the level gelatin is consumed as a food or a food ingredient."

As taken from FDA, 2015.

"The current methodology to identify allergenic food proteins is effective in identifying those that are likely to cross-react with known allergens. However, most assays show false positive results for low/non-allergens. Therefore, an ex vivo/in vitro DC-T cell assay and an in vivo mouse model were used to distinguish known allergenic food proteins (Ara h 1, β-Lactoglobulin, Pan b 1, bovine serum albumin, whey protein isolate) from low/non allergenic food proteins (soy lipoxygenase, gelatin, beef tropomyosin, rubisco, Sola t 1). CD4+ T cells from protein/alum-immunized mice were incubated with corresponding protein-pulsed bone marrow-derived DC and analyzed for cytokine release. All known allergens induced Th2 responses in vitro, whereas soy lipoxygenase, gelatin or beef tropomyosin did not. Sola t 1 and rubisco induced a more generalized T cell response due to endotoxin contamination, indicating the endotoxin-sensitivity of the DC-T assay. To analyze responses in vivo, mice were orally sensitized on days 0 and 7. Known allergens induced IgE and mMCP-1 release upon oral challenge at day 16, whereas the low/non-allergens did not. Both the DC-T cell assay and the mouse model were able to distinguish 5 known allergens from 5 low/non-allergens and may be useful to identify novel allergenic food proteins." As taken from Smit J et al. 2016. Toxicol. Lett. 262, 62-69. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27663974

"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). Codfish-specific IgE was also measured in the patients and they underwent double-blinded, placebo-controlled food challenges with fish Gelatin. The fish Gelatin used for the study was made through acid extraction of codfish skins and had an average molecular weight of 60,000 Da. 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. Serum IgE antibodies were tested against Gelatin and 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”

“A 30-year-old woman with a history of atopic dermatitis experienced anaphylaxis twice on separate occasions, once after consuming a fortified yogurt containing fish-sourced Hydrolyzed Collagen and once after consuming a gummy candy containing fish-sourced Hydrolyzed Collagen. Fifteen months prior to the anaphylactic episodes, the patient had been applying a moisturizer containing Atelocollagen derived from fish 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 fish-sourced Hydrolyzed Collagen in the food products, the moisturizer, 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 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 fish-sourced Hydrolyzed Collagen protein. The researchers of this case study speculated that the 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”

CIR (2017)

“The measles-mumps-rubella (MMR) vaccine is generally well tolerated, and reports of anaphylaxis to the vaccine are rare. IgE-mediated reactions to vaccines are often caused by additives or residual vaccine components. An inability to obtain proper immunizations can be a disqualifying component to military service. We report a case of anaphylaxis to the MMR vaccine in a new military recruit sensitized to gelatin IgE.” As taken from Miller CK et al. 2020. Mil. Med. 185(9-10), e1869-e1871. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32395766/

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

CIR (2022)

5.8. All other relevant types of toxicity

“STUDY DESIGN: Prospective porcine animal model. OBJECTIVE: Determine if injecting FloSeal into pedicles for hemostasis causes emboli. SUMMARY OF BACKGROUND DATA: Bleeding from spinal deformity cases can be substantial, especially when surgical procedures involve bilateral fixation at multiple segments. It is not unusual to observe hemorrhage from vascular pedicles during each step of pedicle screw tract preparation. When multiple fixation points are required, blood loss can be excessive. To minimize estimated blood loss and associated morbidity, surgeons have injected liquefied gelatin into pedicles after drilling, palpating, and/or tapping. FloSeal is one of the most popular commercially available injectable agents and we sought to investigate the potential for embolization when used as an intrapedicular hemostatic agent. METHODS: Two adult minipigs were anesthetized and underwent sequential bilateral pedicle cannulation from T-spine to sacrum. At every level, tracts were cannulated, palpated, and tapped. In every tract, FloSeal was injected into each pedicle until back pressure was detected on the syringe or to a maximum volume

of 2 mL, then pedicle screws were inserted. The right ventricular outflow tract was visualized real time using transesophageal echocardiography. Postmortem evaluation of heart and lungs was performed. RESULTS: FloSeal injected into pedicles caused a consistent large showering of the right ventricular outflow tract in both pigs as visualized on intraoperative transesophageal echocardiography. A second large showering occurred during screw insertion after FloSeal was injected. Microscopic examination of lungs clearly identified amphophilic amorphous material in many small vessels consistent with FloSeal. CONCLUSION: This study suggests caution when injecting gelatin hemostatic agents into pedicles to stop bleeding during spinal surgery as we saw clear evidence of fat and gelatin emboli when used in this animal model. Further investigation into how to minimize this embolic showering may help the cardiopulmonary at risk patient who requires spinal surgery, especially when multiple points of pedicle screw fixation are used." As taken from Kuhns CA et al. 2015. Spine (Phila. Pa. 1976) 40(4), 218-23. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25494314 "The Panel was also concerned about the inherent risks of using animal-derived ingredients in cosmetic products, namely the potential for transmission of infectious agents. While Gelatin and Collagen prepared exclusively from hides and skins do not have the propensity to carry disease, the Panel stressed that these ingredients must be free of detectible infectious pathogens (ie, BSE) if these materials are derived from other bovine materials. Raw material suppliers and formulators of these ingredients must assure that these ingredients are free from pathogenic viruses and other infectious agents."

CIR (2022)

6. Functional effects on

6.1. Broncho/pulmonary system

"Recently, side effects of plasma expanders like hydroxyethyl starch and gelatine gained considerable attention. Most studies have focused on the kidneys; lungs remain unconsidered. Isolated mouse lungs were perfused for 4 hours with buffer solutions based on hydroxyethyl starch (HES) 130/0.4, HES 200/0.5 or gelatine and ventilated with low or high pressure under physiological pH and alkalosis. Outcome parameters were cytokine levels and the wet-to-dry ratio. For cytokine release, murine and human PCLS were incubated in three different buffers and time points. In lungs perfused with the gelatine based buffer IL-6, MIP-2 and KC increased when ventilated with high pressure. Wet-to-dry ratios increased stronger in lungs perfused with gelatine - compared to HES 130/0.4. Alkalotic perfusion resulted in higher cytokine levels but normal wet-to-dry ratio. Murine PCLS supernatants showed increased IL-6 and KC when incubated in gelatine based buffer, whereas in human PCLS IL-8 was elevated. In murine IPL HES 130/0.4 has lung protective effects in comparison to gelatine based infusion solutions, especially in the presence of high-pressure ventilation. Gelatine perfusion resulted in increased cytokine production. Our findings suggest that gelatine based solutions may have side effects in patients with lung injury or lung oedema." As taken from Krabbe J et al. 2018. Sci. Rep. 8(1), 5123. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1038%2Fs41598-018-23513-0

6.2. Cardiovascular system

Drug Warnings:

"In large amt.../gelatines/ can cause pseudo-agglutinations, incr sedimentation rate, & cause fixation of blood stream proteins in tissues. such effects are reversible & disappear simultaneously with elimination of the gelatine." [Lefaux, R. Practical Toxicology of Plastics. Cleveland: CRC Press Inc., 1968., p. 285] **PEER REVIEWED**

"Applications: when /gelfoam/...is packed into cavities or closed tissue spaces, care should be exercised to avoid overpacking because, as material absorbs fluid, it expands & may press on

neighboring structures." [American Medical Association, AMA Department of Drugs. AMA Drug Evaluations. 4th ed. Chicago: American Medical Association, 1980., p. 1115] **PEER REVIEWED**

"Adverse effects of plasma vol expanders are discussed." [ISBISTER JP, FISHER MM; ADVERSE EFFECTS OF PLASMA VOLUME EXPANDERS; ANAESTH INTENSIVE CARE 8(2) 145 (1980)] **PEER REVIEWED**

As taken from HSDB, 2002

Gelatin use impairs platelet adhesion during cardiac surgery (Abstract). Artificial colloids based on gelatin are used as plasma expander to replace donor blood products. In laboratory experiments, gelatin reduced both the velocity and extend of platelet agglutination by ristocetin, and only the agglutination velocity by polybrene ($p < 0.05$). Furthermore, gelatin delayed the in-vitro platelet plug formation under shear-stress in the absence of ADP ($p < 0.05$), whereas gelatin induced no delay in the presence of ADP. Thus, after induction of vWF release from platelets by polybrene or ADP, platelet function was normal. These results indicate that gelatin affects in particular the functionality of plasma-vWF and partly inhibits platelet adhesion. These negative effects of gelatin on hemostasis were demonstrated in two clinical studies during cardiac surgery. In a randomized study of sixty patients undergoing cardiac surgery, gelatin as prime in the heart-lung machine appeared to result in diminished efficacy of aprotinin on hemostasis, whereas it did not affect hemostasis in non-aprotinin patients. An additional retrospective clinical study showed that only high dose of gelatin affected hemostasis. This suggests a limited role of plasma-vWF and a strong back-up mechanism of platelet-vWF in achieving hemostasis.

As taken from Tabuchi N et al. Thromb Haemost. 1995 Dec; 7 4(6):1447-51. Pubmed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/8772218

"We investigated the in vitro viscoelastic changes of progressive haemodilution with succinylated gelatin (SG) solution compared with normal saline (NS) using rotational thromboelastometry (ROTEM®). Whole blood (WB) samples obtained from 20 healthy volunteers were diluted in vitro with SG solution or NS by 10%, 20% and 40%. Fibrinogen concentration and ROTEM (EXTEM, FIBTEM) variables including coagulation time (CT), clot formation time (CFT), α-angle, and maximum clot firmness (MCF) were measured in the undiluted sample and at each degree of haemodilution. Haemodilution with SG decreased FIBTEM MCF by 34.8% at 20% dilution (SG 20% haemodilution mean 9.1 [standard deviation, SD 2.7] mm versus WB, mean 13.9 [SD 3.4] mm) whereas this was observed only at 40% haemodilution with NS (mean 8.5 [SD 2.7], 38.7% decrease). We found that 40% haemodilution with SG slowed clot formation (EXTEM CFT; SG 40%, mean 179 [SD 39] seconds versus WB mean 87.9 [SD 13.7] seconds; increased CFT by 103%), reduced clot strength by 23.5% (EXTEM MCF; SG 40% mean 47.7 [SD 3.4] mm versus WB mean 62.4 [SD 2.5] mm), and decreased fibrin formation (FIBTEM MCF; SG 40% mean 5.8 [SD 1.6] mm versus WB mean 13.9 [SD 3.4] mm); 58.4% decrease). The platelet contribution to clot strength (EXTEM MCF–FIBTEM MCF) was not changed by SG. We found that haemodilution more than 20% with SG impaired coagulation greater than that observed with NS haemodilution in this in vitro study. This suggests that at 40% haemodilution with SG, a clinical scenario that could occur during resuscitation of a patient in grade IV haemorrhagic shock, impaired coagulation could occur. Frequent monitoring of coagulation is advised when SG solutions are administered rapidly during volume resuscitation." As taken from Kam PCA et al. 2018. Anaesth. Intensive Care 46(3), 1-6. Available at https://www.researchgate.net/publication/324181288_

6.3. Nervous system

"OBJECTIVE: One significant drawback during a cranial reoperation is the presence of meningocerebral adhesions. The appearance of connective tissue bridges between the inner surface of the dura and the pia-arachnoid is mostly related to dural closure and the condition in which the surgical field was left in the previous surgery. This study was done to determine the

benefit of placing a thin-layer gelatin sponge of polypeptides subdurally to prevent meningocerebral adhesions. METHODS: From September 2005 through May 2012, 902 craniotomies were performed for various lesions by the senior author (U.T.). Beginning in February 2009, we began placing a gelatin sponge under the dural flap to isolate the dural healing process from the cortical surface. To compare the degree of meningocerebral adhesions statistically, reoperation cases between February 2009 and May 2012 were divided into 2 groups as group G (gelatin) and group C (Control) in which the dural closure was made with and without subdural application of the gelatin sponge, respectively. RESULTS: In all patients of group G (n = 15), a neomembrane was found when the dura was opened. This layer was easily dissected and showed no or minimal attachment to the underlying cerebral cortex. However, in group C (n = 14), meningocerebral adhesions in various degrees were detected. Adhesion scores were significantly greater in group C than in group G ($P < 0.001$). CONCLUSION: This study proves that, during the dural closure, placing a thin layer of gelatin sponge in the subdural space is a safe and effective method for preventing meningocerebral adhesions." As taken from Gonzalez-Lopez P et al. 2015. World Neurosurg. 83(1), 93-101. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/24560706

6.4. Other organ systems, dependent on the properties of the substance

"MAY CAUSE REVERSIBLE NEPHROSIS." [Rossoff, I.S. Handbook of Veterinary Drugs. New York: Springer Publishing Company, 1974., p. 237] **PEER REVIEWED**

As taken from HSDB, 2002.

Liver tissue responses to gelatin and gelatin/chitosan gels (Abstract). Gelatin and gelatin/chitosan gels, crosslinked using glutaraldehyde, were previously developed as substrates for three-dimensional cell-assembly techniques. In this study, the biocompatibility and biodegradation of gelatin and gelatin/chitosan gels were evaluated following implantation in rat livers for periods up to 16 weeks. The two gels were characterized by different inflammatory responses and degradation rates. The gelatin/chitosan gel is more efficient in inducing fibrin formation and vascularization at the implant-host interface. The degrees of inflammatory reaction for the gelatin/chitosan gel were significantly stronger than the gelatin gel. Advanced biodegradation of the gelatin gels was observed. These data indicate that the gelatin gel has better liver tissue biocompatibility and a faster biodegraded rate than the gelatin/chitosan gel.

As taken from Wang X et al. J Biomed Mater Res A. 2008 Oct; 87(1):62-8. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/18080311?dopt=AbstractPlus

"Collagen hydrolysates (CHs) are mixtures of peptides obtained by partial hydrolysis of gelatin that are receiving scientific attention as potential oral supplements for the restoration of osteoarticular tissues. The aim of this study was to evaluate the effectiveness of CHs for promoting longitudinal bone growth in growing rats. An in vitro study was carried out in osteoblast-like MG63 cells and the most effective CH on bone formation was selected among 36 various CHs. An in vivo study confirmed the functional effects of a selected CH with molecular weight of <3 kDa on longitudinal bone growth. CHs dose-dependently promoted the longitudinal bone growth and height of the growth plate in adolescent male rats, whereas gelatin failed to affect longitudinal bone growth. Insulin-like growth factor-1 and bone morphogenetic protein-2 in the CH treated group were highly expressed in the growth plate. These results suggest that CHs isolated in this study may provide beneficial effects on bone metabolism of growing animals and humans." As taken from Leem KH et al. 2013. J. Med. Food. 16(5), 447-53. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23631489

"PURPOSE: To report initial experience of temporary portal vein embolization (PVE) with a powdered form of absorbable gelatin sponge before major liver resection. MATERIALS AND METHODS: From 2009-2013, 20 patients (6 women and 14 men; median age, 61.5 y ± 2.8; range, 49-80 y) considered for major liver resections for both primary and secondary hepatic malignancies

underwent temporary PVE. Data were retrospectively reviewed. Embolization of selected portal vein segments was performed using the powdered form of an absorbable gelatin sponge. All patients underwent volumetric computed tomography (CT) assessment before and at 4-6 weeks after PVE. Liver histology was normal in 13 patients; 1 patient had steatosis, and 6 patients had cirrhosis. RESULTS: Subsegmental, segmental, and sectorial embolization was successfully performed in all patients. None of the patients developed liver insufficiency or fever after embolization. Volumetric CT assessment showed the disappearance of all portal thrombosis in 14 patients. The median hypertrophy ratio of the nonembolized liver was 29.4% ± 6.9 (range, 3.3-127.2%). Of 20 patients, 15 underwent surgery 1-2 months after temporary PVE. One (6.7%) patient presented with liver decompensation in the postoperative period. Five patients were not eligible for surgery because of tumor progression. Histologic examination of the resected liver revealed the presence of absorbable gelatin sponge powder in a few distal portal tracts in four patients. No residual absorbable gelatin sponge powder was observed in portal vessels in the remaining 11 patients. CONCLUSIONS: Temporary PVE resulted in sufficient hypertrophy of the liver that did not receive embolization to enable surgical planning in all patients in our series." As taken from Tranchart H et al. 2015. J. Vasc. Interv. Radiol. 26(4), 507-15. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25640643

"Green tea catechins had an in vitro antibacterial effect against periodontopathic bacteria and were able to inhibit destruction of the periodontal tissue. In this study, we aimed to evaluate the effect of locally delivered gel containing green tea extract as an adjunct to non-surgical periodontal treatment. Forty-eight subjects who had teeth with probing pocket depth of 5-10 mm were randomly allocated into the test or control group. Probing pocket depth, clinical attachment level, gingival index (GI), bleeding on probing (BOP) and full mouth plaque score were measured at baseline. Subjects received oral hygiene instruction, single episode of scaling and root planing and subgingival application of the green tea gel (test group) or the placebo gel (control group). The gel was repeatedly applied at 1 and 2 weeks later. The parameters were recorded again at the 1st, 3rd and 6th month after the last gel application. The results showed that all parameters were improved in both groups compared to baseline. The test group exhibited significantly higher reduction in BOP at the 3rd month ($p = 0.003$) and significantly lower GI at the 1st month ($p < 0.001$) and 3rd month ($p < 0.001$) when compared with the control group. Thus, green tea gel could provide a superior benefit in reducing bleeding on probing and gingival inflammation when used as an adjunct to non-surgical periodontal treatment." As taken from Rattanasuwan K et al. 2016. Odontology 104, 89-97. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25523604

"OBJECTIVES: To examine the usefulness of an absorbable hemostatic gelatin sponge for hemostasis after transrectal prostate needle biopsy. SUBJECTS AND METHODS: The subjects comprised 278 participants who underwent transrectal prostate needle biopsy. They were randomly allocated to the gelatin sponge insertion group (group A: 148 participants) and to the non-insertion group (group B: 130 participants). In group A, the gelatin sponge was inserted into the rectum immediately after biopsy. A biopsy-induced hemorrhage was defined as a case in which a subject complained of bleeding from the rectum, and excretion of blood clots was confirmed. A blood test was performed before and after biopsy, and a questionnaire survey was given after the biopsy. RESULTS: Significantly fewer participants in group A required hemostasis after biopsy compared to group B (3 (2.0%) vs. 11 (8.5%), P=0.029). The results of the blood tests and the responses from the questionnaire did not differ significantly between the two groups. In multivariate analysis, only "insertion of a gelatin sponge into the rectum" emerged as a significant predictor of hemostasis. CONCLUSION: Insertion of a gelatin sponge into the rectum after transrectal prostate needle biopsy significantly increases hemostasis without increasing patient symptoms, such as pain and a sense of discomfort." As taken from Kobatake K et al. 2015. Int. Braz. J. Urol. 41(2), 337-43. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26005977

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

No data available to us at this time.

9. Heated/vapor emissions toxicity

No data available to us at this time.

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that gelatins are of uncertain persistence in the environment. Data accessed May 2017 on the OECD website.

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that gelatins are not inherently toxic to aquatic organisms and are of low ecotoxicological concern. Data accessed May 2017 on the OECD website.

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

No data available to us at this time.

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that gelatins are of uncertain bioaccumulative potential in the environment. Data accessed May 2017 on the OECD website.

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

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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 Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer.

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ABSTRACT

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) reviewed the safety of 19 skin and connective tissue-derived proteins and peptides, which function mainly as skin and/or hair conditioning agents in cosmetics. The Panel reviewed the relevant data provided and concluded that these ingredients are safe in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

The skin and connective tissue-derived proteins and peptides detailed in this report are described in the *International Cosmetic Ingredient Dictionary and Handbook* (Dictionary) to function mainly as skin and hair conditioning agents in cosmetics.[1] This report assesses the safety of the following 19 skin and connective tissue-derived ingredients:

Ammonium Hydrolyzed Collagen
Atelocollagen
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 Panel previously reviewed the ingredient Hydrolyzed Collagen, and concluded that it is safe for use in cosmetics; the report was published in 1985 and the conclusion was reaffirmed in a re-review that was published in 2006.[2,3] This ingredient was included in this safety assessment because of the relevance of the information in regards to reviewing the safety of the other ingredients in the report. Summary data from the original safety assessment have been included in this report in *italics*.

Additionally, the safety of several other hydrolyzed proteins as used in cosmetics has been reviewed by the Panel in several previous assessments. The Panel concluded that Hydrolyzed Keratin (finalized in 2016), 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.[4-8] 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 bovine milk-derived proteins, which have tentative conclusions of safe as used, in separate reports. In addition to the review of these other protein-derived ingredients, the Panel has assessed the safety of Ethanolamine (also known as monoethanolamine or MEA) and Ethanolamine Salts and concluded these ingredients are safe when formulated to be nonirritating (rinse-off products only) and should not be used in cosmetic products in which *N*-nitroso compounds may be formed.[10]

Actin, Collagen, Elastin, Fibronectin, Gelatin, and reticulin all are derived from essential components in animal 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 skin and connective 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 skin and connective 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).

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that CIR typically evaluates, is provided on the CIR website (http://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; http://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

CHEMISTRY

Definition

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

Skin and connective 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 abundant protein in mammals is collagen, making up approximately 30% of all proteins by mass.[11,12] 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).[12] 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.[11] 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 accomplished via acid, enzyme, or other methodologies. These methodologies, and the degree to which they are utilized, may profoundly affect the size and biological activity 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.[11] 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.[11,13] 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.[12,14] 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.[11] 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 (as reported in 1987), skin, and related tissues; Type 2 is derived from cartilage and its derivatives.[15] In skin, Elastin is the intact elastic fiber network that comprises approximately 2% to 4% of the dermis by volume.[13]

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.[11]

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.[11] 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[11]

Physical and Chemical Properties

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

Collagen

Solutions of Collagen for cosmetic use have a pH range of 3.8 to 4.7[14]

Hydrolyzed Collagen

Hydrolyzed Collagen may be a powder or solution.[2] A 10% aqueous solution has a pH of 4.0-6.5.

Elastin

Purified Elastin is a pale yellow color and exhibits a bluish fluorescence in UV light.[11] It resists acid and alkaline hydrolysis. It is practically insoluble even in hydrogen-bond-breaking solvents at temperatures up to 100 ºC, and is nearly impossible to bring into solution except by using reagents capable of hydrolyzing peptide bonds. Unprocessed or native elastin is reported to be too insoluble for use in cosmetic formulations. [15]

Fibronectin

Fibronectin can be provided in a solution or as a lyophilized powder.[16]

Gelatin

Gelatin is a vitreous, brittle solid that is colorless to faintly yellow.[11,17] 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.[15]

Method of Manufacturing

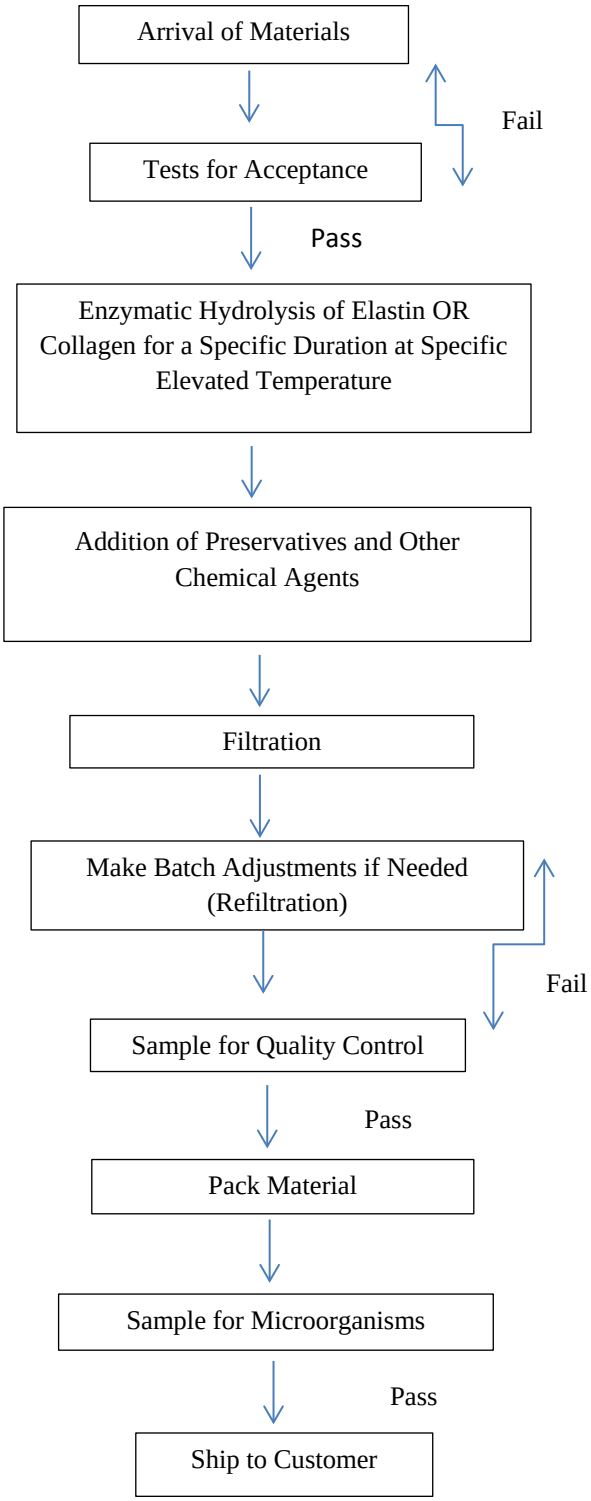
Methods used to manufacture protein hydrolysates typically yield broad MW distributions of peptides, ranging from 500 to 30,000 daltons (Da), equating to 4 to 220 amino acids in length.[18,19] Treatment with certain enzymes, such as papain, can routinely yield narrower distributions of 500 to 10,000 Da, equating to 4 to 74 amino acids in length. The available methods of manufacturing for the skin and connective tissue-derived proteins and peptides are summarized in Table 3.

Hydrolyzed Collagen

A representative manufacturing flow chart for Hydrolyzed Collagen is found in Scheme 1. This process may vary slightly between specific products with the elimination of the use of preservatives after hydrolysis and the addition of filtration and concentration of solution before the first quality control.[20]

A supplier has reported that their Hydrolyzed Collagen products (6 products with MW ranges of 400 to 2000 Da, concentration up to 50% in water) are prepared by acidic, alkalic, and/or enzymatic hydrolysis of bovine gelatin, swine gelatin, or fish scale until the molecular weight reaches the target range.[21]

Scheme 1. Representative manufacturing flow chart for Hydrolyzed Elastin or Hydrolyzed Collagen (bovine and fish sourced)[22-26]



Soluble Collagen

A representative manufacturing flow chart for Soluble Collagen is found in Scheme 2.

Hydrolyzed Elastin

A representative manufacturing flow chart for Hydrolyzed Elastin is found in Scheme 1. This process may vary slightly between specific products with the use of pH adjustment during hydrolysis.

Gelatin

According to 21 CFR§700.27, Gelatin is "…a product that has been obtained by the partial hydrolysis of collagen derived from hides, connective tissue, and/or bones of cattle and swine. Gelatin may be either Type A (derived from an acid-treated precursor) or Type B (derived from an alkali-treated precursor) that has gone through processing steps that include filtration and sterilization or an equivalent process in terms of infectivity reduction."

Composition

The typical amino acid compositions for Collagen, Soluble Collagen, and Elastin are presented in Table 4.

Impurities

Several of the ingredients in this safety assessment, including Hydrolyzed Collagen, Hydrolyzed Elastin, and Gelatin, may be bovine sourced. Some bovine materials may be considered risk materials for transmission of infectious agents (e.g., bovine spongiform encephalopathy (BSE) prions). According to 21 CFR§700.27, "no cosmetic shall be manufactured from, processed with, or otherwise contain, prohibited cattle materials." Prohibited cattle materials "mean specified risk materials, small intestine of all cattle…, material from non-ambulatory disabled cattle, material from cattle not inspected and passed, or mechanically separated." Gelatin or hides and hide-derived products are not prohibited cattle materials. Cosmetic manufacturers must follow record keeping requirements that "demonstrate that the cosmetic is not manufactured from, processed with, or does not otherwise contain prohibited cattle materials."

The World Organization for Animal Health (OIE) recommends that "when authorizing import or transit of…gelatin and collagen prepared exclusively from hides and skins…and any products made from these commodities and containing no other tissues from cattle, veterinary authorities should not require any BSE related conditions [i.e. restrictions], regardless of the BSE risk status of the cattle population of the exporting country, zone, or compartment."[27]

Collagen

An analysis for 3 different Collagen products found the level of arsenic to be less than 1 ppm. [14]

Hydrolyzed Collagen

The maximum concentrations of iron and heavy metals reported in Hydrolyzed Collagen were 3 ppm and 25 ppm, respectively. [2]

A supplier reported that their Hydrolyzed Collagen products (6 products with MW ranges of 400 to 2000 Da, concentration up to 50% in water) sourced from bovine gelatin, swine gelatin, and fish scales contain not more than 10 ppm heavy metals and not more than 1 ppm arsenic.[21]

A supplier reported that their Hydrolyzed Collagen products are BSE-free.[28,29]

Soluble Collagen

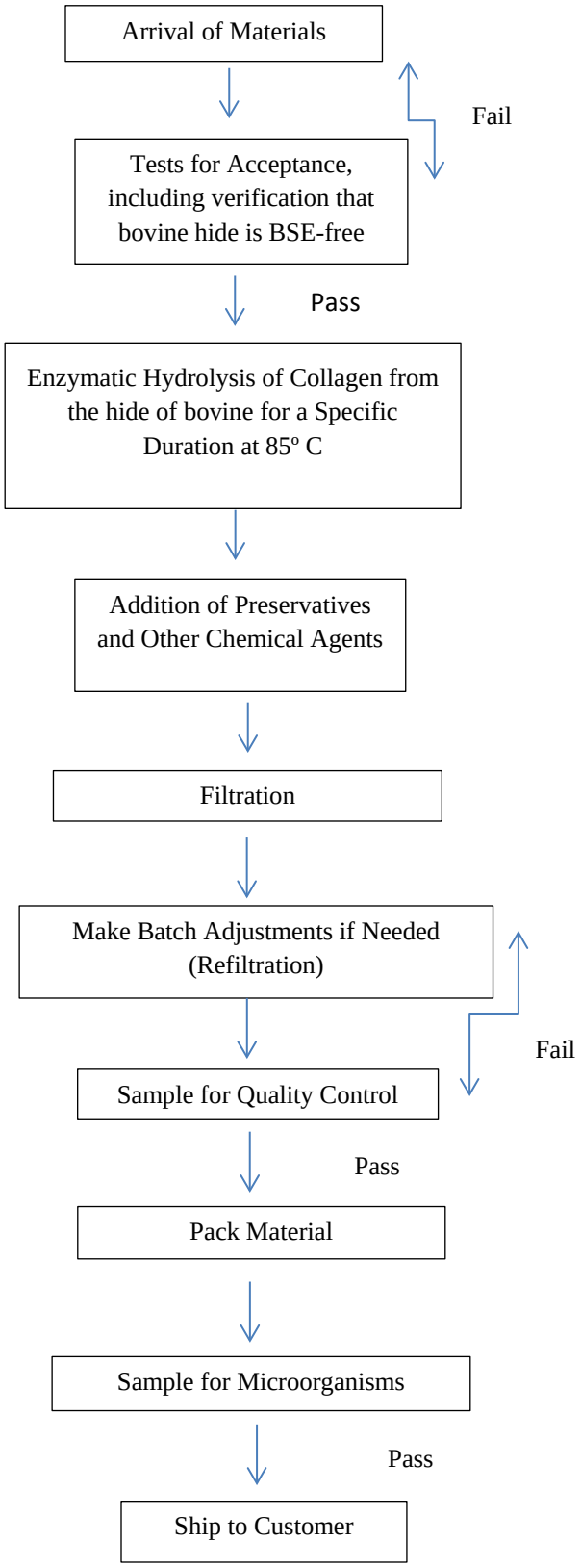
A supplier reported that their Soluble Collagen product is BSE-free.[30]

Elastin and Hydrolyzed Elastin

Impurities in commercial Elastin-based preparations include contamination by lipoid substances from the raw materials and products of Collagen degradations.[31]

A supplier certified that their Hydrolyzed Elastin products are BSE-free.[32-34]

Scheme 2. Manufacturing flow chart for Soluble Collagen (bovine sourced)[35]



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.[17]

A supplier certified that their Gelatin product is BSE-free.

USE

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the U.S. Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in 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, the ingredients with the greatest number of uses are Hydrolyzed Collagen (543 formulations) and Soluble Collagen (425 formulations); the majority of uses are in leave-on skin care products (Table 5 and Table 6).[36] Gelatin is used in a total of 334 formulations; 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.[37] Gelatin is used at up to 66% in bath oils, tablets, and salts. The other in-use ingredients are used at much lower concentrations.

Historic and current use data for Hydrolyzed Collagen is reported in Table 6. 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[2,3]). The maximum use concentration of Hydrolyzed Collagen was reported to be 16.5% in hair tonics and dressings in 2016; it was previously reported to be used at concentrations greater than 50% (in rinse-off formulations).[2,37]

Ingredients with no reported uses in the VCRP or by Council are listed in Table 7.

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, Gelatin is used in bath oils, tablets and salts at up to 66% and Hydrolyzed Collagen is used in an eyeliner at up to 3.2%.[37] 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.[38-41] 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.[38,39] 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.[42-44]

The skin and connective 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.[45]

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.[46]

Collagen

Non-cosmetic uses of Collagen include fibers in sutures, leather substitutes, coatings as a gel in photographic emulsions, and food casings.[11]

Gelatin

Non-cosmetic uses of Gelatin include uses in food as a stabilizer, thickener, texturizer, firming agent, surface-active agent, or surface-finishing agent.[11,17] 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.[11] 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.[47] 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 di- and tri-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 in rabbits.[2]

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.[48] 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 of mice and rats.[2]

The oral LD_{50} of Hydrolyzed Collagen (30% solution in water; fish scale sourced; MW ~ 400 Da) was estimated to be greater than 2500 mg/kg body weight in Sprague-Dawley CD rats.[21] This acute toxicity test was performed in accordance to Organization for Economic Co-operation and Development test guideline (OECD TG) 423. A group of 3 female rats were treated orally with the test material at a dose level of 2000 mg/kg body weight, with another group of 3 fasted female rats receiving also receiving the material at the same dose level. No deaths or signs of systemic toxicity were observed during the 14 days of monitoring post-dosing. All animals exhibited expected gains in body weight. No abnormalities were observed at necropsy.

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.[49] 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 in rabbits and pigs on 2 cosmetic formulations containing 2% Hydrolyzed Collagen were negative for systemic toxicity.[2]

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.[48] 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 small but statistically significant 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 was tolerated well in this rat study.[48]

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.[50] 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 skin and connective tissue-derived proteins and peptides were discovered and no unpublished data were submitted.

GENOTOXICITY

In Vitro

Hydrolyzed Collagen

No mutagenicity was observed in an Ames test of Hydrolyzed Collagen (30% solution with water; sourced from fish scales; MW ~400 Da).[21] *Salmonella typhimurium* strains TA 1535, TA1537, TA98, and TA100 and

Escherichia coli strain WP2uvrA were used in this test, which was performed in accordance to OECD TG 471. The dose range was 50 to 5000 µg/ plate, with and without metabolic activation.

In another Ames test performed in accordance to OECD TG 471, Hydrolyzed Collagen (20% solution with water; source from fish scales; MW ~400 Da) was not mutagenic in *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 and *E. coli* strain WP2uvrA, with or without metabolic activation.[21] The dose range was 50 to 5000 µg/ plate.

Hydrolyzed Collagen (30% solution with water; sourced from fish scales; MW ~400 Da) was not clastogenic in a Chinese hamster lung (CHL) cell line chromosome aberration test.[21] The cells were tested with and without metabolic activation.

CARCINOGENICITY

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

OTHER RELEVANT STUDIES

Type 1 Hypersensitivity

Type 1 (i.e., immediate) hypersensitivity reactions can occur in individuals allergic to certain proteins, such as those found in fish. An allergen must have at least 2 IgE-binding epitopes, and each epitope must be at least 15 amino-acid residues long, to trigger a Type 1 hypersensitivity reaction.[51] Type 1 responses can be elicited in sensitized patients when pairs of IgE molecules against a specific allergen are bound to receptors on the surface of mast cells and other cells that mediate immune reactions. The binding of an allergen molecule to two receptor-bound IgE molecules results in the crosslinking of the pair of IgE molecules. The crosslinking of sufficient numbers of IgE pairs bound to the receptors on the surface of a mast cell results in degranulation of the mast cell and the release of vasoactive amines, which are responsible for the Type 1 reaction. For some hydrolyzed proteins, the minimum number of amino acids (or weight-average MW) to elicit Type 1 hypersensitivity has been demonstrated with experimental data. For example, studies on hydrolyzed wheat protein show that hydrolysates with MWs less than 3500 Da do not have the properties required to induce Type 1 hypersensitivity.[9] Conclusive studies that detail the number of amino acids needed to trigger mast cell degranulation for hydrolyzed fish proteins, however, were not identified.

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).[52] Codfish-specific IgE was also measured in the patients and they underwent double-blinded, placebo-controlled food challenges with fish Gelatin. The fish Gelatin used for the study was made through acid extraction of codfish skins and had an average molecular weight of 60,000 Da. 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. [53] Serum IgE antibodies were tested against Gelatin and 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

Dermal irritation studies are presented in Table 8.[21,54-63] No irritation was predicted in in vitro studies of Hydrolyzed Collagen (bovine sourced; up to 55%), Soluble Collagen (fish and bovine sourced; undiluted), and Hydrolyzed Elastin (fish and bovine sourced; undiluted). No irritation was observed in rabbits or guinea pigs treated with Hydrolyzed Collagen (fish sourced; 30% solution) and Hydrolyzed Elastin (source not reported; tested neat). Hydrolyzed Collagen (bovine, swine, and fish sourced) was not irritating in human studies at concentration up to 50% in water solution.

Animal

Hydrolyzed Collagen

Primary skin irritation tests in rabbits indicated that Hydrolyzed Collagen was nonirritating or minimally irritating when tested at up to 100%.[2]

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.[2]

Sensitization

Animal

Hydrolyzed Collagen

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

Hydrolyzed Collagen (30% solution in water; fish scale sourced; MW ~400 Da) was considered to be non-sensitizing in a guinea pig maximization test using 15 Hartley guinea pigs and performed in accordance to OECD TG 406.[21] The animals received 7.5% active ingredient intradermally with Freund's complete adjuvant during the first induction, while undiluted test material was applied to clipped dorsal skin under occlusive 48 h patch during the second induction. The animals were challenged with undiluted test material on clipped flank skin under occlusive 24 h patch. No reactions were observed in any animal 24 h and 48 h post-challenge patching.

Human

Hydrolyzed Collagen

Formulations containing 0.5% to 28% Hydrolyzed Collagen produced some irritation but no sensitization in human repeated insult patch tests (HRIPTs).[2]

In a HRIPT with 50 subjects, Hydrolyzed Collagen (20% solution in water; fish scale sourced; MW ~400 Da) was not sensitizing.[21] The test material (0.2 ml) was applied to infrascapular skin with occlusive patches.

A study of sensitization to protein hydrolysates in hair care products was performed in 3 groups of patients.[64] 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.[64]

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.[65] The test patches were occlusive.

Phototoxicity

Hydrolyzed Collagen

Hydrolyzed Collagen at up to 2% was not phototoxic to guinea pigs and rabbits, nor was it phototoxic or photosensitizing to humans at up to 0.5%.[2] *UV-induced erythema was decreased after application of 10% solution of Hydrolyzed Collagen (MW = 1500 Da) onto the skin after irradiation.*

OCULAR IRRITATION STUDIES

Ocular irritation studies are presented in Table 9.[21,54-63] Hydrolyzed Collagen (fish and bovine sourced; up to 55%), Soluble Collagen (fish and bovine sourced; undiluted), and Hydrolyzed Elastin (fish and bovine sourced; undiluted) were predicted to be minimally or non-irritating in in vitro studies. Hydrolyzed Collagen (fish, swine,

and bovine sourced; up to 30%) and Hydrolyzed Elastin (source not reported; tested neat) were not irritating in rabbit studies.

Animal

Hydrolyzed Collagen

Hydrolyzed Collagen was minimally irritating to rabbit eyes when tested full-strength.[2]

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.[66] The patient's serum total IgE level was 442 kU/L, and strong radioallergosorbent test (RAST) 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.

Atelocollagen and Hydrolyzed 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 fish-sourced Hydrolyzed Collagen and once after consuming a gummy candy containing fish-sourced Hydrolyzed Collagen.[67] Fifteen months prior to the anaphylactic episodes, the patient had been applying a moisturizer containing Atelocollagen derived from fish 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 fish-sourced Hydrolyzed Collagen in the food products, the moisturizer, 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 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 fish-sourced Hydrolyzed Collagen protein. The researchers of this case study speculated that the 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.

Hydrolyzed Collagen

A 22-year-old female reported contact urticaria following use of a hair conditioner that contained steartrimonium hydrolyzed animal protein.[68] 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 kU/L. RASTs were negative to pork, beef, chicken, and mutton.

SUMMARY

This report assesses the safety of 19 skin and connective 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 is not repeated below.

Ingredients with the greatest number of reported uses in 2017 are Hydrolyzed Collagen (543 formulations) and Soluble Collagen (425 formulations); the majority of uses are in leave-on skin care products. Gelatin is used in a total of 334 formulations; 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. The other in-use ingredients are used at much lower concentrations.

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

No dermal irritation was predicted based on in vitro studies of Hydrolyzed Collagen (bovine sourced; up to 55%), Soluble Collagen (fish and bovine sourced; undiluted), and Hydrolyzed Elastin (fish and bovine sourced; undiluted). No dermal irritation was observed in rabbits or guinea pigs treated with Hydrolyzed Collagen (fish sourced; 30% solution) and Hydrolyzed Elastin (source not reported; tested neat). Hydrolyzed Collagen (bovine, swine, and fish sourced) was not irritating in human dermal studies at concentrations up to 50% in water solution.

A guinea pig maximization test found Hydrolyzed Collagen (fish sourced; 30% solution in water) to be non-sensitizing. In HRIPT studies, Hydrolyzed Elastin (25% w/v in corn oil) and Hydrolyzed Collagen (fish sourced; 20% solution in water) did not produce dermal irritation or dermal sensitization. Hydrolyzed Collagen produced positive results in skin prick tests of dermatitic patients.

Hydrolyzed Collagen (fish and bovine sourced; up to 55%), Soluble Collagen (fish and bovine sourced; undiluted), and Hydrolyzed Elastin (fish and bovine sourced; undiluted) were predicted to be minimally or non-irritating in ocular in vitro studies. Hydrolyzed Collagen (fish, swine, and bovine sourced; up to 30%) and Hydrolyzed Elastin (source not reported; tested neat) were not irritating in rabbit ocular studies.

Case reports of dermal sensitization to cosmetics containing Elastin, Atelocollagen, 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 or carcinogenicity studies on skin and connective tissue-derived proteins and peptides were identified in a literature search for these ingredients, and no unpublished data were submitted.

DISCUSSION

The Panel noted that there was a lack of systemic toxicity data (i.e. reproductive and developmental toxicity, genotoxicity, and carcinogenicity data); however, the Panel was not concerned that these proteins and peptides would cause adverse systemic effects in the general population. These proteins and peptides, similar to the other proteins and peptides reviewed by the Panel, are found in food, and daily exposures from the consumption of food can be expected to yield much larger systemic exposures to these ingredients than those from use in cosmetic products. The Panel also found that the earlier assessments of Hydrolyzed Collagen supported the safety of these ingredients in cosmetic products.

The Panel noted that fish proteins are known food allergens that can elicit Type 1 immediate hypersensitivity reactions when ingested by sensitized individuals. The Panel expressed concern that sensitized individuals would not easily recognize cosmetic products containing fish-derived collagen based on the current naming conventions used in the ingredient lists on product labels (e.g., Collagen and Hydrolyzed Collagen may be sourced from fish, though “fish” is not in the ingredient name). In the absence of negative Type 1 immediate hypersensitivity data for fish-derived protein ingredients (or other information supporting an inability of the supplied ingredient to elicit such sensitization (e.g., a maximum peptide length that is shorter than the minimum IgE-binding epitopes)), the Panel advised manufacturers to label products containing these fish-derived ingredients to inform individuals sensitized to fish proteins.

The Panel was also concerned about the inherent risks of using animal-derived ingredients in cosmetic products, namely the potential for transmission of infectious agents. While Gelatin and Collagen prepared exclusively from hides and skins do not have the propensity to carry disease, the Panel stressed that these ingredients must be free of detectible infectious pathogens (i.e., BSE) if these materials are derived from other bovine materials. Raw material suppliers and formulators of these ingredients must assure that these ingredients are free from pathogenic viruses and other infectious agents.

The Panel discussed the issue of incidental inhalation exposure from hair care products, skin care preparations, face powders, and fragrances. There were no inhalation toxicity data available. Although the Panel noted that droplets/particles from spray and loose-powder cosmetic products would not be respirable to any appreciable amount, the potential for inhalation toxicity is not limited to respirable droplets/particles deposited in the lungs. In principle, inhaled droplets/particles deposited in the nasopharyngeal and thoracic regions of the respiratory tract may cause toxic effects depending on their chemical and other properties. However, coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

CONCLUSION

The Panel concluded that the 19 skin and connective tissue-derived proteins and peptides listed below are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

Ammonium Hydrolyzed Collagen
Atelocollagen
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*

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

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.
Atelocollagen 55963-88-7	Atelocollagen is the protein obtained when the telopeptides are enzymatically removed from collagen.	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 skin and connective tissue-derived proteins[2,11,15,21,31,69-71]

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	400 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. Method of manufacturing

Ingredient	Source	Procedure	Reference
Collagen	Not reported	Prepared by dissolving the mineral part of bones with phosphoric acid.	[11]
Hydrolyzed Collagen	*Bovine or fish*	*Prepared by alkaline hydrolysis followed by enzymatic hydrolysis to the desired molecular weight*	[2,3]
Hydrolyzed Collagen (MW = 2000 Da)	Bovine	Prepared by combination of alkaline and enzymatic hydrolysis	[71]
Elastin	Farm animals such as cattle or goats	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.	[15]
Elastin	Collagen (unspecified)	Elastin may be a byproduct of the purification of Collagen	[15]
Hydrolyzed Elastin (MW=1000-4000 Da)	Codfish skin or bovine neck tendons	Prepared by washing and purifying to remove soil and other residual material and then dried. Dried material is 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.	[72,73]
Hydrolyzed Elastin (MW = 3000-4000 Da)	Numerous sourced animal ligaments or hides	Prepared by enzymatic hydrolysis (by pancreatic elastase, ficin, pepsin or trypsin) or acid hydrolysis at high temperatures (70-100º C, depending on acid) at several 1 hour intervals.	[15]
Hydrolyzed Elastin (MW=2000-4000 Da)	Not reported	Manufactured by enzymatic hydrolysis for a specific duration of time and at an elevated temperature (details not provided). Resultant hydrolyzed protein composed of di- and tri-peptides.	[62]
Gelatin	Collagen (unspecified)	Prepared by the acid, alkaline or enzymatic hydrolysis of Collagen. 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.	[17]
Soluble Collagen	Bovine dermal tissues, bony fish skins, or tropical fish swim bladders	Extracted by neutral salt solutions	[74]
Soluble Elastin	Cattle ligaments	Obtained by acid treatment 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.	[15]

Table 4. Amino acid residue profile of Collagen, soluble Collagen, and Elastin (residues per 1000).[14,15,75]

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 5. Frequency (2017) and concentration of use (2016) according to duration and type of exposure for skin and connective tissue-derived proteins and peptides.[36,37]

	# 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		**Atelocollagen**		**Collagen**		**Elastin**	
Totals[†]	**NR**	**0.03-0.12**	**118**	**0.0000001-0.099**	**299**	**0.00005-96**	**46**	NR
Duration of Use								
Leave-On	NR	0.1-0.12	114	0.0000001-0.005	241	0.0001-96	40	NR
Rinse Off	NR	0.03-0.05	4	0.0000001-0.099	58	0.00005-1.3	6	NR
Diluted for (Bath) Use	NR	NR	NR	NR	NR	NR	NR	NR
Exposure Type								
Eye Area	NR	NR	17	0.0000022	31	0.00012-0.2	2	NR
Incidental Ingestion	NR	NR	39	0.0004	5	0.0035-0.05	NR	NR
Incidental Inhalation-Spray	NR	NR	19[b]; 18[c]	0.0000001[b]	55[b]; 94[c]	0.00095[b]	11[b]; 11[c]	NR
Incidental Inhalation-Powder	NR	0.1[a]	1; 18[c]	0.000022; 0.00002-0.000033[a]	94[c]	0.0018-96[a]	11[c]	NR
Dermal Contact	NR	0.03-0.12	66	0.0000001-0.099	240	0.00005-96	42	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	0.05	1	NR	39	0.00095-1.3	4	NR
Hair-Coloring	NR	NR	NR	NR	1	NR	NR	NR
Nail	NR	NR	NR	NR	4	NR	NR	NR
Mucous Membrane	NR	NR	39	0.0004	8	0.0035-0.67	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

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

Table 5. Frequency (2017) and concentration of use (2016) according to duration and type of exposure for skin and connective tissue-derived proteins and peptides.[36,37]

	# 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 Fibronectin		**Hydrolyzed Reticulin**		**MEA-Hydrolyzed Collagen**		**Soluble Collagen***	
Totals†	**10**	**0.025-0.05**	NR	**0.025-0.05**	NR	**0.03-0.12**	**425**	**0.0000005-0.7**
Duration of Use								
Leave-On	9	0.05	NR	0.05	NR	0.1-0.12	368	0.0000005-0.7
Rinse Off	1	0.025	NR	0.025	NR	0.03-0.06	57	0.000025-0.014
Diluted for (Bath) Use	NR	NR	NR	NR	NR	NR	NR	0.000035-0.005
Exposure Type								
Eye Area	1	NR	NR	NR	NR	NR	51	0.00003-0.05
Incidental Ingestion	NR	NR	NR	NR	NR	NR	6	0.00035-0.01
Incidental Inhalation-Spray	4[b]; 3[c]	NR	NR	NR	NR	NR	104[b]; 141[c]	0.000005-0.0035; 0.00035-0.0035[b]
Incidental Inhalation-Powder	3[c]	NR	NR	NR	NR	0.1[a]	141[c]	0.0035; 0.0001-0.7[a]
Dermal Contact	10	0.025-0.05	NR	0.025-0.5	NR	0.03-0.12	379	0.000005-0.7
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	0.06	15	0.00001-0.014
Hair-Coloring	NR	NR	NR	NR	NR	NR	1	0.000025-0.0005
Nail	NR	NR	NR	NR	NR	NR	NR	0.0000005-0.01
Mucous Membrane	NR	NR	NR	NR	NR	NR	9	0.000035-0.01
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

	Soluble Collagen Extract‡	
Totals†	**2**	NR
Duration of Use		
Leave-On	2	NR
Rinse Off	NR	NR
Diluted for (Bath) Use	NR	NR
Exposure Type		
Eye Area	NR	NR
Incidental Ingestion	NR	NR
Incidental Inhalation-Spray	1[b]; 1[c]	NR
Incidental Inhalation-Powder	1[c]	NR
Dermal Contact	2	NR
Deodorant (underarm)	NR	NR
Hair - Non-Coloring	NR	NR
Hair-Coloring	NR	NR
Nail	NR	NR
Mucous Membrane	NR	NR
Baby Products	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 Dictionary, possibly the same as Collagen Extract.

Table 6. Historic and current frequency and concentration of use according to duration and type of exposure for Hydrolyzed Collagen.[36,37]

	# 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 7. Ingredients not reported in use.

Calcium Hydrolyzed Collagen
Zinc Hydrolyzed Collagen
Hydrolyzed Collagen Extract
Hydrolyzed Gelatin
Soluble Elastin
Hydrolyzed Spongin

Table 8. Dermal irritation studies of Hydrolyzed Collagen, Soluble Collagen, and Hydrolyzed Elastin.

Ingredient	Concentration	Method	Result	Reference
		In Vitro		
Hydrolyzed Collagen (MW ~ 2000 Da; source = bovine)	25, 50, 75, 100, or 125 µl	Irritection® dermal model	Predicted to be non-irritating	[54]
Hydrolyzed Collagen (source = bovine)	55% solution, undiluted	EpiDerm™ Assay	Predicted to be non-irritating	[57]
Soluble Collagen (source = Atlantic cod)	25, 50, 75, 100, or 125 µl	Irritection® dermal model	Predicted to be non-irritating	[56]
Soluble Collagen (source = bovine)	Undiluted	EpiDerm™ Assay	Predicted to be non-irritating	[61]
Hydrolyzed Elastin (MW ~4000 Da; source = fish)	Concentration not reported	EpiDerm™ Assay	Predicted to be non-irritating	[62]
Hydrolyzed Elastin (MW ~4000 Da; source not reported)	Concentration not reported	EpiDerm™ Assay	Predicted to be non-irritating	[62]
Hydrolyzed Elastin (source = Atlantic cod)	Undiluted	EpiDerm™ Assay	Predicted to be non-irritating	[58]
Hydrolyzed Elastin (source = young cattle)	25, 50, 75, 100, or 125 µl	Irritection® dermal model	Predicted to be non-irritating	[55]
Hydrolyzed Elastin (source = cow skin)	Undiluted	EpiDerm™ Assay	Predicted to be non-irritating	[60]
Hydrolyzed Elastin (source = cow skin)	Undiluted	EpiDerm™ Assay	Predicted to be non-irritating	[59]
		Animal		
Hydrolyzed Collagen (MW ~400 Da; source = fish scale)	30 % solution in water, tested neat	Primary skin irritation test in 3 New Zealand White rabbits; occluded 2.5 cm^2 patches with 0.5 ml test material; test performed in accordance to OECD TG 404	Very slight erythema noted in one treated skin 1 h post-patch removal; primary irritation index was 0.0; test material non-irritating and not corrosive	[21]
Hydrolyzed Collagen (MW ~400 Da; source = fish scale)	30 % solution in water, tested neat	Cumulative skin irritation test in 3 male and 3 female Hartley guinea pigs; once daily treatments for 14 days to 2.0 cm^2 clipped dorsal skin	Non-irritating	[21]
Hydrolyzed Elastin (MW ~3000 Da; source not reported)	Tested neat	Draize primary dermal irritation study in 6 New Zealand white rabbits; test sites occluded for 24 h	Not a primary irritant; primary irritation index was 0.38	[63]
		Human		
Hydrolyzed Collagen (MW ~400 Da; source = bovine gelatin)	50% solution in water, tested neat	24 h occlusive patch test in 60 subjects (50 healthy, 10 allergic); 0.5 ml applied to left front arms	Non-irritating	[21]
Hydrolyzed Collagen (MW ~400 Da; source = bovine gelatin)	30% solution in water, tested neat	24 h occlusive patch test in 60 subjects (50 healthy, 10 allergic); 0.5 ml applied to left front arms	Non-irritating	[21]
Hydrolyzed Collagen (MW ~1000 Da: source = swine gelatin)	30% solution in water, tested neat	24 h occlusive patch test in 60 subjects (50 healthy, 10 allergic); 0.5 ml applied to left front arms	Non-irritating	[21]
Hydrolyzed Collagen (MW ~2000 Da; source = swine gelatin)	30% solution in water, tested neat	24 h occlusive patch test in 60 subjects (50 healthy, 10 allergic); 0.5 ml applied to left front arms	Non-irritating	[21]
Hydrolyzed Collagen (MW ~400 Da; source = fish scale)	30% solution in water, tested neat	24 h occlusive patch test in 21 healthy subjects; 0.03 g applied to backs	Non-irritating	[21]
Hydrolyzed Collagen (MW ~400 Da; source = fish scale)	20% solution in water, tested neat	24 h occlusive patch test in 20 healthy subjects	Slight erythema in one subject; non-irritating	[21]

Table 9. Ocular irritation studies of Hydrolyzed Collagen, Soluble Collagen, and Hydrolyzed Elastin

Ingredient	Concentration	Method	Result	Reference
In Vitro				
Hydrolyzed Collagen (MW ~ 2000 Da; source = bovine)	25, 50, 75, 100, or 125 µl	Irritection® ocular model	Predicted to be a minimal irritant	[54]
Hydrolyzed Collagen (MW ~400 Da; source = fish scale)	10% active in purified water	BCOP in accordance to OECD TG 437	Predicted to be non-irritating	[21]
Hydrolyzed Collagen (source = bovine)	55% solution, undiluted	EpiOcular™ Assay	Predicted to be non-irritating	[57]
Soluble Collagen (source = Atlantic cod)	25, 50, 75, 100, or 125 µl	Irritection® ocular model	Predicted to be a minimal irritant	[56]
Soluble Collagen (source = bovine)	Undiluted	EpiOcular™ Assay	Predicted to be non-irritating	[61]
Hydrolyzed Elastin (source = Atlantic cod)	Undiluted	EpiOcular™ Assay	Predicted to be non-irritating	[58]
Hydrolyzed Elastin (source = young cattle)	25, 50, 75, 100, or 125 µl	Irritection® ocular model	Predicted to be a minimal irritant	[55]
Hydrolyzed Elastin (source = cow skin)	Undiluted	EpiOcular™ Assay	Predicted to be non-irritating	[60]
Hydrolyzed Elastin (source = cow skin)	Undiluted	EpiOcular™ Assay	Predicted to be non-irritating	[59]
Hydrolyzed Elastin (MW = 2000-4000 Da; 2 products, one source = fish, other source not reported)	Concentration not reported	EpiOcular™ Assay	Predicted to be non-irritating	[62]
Ocular - Animal				
Hydrolyzed Collagen (MW ~400 Da; source = bovine gelatin)	25% active diluted by 1%, 5%, 15%, 25%, and 50% v/v in saline	Ocular irritation study in 15 male rabbits; test material instilled in one eye while other eye served as control; eyes observed at instillation, 1 h and 24 h post	Non-irritating	[21]
Hydrolyzed Collagen (MW ~400 Da; source = bovine gelatin)	15% active diluted by 1%, 5%, 15%, 25%, and 50% v/v in saline	Ocular irritation study in 15 male rabbits; test material instilled in one eye while other eye served as control; eyes observed at instillation, 1 h and 24 h post	Non-irritating	[21]
Hydrolyzed Collagen (MW ~1000 Da; source = swine gelatin)	3% active diluted by 0.1%, 0.5%, 1%, 5%, and 10% v/v in saline	Ocular irritation study in 15 male rabbits; test material instilled in one eye while other eye served as control; eyes observed at instillation, 1 h and 24 h post	Non-irritating	[21]
Hydrolyzed Collagen (MW ~2000 Da; source = swine gelatin)	15% active diluted by 1%, 5%, 15%, 25%, and 50% v/v in saline	Ocular irritation study in 15 male rabbits; test material instilled in one eye while other eye served as control; eyes observed at instillation, 1 h and 24 h post	Non-irritating	[21]
Hydrolyzed Collagen (MW ~400 Da; source = fish scale)	30% active, tested neat	Ocular irritation study in 3 New Zealand White rabbits; test performed in accordance with OECD TG 405	Maximum group mean score was 4.0; minimally irritating	[21]
Hydrolyzed Elastin (MW = 3000 Da; source not reported)	tested neat	Draize ocular irritation study in 6 New Zealand white rabbits; treated eye was not rinsed	Not a primary irritant	[63]

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Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to a notification from DSM on fish gelatine for use as a formulation aid (carrier) in vitamin and carotenoid preparations pursuant to Article 6, paragraph 11 of Directive 2000/13/EC- for permanent exemption from labelling

(Request Nº EFSA-Q-2006-161)

(Adopted on 15 October 2007 by written procedure)

SUMMARY

Fish is an important allergenic food. Fish allergy has been reported to affect 0.2%-2.2% of the population in European countries. Allergic reactions to fish can be severe. The major allergen of fish is the muscle protein parvalbumin. The present application concerns fish gelatine produced from fish skin for use as a formulation aid (carrier) in vitamin and carotenoid preparations. Gelatine is made by denaturation of collagen.

Allergens of concern are residual amounts of parvalbumin, and gelatine itself. The information provided by the applicant indicates that the production process of gelatine from fish skins for this particular purpose is well standardized. A monoclonal and a polyclonal ELISA assay for measuring parvalbumin in fish gelatine have been developed, with a limit of detection of 1µg/g and 0.04µg/g, respectively. None of the assays detected parvalbumin in ten commercial lots of gelatine. According to the applicant, daily fish gelatine intake from vitamin preparations intended for use in food supplements, colourings and beverages is in the low milligram range. Estimation of the highest concentration of fish gelatine in vitamin containing preparations available on the market, indicates a concentration of 30mg per litre, or 7.5mg per 250ml serving. Assuming a parvalbumin content in gelatine of 0.04µg/g, the estimated intake of parvalbumin with one serving will be 0.0003µg.

In a double blind placebo controlled food challenge study, none of 30 fish-allergic patients tested reacted to a cumulative dose of 3.6g of codfish gelatine, while a confirmed mild reaction was experienced by one individual at a cumulative dose of 7.6g.

Taking into account the information available, the Panel considers that it is unlikely that fish gelatine used as a formulation aid (carrier) in vitamin and carotenoid preparations will trigger an adverse allergic reaction in susceptible individuals under the conditions of production and use specified by the applicant.

KEY WORDS

Fish gelatine, carrier, vitamin preparations, carotenoid preparations, parvalbumin, food allergy.

BACKGROUND

In November 2003, the European Parliament and the Council adopted Directive 2003/89/EC[1] amending Directive 2000/13/EC, as regards indication of the ingredients present in foodstuffs.

Annex IIIa of the Directive specifies a list of food ingredients or substances that are known to trigger allergic reactions or intolerances in sensitive individuals for which no labelling exemptions are allowed. Whenever the listed ingredients/substances or their derivatives are used in the production of foodstuffs, they must be labelled.

Article 1, paragraph 11, subparagraph 2 of the Directive establishes a procedure allowing for temporary labelling exemption of derivatives from ingredients listed in Annex IIIa for which it has been scientifically established that it is not possible for them to cause adverse reactions. In accordance with this provision, submissions of request for temporary labelling exemption were notified to the Commission before 25 August 2004. The Commission, after consultation with the European Food Safety Authority, adopted a list (Directive 2005/26/EC[2]) of those ingredients which are temporarily excluded from Annex IIIa until 25 November 2007, pending the final results of the notified studies.

Consequently, applicants who submitted a dossier in 2004 on the basis of subparagraph 2, resulting in the inclusion of a product in the list of Directive 2005/26/EC, and who are seeking exclusion of that product from Annex IIIa beyond 25 November 2007 will have to submit a request enclosing the final results of the notified scientific studies. Therefore in the context of the permanent labelling exemption procedure, the European Food Safety Authority is asked to provide scientific opinions on the submissions in accordance with the present terms of reference.

TERMS OF REFERENCE

In accordance with Article 29 (1) (a) of Regulation (EC) N° 178/2002, the European Commission requests the European Food Safety Authority to evaluate the scientific data submitted by DSM Nutritional Products in the framework of the procedure laid down in Article 6, paragraph 11 of Directive 2000/13/EC. On the basis of that evaluation, EFSA is requested to issue an opinion on the information provided, and particularly to consider the likelihood of adverse reactions triggered in susceptible individuals by the consumption of the following ingredients/substances used under the conditions specified by the applicant: fish gelatine for use as a formulation aid (carrier) in vitamin and carotenoid preparations.

ASSESSMENT

The extent of the fish-allergic population is uncertain. A Danish population-based study with double blind placebo controlled food challenge (DBPCFC) verification of allergy (Osterballe *et al*., 2005) found 0.2% of the adult population allergic to codfish. The total number of fish-

[1] Directive 2003/89/EC of the European Parliament and of the Council amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs. OJ L 308. 25.11.2003, p. 15.
[2] Commission Directive 2005/26/EC of 21 March 2005 establishing a list of food ingredients or substances provisionally excluded from Annex IIIa of Directive 2000/13/EC of the European Parliament and of the Council. OJ L 75, 22.03.2005, p. 33-34.

allergic individuals may be somewhat higher, because some fish-allergic individuals do not react to codfish. Two population-based American studies based on telephone surveys (Sicherer *et al.*, 2004; Vierk *et al.*, 2007) gave numbers in the same range, whereas in a European questionnaire-based study (Woods *et al.*, 2001) 2.2% reported (self-perceived) fish allergy or intolerance.

Taking into account the numerous and well documented reports of allergic individuals (NDA, 2004a) reacting to fish, it is appropriate for the Panel to assess the likelihood of adverse reactions in allergic individuals consuming products containing gelatine made from fish skins.

A dossier submitted by DSM Nutritional Products to the European Commission pursuant to Article 6, Paragraph 11 of Directive 2000/13/EC as amended by Directive 2003/89/EC on 23 August 2004 for temporary exemption from labelling was the basis for an earlier assessment of gelatine by the NDA panel (NDA, 2004b). The present opinion is based on an updated dossier from the same organization, with an application for permanent exemption from labelling. The updated dossier contains new data mainly with regard to analytical studies on residual amounts of parvalbumin in gelatine.

1. Manufacturing process

1.1 Procedures and quality control

Gelatine is produced by extraction and hydrolysis of fibrous, insoluble collagen from fish skin, as described in the opinion regarding temporary exemption (NDA, 2004b). The production process consists of several steps of extensive washing, acidification, heating and filtration. Thereafter, dry gelatine is produced by evaporation of water from a thin film of liquid concentrate. Representative samples are taken during the subsequent grinding process and sent to an independent laboratory for testing to comply with set specifications. The applicant follows a Quality Management Plan and refers to a standardised manufacturing process for the specified use of the product.

1.2 Removal of parvalbumin during production

Mostly parvalbumins have been isolated from fish muscle. However, it is uncertain whether parvalbumin has been looked for in fish skin and fish scales. The allergenicity of fish parvalbumin is not easily destroyed by heat, proteolytic activity or denaturation with chemicals. The applicant states that during washing of fish skins as part of the production process “the water-soluble allergens are substantially reduced”. Documentation is provided in the form of data on falling levels of parvalbumin in the washing water with increasing numbers of wash cycles. However, the data are derived from a model system measuring parvalbumin in washing water and not in skin extracts. Quantification in relation to washing was attempted only by SDS-PAGE and Western blotting using a monoclonal antibody to tuna parvalbumin. The Panel notes that the described procedures are not sensitive or specific enough to enable the determination of low amounts of parvalbumin in fish skin extracts.

2. Characterisation of the product and its use

2.1 Characterisation of fish gelatine

Fish gelatine is denatured collagen from fish skins. Only the skins of certain cold water fish are used, with cod, pollock, and haddock being the most common species.

Fish gelatine has an approximate molecular weight of 60,000Da. The CAS (Chemical Abstract Services) number is the same for all gelatines including fish gelatine: CAS # 9000-70-8. The applicant provides specifications of the product they use, and states that they are in compliance with EU specifications, European Pharmacopoeia, US Pharmacopoeia and Japanese Pharmacopoeia.

2.2 Conditions of use and exposure levels

2.2.1 Conditions of use

Under the present application, fish gelatine is used for the micro-encapsulation of oil soluble substances, specifically vitamins A, D, E and carotenoids (e.g. β-carotene, canthaxanthin, lutein, lycopene) to bring the substances into suitable form for incorporation into processed foods like fruit-based beverages (so-called ACE drinks) and multivitamin drinks. Carotenoids are also used as food colours. The encapsulation process is described by the applicant. The products are water-dispersible and the nutrients are bioavailable. The use of fish gelatine as a formulation aid (carrier) in vitamin/carotenoid preparations is protected by a patent owned by the applicant.

2.2.2 Exposure levels

The applicant claims that the amount of fish gelatine in the final products introduced by vitamin and carotenoid preparations is very low (see below). The amount of fish gelatine present in a number of fortified or coloured foods or beverages usually ranges from 2 to 20 mg/kg.

2.2.2.1 Fish gelatine intake calculated on the basis of typical reference values for vitamins

Reference values for nutrition labelling are currently laid down for vitamins and minerals (Council Directive 90/496/EEC). Relevant for this application are reference values for vitamins A (β-carotene), D and E. For a set of product forms listed by the applicant, estimated potential intakes of fish gelatine per day are as follows: 2.0-7.2mg fish gelatine for vitamin A/β-carotene preparations, 6.0mg fish gelatine for vitamin E and 1.5mg fish gelatine for vitamin D preparations. Based on these calculations, the applicant states that the intake of fish gelatine per day will be below the tolerated dose of 3.6g reported by Hansen *et al*. (2004).

2.2.2.2 Fish gelatine intake calculated on the basis of existing products on the European market

German data are used, as this country has the highest consumption of the relevant products and is where the highest-dosed products can be found. According to the applicant, ACE and multivitamin beverages contain maximum 20mg β-carotene per litre (self-limitation of the German industry). The applicant estimates that the concentration of fish gelatine is 30mg per litre, or 7.5mg per 250ml serving. Consumption of 1.5 litres per day would result in 45 mg fish gelatine daily. Additional calculations based on seven different beverages on the German market give fish gelatine contents per 250ml serving over a range from 6.3 to 7.2mg, which are below the claimed lowest reported triggering dose. However, it must be taken into

consideration that a number of other sources (e.g. yogurt and cakes) may contribute to the total fish gelatine intake.

2.2.2.3 Parvalbumin intake with fish gelatine

Assuming a maximum parvalbumin content in gelatine of 0.04µg/g (LOD of the most sensitive ELISA, see below), and an intake of one serving with 7.5mg gelatine (see above) the estimated intake of parvalbumin with one serving will be 0.3 ng. Considering that the extractable amount of parvalbumin has been found to be 1.3µg/mg in cod (study in the dossier), 1.6µg/mg in mackerel (Kobayashi *et al.*, 2006), and 3.7-4.9µg/mg in carp (Vornanen *et al.*, 1999), the dose of parvalbumin in one serving appears to be lower than the lowest dose of parvalbumin till now reported to elicit a reaction in a DBPCFC study (low mg range of fish (Taylor *et al.*, 2002)). The Panel notes, however, the uncertainty with regard to the yield when extracting parvalbumin from fish meat, the uncertainties with regard to the accuracy of both the ELISA assays used, and the uncertainty with regard to establishing a triggering dose in allergic reactions to fish.

3. Evidence of non-allergenicity

3.1 History of non-allergenicity of the product

3.1.1 Literature search strategy

No formal literature search is described by the applicant, neither in the previous nor the present application. However, the applicant states that a literature search with a focus on new papers since 2003 was undertaken.

3.1.2 Historical evidence of safe use

The applicant claims that a history of safe use is established. Fish gelatine-containing vitamin and carotenoid preparations have been on the market since 1992. The applicants state their unawareness of any documented report about allergic reactions due to the presence of fish gelatine in the products in question.

It should, however, be mentioned that lack of clear labelling may be the reason for the absence of reports as consumers and health professionals would not be aware of the constituents of the products consumed, and therefore would not have suspected or reported fish gelatine as a cause for an allergic reaction.

3.2 Preclinical studies on allergenicity

3.2.1 Parvalbumin cross-reactivity

An important factor in allergenicity studies of fish parvalbumins is the degree of cross-reactivity between different fish species, and the species specificity of antibodies used. At least 31 parvalbumins have been described (Allergome database, 2007). Fish parvalbumins are assumed to be broadly cross-reactive (NDA, 2004a). However, clinical practice as well as laboratory experience indicate that cross-reactivity between fish species sometimes is limited or absent. An example was found in the present application, where an anti-carp monoclonal antibody employed in an ELISA showed binding to only five of seven fish species tested.

Bioinformatics analysis was undertaken to compare available amino acid sequences from commonly consumed fish parvalbumins, among them carp, cod and pollack. Homology studies of 18 fish parvalbumins from 10 species, based on publicly available sequences (NCBI, 2007), are reported. A given fish species contains usually 2-5 different parvalbumins. Two Atlantic and one Baltic cod parvalbumins were 63% to 74% identical, and parvalbumins from the other fish were from 53% to 92% identical to the three codfish parvalbumins. Carp parvalbumin shared 65%-85% identical sequences with cod parvalbumins. The three fish species that according to the applicant are most used for gelatine production (cod, haddock and pollack) all belong to the same family, *Gadidae*, and therefore are presumed to be more cross-reactive than more distantly related fish. Referring to the literature (Albersee, 2000), the applicants conclude that high levels of sequence identity were observed that indicate a high likelihood of IgE cross-reactivity. This suggests that antibodies to one or two of these parvalbumins could serve as reagents for detection of parvalbumins from a wide range of fish species. The Panels notes that the use of antibodies raised against parvalbumin from one species of fish entails a certain uncertainty, which may affect the detection and measurement of parvalbumin from other fish species.

No data have been presented regarding fish gelatine cross-reactivity.

3.2.3 Analytical data on parvalbumin levels in fish gelatine

A commercially available monoclonal antibody against frog parvalbumin was found to react in immunoblotting studies only with parvalbumin from some less relevant fishes and not codfish.

3.2.3.1 Monoclonal anti-carp parvalbumin

A commercially available monoclonal antibody against carp parvalbumin was used to develop a competitive ELISA. This ELISA was found to have an LOD of 0.05µg/ml, which according to the applicant is equivalent to 1µg/g in a solid sample. The sensitivity was verified by spiking experiments with known amounts of carp parvalbumin. Ten commercial batches of gelatine were analysed with this ELISA, none of them showing parvalbumin levels above the detection limit.

3.2.3.2 Polyclonal anti-cod parvalbumin ELISA

A rabbit polyclonal antibody made against specially purified cod parvalbumin was used as capture antibody. Due to technical difficulties an inhibition ELISA was not achieved, and a sandwich ELISA was developed. The LOD for cod parvalbumin with this ELISA is reported to be 0.04µg/g. Only cod parvalbumin was tested. Ten commercial batches of gelatine were analysed also with this ELISA, none of them showing parvalbumin levels above the LOD.

3.2.4 IgE binding studies with fish gelatine

A comprehensive overview over fish collagen and gelatine IgE reactivity was given with the opinion in relation to the application for temporary exemption (NDA, 2004b).

In brief, experimental studies showed that collagen's IgE binding capacity was thermostable and preserved also in peptide fragments. A number of studies, in particular from Japan, have reported human IgE binding to fish collagen and gelatine (NDA, 2004b), but in none of these studies were DBPCFC with collagen or gelatine performed. There are no reports of challenges

with fish collagen or gelatine apart from the two studies cited below. It must be emphasised that the presence of IgE-binding to gelatine does not equate with clinical allergy, and the clinical importance of fish gelatine as an allergen is uncertain.

3.2.5 Digestibility of fish gelatine

Fish gelatine was analysed for digestibility by pepsin. Peanut allergens were used as a reference. Compared to certain peanut allergens, fish gelatine digested quickly. The Panel notes that this information alone is of limited value when considering allergenicity.

3.3 Clinical studies

The two clinical studies outlined below are described and discussed in more detail in the opinion for temporary exemption (NDA, 2004b). Regarding challenge studies in fish allergic individuals with specific IgE to fish gelatine, the applicant states that it would in practice be impossible to find a sufficient number of such individuals available for testing, since the frequency of IgE seropositivity to fish gelatine in Europe appears to be in the order of 3% among fish allergic individuals.

3.3.1 Food challenge studies with fish gelatine

In a DBPCFC study by a Danish group (Hansen *et al.*, 2004), no adverse reactions were observed when 30 codfish allergic patients were given a cumulative dose of 3.6 g of codfish skin-derived fish gelatine. When given a cumulative dose of 7.6 g, two patients experienced a mild reaction, confirmed in repeated challenges in one of the two. There was no reaction to the next higher dose.

In a French single-blind study (André *et al.*, 2003) of 100 children and adults with IgE reactivity to various species of fish, fish (tuna) skin-derived collagen was used. It was found that 3 out of 100 sera showed IgE binding to tuna skin-derived gelatine. The three seropositive individuals were skin tested with fish gelatine, and the tests were negative. Similarly, a food challenge test using 5 g of tuna gelatine was also negative in these three subjects. The Panel notes that the allergenicity of tuna in several clinical and laboratory studies appears to be comparatively low.

CONCLUSIONS

Taking into account the information available, the Panel considers that it is unlikely that fish gelatine used as a formulation aid (carrier) in vitamin and carotenoid preparations will trigger an adverse allergic reaction in susceptible individuals under the conditions of production and use specified by the applicant.

DOCUMENTATION PROVIDED TO EFSA

Dossier submitted by DSM Nutritional Products to the European Commission pursuant to Article 6 Paragraph 11 of Directive 2000/13/EC as amended by Directive 2003/89/EC, on 29 September 2006.

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PANEL MEMBERS

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I U C L I D

D a t a s e t

Existing Chemical	Substance ID: 9000–70–8
CAS No.	9000–70–8
EINECS Name	Gelatins
EINECS No.	232–554–6
Structural Formula	Polimero biologico composto di catene lineari di polipeptidi
Molecular Formula	Polipeptide con peso molecolare tra i 500 e 500000 D

Dataset created by: EUROPEAN COMMISSION – European Chemicals Bureau

This dossier is a compilation based on data reported by the European Chemicals Industry following 'Council Regulation (EEC) No. 793/93 on the Evaluation and Control of the Risks of Existing Substances'. All (non–confidential) information from the single datasets, submitted in the IUCLID/HEDSET format by individual companies, was integrated to create this document.

The data have not undergone any evaluation by the European Commission.

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1.0.1 OECD and Company Information

Name: Agfa–Gevaert AG
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Country: Germany

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Name: Ewald–Gelatine GmbH
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Country: Germany
Phone: 06751/860
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Phone: +46 435 265 00
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Telex: 72496

Name: Gelatine Delft B.V.
Street: Rotterdamseweg 270
Town: 2628 AT Delft
Country: Netherlands
Phone: 31 15 569301
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Name: Gelatine Products Ltd.
Street: Sutton–Weaver
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Telex: 629303

Name: Henkel KGaA
Street: Henkelstr. 67
Town: 40589 Duesseldorf
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Name: ITALGELATINE S.P.A.
Street: S. Statale Alba–Bra 201
Town: 12060 Santa Vittoria D'Alba (CN)
Country: Italy
Phone: 0172/478047
Telefax: 0172/478715
Telex: 211551 ITALGE I

Name: NEUBER GES.M.B.H.
Street: BRÜCKENGASSE 1
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Country: Austria
Phone: 0222/599950
Telefax: 0222/5970200

Name: SYSTEMS BIO–INDUSTRIES
Street: 4, Place des Ailes
Town: 92641 BOULOGNE–BILLANCOURT
Country: France
Phone: 33 1 47122525
Telefax: 33 1 47122656
Telex: 633020 SBI PAR

1.0.2 Location of Production Site

–

1.0.3 Identity of Recipients

–

1.1 General Substance Information

Substance type: inorganic
Physical status: liquid

Substance type: inorganic
Physical status: solid

Substance type: natural substance
Physical status: solid

Substance type: organic
Physical status: solid

1.1.1 Spectra

-

1.2 Synonyms

Collagens, gelatins
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

DAB 7
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

DAB 7 (gelatin)
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

E 260
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

E 300
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

E 300 (gelatin)
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Emagel
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

F 275
Source: Henkel KGaA Duesseldorf

FCR 500
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Gelafusal
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

gelatin
Source: Croda Colloids Ltd Widnes, Cheshire

Gelatin
Source: Gelatine Products Ltd. Runcorn,Cheshire

GELATIN (INCI) (INN)
Source: Henkel KGaA Duesseldorf

gelatina (INN)
Source: Henkel KGaA Duesseldorf

gelatine
Source: SYSTEMS BIO-INDUSTRIES BOULOGNE-BILLANCOURT
Croda Colloids Ltd Widnes, Cheshire
Ewald-Gelatine GmbH Bad Sobernheim

Gelatine
Source: Gelatine Products Ltd. Runcorn,Cheshire
BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Gelatine Bloom 250
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

GELATINE, GELATIN
Source: EXTRACO AB KLIPPAN

Gelatine, gelatin
Source: DGF Stoess AG Eberbach

Gelatins
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Gelfoam
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

gelificante proteico di origine animale
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

Gelita Sol E
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Gelita-Collagel
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Gelita-Sol P
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Gelrite
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Glutins (gelatins)
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

M 394
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

M 396
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

M 400 (gelatin)
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Neosoft GE 82
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Nikkol CCP 4
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Nittait GF 600A
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Ossein Gelatin HOS 1
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

P 104
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

P 2115
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

P 2225
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

PA 10 (gelatin)
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Pharmagel A
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Pharmagel AdB
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Pharmagel B
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Rousselot 3046
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

S 692
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Spongiofort
Source: BASF AG Ludwigshafen
Henkel KGaA Duesseldorf

Source: Gelatine Delft B.V. Delft

(1)

1.3 Impurities

-

1.4 Additives

-

1.5 Quantity

Quantity 100 000 - 500 000 tonnes

1.6.1 Labelling

-

1.6.2 Classification

-

1.7 Use Pattern

Type: type
Category: Non dispersive use

Type: type
Category: Use resulting in inclusion into or onto matrix

Type: type
Category: Wide dispersive use

Type: industrial
Category: Basic industry: basic chemicals

Type: industrial
Category: Personal and domestic use

Type: industrial
Category: Photographic industry

Type: industrial
Category: Polymers industry

Type: industrial
Category: Textile processing industry

Type: industrial
Category: other: Lebensmittel

Type: industrial
Category: other: Medizinische Anwendungen

Type: industrial
Category: other: food

Type: industrial
Category: other: medical

Type: use
Category: Adhesive, binding agents

Type: use
Category: Cosmetics

Type: use
Category: Food/foodstuff additives

Type: use
Category: Pharmaceuticals

Type: use
Category: Photochemicals

Type: use
Category: Stabilizers

Type: use
Category: other: (industria alimentare, enologica e tecnica)

Type: use
Category: other: Sprengmittel für geformte Reinigungsmittel

Type: use
Category:

1.7.1 Technology Production/Use

-

1.8 Occupational Exposure Limit Values

Type of limit: other: nessun limite di esposizione, prodotto alimentare
Limit value:
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

Type of limit:
Limit value:
Source: Gelatine Delft B.V. Delft

(2)

Type of limit:
Limit value:
Remark: In the absence of a specific exposure limit for a particular dust, personal exposure should be kept below both 10mg/m3 8 hour TWA total inhalable dust and 5mg/m3 8 hour TWA respirable dust.
Source: Croda Colloids Ltd Widnes, Cheshire

(3)

Type of limit:
Limit value:
Remark: Kein MAK–Wert festgelegt
Source: BASF AG Ludwigshafen

(4)

Type of limit:
Limit value:
Source: DGF Stoess AG Eberbach

1.9 Source of Exposure

Source: Gelatine Delft B.V. Delft

(5)

Remark: Sources of exposure to gelatin include ingestion, from its use in food, and skin and eye contact, from its use in cosmetics and pharmaceuticals etc.
Source: Croda Colloids Ltd Widnes, Cheshire

Remark: Sources of exposure to gelatin include ingestion, from its use in food, and skin and eye contact, from its use in cosmetics and pharmaceuticals etc.
Source: Gelatine Products Ltd. Runcorn,Cheshire

Remark: Sources of exposure to gelatin include ingestion from its use in food and pharmaceuticals. Skin/eye contact from its use in cosmestics and pharmaceuticals.
Source: EXTRACO AB KLIPPAN

Remark: Verschlucken bei der Verwendung als Lebensmittel und in Pharmazeutika.
Haut– und Augenkontakt bei der Verwendung in Kosmetika und Pharmazeutika.
Source: DGF Stoess AG Eberbach

1.10.1 Recommendations/Precautionary Measures

–

1.10.2 Emergency Measures

–

1.11 Packaging

–

1.12 Possib. of Rendering Subst. Harmless

–

1.13 Statements Concerning Waste

–

1.14.1 Water Pollution

–

1.14.2 Major Accident Hazards

–

1.14.3 Air Pollution

Classified by: TA–Luft (DE)
Labelled by: TA–Luft (DE)
Number: 3.1.7 (organic substances)
Class of danger: III
Remark: Vorläufige Zuordnung
Zusätzlich Regelung nach 3.1.3
Source: BASF AG Ludwigshafen

1.15 Additional Remarks

Source: Gelatine Delft B.V. Delft

(6)

1.16 Last Literature Search

–

1.17 Reviews

–

1.18 Listings e.g. Chemical Inventories

–

2.1 Melting Point

Value:
Remark: Not applicable in solid state (melting is obtained in aqueous solutions)
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Value:
Decomposition: yes
Sublimation: no
Remark: Non applicabile allo stato solido
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

2.2 Boiling Point

Value:
Remark: Not applicable in solid state.
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Value:
Decomposition: yes
Remark: Non applicabile allo stato solido
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

2.3 Density

Type: relative density
Value: .5 – .8 g/cm3 at 25 degree C
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

Type:
Value:
Remark: Between 0,5 and 0,8 (powder or granulous : depending of the size of the grains).
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

2.3.1 Granulometry

–

2.4 Vapour Pressure

Value:
Remark: Not applicable (solid state).
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Value:
Remark: Non applicabile, prodotto solido
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

2.5 Partition Coefficient

log Pow:
Method:
Year:
Remark: not applicable
Source: SYSTEMS BIO-INDUSTRIES BOULOGNE-BILLANCOURT

log Pow:
Method:
Year:
Remark: Non applicabile
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

2.6.1 Water Solubility

Value: <= 500 g/l at 60 degree C
Qualitative: of high solubility
pH: 0 - 14 at 500 g/l and 60 degree C
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

Remark: Soluble in water at 40°C or above.
Source: SYSTEMS BIO-INDUSTRIES BOULOGNE-BILLANCOURT

2.6.2 Surface Tension

-

2.7 Flash Point

Value:
Type:
Method:
Year:
Remark: Not applicable
Source: SYSTEMS BIO-INDUSTRIES BOULOGNE-BILLANCOURT

Value:
Type:
Method:
Year:
Remark: Non applicabile
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

2.8 Auto Flammability

Value:
Remark: None
Source: SYSTEMS BIO-INDUSTRIES BOULOGNE-BILLANCOURT

Value:
Remark: Nessuna
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

2.9 Flammability

Result: non flammable
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

Result:
Remark: None
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

2.10 Explosive Properties

Result: not explosive
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

Result:
Remark: None
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

2.11 Oxidizing Properties

Result: no oxidizing properties
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

Result:
Remark: None
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

2.12 Additional Remarks

Remark: None
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Remark: Nessuna
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

Remark: Gelatin is a natural occuring polymer.
Polymers should not have been included in EINECS, therefore only chapter 1 of Hedset dossier will be submitted.
Source: Agfa–Gevaert AG Leverkusen

3.1.1 Photodegradation

Type:
Method:
Year: **GLP:**
Test substance:
Remark: Good stabililty
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Type:
Method:
Year: **GLP:**
Test substance:
Remark: Buona stabilità
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

3.1.2 Stability in Water

Type:
Method:
Year: **GLP:**
Test substance:
Remark: Susceptible to microbiological decomposition
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Type:
Method:
Year: **GLP:**
Test substance:
Remark: Decomposizione microbiologica
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

3.1.3 Stability in Soil

Type: **Radiolabel:**
Concentration:
Cation exch. capac.
Microbial biomass:
Method:
Year: **GLP:**
Test substance:
Remark: natural/organical decomposition
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Type: **Radiolabel:**
Concentration:
Cation exch. capac.
Microbial biomass:
Method:
Year: **GLP:**
Test substance:
Remark: Decomposizione microbiologica
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

3.2 Monitoring Data (Environment)

Type of measurement:
Medium:
Remark: not available
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Type of measurement:
Medium:
Remark: Non disponibile¿
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

3.3.1 Transport between Environmental Compartments

Type:
Media:
Method:
Year:
Remark: Non disponibile
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

3.3.2 Distribution

Media:
Method:
Year:
Remark: Non disponibile
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

3.4 Mode of Degradation in Actual Use

Remark: Totalmente naturalmente biodegradabile
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge
Concentration: 10 g/l related to Test substance
Degradation: = 100 % after 24 hour(s)
Result: inherently biodegradable
Method:
Year: **GLP:**
Test substance: as prescribed by 1.1 - 1.4
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

Type:
Inoculum:
Method:
Year: **GLP:**
Test substance:
Remark: Totally naturally biodegradable
Source: SYSTEMS BIO-INDUSTRIES BOULOGNE-BILLANCOURT

3.6 BOD5, COD or BOD5/COD Ratio

B O D 5

Concentration: 10 g/l related to Test substance
BOD5: ca. 1600 mgO2/l

C O D

COD: ca. 200 mg/g substance

R A T I O B O D 5 / C O D

BOD5/COD: ca. 8

Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

3.7 Bioaccumulation

Species:
Exposure period:
Concentration:
BCF:
Elimination:
Method:
Year: **GLP:**
Test substance:
Remark: None
Source: SYSTEMS BIO-INDUSTRIES BOULOGNE-BILLANCOURT

Species:
Exposure period:
Concentration:
BCF:
Elimination:
Method:
Year: **GLP:**
Test substance:
Remark: Nessuno
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

3.8 Additional Remarks

Remark: Gelatin is a natural occuring polymer.
Polymers should not have been included in EINECS, therefore only chapter 1 of Hedset dossier will be submitted.
Source: Agfa–Gevaert AG Leverkusen

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type:
Species:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: Ingredient in fish food. Expected to be non–toxic
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Type:
Species:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D’’Alba (CN)

4.2 Acute Toxicity to Aquatic Invertebrates

Species:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: Being a protein, expected to be non–toxic
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Species:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D’’Alba (CN)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species:
Endpoint:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: Being a protein, expected to be non–toxic
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Species:
Endpoint:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type:
Species:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: Non–toxic since gelatin will support growth of bacteria
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Type:
Species:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

Species:
Endpoint:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species:
Endpoint:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

Type:
Species:
Endpoint:
Exposure period:
Unit:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

4.6.2 Toxicity to Terrestrial Plants

Species:
Endpoint:
Expos. period:
Unit:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

4.6.3 Toxicity to other Non–Mamm. Terrestrial Species

Species:
Endpoint:
Expos. period:
Unit:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

4.7 Biological Effects Monitoring

Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

4.8 Biotransformation and Kinetics

Type:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

4.9 Additional Remarks

Remark: Gelatin is a natural occuring polymer.
Polymers should not have been included in EINECS, therefore only chapter 1 of Hedset dossier will be submitted.
Source: Agfa–Gevaert AG Leverkusen

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type:
Species:
Sex:
Number of Animals:
Vehicle:
Value:
Method:
Year: **GLP:**
Test substance:
Remark: No acute oral toxicity (LD50<5g/kg bodyweight (rat)
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Type:
Species:
Sex:
Number of Animals:
Vehicle:
Value:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D’’Alba (CN)

5.1.2 Acute Inhalation Toxicity

Type:
Species:
Sex:
Number of Animals:
Vehicle:
Exposure time:
Value:
Method:
Year: **GLP:**
Test substance:
Remark: No acute inhalation toxicity expected based on nature of gelatin (protein)
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Type:
Species:
Sex:
Number of Animals:
Vehicle:
Exposure time:
Value:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

5.1.3 Acute Dermal Toxicity

Type:
Species:
Sex:
Number of Animals:
Vehicle:
Value:
Method:
Year: **GLP:**
Test substance:
Remark: No acute dermal toxicity (see 5.2.1)
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Type:
Species:
Sex:
Number of Animals:
Vehicle:
Value:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

5.1.4 Acute Toxicity, other Routes

Type:
Species:
Sex:
Number of Animals:
Vehicle:
Route of admin.:
Value:
Method:
Year: **GLP:**
Test substance:
Remark: No evidence of acute toxicity
Source: SYSTEMS BIO-INDUSTRIES BOULOGNE-BILLANCOURT

Type:
Species:
Sex:
Number of Animals:
Vehicle:
Route of admin.:
Value:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species:
Concentration:

Exposure:
Exposure Time:
Number of Animals:
PDII:
Result:
EC classificat.:
Method:
Year: **GLP:**
Test substance:
Remark: Non-irritating to skin, based on repeat insult patch tests on human volunteers, using à 50% solution of (non-gelling) gelatin. (CRODA data)
Source: SYSTEMS BIO-INDUSTRIES BOULOGNE-BILLANCOURT

Species:
Concentration:

Exposure:
Exposure Time:
Number of Animals:
PDII:
Result:
EC classificat.:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
La gelatina viene manipolata nelle proprie confezioni nel rispetto delle regolamentazioni sulla protezione dei lavoratori, applicabile negli Stati Membri, al fine di prevenire ogni tipo di irritazione
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

5.2.2 Eye Irritation

Species:
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result:
EC classificat.:
Method:
Year: **GLP:**
Test substance:
Remark: Essentially non–irritating (rabbit eye), based on 50% solution of (non–gelling) gelatin. (CRODA data)
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Species:
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result:
EC classificat.:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
La gelatina viene manipolata nelle proprie confezioni nel rispetto delle regolamentazioni sulla protezione dei lavoratori, applicabile negli Stati Membri, al fine di prevenire ogni tipo di irritazione
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

5.3 Sensitization

Type:
Species:
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: **GLP:**
Test substance:
Remark: Non sensitizer, based on repeat insult patch tests on human volunteers, using a 50% solution of (non–gelling) gelatin.
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Type:
Species:
Number of Animals:
Vehicle:
Result:
Classification:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

5.4 Repeated Dose Toxicity

Species: **Sex:**
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method:
Year: **GLP:**
Test substance:
Remark: No hazards expected based on the nature of gelatin and its food use.
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Species: **Sex:**
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

5.5 Genetic Toxicity 'in Vitro'

Type:
System of testing:
Concentration:
Metabolic activation:
Result:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

5.6 Genetic Toxicity 'in Vivo'

Type:
Species: **Sex:**
Strain:
Route of admin.:
Exposure period:
Doses:
Result:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

5.7 Carcinogenicity

Species: **Sex:**
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses:
Result:
Control Group:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

5.8 Toxicity to Reproduction

Type:
Species: **Sex:**
Strain:
Route of admin.:
Exposure Period:
Frequency of treatment:
Duration of test:
Doses:
Control Group:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

5.9 Developmental Toxicity/Teratogenicity

Species: **Sex:**
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Duration of test:
Doses:
Control Group:
Method:
Year: **GLP:**
Test substance:
Remark: Dati non disponibili
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

5.10 Other Relevant Information

Type:
Remark: No hazards expected based on the nature of gelatin and its food use.
Source: SYSTEMS BIO–INDUSTRIES BOULOGNE–BILLANCOURT

Type:
Remark: La gelatina è inscritta come alimento nel Codice Alimentare (FAO/WHO) e approvata dal JECFA (1970)
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)
(7)

Type:
Remark: Gelatin is a natural occuring polymer.
Polymers should not have been included in EINECS, therefore only chapter 1 of Hedset dossier will be submitted.
Source: Agfa–Gevaert AG Leverkusen

5.11 Experience with Human Exposure

Remark: L'esposizione accidentale o professionale alla sostanza in esame non ha prodotto, per quanto è a nostra conoscenza, alcun effetto patologico o epidemiologico sull'uomo
Source: ITALGELATINE S.P.A. Santa Vittoria D''Alba (CN)

(1) data are submitted by others

(2) data wille be submitted by others

(3) HSE, (1995), EH40/95 Occupational Exposure Limits – 1995, HMSO

(4) TRGS 900 von 10/1996 und 905 von 4/1995

(5) data will be submittedby others

(6) data will be submitted by others

(7) Codice Alimentare (FAO/WHO)

7.1 Risk Assessment

-

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization or of the Food and Agriculture Organization of the United Nations.

WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES
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No. 48

EVALUATION OF FOOD ADDITIVES

SPECIFICATIONS FOR THE IDENTITY AND PURITY OF FOOD ADDITIVES AND THEIR TOXICOLOGICAL EVALUATION: SOME EXTRACTION SOLVENTS AND CERTAIN OTHER SUBSTANCES; AND A REVIEW OF THE TECHNOLOGICAL EFFICACY OF SOME ANTIMICROBIAL AGENTS

Fourteenth Report
of the Joint FAO/WHO Expert Committee
on Food Additives

Geneva, 24 June–2 July 1970

Published by
FAO and WHO

WORLD HEALTH ORGANIZATION
GENEVA
1971

Specifications for the substances considered in this report, monographs containing summaries of relevant biological data and toxicological evaluations, and a review of the technological efficacy of some antimicrobial agents will be issued by FAO and WHO in separate publications entitled :

1. *Toxicological evaluation of some extraction solvents and certain other substances*

   FAO Nutrition Meetings Report Series, 1971, No. 48 A

   WHO/Food Add./70.39

2. *Specifications for the identity and purity of some extraction solvents and certain other substances*

   FAO Nutrition Meetings Report Series, 1971, No. 48 B

   WHO/Food Add./70.40

3. *A review of the technological efficacy of some antimicrobial agents*

   FAO Nutrition Meetings Report Series, 1971, No. 48 C

   WHO/Food Add./70.41

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Geneva, 24 June - 2 July 1970

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Wld Hlth Org. techn. Rep. Ser., 1971, No. 462

EVALUATION OF FOOD ADDITIVES

SPECIFICATIONS FOR THE IDENTITY AND PURITY OF FOOD ADDITIVES AND THEIR TOXICOLOGICAL EVALUATION: SOME EXTRACTION SOLVENTS AND CERTAIN OTHER SUBSTANCES; AND A REVIEW OF THE TECHNOLOGICAL EFFICACY OF SOME ANTIMICROBIAL AGENTS

Fourteenth Report of the Joint FAO/WHO Expert Committee on Food Additives

A Joint FAO/WHO Expert Committee on Food Additives met in Geneva from 24 June to 2 July 1970. The meeting was opened by Dr L. Bernard, Assistant Director-General, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and of the World Health Organization.

1. INTRODUCTION

As a result of the recommendations of the Joint FAO/WHO Conference on Food Additives held in September 1955,[1] thirteen Joint FAO/WHO Expert Committees on Food Additives have met (see Annex 1).

The present meeting was convened on the recommendations made in the thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives. Its terms of reference were: (1) to draw up specifications for and to make a toxicological evaluation of certain food additives, and (2) to review the technological efficacy of certain antimicrobial agents (see Annex 2). Most of the substances considered had been suggested by the Codex Committee on Food Additives, to which the Expert Committee acts as an advisory body on questions of toxicity, specifications for identity and purity, and methods of analysis. Some of these substances, notably cyclamates, monosodium glutamate, and mercury, have achieved so much notoriety that the delegates to the Twenty-third World Health Assembly adopted a resolution concerning the health hazards of food additives (see Annex 4).

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1956, No. 107; *FAO Nutrition Meetings Report Series*, 1956, No. 11.

In order to facilitate the discussions, the Committee constituted itself into two groups, one of which gave major attention to toxicological evaluation and the other to chemical specifications and technological efficacy-

2. PRINCIPLES GOVERNING THE ESTABLISHMENT OF SPECIFICATIONS

As stressed in previous reports, the specifications of a food additive play an important role in its toxicological evaluation.

2.1 Scope

As in the past, the specifications have been developed for use by toxicologists and others concerned with the identity and purity of food additives, their purpose being to prescribe an adequate degree of purity that should be met by the substances. The specifications are not necessarily suitable for commercial use, since they may not take into account all the criteria that are of interest to the commercial user. However, references are made in the individual specifications to some of the criteria that may be of interest in commerce. Since the specifications for additives are intended for use at an international level, each specification should be drawn up in such a way as to encompass suitable products of manufacturers all over the world.

As in previous meetings,[1] the Committee agreed that specifications would be developed for those substances that are manufactured commercially and that have been recognized by the Committee as being used in food processing. The specifications for identity and for purity, together with the methods of analysis where applicable, will be set out in a separate publication (see page 2). Where a complete specification cannot be developed owing to lack of information, a tentative specification will be given, indicating the additional information required.

2.2 Microbiological requirements

Attention is drawn to the recommendation made in the thirteenth report [2] regarding microbiological contamination of food additives produced from natural sources. The Committee agreed to include some criteria for microbiological quality when considering proteinaceous substances, such as gelatin and sodium caseinate.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1964, No. 281 (*FAO Nutrition Meetings Report Series*, 1964, No. 35); *Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 445 (*FAO Nutrition Meetings Report Series, 1970* No. 46).

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 445 (*FAO Nutrition Meetings Report Series*, 1970, No. 46).

2.3 Analytical methods

Although newer analytical methods are being continually developed and the sensitivity and reliability of existing methods are being improved, the methods cited, or others based on similar principles, should be considered adequate at present for international purposes.

3. PRINCIPLES GOVERNING TOXICOLOGICAL EVALUATIONS

3.1 General guidelines and acceptable daily intakes

The Committee again adopted the same general principles for the establishment of acceptable daily intakes (ADIs) set out in previous relevant reports of the Joint FAO/WHO Expert Committee on Food Additives. The definitions of ADIs have already been stated in the thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives.[1] Emphasis was placed on the more recent advances in toxicological and biochemical methodology and interpretation set forth in the report of the WHO Scientific Group on Procedures for Investigating Intentional and Unintentional Food Additives.[2]

3.2 Special considerations

A number of items on the agenda gave rise to consideration of matters of principle.

3.2.1 *Evaluation of a toxic metabolite formed by intestinal microflora*

The formation of cyclohexylamine from cyclamate again exemplifies the possibility of the generation of toxic metabolites from food additives. In this instance, the microflora of the alimentary tracts of man and of the animal species used in toxicological testing behave similarly. In other instances, however, it is possible that the different micro-organisms colonizing human and animal intestinal tracts could generate different toxic metabolites from the same food additive.

3.2.2 *Food additives that are also natural constituents of the diet*

Since no general principles could be formulated in connexion with the evaluation of glutamates, phosphates and copper, each substance had to be evaluated on an individual basis.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 445, p. 8 (*FAO Nutrition Meetings Report Series*, 1970, No. 46, p. 8).

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 348.

3.2.3 *Extrapolation to man of animal data indicating a "vulnerable age" of toxicological reactivity*

The possibility exists that high doses of glutamate produce brain lesions in newborn animals. Any attempt to interpret these data in terms of human neonates and infants involves the problem of how far developmental stages in animal species and in man can be considered equivalent in relation to vulnerability to possible effects of food additives. Relevant information would be of considerable value.

3.2.4 *Relationship between chemical and biological reactivity of food additives*

The suggestion has sometimes been made that because a food additive was chemically inert it would be without long-term toxic effects. There is, however, sufficient experimental evidence to demonstrate that such assumptions are not always valid and therefore adequate toxicological studies are always indispensable.

3.2.5 *Formation of toxic products by interaction between an additive and a food constituent*

If a toxic reaction product arising from interaction between an additive and a food constituent can be identified, then it can be subjected to appropriate toxicological investigation and the results can be evaluated. Furthermore, it is possible that unidentified toxic products may be formed. In this case, foodstuffs treated with the additive should be used in experiments. A number of such experiments with foodstuffs extracted with solvents are described in the monographs.

3.2.6 *The significance of results of mutagenicity studies on food additives*

This question was considered in 1966 by a WHO Scientific Group on Procedures for Investigating Intentional and Unintentional Food Additives,[1] which pointed out the difficulties in extrapolating such experimental results to man, and mentioned also the mutagenic effects of some foods. During the evaluation of cyclamates, the Committee drew attention to the possibility that chromosomal breaks might be produced by physical changes as well as by chemical agents. These observations cannot yet be interpreted in terms of human health hazards.

3.2.7 *Data derived from unconventional studies*

Some of the toxicological data in the monographs considered by the present Committee were obtained from experiments in which non-oral

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 348.

routes of administration, unusual species, or unconventional procedures were used. Although these data were not of direct value in making toxicological assessments, they provided useful background information and, while of little prospective use, may contribute in retrospect to the understanding of toxic effects.

3.2.8 *The need for priorities in the testing of food additives*

The Committee had before it a plethora of experimental evidence relating to some food additives, but there was a paucity of data on which to evaluate solvents used in food processing. It would therefore be desirable to apply a more balanced judgement when commissioning toxicological studies, having regard to the expense of toxicological testing, the shortage of suitable facilities and competent personnel, and the need for protection of the health of the public. Priority should be given to investigations on solvents and other substances which are valuable in food technology and beneficial to the public.

4. SPECIAL CONSIDERATIONS RELATING TO SOLVENTS

The solvents used in the food industry can be divided into two broad groups according to their function: (*a*) Carrier solvents, used to aid the dispersion of colours, flavours, emulsifying agents and other intentional food additives. Such solvents were not on the agenda, but it was noted that some of them are also used as extraction solvents. (*b*) Extraction solvents, used principally for extracting oils and fats from unprocessed and semi-processed produce, for defatting of fish and other meals in the preparation of protein concentrates, and for the decaffeination of coffee.

4.1 Chemical aspects

For a number of solvents, the Committee was able to draw on information provided in an interim report prepared by the Food Additives and Contaminants Commission of the Food Section of the International Union of Pure and Applied Chemistry.

Specifications were drawn up covering identity and freedom from impurities, but they do not necessarily call for chemically pure compounds since some of the solvents are mixtures of homologous compounds.

Stabilizers may be added to some solvents (see page 22), but there were insufficient data to permit their inclusion in the specifications.

4.2 Toxicological aspects

The use of solvents in food technology raises 4 toxicological issues:

(*a*) Treatment with solvents may affect the nutritive value of foodstuffs.

(*b*) Residues of solvents may have toxic effects.

(*c*) Impurities in solvents and additives to solvents may remain in the extracted food and have toxic effects.

(*d*) A solvent may react with the constituents of a foodstuff to form toxic products.

The present usage of certain solvents could be accepted only tentatively in the light of what is known about their toxicity, even bearing in mind their limited use. Further information on the uses of solvents, the levels of residues, and the nature and the levels of stabilizers is required. The Committee considered that as more toxicological data became available the solvents should be re-evaluated and, as far as possible, acceptable daily intakes should be assigned.

In considering the safety of extraction solvents, some recognition was given to the Threshold Limit Values (TLV) for the vapour phase set out by the American Conference of Governmental and Industrial Hygienists, although the Committee was aware of the limitations inherent in the application of data on inhalation to any assessment of safety of residues in foods. In addition to the obvious differences arising because inhaled substances do not pass into the portal vein and immediately through the liver, there is a paucity of data about rates of absorption, preferential storage sites and the like. It was also recognized that TLVs were industrial standards designed to apply to healthy adults subject to exposure during working hours. For this reason, TLVs could be regarded as providing only a rough guide to potential oral toxicity.

4.3 Solvent residues

With good manufacturing practice, extraction solvents would, in most cases, be largely recovered after use, and residues in foodstuffs would be expected to be reduced to a minimum, but there are a few exceptions, such as ethanol. Minimization of residues of solvents to toxicologically negligible amounts was considered by the Committee to be of particular importance with halogenated hydrocarbons and methanol.

The term "good manufacturing practice" allows considerable latitude in interpretation. The Committee believed that the level of solvent residues should be reduced below that required by purely economic considerations.

A number of methods have been published for the detection and measurement of residues of the various solvents under consideration. Not all of them, however, are suitable for the detection and measurement of residues in food.

It is necessary to consider these methods in relation to the technological use of the solvent; separate methods for residue analysis or separate modifications of a common basic method may be necessary for the same

solvent where it has two or more distinct extraction uses. For the chlorinated solvents and carbon disulfide the problem of solvent residue estimation is virtually identical to that of fumigant residue estimation. For these, general gas chromatographic "multidetection" systems of residue analysis are being developed. A multidetection system developed specifically for solvent residues has been used for hexane and chlorinated hydrocarbons.[1]

4.4 Impurities in solvents

The main problem centres on petroleum hydrocarbon fractions that may contain aromatics and polynuclear hydrocarbons. These impurities raise a toxicological problem, particularly as some polynuclear aromatic hydrocarbons are carcinogenic. They may become concentrated in the extracted oil or may be transferred to the residual material when the solvent is removed from the processed food.

Little information is available at present about the carcinogenic constituents of polynuclear aromatic hydrocarbons. Some work is now in progress and more precise information may become available in the near future. In the meantime, it was decided to develop only tentative specifications for hexane and heptane.

Some additional information on this important problem is given in Annex 3.

4.5 Interaction with food

Although solvents do not normally react with food, there are particular circumstances in which an interaction is possible between chlorinated solvents (e.g., dichloroethane, trichloroethylene, dichloromethane, trichloroethane) and the protein or other food constituents. For example, cysteine can react with trichloroethylene to form dichlorovinylcysteine,[2] and dichloroethane can react with trimethylamine to form choline chloride (a reaction that has been observed in the extraction of fish-meal).[3] The alcohols and acetone may denature protein. No interaction with food has been detected when petroleum hydrocarbon fractions are used. The products formed by the interaction of certain halogenated hydrocarbons with certain food constituents are toxic. The principles of toxicological assessment are discussed elsewhere (see section 3.2.4).

---

[1] Dean, A. C., Bradford, E., Hubbard, A. W., Pocklington, W. D. & Thomson, J. (1969) *J. Chromatog.*, **44**, 465–471.

[2] McKinney, L. L., Elridge, A. C. & Cowan, Y. C. (1959) *J. Amer. chem. Soc.*, **81**, 1423.

[3] Munro, L. C. & Morrison, A. B. (1967) *Canad. J. Biochem.*, **45**, 1049.

5. COMMENTS ON SUBSTANCES ON THE AGENDA

5.1 Items not considered further

A number of items were briefly considered but were not evaluated for the reasons given below, and no monographs were prepared for them.

(*a*) *Carbon disulfide and 1,1,1-trichloroethane* (methylchloroform)

These two substances were on the agenda but there was no evidence that they were used for food extraction; therefore the toxicological data available were not considered and specifications were not prepared.

(*b*) *Ethyl acetate*

Although this is a solvent, there was no evidence of its use as such in food processing. The specification for and the toxicological evaluation of ethyl acetate as a flavouring agent remained unchanged.[1]

(*c*) *2-Methylpropan-1-ol (isobutyl alcohol)*

Although a request was received to consider this substance as a potential extraction solvent for fish protein concentrates, the data submitted were inadequate for preparation of a specification and for toxicological evaluation.

5.2 Items briefly considered

(*a*) *Caseinates*

Casein is recognized as the major nutritional component in milk. When isolated according to good manufacturing practice employing food-grade reagents, it is essentially unchanged and may be regarded as a food. Therefore a toxicological evaluation was deemed unnecessary. A specification for sodium caseinate was prepared.

(*b*) *Edible gelatin*

It was recognized by the Committee that edible gelatin is a protein of low nutritive value. As such, its use should be limited to foodstuffs constituting a trivial portion of the diet unless supplemented to achieve nutritional adequacy. In view of these facts the Committee recognized that edible gelatin is a food and could be used in accordance with good manufacturing practice. A specification was prepared.

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[1] Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Flavouring Substances and Non-Nutritive Sweetening Agents: Eleventh Report, *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 383 (*FAO Nutrition Meetings Report Series*, 1968, No. 44).

(*c*) *Curcumin*

No significant information was available on which to base an evaluation. Revised specifications were prepared for turmeric and curcumin.

(*d*) *Carrageenan and furcellaran*

New evidence suggests a need to re-evaluate carrageenan; however, since a number of studies are known to be in progress, the Committee decided to postpone consideration until their completion.

5.3 Evaluation

5.3.1 *Miscellaneous food additives*

A number of substances were assessed in the light of toxicological data. The results of the evaluation are summarized in Annex 5.

(*a*) *Brominated vegetable oils*

Although these substances have been used for some years in soft drinks and fruit juices, no evaluation was made at the ninth committee meeting in 1966 because of lack of suitable data. Since then short-term studies in animals have demonstrated that high doses cause degenerative cardiac lesions. Furthermore, accumulation of lipid and lipid-bound bromine has been demonstrated in adipose tissue and in intracellular fat of various other tissues, both in man and in experimental animals. This evidence suggests that a human epidemiological problem could arise from the use of brominated vegetable oils. It was concluded that they should not be used as food additives in the absence of evidence indicating their safety; therefore, specifications are limited to an identification test.

(*b*) *Cyclamates*

The evaluation of cyclamates by the eleventh committee led to the assignment of only a temporary ADI because of reservations concerning their safety, and further work was requested by 1970. The publicity given to results indicating a possible toxicological hazard led to much anxiety amongst the public.

Consideration of cyclamates encompassed cyclohexylamine and mixtures of cyclamates and saccharin, since some relevant toxicological studies have been carried out with these substances. The data are included in the body of the monograph on cyclamates.

Although there has been a considerable amount of further work, some questions have still not been fully answered.

(i) The laxative action of high doses of cyclamates has been attributed to an osmotic effect, but other mechanisms have not been excluded: in

the absence of a definitive study the question of its long-term toxic effect on the intestinal tract remains open.

(ii) Because cyclamates are converted to cyclohexylamine by intestinal flora, this metabolite has been extensively studied. This phenomenon also poses a fundamental problem which is considered elsewhere (see section 3.2.1).

(iii) The mutagenic effects of cyclamates and cyclohexylamine were regarded as indications for further experiments, but their immediate relevance in toxicological evaluation is not clear. The question is discussed elsewhere (see section 3.2.6).

(iv) The Committee discussed the production of tumours of the urinary bladder following implantation of cholesterol pellets containing cyclamate. It held that these results were not in themselves definitive for the assessment of a toxic effect, since ingestion of the substance is the only route of administration of food additives : they may, however, provide a warning sign justifying further studies. A similar approach had been employed in assessing the significance of findings of tumours in studies carried out with subcutaneous injections of certain food colours.[1]

Since tumours were also found in experiments in which cyclamate alone or in combination with saccharin was given orally, no ADI was assigned to cyclamates. Attention is drawn to numerous studies, in progress or planned, on cyclamates and cyclohexylamine. These studies will be evaluated as they become available. The Committee's evaluation recognizes that there are benefits in the use of cyclamates in the management of diabetics and the grossly obese, against which possible risks have to be balanced.

The Committee also reviewed the specifications for calcium cyclamate and sodium cyclamate prepared at the eleventh meeting.[2] After taking into consideration the quality of the products commercially available it was agreed that the limit of cyclohexylamine should be reduced from 100 mg/kg to 25 mg/kg. The Committee further felt that the same limit of cyclohexylamine should be prescribed for cyclohexylsulfamic acid, which was also considered at the eleventh meeting. A method for the estimation of cyclohexylamine in cyclamates and in cyclohexylsulfamic acid was developed. Analytical methods now available for the detection and estimation of dicyclohexylamine are sensitive to 1–2 mg/kg.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 445.

[2] Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Flavouring Substances and Non-Nutritive Sweetening Agents: Eleventh Report, *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 383 (FAO *Nutrition Meetings Report Series*, 1967, No. 44).

(*c*) *Monosodium glutamate*

At the thirteenth meeting of the Committee consideration of monosodium glutamate was deferred, and it was noted that its use as a condiment raised a problem for its assessment as a food additive. As with cyclamate, considerable publicity has been given to laboratory data purporting to have toxicological significance for the addition of glutamate to food, generally without a balanced consideration of all the available evidence. The papers referred to in the monograph on glutamate were selected from the extensive literature on this compound because of their relevance to its evaluation as an additive. As a result of the publicity given to glutamate, a considerable amount of work is now in progress.

Much of the recent work on the effects of glutamate is concerned with high doses which produce acute pharmacological reactions, but there is little in these data to suggest a long-term toxic hazard. A more serious potential toxic hazard of glutamate is the finding that it produced brain damage in new-born animals.[1] Although the weight of the evidence does not support the contention that glutamate as an additive in the diet can lead to brain damage, caution is desirable in the use of glutamate as an additive in infant diets.

It has been suggested that the main reason for the addition of monosodium glutamate to baby food is to improve the taste from the mother's point of view; there is, however, no evidence that infants like the taste —which would provide a more cogent reason for its use. The sixth report suggested that foods prepared specifically for infant diets require special consideration, urging that food additives should be avoided where possible and that great care should be exercised both in the choice of additive and the level of use.

The Committee concluded that monosodium glutamate could be given an unconditional ADI of 0–120 mg/kg applicable to the general population except infants under one year of age.

A tentative specification had been prepared for monosodium glutamate at the thirteenth meeting. This was reviewed and a final specification was developed. The Committee felt that similar specifications should also be prepared for other glutamates, such as potassium or calcium glutamate. This could not be done, however, as they were not on the agenda and no information on these substances was available.

(*d*) *Phosphoric acid and phosphates*

The Committee is aware of the need to revise the specifications for phosphoric acid and a number of phosphates that had been developed in earlier meetings.

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[1] Olney, J. W. (1969) *Science*, **164**, 719.

A new monograph on biological data was prepared to replace the previous individual monographs on phosphoric acid and the various phosphates of sodium, potassium, calcium, and magnesium. There are no toxicological grounds for treating these substances separately. Phosphoric acid itself carries no special hazards that distinguish it from other acids used in foods.

5.3.2 *Filtration aids and clarifying agents*

Consideration was given to a filtration medium (asbestos), a filtration aid and decolourizer (activated vegetable carbon), and a flocculant (tannin). The results of the evaluations are summarized in Annex 6.

(*a*) *Asbestos*

It was not possible to prepare a specification since information concerning the physical forms of asbestos used in the food industry was lacking; there is a need for methods to distinguish between these forms. Information is also required concerning any significant changes in the chemical or physical properties of asbestos that may occur if it is exposed to acidic or alkaline media in food processing.

It was noted that asbestos is a contaminant in some talcs. It was recommended that new methods of detection should be used to eliminate contamination of food by asbestos from this source.

The many industrial uses of asbestos have made it a ubiquitous environmental contaminant. There was some discussion on the possibility that the ingestion of foods contaminated with asbestos fibres might add to the risk of carcinogenicity.

Evidence was considered on persorption and lymphatic transport of asbestos fibres injected subcutaneously. It was felt that there was a real possibility of persorption of asbestos fibres ingested with foods that had been exposed to asbestos-containing filter pads during their processing. It was decided that no definitive evaluation could be made until relevant experimental data concerning this possibility had been submitted.

In spite of these reservations, it was concluded that the use of asbestos in processes in which it is essential would cause no hazards to the consumer, provided that crocidolite is not used and that residues in food are kept at negligible levels in accordance with good manufacturing practice. Nevertheless, it was felt that suitable alternative filtration media ought to be developed.

(*b*) *Activated vegetable carbon* (food grade)

Activated vegetable carbon differs from carbon blacks in that the latter are derived from the incomplete combustion of hydrocarbons from fossil fuels, whereas the former is derived from vegetable matter or lignites.

Certain carbon blacks are used as food colours,[1] and activated vegetable carbons are largely used as clarifying agents or decolourizers. Virtually no activated vegetable carbon is present in the finished food product, and the data on this type of carbon did not demonstrate toxicity. A specification for activated vegetable carbon was prepared.

(*c*) *Tannins* (food grade)

A tentative specification for tannins prepared by the Committee relates only to tannins that yield gallotannins (derivatives of gallic acid) on hydrolysis. Many other kinds of tannin also occur in nature, including those that on hydrolysis yield ellagitannins (derivatives of hexahydroxy *o*-dibenzoic acid) and condensed (non-hydrolysable) tannins (e.g., in the Douglas fir). The hydrolysable tannins can also be distinguished from the condensed tannins: on dry distillation the former yield pyrogallol (1,2,3-trihydroxybenzene) whereas the latter yield catechol (1,2-dihydroxybenzene). Hydrolysable gallotannins may be obtained from nutgalls, the excrescences that form on young twigs of various *Quercus* spp. (e.g., *Q. infectoria*, *Q. linnaei*); these include Chinese and aleppo tannins. They may also be obtained from the leaves of various sumac species (e.g., *Rhus coriaria*, *R. glabra*, and *R. typhina*; these include Sicilian and American sumac). All consist of the polydigalloyl esters of glucose. A further source of hydrolysable gallotannins is the seed pods of tara (*Caesalpinia spinosa*); these consist essentially of the polydigalloyl esters of quinic acid. Recognizing that the Committee was recommending two types of ADI, one for tannins derived from Peruvian tara and the other from Turkish aleppo, Chinese tara, and Sicilian sumac, it was considered necessary to collect more information to distinguish between tannins derived from these various sources.

Evaluation of the toxicity of tannins was complicated by the fact that they are naturally occurring constituents of many foods, such as wine and brewed tea. It was also noted that the addition of tannins as flocculants in food processing should result in virtually no residues in finished foods, providing good manufacturing practices are followed.

5.3.3 *Heavy metal contaminants and related additives*

Three heavy metals were considered, namely copper, mercury, and tin. Mercury is always a contaminant, never an additive. However, copper and tin may be both additives and contaminants. The Committee considered that the intentional addition of small amounts of tin and copper for technological purposes would not significantly increase the total intakes of

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[1] Specifications for Identity and Purity of Food Additives and their Toxicological Evaluation: Food Colours and Some Antimicrobials and Antioxidants: Eighth Report, *Wld Hlth Org. techn. Rep. Ser.*, 1965, No. 309 (*FAO Nutrition Meetings Report Series*, 1965, No. 38).

these metals and would therefore not be objectionable from a toxicological point of view (see also Annex 5).

(*a*) *Copper and cupric sulfate*

Copper is recognized as an essential trace element and a required dietary constituent. In the past a major source of dietary copper was from utensils used in food processing. When stainless steel vessels are used less copper appears in the food, but the presence of copper is sometimes necessary, as for example in controlling fermentation in the manufacture of Emmentaler cheese. The addition of copper has also been proposed for colour fixation in some processed vegetables. Another source of dietary copper arises from its use as a pesticide.

Toxicological data before the Committee were not amenable to the assignment of an ADI, since a no-effect level could not be determined. Data on the use of copper as a food supplement for growth promotion in pigs were considered of little relevance in assessing toxicity, and therefore were not included in the monograph. It was noted that toxicity at 30 mg/kg body weight in rats was minimal. The Committee concluded that reliance could be placed on the long human exposure to background levels of copper and, provided that intake does not exceed 0.5 mg/kg/day (as indicated in the tenth report), no deleterious effects would be expected. In this connexion, the Committee was presented with recent data indicating that certain foodstuffs naturally have a high content of copper which has not apparently produced any toxic effects.

A specification for cupric sulfate has been prepared.

(*b*) *Mercury*

Analyses in certain countries have shown that some foods are contaminated with mercury to an extent liable to cause human intoxication. The source of the mercury is environmental pollution, the major sources being chloralkali industries using mercury cells, paper pulp factories using phenylmercury salts as slimicides, wood pulp factories using phenylmercury salts as fungicides, the industrial use of inorganic mercurials, and the agricultural use of various organomercurial fungicides. Whatever the source of pollution, most mercury compounds can be converted in nature to methylmercury, traces of which may then occur in fish and other foods of animal origin.

The methylmercury radical is very stable. It is only slowly excreted after ingestion by man and its elimination by fish is even slower, the half-life exceeding one year.

Surveys of mercury levels in foods have been carried out in only a few countries.[1] There is urgent need for such surveys, especially in regard to

[1] Abbott, D. C. & Tatton, J. O'G. (1970) *Pesticide Sci.*, **1**, 99–100.

fish and shellfish. It would also be desirable to have analytical data on the level of total mercury and, where possible, the forms in which it occurs. As a guide to the choice of analytical methods, it should be noted that fish from uncontaminated areas usually contain less than 0.1 ppm and in other foods there is less than 0.05 ppm ; [1] in contrast, fish and shellfish from areas contaminated by mercury may contain more than 1 ppm.

Methylmercury compounds produce serious and sometimes fatal neurotoxicity and embryopathy. Over 200 cases of methylmercury intoxication have been reported in the literature. Of these more than 100 occurred in Minamata and Niigata in Japan, during the last two decades, due to ingestion of contaminated fish and shellfish : the syndrome of intoxication has been termed Minamata disease.[2] Approximately 20 cases of " congenital " Minamata disease were due to consumption of contaminated fish and shellfish by pregnant women, who themselves were usually asymptomatic.[3]

The biological data available to the Committee do not allow an ADI for methylmercury to be established. The Committee has, however, taken notice of a number of alarming points : (1) the epidemics of poisoning, (2) the high sensitivity of the fetus, (3) the occurrence, among fish-eaters in non-epidemic areas, of mercury levels in blood and hair approaching those associated with symptoms of poisoning,[4] and (4) a correlation in man between exposure to mercury as a contaminant of fish and the incidence of chromosome breaks in circulating lymphocytes.[5]

Although a considerable amount of data were available, the Committee thought it would be premature to prepare a monograph, especially as publication of a detailed review of the risks associated with mercury in fish was expected within a few months. This review should prove of value in putting the problem in perspective and may stimulate further work.

There are no data on which to assign an ADI to mercury : this is urgently required as a guide to the levels of contamination above which food should be discarded. It is strongly urged that environmental pollution by mercury should be reduced to the minimum possible. Such measures have already been put into effect in some countries.

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[1] Westoö, G. (1969) In : Mille, M. W. & Berg, G. G., eds., *Chemical fallout*, Springfield, Thomas, pp. 75–90.

[2] Tokuomi, H., Okajima, T., Kanai, J., Tsunoda, M., Ichiyasu, Y., Misumi, H., Shimomura, K. & Takaba, M. (1961) *Wld Neurol.*, **2**, 536–545, & *Kumamoto med. J.*, **14**, 47–61.

[3] Matsumoto, H., Koya, G. & Takeuchi, T. (1965) *J. Neuropath. exp. Neurol.*, **24**, 563–574.

[4] Birke, G., Johnels, A. G., Plantin, L. O., Sjöstrand & Westermark, T. (1967) *Laek.-Tidn.*, **64**, 3628–3637.

[5] Skerfving, S., Hansson, K. & Lindsten, J. (1970) *Arch. environm. Hlth*, **21**, 133–139.

(*c*) *Tin and stannous chloride*

A specification on stannous chloride was prepared. The Committee noted that the substance had only a limited application as a food additive : it is used as an additive when asparagus and peas are packed in glass containers or in lacquered cans, and it is also used in soda waters.

The presence of tin in foods may be due either to contamination or its use as an additive. Stannous ions prevent the migration of other heavy metals into canned food, inhibit the oxidation of ascorbic acid, and may impart a characteristic flavour.

In solid foods, tin is mostly protein bound and in the quantities likely to occur it has no apparent toxic effect. However, in acidic liquid food and beverages, high levels may occur which have occasionally produced acute toxic effects.

The absence of chronic or severe toxic effects due to tin in man, despite a long history of consumption of canned foods, indicates that there is no need to depart from the limits set by good manufacturing practice as recommended in the tenth report. Work at present in progress could lead to evaluation and the assignment of an ADI in future.

5.3.4 *Extraction solvents*

The results of evaluations are summarized in Annex 7.

(*a*) *Alcohols and acetone*

The solvents in this group are hydrophilic and are used in the extraction of oils and fats from wet materials. Specifications for them have been prepared for publication.

Ethanol. This substance, being the principal non-aqueous constituent of alcoholic beverages and a common constituent of the diet, is subject to different considerations from most of the other solvents. In certain countries, the economic penalties, in the form of excise duty, attached to the use of ethanol have led to the use of alternative solvents whose residues pose greater problems than those of ethanol. The relaxation of restraints on the use of ethanol in food technology therefore seems desirable.

Methanol. This substance is present in certain spirits and liqueurs in concentrations of up to 200 ppm. The intake of high doses of methanol is known to produce severe ocular damage in susceptible individuals.

When used as an extraction solvent in food technology the residues of methanol should be reduced to a minimum by observing good manufacturing practice. The Committee was informed that methanol was used only for extracting spice oils and hop oils, and that residues from these sources are insignificant in the diet.

Propan-2-ol (isopropyl alcohol). This is a minor constituent of some alcoholic beverages. It is used in certain countries as a substitute for ethanol as an extraction and carrier solvent, and as a substitute for halogenated hydrocarbon solvents in the processing of fish-protein concentrate. There have been a number of short-term human studies with propan-2-ol, and long-term animal studies are in progress. The results of these studies may lead to the assignment of an ADI when they become available. Meanwhile, with good manufacturing practice, the residues in foodstuffs should be negligible.

Acetone. This substance is formed in human intermediary metabolism and small amounts are readily metabolized. As a solvent it apparently has only minor uses in food technology. Since it has a high vapour pressure it was considered that, with good manufacturing practice, residues should be toxicologically negligible. Traces of acetone have been detected in ground pepper.

(*b*) *Petroleum hydrocarbon fractions (hexane and heptane)*

Aliphatic hydrocarbon extraction solvents, which are lipophilic, have been widely used in the food industry. While the Committee recognized that the main hydrocarbon solvent used is the hexane fraction of petroleum ether, it was felt that the specifications for commercial hexane should not exclude the use of pure *n*-hexane or commercial hexane with higher proportions of *n*-hexane than specified. The Committee did not have precise information regarding other petroleum hydrocarbon fractions used as extraction solvents, although it was reported that heptane, and certain cuts with a boiling range below that of the hexane fraction, were in use for some specific food extraction requirements. Since the Committee was not aware of any food extraction use for fractions higher than heptane, it was decided to develop a specification for heptane instead of for a general range of petroleum hydrocarbon fractions. At the same time it was understood that any lower boiling fractions used should conform to the general purity criteria prescribed for hexane and heptane.

The amounts of solvent residues in the food depend upon the desolventizing conditions and the subsequent treatment. Pritchard et al.[1] found total hexane residues of 0.01–0.15% in extracted freshly produced palm-kernel, soya-bean, and groundnut meals. No residues were found by Watts & Holswade,[2] using a method of detection sensitive to 10 mg/kg, in samples of cottonseed, corn, groundnut, soya-bean, and safflower oil.

There is a scarcity of information on which to base an assessment of the toxicological significance of the residues of these solvents; this is

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[1] Pritchard, J. L. R., Ferner, S. N. & Wong, D. R. (1964) *Chem. & Ind.*, 2062–2065.

[2] Watts, J. O. & Holswade, W. (1967) *J. Ass. off. agric. Chem.*, **50**, 717.

unfortunate as they are amongst the most widely used in food technology. The argument that their chemical inertness implies biological inertness was considered to be suspect and no substitute for direct experimentation. The Committee held that data relating to oral administration of these substances were required in order to arrive at an ADI. With good manufacturing practice, the residues remaining in foods are less than 1 ppm.

(*c*) *Halogenated hydrocarbons*

These solvents are often used as alternatives to petroleum hydrocarbon fractions to avoid high fire risks, but they have the technological disadvantages that they present greater occupational health hazards and may cause more corrosion of equipment. They also have specific uses in the extraction of hops, paprika, and coffee.

A stabilizer is often added to halogenated hydrocarbons in a concentration of up to 100 mg/kg in order to prevent decomposition, particularly if they should be exposed to strong sunlight or other sources of ultraviolet radiation. Although it was not clear which stabilizers are present in the solvents used for food extraction, the following are generally added to this group of solvents: thymol and cresol, branched aliphatic hydrocarbons, triethylamine, di-isopropylamine and other amines, stearates, and ammonium carbamate. More information is needed about the nature and amount of stabilizers added to halogenated hydrocarbons used in processing foods. There are toxicological objections to the phenolic stabilizers and they should not be used: there is, however, little or no evidence on the toxicological significance of the others.

Dichloromethane (methylene chloride). This solvent can react with thiol groups of sulfur-containing amino acids, but only under extreme conditions that do not obtain in food processing. Long-term feeding studies have been carried out with hops and coffee that have been treated with the solvent, but as some of the reports of this work were available to the Committee only in an abridged form, there was insufficient information for establishing an ADI. The Committee considered that residues could be kept to toxicologically negligible levels by good manufacturing practice.

1,2-Dichloroethane (ethylene dichloride). This substance is used as a fumigant of foodstuffs and as a solvent in food processing: identical considerations apply to residues in foods arising from both uses. A toxicological evaluation of the fumigant residues is given elsewhere.[1] New data before the Committee led to the drafting of another monograph but did not change the previous evaluation, that residues should be restricted to a minimum by observing good manufacturing practice.

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[1] Evaluation of the Hazards to Consumers Resulting from the Use of Fumigants in the Protection of Food. WHO/Food Add./28.65 (*FAO Meeting Report* No. PL/1965/10/2).

1,1,2-Trichloroethylene. The Committee noted that trichloroethylene reacts with cysteine in proteins to form a toxic product. A feeding trial with coffee extracted by the solvent has been carried out, and although no adverse effects were noted, the data were not considered adequate for establishing a formal ADI. However, it was considered that with good manufacturing practice the residues in extracted coffee are toxicologically negligible.

Specifications for the above three halogenated hydrocarbons were prepared for publication.

5.3.5 *Miscellaneous items*

The results of evaluations are also summarized in Annex 5.

(*a*) *Ethyl maltol.* It was noted that ethyl maltol is intended as an alternative to its homologue, maltol, which was evaluated at the eleventh meeting and for which a monograph is already available.[1] Data on ethyl maltol were sufficient to assign an ADI and a specification has been prepared for publication.

(*b*) *Food grade mineral oil.* Since polycyclic aromatic hydrocarbons occur in mineral oils, it is important that limits be set for them in the specifications. The Committee noted that stabilizers are sometimes added to food grade mineral oil. Addition of stabilizers was likely to interfere with the test for the determination of polynuclear aromatic hydrocarbons. As the type and quantity of the stabilizers used in the product were not known, it was decided to prepare for publication a specification for " food grade mineral oil ". This specification does not include other types of mineral oil used in the food industry to which stabilizers are added for certain technological purposes. It may be possible to develop a separate specification for white mineral oil containing stabilizers as more information becomes available.

The Committee noted that ingested mineral oil is absorbed and stored in tissues. However, there was no evidence that this had deleterious consequences. It was recognized that mineral oil could interfere with the absorption of fat-soluble vitamins, but with modest levels of intake the consequences are not significant. It was also noted that the use of mineral oil in food was self-limiting in that excessive amounts are laxative.

(*c*) *Oleoresins of paprika.* These are derived from a widely consumed natural foodstuff, and there were no data indicative of a toxic hazard. The use of the oleoresins as a spice was self-limiting and obviates the need for an ADI. A specification has been prepared for publication.

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[1] Toxicological Evaluation of Some Flavouring Substances and Non-Nutritive Sweetening Agents, WHO/Food Add./68.33 (*FAO Nutrition Meetings Report Series*, 1968, No. 44A).

6. REVIEW OF TECHNOLOGICAL EFFICACY OF SOME ANTIMICROBIAL AGENTS

At previous meetings the Committee had prepared specifications for, and made toxicological evaluations of, some chemical preservatives. The Committee has now prepared monographs on the technological efficacy of the following antimicrobial agents : benzoic acid and benzoates, nitrates and nitrites, esters of *p*-hydroxybenzoic acid, propionic acid and propionates, sodium diacetate, sorbic acid and sorbates, sulfur dioxide and related substances, and diethylpyrocarbonate.[1] The monographs do not contain recommendations for use, " tolerances ", legal restrictions, or clearances, but constitute a review of data available in the literature. The use levels given in the monographs do not necessarily correspond to those specified by legislation or to the optimum concentrations for technological purposes.

6.1 Methods of analysis in food

Methods for assaying the pure substance are given in the monographs on specifications. Although the Committee felt that the monographs on technological efficacy should also contain methods of analysis for determining the additives in foods, it was advised that the elaboration of such methods was already being undertaken by the Joint FAO/WHO Codex Alimentarius Commission.

6.2 Review of efficacy

Only a limited number of antimicrobial agents are acceptable from a toxicological point of view. Because the antimicrobial activity of these agents is dependent on pH, their use is limited to acidic foods such as fruits, fruit juices, and jams, as well as salads and certain other foods. Even then their use may not be fully effective, as antibacterial agents used singly have a limited spectrum of antibacterial activity and may induce resistance in the flora exposed to them. Adequate chemical preservation of foods with nearly neutral pH values therefore presents difficulties pending the development of a wide-spectrum, non-pH-dependent, and organoleptically acceptable preservative of low mammalian toxicity. Meanwhile for the preservation of foods of medium and high pH one has to rely on physical methods such as drying, freezing, refrigeration, or heating. In view of the above considerations the efficacy of antibiotics such as nisin, pimaricin, and tylosin deserves full attention.

[1] The specifications for diethylpyrocarbonate and certain other antimicrobial agents that were elaborated at the ninth meeting of the Committee are attached to the monograph on technological efficacy of these compounds because they have not been previously published.

7. ESTIMATION OF FOOD ADDITIVE INTAKE

At the request of the sixth session of the Codex Committee on Food Additives, the Committee considered a study by WHO entitled " Estimation of Food Additive Intake, 1969/70 Computerized Calculation of Potential Food Additive Intake ". The method employed in this study relied mainly on average food consumption data already available except in the case of beverages and confectionery products, for which high-consumption parameters were used.

The Committee considered that, with the material available, this method for assessing the potential intake of food additives was the best, even though many assumptions had to be made. It recognized that the intake of food additives by heavy consumers of particular foods cannot be accurately assessed. Nevertheless the results are useful for selecting priorities for further extensive investigation.

The Committee was aware that dietary surveys of the weekly food intake of individuals are in progress (in some cases the period is longer than one week). From these surveys, an independent assessment can be made of maximum intakes, at least in the age and sex groups surveyed. Population groups specially vulnerable to excessive intake of particular food additives should be identified. To do so will require a planned survey which takes into account sociological and economic factors as well as unusual dietary patterns.

The Committee was aware that care needs to be taken to itemize the food records so that intakes of each individual food can be identified. It also recognized that unless those responsible for the design of the study are aware of the total information needed, the basic data needed might not be collected and storage of the data might not be such as to permit ready retrieval for the calculation of food additive intake.

At the fifth session of the Codex Committee on Food Additives it had been recommended that each interested country should designate an officer for liaison with WHO on the question of the calculation of food additive intake. The Committee recommended that these liaison officers should be informed of the various aspects of the problem discussed above.

The estimated potential intake of food additives is likely to exceed the actual average intakes. This is because additives are not always present in all the foods in which their use has been proposed, and equally they may not be used to the extent of the maximum permitted. The most reliable way of determining this difference would be by sampling over an extended period and analysing the foods containing the permitted additives.

The Committee recommended that the problem of assessing high persistent consumption of additives by individuals should be the subject of further study.

8. RECOMMENDATIONS

8.1 Recommendations to FAO and WHO

(1) In view of the large numbers of food additives requiring consideration the Committee considers it desirable that further meetings of this Committee should be held annually. Future meetings should also give consideration to (*a*) evaluating the technological efficacy of further classes of food additives, priority to be given to certain specified antibiotics and to antioxidants, (*b*) preparing guidelines for evaluating the efficacy of antimicrobial food preservatives under practical conditions and (*c*) revising outdated specifications of food additives on a systematic basis.

(2) In view of both the seriousness and the extreme complexity of the problem of environmental pollution by mercury and mercury compounds, the Committee recommends that consideration should be given to convening a special meeting for the evaluation of the data available.

(3) The Committee reaffirmed the need for the publication of a compendium containing all its previous specifications for food additives.

8.2 General recommendations

(1) Recognizing the extreme seriousness of the problem of environmental contamination by mercury, the Committee recommends that all possible measures should be taken to reduce this form of pollution. In addition, the Committee recognized the urgent need for surveys of the levels and forms of mercury in foods.

(2) More information is needed on the level of solvent residues in some foods arising from the use of extraction solvents. More versatile, simple, and less expensive methods are desirable for detecting, and estimating the levels of, aromatic hydrocarbons and carcinogenic polynuclear aromatic hydrocarbons in the petroleum hydrocarbon solvents used for food extraction.

(3) Further information is desirable on the potential synergistic effects of antimicrobial agents previously considered by the Committee, in the hope of lowering use levels in individual foods. In addition, further work is necessary in order to develop antimicrobial agents effective at pH values greater than 6.0.

(4) Recognizing the importance of assessing food additive intake the Committee recommended that those responsible for the design and analysis of food consumption surveys should consult FAO and WHO so that the collective data can be used to the maximum extent for calculating the intake of food additives on an individual basis.

Annex 1

REPORTS AND OTHER DOCUMENTS RESULTING FROM PREVIOUS MEETINGS OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

1. General Principles Governing the Use of Food Additives: First Report, *FAO Nutrition Meetings Report Series*, 1956, No. 11: *Wld Hlth Org. techn. Rep. Ser.*, 1956, No. 129.
2. Procedures for the Testing of International Food Additives to Establish their Safety for Use; Second Report, *FAO Nutrition Meetings Report Series*, 1958, No. 17; *Wld Hlth Org. techn. Rep. Ser.*, 1958, No. 144.
3. Specifications for Identity and Purity of Food Additives (Antimicrobial Preservatives and Antioxidants): Third Report. These specifications were subsequently revised and published as *Specifications for Identity and Purity of Food Additives*, vol. I. *Antimicrobial Preservatives and Antioxidants*, Rome, Food and Agriculture Organization of the United Nations, 1962.
4. Specifications for Identity and Purity of Food Additives (Food Colours): Fourth Report. These specifications were subsequently revised and published as *Specifications for Identity and Purity of Food Additives*, vol. II. *Food Colors*, Rome, Food and Agriculture Organization of the United Nations, 1963.
5. Evaluation of the Carcinogenic Hazards of Food Additives: Fifth Report, *FAO Nutrition Meetings Report Series*, 1961, No. 29; *Wld Hlth Org. techn. Rep. Ser.*, 1961, No. 220.
6. Evaluation of the Toxicity of a Number of Antimicrobials and Antioxidants: Sixth Report, *FAO Nutrition Meetings Report Series*, 1962, No. 31; *Wld Hlth Org. techn. Rep. Ser.*, 1962, No. 228.
7. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Emulsifiers, Stabilizers, Bleaching and Maturing Agents: Seventh Report, *FAO Nutrition Meetings Report Series*, 1964, No. 35; *Wld Hlth Org. techn. Rep. Ser.*, 1964, No. 281.
8. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Food Colours and Some Antimicrobials and Antioxidants: Eighth Report, *FAO Nutrition Meetings Report Series*, 1965, No. 38; *Wld Hlth Org. techn. Rep. Ser.*, 1965, No. 309.

*9. Specifications for Identity and Purity and Toxicological Evaluation of some Antimicrobials and Antioxidants, *FAO Nutrition Meetings Report Series*, 1965, No. 38A; WHO/Food Add/24.65.

*10. Specifications for Identity and Purity and Toxicological Evaluation of some Food Colours, *FAO Nutrition Meetings Report Series*, 1966, No. 38B; WHO/Food Add/66.25.

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* These documents can be obtained on request from: Food Additives, World Health Organization, Avenue Appia, 1211 Geneva, Switzerland, or: Food Policy and Food Science Service, Food and Agriculture Organization of the United Nations, 00100 Rome, Italy.

11. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Antimicrobials, Antioxidants, Emulsifiers, Stabilizers, Flour-treatment Agents, Acids and Bases: Ninth Report, *FAO Nutrition Meetings Report Series*, 1966, No. 40: *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 339.

*12. Toxicological Evaluation of Some Antimicrobials, Antioxidants, Emulsifiers, Stabilizers, Flour-Treatment Agents, Acids and Bases, *FAO Nutrition Meetings Report Series*, No. 40 A, B, C; WHO/Food Add/67.29.

13. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Emulsifiers and Stabilizers and Certain Other Substances: Tenth Report, *FAO Nutrition Meetings Report Series*, 1967, No. 43; *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 373.

14. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Flavouring Substances and Non-Nutritive Sweetening Agents: Eleventh Report, *FAO Nutrition Meetings Report Series*, 1968, No. 44; *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 383.

*15. Toxicological Evaluation of Some Flavouring Substances and Non-Nutritive Sweetening Agents, *FAO Nutrition Meetings Report Series*, 1968, No. 44A; WHO/Food Add/68.33.

*16. Specifications and Criteria for Identity and Purity of Some Flavouring Substances and Non-Nutritive Sweetening Agents. *FAO Nutrition Meetings Report Series*, 1969, No. 44B; WHO/Food Add/69.31.

17. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Antibiotics. Twelfth Report. *FAO Nutrition Meetings Report Series*, 1969, No. 45; *Wld Hlth Org. techn. Rep. Ser.*, 1969, No. 430.

*18. Specifications for the Identity and Purity of Some Antibiotics. *FAO Nutrition Meetings Report Series*, 1969, No. 45A; WHO/Food Add/69.34.

19. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Food Colours, Emulsifiers, Stabilizers, Anticaking Agents, and Certain Other Substances. Thirteenth Report. *FAO Nutrition Meetings Report Series*, 1970, No. 46; *Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 445.

*20. Toxicological Evaluation of Some Food Colours, Emulsifiers, Stabilizers, Anti-caking Agents and Certain Other Substances. *FAO Nutrition Meetings Report Series*, No. 46A; WHO/Food Add/70.36.

*21. Specifications for the Identity and Purity of some Food Colours, Emulsifiers and Stabilizers, Anti-caking Agents and Certain Other Substances. *FAO Nutrition Meetings Report Series*, No. 46B; WHO/Food Add/70.37.

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* These documents can be obtained on request from: Food Additives, World Health Organization, Avenue Appia, 1211 Geneva, Switzerland, or: Food Policy and Food Science Service, Food and Agriculture Organization of the United Nations, 00100 Rome, Italy.

Annex 2

LIST OF FOOD ADDITIVES ON THE AGENDA

CATEGORY 1

Food additives for re-evaluation

Brominated vegetable oils
Curcumin
Cyclamates
Monosodium glutamate
Phosphoric acid and phosphates

CATEGORY 2

Food additives for establishment of specifications and toxicological evaluation

Extraction solvents

Acetone
Carbon disulfide
1,2-Dichloroethane (ethylene dichloride)
Dichloromethane (methylene chloride)
Ethanol
Ethylacetate
Isobutyl alcohol
Methanol
Methylchloroform (1,1,1-trichloroethane)
n-Hexane
Petroleum ether
Propan-2-ol
1,1,2-Trichloroethylene

Other food additives

Asbestos (filtration aid)
Caseinates, sodium, potassium, calcium and ammonium
Cupric sulfate
Edible gelatin
Ethyl maltol
Mineral oil

Oleoresins of paprika
Pure vegetable carbon
Stannous chloride and tin
Tannins (filtration aid)
Carrageenan and furcellaran

Contaminant

Mercury (toxicological evaluation only)

CATEGORY 3

Food additives for evaluation of the technological efficacy

Benzoic acid and its salts
Diethylpyrocarbonate
Nitrates and nitrites
p-Hydroxybenzoate esters
Propionic acid and its salts
Sodium diacetate
Sorbic acid and its salts
Sulfurous acid and its salts

Annex 3

IMPURITIES IN SOLVENTS

1. *Aromatics*

The main aromatic impurity in hydrocarbon solvents is benzene. Food-grade hexane contains less than 0.2% of benzene because it has been hydrogenated in order to convert any benzene it may contain into cyclohexane. In an experiment where a high percentage (3%) of benzene was intentionally added to hexane, the residues in cocoa butter were less than 0.1 ppm, and extracted groundnut meal contained only a few parts per million of benzene.[1] Johnson, Nursten & Self [2] have reviewed the literature on the occurrence of aromatic hydrocarbons (including polynuclear ones) in foods at levels substantially below 1 mg/kg.

2. *Polynuclear aromatic hydrocarbons*

The occurrence of polynuclear aromatic hydrocarbons in foods has been reviewed by Gunther & Buzzetti,[3] in crude vegetable oils by Grimmer & Hildebrandt [4] and in coconut oil by Biernoth & Rost.[5]

The methods for detecting and estimating polynuclear aromatic hydrocarbons have been reviewed by Haenni.[6] The method of Howard et al.[7] was used by Howard, Fazio & White [8] for the estimation of residues of individual polynuclear compounds in commercial hexane solvents used in edible oil extraction. The hydrocarbons are isolated by partition, column and thin-layer chromatography and measured by ultraviolet and spectrophotofluorometric procedures with recoveries of about 90% when present in the hexane at the 2 µg/kg level. An alternative method is to specify the maximum absorption value at selected wavelengths in the ultraviolet region ; although this is a less specific and less sensitive method than that of Howard et al., it has been included in the specifications since it is much simpler.

Alders [9] has shown on theoretical grounds that, after refining, even raw hexane with an initial content of 3% of aromatic hydrocarbons will not

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[1] Shell Chemical—unpublished report.

[2] Johnson, A. E., Nursten, H. E. & Self, R. (1969) *Chem. & Ind.*, pp. 10–12.

[3] Gunther, F. A. & Buzzetti, F. (1965) *Residue Rev.*, **9**, 90.

[4] Grimmer, G. & Hildebrandt, A. (1967) *Chem. & Ind.*, pp. 2000–2002.

[5] Biernoth, G. & Rost, H. E. (1967) *Chem. & Ind.*, pp. 2002–2003.

[6] Haenni, E. O. (1968) *Residue Rev.*, **24**, 41–78.

[7] Howard, J. W., Teague, R. T., White, R. H. & Fry, B. E. (1966) *J. Ass. off. agric. Chem.*, **49**, 595.

[8] Howard, J. W., Fazio, T. & White, R. H. (1968) *J. Agric. Food Chem.*, **16**, 72–76.

[9] Alders, L., unpublished data.

contain more than 10 µg/kg of residue. A simple estimation of the polynuclear aromatic hydrocarbon levels in the extracted oil can be obtained by calculating these from the amount of " make-up " solvent required in an extraction plant. " Make-up " solvent is necessary to replace solvent losses, which are mainly caused by evaporation through leaks in the equipment and incomplete recovery of solvent from the air. These losses range from 2 to 10 litres (1.4–7 kg) per ton of extracted oil and the detection limit of the polynuclear hydrocarbons in a solvent by the proposed ultraviolet absorption test is 0.5 mg/kg. Consequently, even if these hydrocarbons were present in the solvent up to the detection limit and if they were left entirely in the extracted oil, this would not contain more than 0.7–3.5 µg/kg of polynuclear aromatic hydrocarbons.

Annex 4

RESOLUTION WHA23.50 OF THE TWENTY-THIRD WORLD HEALTH ASSEMBLY

Health Hazards of Food Additives

The Twenty-third World Health Assembly,

Being concerned about the potential hazards of food additives to the consumer ;

Aware of the increasing research done on toxicity of food additives ;

Having noted the intensive publicity commonly given by the lay press to questions of safety of food additives and the widespread repercussions which follow action by any country to limit or prohibit the use of a generally used food additive ;

Noting that the matter has been raised at the forty-fifth session of the Executive Board ; and

Agreeing that there is an urgent need for rapid dissemination of the results of toxicity research of food additives, including the results and consequences of evaluation of such studies,

1. REQUESTS Member States :

   (i) to communicate immediately to WHO any decision to limit or prohibit the use of a food additive ; and

   (ii) to supplement as soon as possible such information with the data in support of the decision taken ; and

2. REQUESTS the Director-General where such action would be useful :

   (i) to transmit immediately to Member States information received under paragraph (1) ;

   (ii) to take expeditious steps to evaluate any significant new evidence of toxicity of a specific food additive, including if necessary the convening of a meeting of experts, where appropriate in consultation with FAO ;

   (iii) to distribute promptly to Member States any conclusions of such a meeting.

Fifteenth plenary meeting, 21 May 1970.

Annex 5

TOXICOLOGICAL EVALUATIONS: MISCELLANEOUS FOOD ADDITIVES AND CONTAMINANTS [a]

Substance	*Acceptable daily intake for man* [1] *(mg/kg body-weight)*
Brominated vegetable oils [b]	No ADI
Carrageenan [c] / Furcellaran [c]	0–50 [2]
Cyclamates, calcium and sodium [d]	No ADI [3]
Copper and cupric sulfate [e]	No ADI [4]
Ethyl maltol [e]	0–2
Food grade mineral oil [e]	Use limited by good manufacturing practice
Mercurial compounds	No ADI
Monosodium L-glutamate [e]	0–120 [5]
Oleoresins of paprika [e]	Self limiting as a spice
Phosphoric acid and phosphates [f]	0–30 [6]
Tin and stannous chloride [e]	No ADI [4]

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[a] Specifications are also available for gelatin and sodium caseinate (page 12).

[b] Identification test only.

[c] Specifications available (Annex 1, ref. 21).

[d] Specifications available (Annex 1, ref. 16; also Section 5.3.1 (*b*)).

[e] Specifications available (see p. 2).

[f] Specifications available (see Annex 1, ref. 7).

[1] Unconditional ADIs unless otherwise indicated.

[2] As carrageenan or furcellaran, or the sum of both. (There was a printing error in the thirteenth report (Annex 1, ref. 19) but the monographs (Annex 1, ref. 20) are correct.)

[3] Use in the management of diabetes and gross obesity not considered in this evaluation.

[4] For evaluation see section 5.3.3.

[5] Additional to the amount naturally occurring in the diet. Applicable to the general population except infants under 1 year of age.

[6] Conditional acceptable daily intake 30–70 mg/kg. Both the unconditional and conditional ADIs include the amount occurring in the diet.

Annex 6

TOXICOLOGICAL EVALUATIONS: FILTRATION AIDS AND RELATED SUBSTANCES

Substance	*Acceptable daily intake for man (mg/kg body-weight)*
Activated vegetable carbon [a] (food grade)	No limit except for good manufacturing practice
Asbestos	Decision postponed [c]
Tannins (food grade) [b]	
Derived from Peruvian tara	0–0.6 [d]
Derived from Turkish aleppo, Chinese tara, and Sicilian sumac	0–0.3 [d]

[a] Specification available (see p. 2).

[b] Tentative specifications available for tannins used as a flocculant or clarifying agent (see p. 2).

[c] See p. 16.

[d] Temporary ADI.

Annex 7

TOXICOLOGICAL EVALUATIONS: EXTRACTION SOLVENTS

1. *Substances considered*

Acetone
1,2-Dichloroethane
Dichloromethane
Ethanol
Methanol
Petroleum hydrocarbon fractions (hexane and heptane)
Propan-2-ol
1,1,2-Trichloroethylene

2. *Evaluations*

(*a*) The evaluations for these solvents, with the exception of trichloroethylene as a caffeine extractant and ethanol, are tentative, and are subject to re-evaluation when the relevant data become available (see section 4.3.1).

(*b*) These solvents should be used only in accordance with good manufacturing practice, in the expectation that this will result in minimal residues.

(*c*) With 1,2-dichloroethane and 1,1,2-trichloroethylene, care must be taken to avoid the formation of toxic interaction products with certain treated foods.

3. Specifications are available for all these solvents with the exception of hexane and heptane, for which only tentative specifications were prepared (see p. 2).

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

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Abstract

The Expert Panel for Cosmetic Ingredient Safety (Panel) reviewed the safety of 19 skin and connective tissue-derived proteins and peptides, which are reported to function mainly as skin and/or hair conditioning agents in cosmetics. The Panel reviewed the relevant data provided and concluded that these ingredients are safe in the present practices of use and concentration described in this safety assessment.

Keywords

tissue proteins, cosmetics, safety

Introduction

The skin and connective tissue-derived proteins and peptides detailed in this report are described in the *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*) to function mainly as skin and hair conditioning agents in cosmetics.[1] This report assesses the safety of the following 19 skin and connective tissue-derived ingredients:

Ammonium Hydrolyzed Collagen
Atelocollagen
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 Panel previously reviewed the ingredient Hydrolyzed Collagen, and concluded that it is safe for use in cosmetics; the report was published in 1985 and the conclusion was reaffirmed in a re-review that was published in 2006.[2,3] This ingredient was included in this safety assessment because of the relevance of the information in regards to reviewing the safety of the other ingredients in the report.

Additionally, the safety of several other hydrolyzed proteins as used in cosmetics has been reviewed by the Panel in several previous assessments. The Panel concluded that Hydrolyzed Keratin (finalized in 2016), 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.[4-8] 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 Panel concurrently

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reviewed the safety of plant-derived and bovine milk-derived proteins, which had, at that time, tentative conclusions of safe as used, in separate reports. In addition to the review of these other protein-derived ingredients, the Panel has assessed the safety of Ethanolamine (also known as monoethanolamine or MEA) and Ethanolamine Salts and concluded these ingredients are safe when formulated to be nonirritating (rinse-off products only) but should not be used in cosmetic products in which *N*-nitroso compounds may be formed.[10]

Actin, Collagen, Elastin, Fibronectin, Gelatin, and reticulin all are derived from essential components in animal 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 skin and connective 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 skin and connective tissue-derived proteins with unique properties, which may result in varying compositions and impurities within a single ingredient (eg, Hydrolyzed Collagen from animals such as cows may have some impurities that are different from Hydrolyzed Collagen obtained from fish).

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Chemistry

Definition

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

Skin and connective 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 abundant protein in mammals is collagen, making up approximately 30% of all proteins by mass.[11,12] The collagen family is comprised of 28 members (named collagen I to collagen XXVIII) that all have at least 1 triple helix in their structure at varying degrees (see further description below).[12] 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.[11] 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 (eg, 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 accomplished via acid, enzyme, or other methodologies. These methodologies, and the degree to which they are utilized, may profoundly affect the size and biological activity 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 (eg, Calcium Hydrolyzed Collagen).

Actin. Actin is a major protein of muscle and an important component of all eukaryotic cells.[11] 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.[11,13] 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 1 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.[12,14] 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

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
Atelocollagen 55963-88-7	Atelocollagen is the protein obtained when the telopeptides are enzymatically removed from collagen	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 100085-10-773 049-73-7 [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 100085-35-673 049-73-7 [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 Skin and Connective Tissue-Derived Proteins.[2,11,15,16-18,27,37]

Ingredient	Molecular weight (Da) Range
Collagen (native)	130 000 to >1 000 000
Soluble collagen	30 000 to 40 000, but may be up to an average of 300 000
Hydrolyzed collagen	400 to 25 000
Hydrolyzed actin	58.4% < 5000; 41.4% > 5000 and <30 000
Hydrolyzed elastin	500 to 150 000
Fibronectin	>200 000

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.[11] 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 2 types of elastin: Type 1 is derived from bovine neck ligaments, aorta (as reported in 1987), skin, and related tissues; Type 2 is derived from cartilage and its derivatives.[15] In skin, Elastin is the intact elastic fiber network that comprises approximately 2% to 4% of the dermis by volume.[13]

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.[11]

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.[11] Glycine or alanine accounts for 1 third to 1 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[11]

Physical and Chemical Properties

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

Collagen

Solutions of Collagen for cosmetic use have a pH range of 3.8 to 4.7[14]

Hydrolyzed Collagen

Hydrolyzed Collagen may be a powder or solution.[2] A 10% aqueous solution has a pH of 4.0-6.5.

Elastin

Purified Elastin is a pale yellow color and exhibits a bluish fluorescence in UV light.[11] It resists acid and alkaline hydrolysis. It is practically insoluble even in hydrogen-bond-breaking solvents at temperatures up to 100°C, and is nearly impossible to bring into solution except by using reagents capable of hydrolyzing peptide bonds. Unprocessed or native elastin is reported to be too insoluble for use in cosmetic formulations.[15]

Fibronectin

Fibronectin can be provided in a solution or as a lyophilized powder.[19]

Gelatin

Gelatin is a vitreous, brittle solid that is colorless to faintly yellow.[11,20] 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°C, while cold-water ocean fish sourced Gelatin has a gel point between 5 and 10°C. 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.[15]

Method of Manufacturing

Methods used to manufacture protein hydrolysates typically yield broad MW distributions of peptides, ranging from 500 to 30 000 daltons (Da), equating to 4 to 220 amino acids in length.[21,22] Treatment with certain enzymes, such as papain, can routinely yield narrower distributions of 500 to 10 000 Da,

Table 3. Method of Manufacturing.

Ingredient	Source	Procedure	Reference
Collagen	Not reported	Prepared by dissolving the mineral part of bones with phosphoric acid	11
Hydrolyzed collagen	Bovine or fish	Prepared by alkaline hydrolysis followed by enzymatic hydrolysis to the desired molecular weight	2,3
Hydrolyzed collagen (MW = 2000 Da)	Bovine	Prepared by combination of alkaline and enzymatic hydrolysis	18
Elastin	Farm animals such as cattle or goats	Prepared from cattle aortas through extraction with sodium hydroxide at 100°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	15
Elastin	Collagen (unspecified)	Elastin may be a byproduct of the purification of collagen	15
Hydrolyzed elastin (MW = 1000-4000 Da)	Codfish skin or bovine neck tendons	Prepared by washing and purifying to remove soil and other residual material and then dried. Dried material is 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	23,24
Hydrolyzed elastin (MW = 3000-4000 Da)	Numerous sourced animal ligaments or hides	Prepared by enzymatic hydrolysis (by pancreatic elastase, ficin, pepsin or trypsin) or acid hydrolysis at high temperatures (70-100°C, depending on acid) at several 1 hour intervals	15
Hydrolyzed elastin (MW = 2000-4000 Da)	Not reported	Manufactured by enzymatic hydrolysis for a specific duration of time and at an elevated temperature (details not provided). Resultant hydrolyzed protein composed of di- and tri-peptides	67
Gelatin	Collagen (unspecified)	Prepared by the acid, alkaline or enzymatic hydrolysis of collagen. 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	20
Soluble collagen	Bovine dermal tissues, bony fish skins, or tropical fish swim bladders	Extracted by neutral salt solutions	25
Soluble elastin	Cattle ligaments	Obtained by acid treatment at 80°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 90°C	15

equating to 4 to 74 amino acids in length. The available methods of manufacturing for the skin and connective tissue-derived proteins and peptides are summarized in Table 3.

Hydrolyzed Collagen

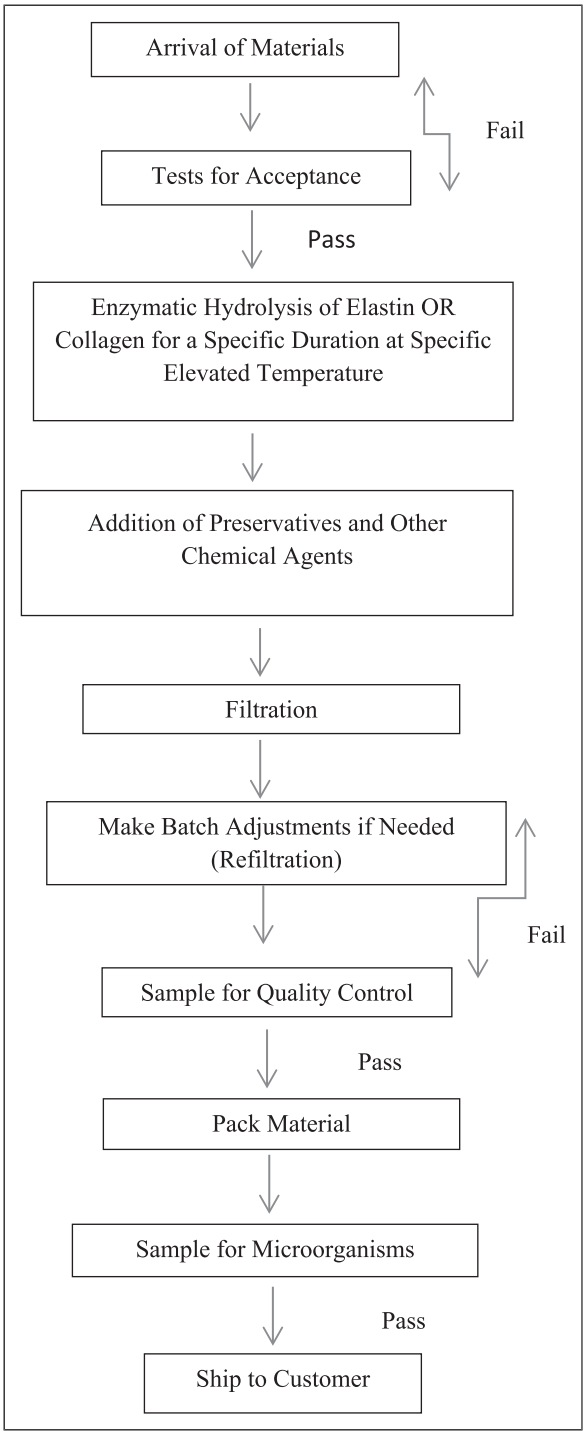
A representative manufacturing flow chart for Hydrolyzed Collagen is found in Scheme 1. This process may vary slightly between specific products with the elimination of the use of preservatives after hydrolysis and the addition of filtration and concentration of solution before the first quality control.[26]

A supplier has reported that their Hydrolyzed Collagen products (6 products with MW ranges of 400 to 2000 Da, concentration up to 50% in water) are prepared by acidic, alkalic, and/or enzymatic hydrolysis of bovine gelatin, swine gelatin, or fish scale until the molecular weight reaches the target range.[27]

Soluble collagen. A representative manufacturing flow chart for Soluble Collagen is found in Scheme 2.

Hydrolyzed elastin. A representative manufacturing flow chart for Hydrolyzed Elastin is found in Scheme 1. This process may vary slightly between specific products with the use of pH adjustment during hydrolysis.

Gelatin. According to 21 CFR§700.27, Gelatin is "… a product that has been obtained by the partial hydrolysis of collagen derived from hides, connective tissue, and/or bones of cattle and swine. Gelatin may be either Type A (derived from an acid-treated precursor) or Type B (derived from an alkali-treated precursor) that has gone through processing steps that include filtration and sterilization or an equivalent process in terms of infectivity reduction."



Scheme 1. Representative manufacturing flow chart for Hydrolyzed Elastin or Hydrolyzed Collagen (bovine and fish sourced).[28-32]

Composition

The typical amino acid compositions for Collagen, Soluble Collagen, and Elastin are presented in Table 4.

Impurities

Several of the ingredients in this safety assessment, including Hydrolyzed Collagen, Hydrolyzed Elastin, and Gelatin, may be bovine sourced. Some bovine materials may be considered risk materials for transmission of infectious agents (eg, bovine spongiform encephalopathy (BSE) prions). According to 21 CFR§700.27, "no cosmetic shall be manufactured from, processed with, or otherwise contain, prohibited cattle materials." Prohibited cattle materials "mean specified risk materials, small intestine of all cattle …, material from non-ambulatory disabled cattle, material from cattle not inspected and passed, or mechanically separated." Gelatin or hides and hide-derived products are not prohibited cattle materials. Cosmetic manufacturers must follow record keeping requirements that "demonstrate that the cosmetic is not manufactured from, processed with, or does not otherwise contain prohibited cattle materials."

The World Organization for Animal Health (OIE) recommends that "when authorizing import or transit of … gelatin and collagen prepared exclusively from hides and skins … and any products made from these commodities and containing no other tissues from cattle, veterinary authorities should not require any BSE related conditions [ie restrictions], regardless of the BSE risk status of the cattle population of the exporting country, zone, or compartment."[33]

Collagen. An analysis for 3 different Collagen products found the level of arsenic to be less than 1 ppm.[14]

Hydrolyzed collagen. The maximum concentrations of iron and heavy metals reported in Hydrolyzed Collagen were 3 ppm and 25 ppm, respectively.[2]

A supplier reported that their Hydrolyzed Collagen products (6 products with MW ranges of 400 to 2000 Da, concentration up to 50% in water) sourced from bovine gelatin, swine gelatin, and fish scales contain not more than 10 ppm heavy metals and not more than 1 ppm arsenic.[27]

A supplier reported that their Hydrolyzed Collagen products are BSE-free.[34,35]

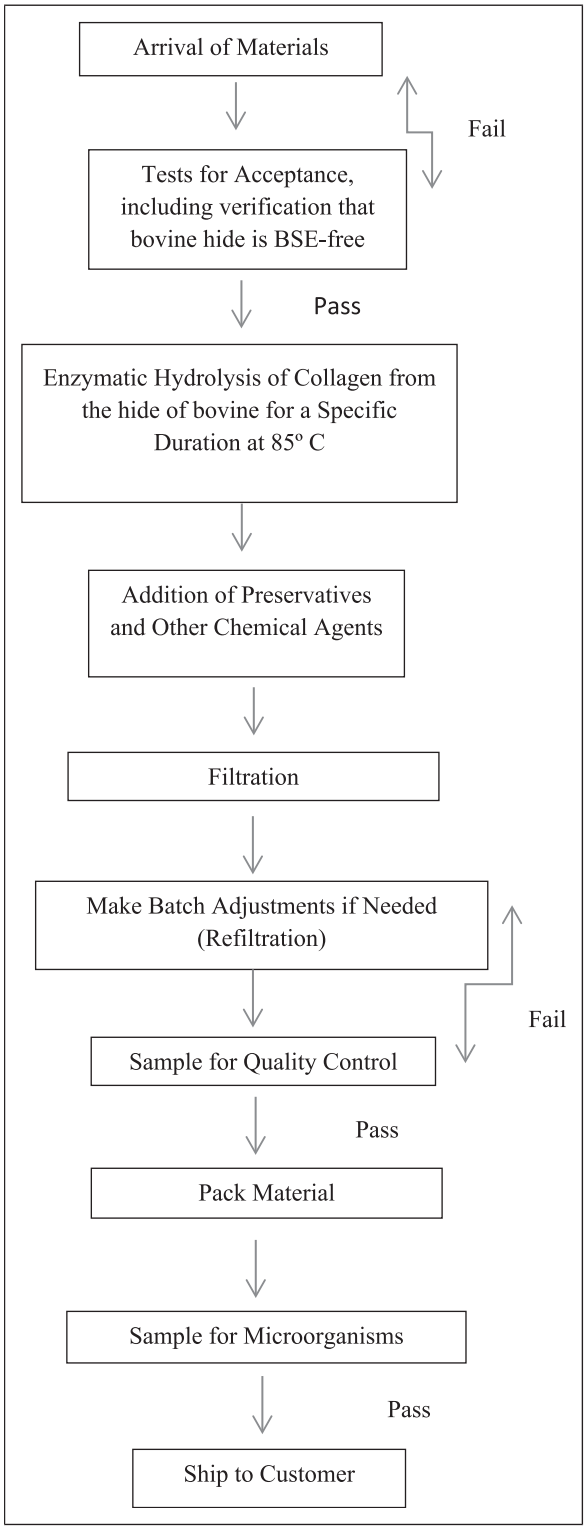
Soluble collagen. A supplier reported that their Soluble Collagen product is BSE-free.[36]

Elastin and hydrolyzed elastin. Impurities in commercial Elastin-based preparations include contamination by lipoid substances from the raw materials and products of Collagen degradations.[37]

A supplier certified that their Hydrolyzed Elastin products are BSE-free.[38-40]

Gelatin

According to the *Food Chemicals Codex*, Gelatin must contain no more than 0.0005% sulfur dioxide,



Scheme 2. Manufacturing flow chart for Soluble Collagen (bovine sourced).[41]

10 mg/kg chromium, 1.5 mg/kg lead, and 0.3 mg/kg pentachlorophenol.[20]

A supplier certified that their Gelatin product is BSE-free.

Use

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in 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, the ingredients with the greatest number of uses are Hydrolyzed Collagen (543 formulations) and Soluble Collagen (425 formulations); the majority of uses are in leave-on skin care products (Table 5 and Table 6).[42] Gelatin is used in a total of 334 formulations; 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.[43] Gelatin is used at up to 66% in bath oils, tablets, and salts. The other in-use ingredients are used at much lower concentrations.

Historic and current use data for Hydrolyzed Collagen is reported in Table 6. The number of uses of Hydrolyzed Collagen has declined since the initial safety assessment in 1981 and the re-review in 2002 (923 and 570 uses, respectively[2,3]). The maximum use concentration of Hydrolyzed Collagen was reported to be 16.5% in hair tonics and dressings in 2016; it was previously reported to be used at concentrations greater than 50% (in rinse-off formulations).[2,43]

Ingredients with no reported uses in the VCRP or by Council are listed in Table 7.

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, Gelatin is used in bath oils, tablets and salts at up to 66% and Hydrolyzed Collagen is used in an eyeliner at up to 3.2%.[43] Additionally, some of these ingredients were reported to be used in hair care products, skin care preparations, face powders, and fragrances and

Table 4. Amino Acid Residue Profile of Collagen, Soluble Collagen, and Elastin (Residues per 1000).[14,15,44]

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

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, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (ie, they would not enter the lungs) to any appreciable amount.[45,46] 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.[47-49] More information may be found in the Panel's Respiratory Exposure Resource Document here https://www.cir-safety.org/sites/default/files/report_InhalationDocument_122021.pdf.

The skin and connective 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, monoalkanol-amine 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.[50]

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 1 or more of the 8 major food allergens, which includes fish, are included in food.[51]

Collagen

Non-cosmetic uses of Collagen include fibers in sutures, leather substitutes, coatings, as a gel in photographic emulsions, and food casings.[11]

Gelatin

Non-cosmetic uses of Gelatin include uses in food as a stabilizer, thickener, texturizer, firming agent, surface-active agent, or surface-finishing agent.[11,20] 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.[11] 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).

Table 5. Frequency (2017) and Concentration of Use (2016) According to Duration and Type of Exposure for Skin and Connective Tissue-Derived Proteins and Peptides.[42,43]

	# 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		Atelocollagen		Collagen		Elastin	
Totals[a]	NR	0.03-0.12	118	0.0000001-0.099	299	0.00005-96	46	NR
Duration of use								
Leave-on	NR	0.1-0.12	114	0.0000001-0.005	241	0.0001-96	40	NR
Rinse off	NR	0.03-0.05	4	0.0000001-0.099	58	0.00005-1.3	6	NR
Diluted for (bath) use	NR	NR	NR	NR	NR	NR	NR	NR
Exposure type								
Eye area	NR	NR	17	0.0000022	31	0.00012-0.2	2	NR
Incidental ingestion	NR	NR	39	0.0004	5	0.0035-0.05	NR	NR
Incidental inhalation-spray	NR	NR	19[c]; 18[d]	0.0000001[c]	55[c]; 94[d]	0.00095[c]	11[c]; 11[d]	NR
Incidental inhalation-powder	NR	0.1[b]	1; 18[d]	0.000022; 0.00002-0.000033[b]	94[d]	0.0018-96[b]	11[d]	NR
Dermal contact	NR	0.03-0.12	66	0.0000001-0.099	240	0.00005-96	42	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - non-coloring	NR	0.05	1	NR	39	0.00095-1.3	4	NR
Hair-coloring	NR	NR	NR	NR	1	NR	NR	NR
Nail	NR	NR	NR	NR	4	NR	NR	NR
Mucous membrane	NR	NR	39	0.0004	8	0.0035-0.67	NR	NR
Baby products	NR	NR	NR	NR	NR	NR	NR	NR

	Fibronectin		Gelatin		Hydrolyzed actin		Hydrolyzed elastin	
Totals[†]	2	NR	334	0.000052-66	20	0.00075-1.6	278	0.00000035-5
Duration of use								
Leave-on	2	NR	33	0.0003-37.8	18	1.6	211	0.00000035-5
Rinse off	NR	NR	301	0.000052-9.5	2	0.00075	67	0.0000035-2.5
Diluted for (bath) use	NR	NR	NR	0.21-66	NR	NR	NR	NR
Exposure type								
Eye area	NR	NR	NR	0.5-1	5	1.6	10	0.00035-0.5
Incidental ingestion	NR	NR	3	NR	NR	NR	2	0.00035-0.15
Incidental inhalation-spray	2[d]	NR	19[c]; 6[d]	0.03; 0.009-0.095[c]; 0.016[d]	7[c]; 6[d]	NR	57[c]; 88[d]	0.00031-0.42; 0.002-0.24[c]
Incidental inhalation-powder	2[d]	NR	1; 6[d]	0.016[d]; 0.03-37.8[b]	6[c]	NR	88[d]; 2[b]	0.0000035; 0.00025-5[b]
Dermal contact	2	NR	286	0.0003-66	10	NR	227	0.00000035-5
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - non-coloring	NR	NR	12	0.000052-0.5	7	0.00075	48	0.00031-2.5

(continued)

Table 5. (continued)

	Fibronectin			Gelatin	Hydrolyzed actin			Hydrolyzed elastin
Hair-coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	33	0.001-0.02	NR	NR	NR	NR
Mucous membrane	NR	NR	257	0.0011-66	NR	NR	5	0.00035-0.15
Baby products	NR	NR	NR	NR	NR	NR	4	NR

	Hydrolyzed fibronectin		Hydrolyzed reticulin		MEA-hydrolyzed collagen			Soluble collagen[e]
Totals[a]	10	0.025-0.05	NR	0.025-0.05	NR	0.03-0.12	425	0.0000005-0.7
Duration of use								
Leave-on	9	0.05	NR	0.05	NR	0.1-0.12	368	0.0000005-0.7
Rinse off	1	0.025	NR	0.025	NR	0.03-0.06	57	0.000025-0.014
Diluted for (bath) use	NR	NR	NR	NR	NR	NR	NR	0.000035-0.005
Exposure type								
Eye area	1	NR	NR	NR	NR	NR	51	0.00003-0.05
Incidental ingestion	NR	NR	NR	NR	NR	NR	6	0.00035-0.01
Incidental inhalation-spray	4[c]; 3[d]	NR	NR	NR	NR	NR	104[c]; 141[d]	0.000005-0.0035; 0.00035-0.0035[c]
Incidental inhalation-powder	3[d]	NR	NR	NR	[d]R	0.1[b]	141[d]	0.0035; 0.0001-0.7[b]
Dermal contact	10	0.025-0.05	NR	0.025-0.5	NR	0.03-0.12	379	0.000005-0.7
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - non-coloring	NR	NR	NR	NR	NR	0.06	15	0.00001-0.014
Hair-coloring	NR	NR	NR	NR	NR	NR	1	0.000025-0.0005
Nail	NR	NR	NR	NR	NR	NR	NR	0.0000005-0.01
Mucous membrane	NR	NR	NR	NR	NR	NR	9	0.000035-0.01
Baby products	NR	NR	NR	NR	NR	NR	NR	NR

	Soluble collagen extract[f]	
Totals[†]	2	NR
Duration of use		
Leave-on	2	NR
Rinse off	NR	NR
Diluted for (bath) use	NR	NR
Exposure type		
Eye area	NR	NR
Incidental ingestion	NR	NR
Incidental inhalation-spray	1[c]; 1[d]	NR
Incidental inhalation-powder	1[d]	NR

(continued)

Table 5. (continued)

	Soluble collagen extract[f]	
Dermal contact	2	NR
Deodorant (underarm)	NR	NR
Hair - non-coloring	NR	NR
Hair-coloring	NR	NR
Nail	NR	NR
Mucous membrane	NR	NR
Baby products	NR	NR

NR = not reported.
[a]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.
[b] It is possible these products may be powders, but it is not specified whether the reported uses are powders.
[c]It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.
[d]Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.
[e]Includes 25 uses listed in the VCRP as "soluble animal collagen".
[f]Not listed in the Dictionary, possibly the same as Collagen Extract.

Table 6. Historic and Current Frequency and Concentration of Use According to Duration and Type of Exposure for Hydrolyzed Collagen.[42,43]

	# 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[a]	923	≤0.1 - >50	570[e]	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[b]; 46[c]	<1; >0.1 - >50[b]; ≤10[c]	3; 108[b]; 38[c]	0.000004-1[b]; 0.06-6[c]	116[b]; 139[c]	0.0017-0.[d]8; 0.0092-16.5[b]
Incidental inhalation-powder	5; 46[c]	≤1; ≤10[c]	4; 38[c]	0.5; 0.06-6[c]	3; 139[c]; 2[d]	0.0015-5[d]
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.

[a]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.

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

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

[d]It is possible theseproducts may be powders, but it is not specified whether the reported uses are powders

[e]Majority of the useswere categorized as "Hydrolyzed Animal Protein" in the VCRP database.

Table 7. Ingredients Not Reported in Use.

Calcium hydrolyzed collagen
Zinc hydrolyzed collagen
Hydrolyzed collagen extract
Hydrolyzed gelatin
Soluble elastin
Hydrolyzed spongin

Toxicokinetics

Gelatin

The bioavailability of Gelatin derived from Nile tilapia scales was determined in an oral pharmacokinetic study in rats.[52] Five groups of 6 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 di- and tri-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 in rabbits.[2]

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.[53] The rats received a single oral dose of 5000 mg/kg body weight and were observed for clinical signs of toxicity for 14 d. 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 of mice and rats.[2]

The oral LD_{50} of Hydrolyzed Collagen (30% solution in water; fish scale sourced; MW ~ 400 Da) was estimated to be greater than 2500 mg/kg body weight in Sprague-Dawley CD rats.[27] This acute toxicity test was performed in accordance to Organization for Economic Co-operation and Development test guideline (OECD TG) 423. A group of 3 female rats were treated orally with the test material at a dose level of 2000 mg/kg body weight, with another group of 3 fasted female rats receiving also receiving the material at the same dose level. No deaths or signs of systemic toxicity were observed during the 14 d of monitoring post-dosing. All animals exhibited expected gains in body weight. No abnormalities were observed at necropsy.

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.[54] 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/d for 2 week. Control animals were given ovalbumin at 20 mg/100 g body weight/d. 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 in rabbits and pigs on 2 cosmetic formulations containing 2% Hydrolyzed Collagen were negative for systemic toxicity.[2]

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.[53] 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 d. 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 small but statistically significant decrease in alkaline phosphatase activity in the 1000 mg/kg/d males was

Table 8. Dermal Irritation Studies of Hydrolyzed Collagen, Soluble Collagen, and Hydrolyzed Elastin.

Ingredient	Concentration	Method	Result	Reference
		In Vitro		
Hydrolyzed collagen (MW ~ 2000 Da; source = bovine)	25, 50, 75, 100, or 125 µl	Irritection® dermal model	Predicted to be non-irritating	59
Hydrolyzed collagen (source = bovine)	55% solution, undiluted	EpiDerm™ Assay	Predicted to be non-irritating	62
Soluble collagen (source = Atlantic cod)	25, 50, 75, 100, or 125 µl	Irritection® dermal model	Predicted to be non-irritating	61
Soluble collagen (source = bovine)	Undiluted	EpiDerm™ Assay	Predicted to be non-irritating	66
Hydrolyzed elastin (MW ~4000 Da; source = fish)	Concentration not reported	EpiDerm™ Assay	Predicted to be non-irritating	67
Hydrolyzed elastin (MW ~4000 Da; source not reported)	Concentration not reported	EpiDerm™ Assay	Predicted to be non-irritating	67
Hydrolyzed elastin (source = Atlantic cod)	Undiluted	EpiDerm™ Assay	Predicted to be non-irritating	63
Hydrolyzed elastin (source = young cattle)	25, 50, 75, 100, or 125 µl	Irritection® dermal model	Predicted to be non-irritating	60
Hydrolyzed elastin (source = cow skin)	Undiluted	EpiDerm™ Assay	Predicted to be non-irritating	65
Hydrolyzed elastin (source = cow skin)	Undiluted	EpiDerm™ Assay	Predicted to be non-irritating	64
		Animal		
Hydrolyzed collagen (MW ~400 Da; source = fish scale)	30% solution in water, tested neat	Primary skin irritation test in 3 New Zealand white rabbits; occluded 2.5 cm^2 patches with 0.5 mL test material; test performed in accordance to OECD TG 404	Very slight erythema noted in 1 treated skin 1 h post-patch removal; primary irritation index was 0.0; test material non-irritating and not corrosive	27
Hydrolyzed collagen (MW ~400 Da; source = fish scale)	30% solution in water, tested neat	Cumulative skin irritation test in 3 male and 3 female Hartley Guinea pigs; once daily treatments for 14 days to 2.0 cm^2 clipped dorsal skin	Non-irritating	27
Hydrolyzed elastin (MW ~3000 Da; source not reported)	Tested neat	Draize primary dermal irritation study in 6 New Zealand white rabbits; test sites occluded for 24 h	Not a primary irritant; primary irritation index was 0.38	68
		Human		
Hydrolyzed collagen (MW ~400 Da; source = bovine gelatin)	50% solution in water, tested neat	24 h occlusive patch test in 60 subjects (50 healthy, 10 allergic); 0.5 mL applied to left front arms	Non-irritating	27

(continued)

Table 8. (continued)

Ingredient	Concentration	Method	Result	Reference
Hydrolyzed collagen (MW ~400 Da; source = bovine gelatin)	30% solution in water, tested neat	24 h occlusive patch test in 60 subjects (50 healthy, 10 allergic); 0.5 mL applied to left front arms	Non-irritating	27
Hydrolyzed collagen (MW ~1000 Da: source = swine gelatin)	30% solution in water, tested neat	24 h occlusive patch test in 60 subjects (50 healthy, 10 allergic); 0.5 mL applied to left front arms	Non-irritating	27
Hydrolyzed collagen (MW ~2000 Da; source = swine gelatin)	30% solution in water, tested neat	24 h occlusive patch test in 60 subjects (50 healthy, 10 allergic); 0.5 mL applied to left front arms	Non-irritating	27
Hydrolyzed collagen (MW ~400 Da; source = fish scale)	30% solution in water, tested neat	24 h occlusive patch test in 21 healthy subjects; 0.03 g applied to backs	Non-irritating	27
Hydrolyzed collagen (MW ~400 Da; source = fish scale)	20% solution in water, tested neat	24 h occlusive patch test in 20 healthy subjects	Slight erythema in 1 subject; non-irritating	27

observed, but was not considered adverse. Minimal but statistically significant increases in albumin in 300 mg/kg/d males and in globulin in 1000 mg/kg/d 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 was tolerated well in this rat study.[53]

Human - Oral

Hydrolyzed Collagen/Gelatin. In a 4-mo 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.[55] The randomized double-blind study divided the children (ages 6-11 yr) 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 skin and connective tissue-derived proteins and peptides were discovered and no unpublished data were submitted.

Genotoxicity

In Vitro

Hydrolyzed collagen. No mutagenicity was observed in an Ames test of Hydrolyzed Collagen (30% solution with water; sourced from fish scales; MW ~400 Da).[21] *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* strain WP2uvrA were used in this test, which was performed in accordance to OECD TG 471. The dose range was 50 to 5000 μg/plate, with and without metabolic activation.

In another Ames test performed in accordance to OECD TG 471, Hydrolyzed Collagen (20% solution with water; source from fish scales; MW ~400 Da) was not mutagenic in *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 and *E. coli* strain WP2uvrA, with or without metabolic activation.[27] The dose range was 50 to 5000 μg/plate.

Hydrolyzed Collagen (30% solution with water; sourced from fish scales; MW ~400 Da) was not clastogenic in a Chinese hamster lung (CHL) cell line chromosome aberration test.[27] The cells were tested with and without metabolic activation.

Carcinogenicity

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

Table 9. Ocular Irritation Studies of Hydrolyzed Collagen, Soluble Collagen, and Hydrolyzed Elastin.

Ingredient	Concentration	Method	Result	Reference
		In Vitro		
Hydrolyzed collagen (MW ~ 2000 Da; source = bovine)	25, 50, 75, 100, or 125 µl	Irritection® ocular model	Predicted to be a minimal irritant	59
Hydrolyzed collagen (MW ~400 Da; source = fish scale)	10% active in purified water	BCOP in accordance to OECD TG 437	Predicted to be non-irritating	27
Hydrolyzed collagen (source = bovine)	55% solution, undiluted	EpiOcular™ Assay	Predicted to be non-irritating	62
Soluble collagen (source = Atlantic cod)	25, 50, 75, 100, or 125 µl	Irritection® ocular model	Predicted to be a minimal irritant	61
Soluble collagen (source = bovine)	Undiluted	EpiOcular™ Assay	Predicted to be non-irritating	66
Hydrolyzed elastin (source = Atlantic cod)	Undiluted	EpiOcular™ Assay	Predicted to be non-irritating	63
Hydrolyzed elastin (source = young cattle)	25, 50, 75, 100, or 125 µl	Irritection® ocular model	Predicted to be a minimal irritant	60
Hydrolyzed elastin (source = cow skin)	Undiluted	EpiOcular™ Assay	Predicted to be non-irritating	65
Hydrolyzed elastin (source = cow skin)	Undiluted	EpiOcular™ Assay	Predicted to be non-irritating	64
Hydrolyzed elastin (MW = 2000-4000 Da; 2 products, 1 source = fish, other source not reported)	Concentration not reported	EpiOcular™ Assay	Predicted to be non-irritating	67
		Ocular - Animal		
Hydrolyzed collagen (MW ~400 Da; source = bovine gelatin)	25% active diluted by 1%, 5%, 15%, 25%, and 50% v/v in saline	Ocular irritation study in 15 male rabbits; test material instilled in 1 eye while other eye served as control; eyes observed at instillation, 1 h and 24 h post	Non-irritating	27
Hydrolyzed collagen (MW ~400 Da; source = bovine gelatin)	15% active diluted by 1%, 5%, 15%, 25%, and 50% v/v in saline	Ocular irritation study in 15 male rabbits; test material instilled in 1 eye while other eye served as control; eyes observed at instillation, 1 h and 24 h post	Non-irritating	27
Hydrolyzed collagen (MW ~1000 Da; source = swine gelatin)	3% active diluted by 0.1%, 0.5%, 1%, 5%, and 10% v/v in saline	Ocular irritation study in 15 male rabbits; test material instilled in 1 eye while other eye served as control; eyes observed at instillation, 1 h and 24 h post	Non-irritating	27
Hydrolyzed collagen (MW ~2000 Da; source = swine gelatin)	15% active diluted by 1%, 5%, 15%, 25%, and 50% v/v in saline	Ocular irritation study in 15 male rabbits; test material instilled in 1 eye while other eye served as control; eyes observed at instillation, 1 h and 24 h post	Non-irritating	27
Hydrolyzed collagen (MW ~400 Da; source = fish scale)	30% active, tested neat	Ocular irritation study in 3 New Zealand white rabbits; test performed in accordance with OECD TG 405	Maximum group mean score was 4.0; minimally irritating	27
Hydrolyzed elastin (MW = 3000 Da; source not reported)	Tested neat	Draize ocular irritation study in 6 New Zealand white rabbits; treated eye was not rinsed	Not a primary irritant	68

Other Relevant Studies

Type I Hypersensitivity

Type 1 (ie, immediate) hypersensitivity reactions can occur in individuals allergic to certain proteins, such as those found in fish. An allergen must have at least 2 IgE-binding epitopes, and each epitope must be at least 15 amino-acid residues long, to trigger a Type 1 hypersensitivity reaction.[56] Type 1 responses can be elicited in sensitized patients when pairs of IgE molecules against a specific allergen are bound to receptors on the surface of mast cells and other cells that mediate immune reactions. The binding of an allergen molecule to 2 receptor-bound IgE molecules results in the crosslinking of the pair of IgE molecules. The crosslinking of sufficient numbers of IgE pairs bound to the receptors on the surface of a mast cell results in degranulation of the mast cell and the release of vasoactive amines, which are responsible for the Type 1 reaction. For some hydrolyzed proteins, the minimum number of amino acids (or weight-average MW) to elicit Type 1 hypersensitivity has been demonstrated with experimental data. For example, studies on hydrolyzed wheat protein show that hydrolysates with MWs less than 3500 Da do not have the properties required to induce Type 1 hypersensitivity.[9] Conclusive studies that detail the number of amino acids needed to trigger mast cell degranulation for hydrolyzed fish proteins, however, were not identified.

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).[57] Codfish-specific IgE was also measured in the patients and they underwent double-blinded, placebo-controlled food challenges with fish Gelatin. The fish Gelatin used for the study was made through acid extraction of codfish skins and had an average molecular weight of 60 000 Da. 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 2 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.[58] Serum IgE antibodies were tested against Gelatin and 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

Dermal irritation studies are presented in Table 8.[27,59-68] No irritation was predicted in in vitro studies of Hydrolyzed Collagen (bovine sourced; up to 55%), Soluble Collagen (fish and bovine sourced; undiluted), and Hydrolyzed Elastin (fish and bovine sourced; undiluted). No irritation was observed in rabbits or guinea pigs treated with Hydrolyzed Collagen (fish sourced; 30% solution) and Hydrolyzed Elastin (source not reported; tested neat). Hydrolyzed Collagen (bovine, swine, and fish sourced) was not irritating in human studies at concentration up to 50% in water solution.

Animal

Hydrolyzed collagen. Primary skin irritation tests in rabbits indicated that Hydrolyzed Collagen was nonirritating or minimally irritating when tested at up to 100%.[2]

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.[2]

Sensitization

Animal

Hydrolyzed collagen. Hydrolyzed Collagen was non-sensitizing in guinea pig studies at up to 2%.[2]

Hydrolyzed Collagen (30% solution in water; fish scale sourced; MW ~400 Da) was considered to be non-sensitizing in a guinea pig maximization test using 15 Hartley guinea pigs and performed in accordance to OECD TG 406.[27] The animals received 7.5% active ingredient intradermally with Freund's complete adjuvant during the first induction, while undiluted test material was applied to clipped dorsal skin under occlusive 48 h patch during the second induction. The animals were challenged with undiluted test material on clipped flank skin under occlusive 24 h patch. No reactions were observed in any animal 24 h and 48 h post-challenge patching.

Human

Hydrolyzed collagen. Formulations containing 0.5% to 28% Hydrolyzed Collagen produced some irritation but no sensitization in human repeated insult patch tests (HRIPTs).[2]

In a HRIPT with 50 subjects, Hydrolyzed Collagen (20% solution in water; fish scale sourced; MW ~400 Da) was not sensitizing.[27] The test material (0.2 mL) was applied to infrascapular skin with occlusive patches.

A study of sensitization to protein hydrolysates in hair care products was performed in 3 groups of patients.[69] 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 1 trademarked version of Hydrolyzed Collagen (1% solution), while 1 other had a reaction to hydroxypropyl trimonium hydrolyzed milk protein.[69]

Hydrolyzed Elastin

In an HRIPT with 52 subjects, Hydrolyzed Elastin (25% w/v in corn oil; MW = 3000 Da) did not produce dermal irritation or dermal sensitization.[70] The test patches were occlusive.

Phototoxicity

Hydrolyzed collagen. Hydrolyzed Collagen at up to 2% was not phototoxic to guinea pigs and rabbits, nor was it phototoxic or photosensitizing to humans at up to 0.5%.[2] UV-induced erythema was decreased after application of 10% solution of Hydrolyzed Collagen (MW = 1500 Da) onto the skin after irradiation.

Ocular Irritation Studies

Ocular irritation studies are presented in Table 9.[27,59-68] Hydrolyzed Collagen (fish and bovine sourced; up to 55%), Soluble Collagen (fish and bovine sourced; undiluted), and Hydrolyzed Elastin (fish and bovine sourced; undiluted) were predicted to be minimally or non-irritating in in vitro studies. Hydrolyzed Collagen (fish, swine, and bovine sourced; up to 30%) and Hydrolyzed Elastin (source not reported; tested neat) were not irritating in rabbit studies.

Animal

Hydrolyzed collagen. Hydrolyzed Collagen was minimally irritating to rabbit eyes when tested full-strength.[2]

Clinical Studies

Case Reports

Elastin. A 26-yr-old woman with a history of fish allergy experienced urticarial eruptions following use of a cosmetic cream containing codfish-derived Elastin.[71] The patient's serum total IgE level was 442 kU/l, and strong radioallergosorbent test (RAST) 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.

Atelocollagen and hydrolyzed collagen. A 30-yr-old woman with a history of atopic dermatitis experienced anaphylaxis twice on separate occasions, once after consuming a fortified yogurt containing fish-sourced Hydrolyzed Collagen and once after consuming a gummy candy containing fish-sourced Hydrolyzed Collagen.[72] Fifteen months prior to the anaphylactic episodes, the patient had been applying a moisturizer containing Atelocollagen derived from fish 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 fish-sourced Hydrolyzed Collagen in the food products, the moisturizer, 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 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 fish-sourced Hydrolyzed Collagen protein. The researchers of this case study speculated that the 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.

Hydrolyzed collagen. A 22-yr-old female reported contact urticaria following use of a hair conditioner that contained steartrimonium hydrolyzed animal protein.[73] 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 kU/l. RASTs were negative to pork, beef, chicken, and mutton.

Summary

This report assesses the safety of 19 skin and connective 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 is not repeated below.

Ingredients with the greatest number of reported uses in 2017 are Hydrolyzed Collagen (543 formulations) and Soluble Collagen (425 formulations); the majority of uses are in leave-on skin care products. Gelatin is used in a total of 334 formulations; 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. The other in-use ingredients are used at much lower concentrations.

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 wk 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-mo study of a dietary supplement containing a 250 mL dose of Hydrolyzed Collagen in human children

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

No dermal irritation was predicted based on in vitro studies of Hydrolyzed Collagen (bovine sourced; up to 55%), Soluble Collagen (fish and bovine sourced; undiluted), and Hydrolyzed Elastin (fish and bovine sourced; undiluted). No dermal irritation was observed in rabbits or guinea pigs treated with Hydrolyzed Collagen (fish sourced; 30% solution) and Hydrolyzed Elastin (source not reported; tested neat). Hydrolyzed Collagen (bovine, swine, and fish sourced) was not irritating in human dermal studies at concentrations up to 50% in water solution.

A guinea pig maximization test found Hydrolyzed Collagen (fish sourced; 30% solution in water) to be non-sensitizing. In HRIPT studies, Hydrolyzed Elastin (25% w/v in corn oil) and Hydrolyzed Collagen (fish sourced; 20% solution in water) did not produce dermal irritation or dermal sensitization. Hydrolyzed Collagen produced positive results in skin prick tests of dermatitic patients.

Hydrolyzed Collagen (fish and bovine sourced; up to 55%), Soluble Collagen (fish and bovine sourced; undiluted), and Hydrolyzed Elastin (fish and bovine sourced; undiluted) were predicted to be minimally or non-irritating in ocular in vitro studies. Hydrolyzed Collagen (fish, swine, and bovine sourced; up to 30%) and Hydrolyzed Elastin (source not reported; tested neat) were not irritating in rabbit ocular studies.

Case reports of dermal sensitization to cosmetics containing Elastin, Atelocollagen, 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 or carcinogenicity studies on skin and connective tissue-derived proteins and peptides were identified in a literature search for these ingredients, and no unpublished data were submitted.

Discussion

The Panel noted that there was a lack of systemic toxicity data (ie reproductive and developmental toxicity, genotoxicity, and carcinogenicity data); however, the Panel was not concerned that these proteins and peptides would cause adverse systemic effects in the general population. These proteins and peptides, similar to the other proteins and peptides reviewed by the Panel, are found in food, and daily exposures from the consumption of food can be expected to yield much larger systemic exposures to these ingredients than those from use in cosmetic products. The Panel also found that the earlier assessments of Hydrolyzed Collagen supported the safety of these ingredients in cosmetic products.

The Panel noted that fish proteins are known food allergens that can elicit Type 1 immediate hypersensitivity reactions when ingested by sensitized individuals. The Panel expressed concern that sensitized individuals would not easily recognize cosmetic products containing fish-derived collagen based on the current naming conventions used in the ingredient lists on product labels (eg, Collagen and Hydrolyzed Collagen may be sourced from fish, though "fish" is not in the ingredient name). In the absence of negative Type 1 immediate hypersensitivity data for fish-derived protein ingredients (or other information supporting an inability of the supplied ingredient to elicit such sensitization (eg, a maximum peptide length that is shorter than the minimum IgE-binding epitopes)), the Panel advised manufacturers to label products containing these fish-derived ingredients to inform individuals sensitized to fish proteins.

The Panel was also concerned about the inherent risks of using animal-derived ingredients in cosmetic products, namely the potential for transmission of infectious agents. While Gelatin and Collagen prepared exclusively from hides and skins do not have the propensity to carry disease, the Panel stressed that these ingredients must be free of detectible infectious pathogens

(ie, BSE) if these materials are derived from other bovine materials. Raw material suppliers and formulators of these ingredients must assure that these ingredients are free from pathogenic viruses and other infectious agents.

The Panel discussed the issue of incidental inhalation exposure from hair care products, skin care preparations, face powders, and fragrances. There were no inhalation toxicity data available. Although the Panel noted that droplets/particles from spray and loose-powder cosmetic products would not be respirable to any appreciable amount, the potential for inhalation toxicity is not limited to respirable droplets/particles deposited in the lungs. In principle, inhaled droplets/particles deposited in the nasopharyngeal and thoracic regions of the respiratory tract may cause toxic effects depending on their chemical and other properties. However, coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

Conclusion

The Expert Panel for Cosmetic Ingredient Safety concluded that the 19 skin and connective tissue-derived proteins and peptides listed below are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

Ammonium Hydrolyzed Collagen
Atelocollagen
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*

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

Author Contributions

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

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Author's Note

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

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.6
Revision Date 28.10.2022
Print Date 08.05.2023
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name	:	Gelatin
Product Number	:	1288485
Brand	:	US Pharmacopeia
REACH No.	:	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No.	:	9000-70-8

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

Identified uses	:	Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Sigma-Aldrich Chemie GmbH Industriestrasse 25 CH-9471 BUCHS
Telephone	:	+41 81 755 2511
Fax	:	+41 81 756 5449
E-mail address	:	technischerservice@merckgroup.com

1.4 Emergency telephone

Emergency Phone #	:	+41 43-508-2011 (CHEMTREC) +41 44-251-5151 (Tox-Zentrum) 145(Tox Info Suisse)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008.

2.2 Label elements

Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008.

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

CAS-No. : 9000-70-8

No components need to be disclosed according to the applicable regulations.

SECTION 4: First aid measures

4.1 Description of first-aid measures

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration.

In case of skin contact
Wash off with soap and plenty of water.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture

Nature of decomposition products not known.

5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information

No data available

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Avoid dust formation. Avoid breathing vapors, mist or gas.
For personal protection see section 8.

6.2 Environmental precautions

No special environmental precautions required.

6.3 Methods and materials for containment and cleaning up

Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Advice on protection against fire and explosion
Provide appropriate exhaust ventilation at places where dust is formed.

Hygiene measures
General industrial hygiene practice.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
Keep container tightly closed in a dry and well-ventilated place. Store in cool place.

Storage class
Storage class (TRGS 510): 13: Non Combustible Solids

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

8.2 Exposure controls

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

The selected protective gloves have to satisfy the specifications of Regulation (EU) 2016/425 and the standard EN 374 derived from it.

Body Protection
Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
No special environmental precautions required.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Physical state	powder
b)	Color	light yellow
c)	Odor	No data available
d)	Melting point/freezing point	No data available
e)	Initial boiling point and boiling range	No data available
f)	Flammability (solid, gas)	No data available
g)	Upper/lower flammability or explosive limits	No data available
h)	Flash point	Not applicable
i)	Autoignition temperature	No data available
j)	Decomposition temperature	No data available
k)	pH	4,0 - 7 at 66,7 g/l at 60 °C
l)	Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
m)	Water solubility	No data available
n)	Partition coefficient: n-octanol/water	No data available
o)	Vapor pressure	No data available
p)	Density	No data available
	Relative density	No data available
q)	Relative vapor density	No data available
r)	Particle characteristics	No data available
s)	Explosive properties	Not classified as explosive.
t)	Oxidizing properties	none

9.2 Other safety information

No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
No data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
No data available

10.5 Incompatible materials
Strong oxidizing agents

10.6 Hazardous decomposition products
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - Rat - > 5.000 mg/kg
Remarks: (External MSDS)
Inhalation: No data available
Dermal: No data available

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitization
No data available

Germ cell mutagenicity
No data available

Carcinogenicity
No data available

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

11.2 Additional Information

Endocrine disrupting properties

Product:

Assessment The substance/mixture does not contain

components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

No toxic effects are to be expected when the product is handled appropriately.

Inhalation of the dusts should be avoided as even inert dusts may impair respiratory organ functions.

Handle in accordance with good industrial hygiene and safety practice.

SECTION 12: Ecological information

12.1 Toxicity
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Endocrine disrupting properties

Product:

Assessment	:	The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

12.7 Other adverse effects
No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product
Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging
Dispose of as unused product.

SECTION 14: Transport information

14.1 UN number

ADR/RID: - IMDG: - IATA: -

14.2 UN proper shipping name

ADR/RID: Not dangerous goods
IMDG: Not dangerous goods
IATA: Not dangerous goods

14.3 Transport hazard class(es)

ADR/RID: - IMDG: - IATA: -

14.4 Packaging group

ADR/RID: - IMDG: - IATA: -

14.5 Environmental hazards

ADR/RID: no IMDG Marine pollutant: no IATA: no

14.6 Special precautions for user

Further information

Not classified as dangerous in the meaning of transport regulations.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of other abbreviations

ADN - European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways; ADR - Agreement concerning the International Carriage of Dangerous Goods by Road; AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RID - Regulations concerning the International Carriage of Dangerous Goods by Rail; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.3
Revision Date 20.07.2023
Print Date 24.11.2023
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name	:	Gelatin, from porcine skin
Product Number	:	G2500
Brand	:	Sigma
REACH No.	:	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No.	:	9000-70-8

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

Identified uses	:	Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Merck Life Science Sp.z.o.o. Szelągowska 30 PL-61-626 POZNAN
Telephone	:	+48 61 8290-100
Fax	:	+48 61 8290-120
E-mail address	:	TechnicalService@merckgroup.com

1.4 Emergency telephone

Emergency Phone #	:	+(48)-223988029 (CHEMTREC) 112 (numer alarmowy)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008.

2.2 Label elements

No hazard pictogram, no signal word, no hazard statement(s), no precautionary statement(s) required

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Ecological information:
The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

Toxicological information:
The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

CAS-No.	:	9000-70-8
EC-No.	:	232-554-6

No components need to be disclosed according to the applicable regulations.

SECTION 4: First aid measures

4.1 Description of first-aid measures

If inhaled
After inhalation: fresh air.

In case of skin contact
In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact
After eye contact: rinse out with plenty of water. Remove contact lenses.

If swallowed
After swallowing: make victim drink water (two glasses at most). Consult doctor if feeling unwell.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Water Foam Carbon dioxide (CO2) Dry powder

Unsuitable extinguishing media
For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture
Nature of decomposition products not known.
Combustible.
Development of hazardous combustion gases or vapours possible in the event of fire.

5.3 Advice for firefighters
In the event of fire, wear self-contained breathing apparatus.

5.4 Further information
none

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Advice for non-emergency personnel: Avoid inhalation of dusts. Evacuate the danger area, observe emergency procedures, consult an expert.
For personal protection see section 8.

6.2 Environmental precautions
No special precautionary measures necessary.

6.3 Methods and materials for containment and cleaning up
Observe possible material restrictions (see sections 7 and 10). Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
Tightly closed. Dry.

Keep in a dry place.

Storage class
Storage class (TRGS 510): 11: Combustible Solids

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

8.2 Exposure controls

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

Skin protection
This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).
Full contact
Material: Nitrile rubber
Minimum layer thickness: 0,11 mm
Break through time: 480 min
Material tested:KCL 741 Dermatril® L

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).
Splash contact
Material: Nitrile rubber
Minimum layer thickness: 0,11 mm
Break through time: 480 min
Material tested:KCL 741 Dermatril® L

Respiratory protection
required when dusts are generated.
Our recommendations on filtering respiratory protection are based on the following standards: DIN EN 143, DIN 14387 and other accompanying standards relating to the used respiratory protection system.
Recommended Filter type: Filter type P1

The entrepeneur has to ensure that maintenance, cleaning and testing of respiratory protective devices are carried out according to the instructions of the producer. These measures have to be properly documented.

Control of environmental exposure
No special precautionary measures necessary.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Physical state	powder
b)	Color	light yellow
c)	Odor	No data available
d)	Melting point/freezing point	No data available
e)	Initial boiling point and boiling range	No data available
f)	Flammability (solid, gas)	No data available
g)	Upper/lower flammability or explosive limits	No data available
h)	Flash point	Not applicable
i)	Autoignition temperature	No data available
j)	Decomposition temperature	No data available
k)	pH	4,0 - 7 at 66,7 g/l at 60 °C
l)	Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
m)	Water solubility	No data available
n)	Partition coefficient: n-octanol/water	No data available
o)	Vapor pressure	No data available
p)	Density	No data available
	Relative density	No data available
q)	Relative vapor density	No data available
r)	Particle characteristics	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	none

9.2 Other safety information

No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
The following applies in general to flammable organic substances and mixtures: in correspondingly fine distribution, when whirled up a dust explosion potential may generally be assumed.

10.2 Chemical stability
The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions
Violent reactions possible with:
strong oxidising agents

10.4 Conditions to avoid
Exposure to moisture may affect product quality.
no information available

10.5 Incompatible materials
No data available

10.6 Hazardous decomposition products
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - Rat - > 5.000 mg/kg
Remarks: (External MSDS)
Inhalation: No data available
Dermal: No data available

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitization
No data available

Germ cell mutagenicity
No data available

Carcinogenicity
No data available

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

11.2 Additional Information

Endocrine disrupting properties

Product:

Assessment	The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

RTECS: LX8580000
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

No toxic effects are to be expected when the product is handled appropriately.

Inhalation of the dusts should be avoided as even inert dusts may impair respiratory organ functions.

Handle in accordance with good industrial hygiene and safety practice.

SECTION 12: Ecological information

12.1 Toxicity
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Endocrine disrupting properties

Product:

Assessment	:	The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

12.7 Other adverse effects
No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods
No data available

SECTION 14: Transport information

14.1 UN number
ADR/RID: - IMDG: - IATA: -

14.2 UN proper shipping name
ADR/RID: Not dangerous goods
IMDG: Not dangerous goods
IATA: Not dangerous goods

14.3 Transport hazard class(es)
ADR/RID: - IMDG: - IATA: -

14.4 Packaging group
ADR/RID: - IMDG: - IATA: -

14.5 Environmental hazards
ADR/RID: no IMDG Marine pollutant: no IATA: no

14.6 Special precautions for user
No data available

Further information

Not classified as dangerous in the meaning of transport regulations.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture
This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.2 Chemical Safety Assessment
For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of other abbreviations

ADN - European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways; ADR - Agreement concerning the International Carriage of Dangerous Goods by Road; AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RID - Regulations concerning the International Carriage of Dangerous Goods by Rail; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative

Further information

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