Safety Assessment of Hyaluronates as Used in Cosmetics

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*All interested persons are provided 60 days from the above release date (i.e., **May 15, 2023**) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to the Cosmetic Ingredient Review (CIR) will be discussed in open meetings, will be available for review by any interested party, and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.*

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ABBREVIATIONS

ADME	absorption, distribution, metabolism, and excretion
ARE	antioxidant response element
BCOP	bovine corneal opacity and permeability
BDDE	1,4-butanediol diglycidyl ether
CAMVA	chorioallantoic membrane vascular assay
CAS	Chemical Abstracts Service
CD44	cluster of differentiation 44
CFR	Code of Federal Regulations
cGMP	current good manufacturing practices
cfu	colony forming units
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Da	dalton
DART	developmental and reproductive toxicity
DMSO	dimethyl sulfoxide
DPRA	direct peptide reactivity assay
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
EU	endotoxin units
FCC	*Food Chemicals Codex*
FDA	Food and Drug Administration
FIGO	International Federation of Gynecology and Obstetrics
FW	formula weight
HA	Hyaluronic Acid
h-CLAT	human cell line activation test
HRIPT	human repeated insult patch test
I-NOSE	Nasal Obstruction Symptom Evaluation Instrument
I_{max}	maximum response value
K_{ow}	n-octanol/water partition coefficient
kDa	kiloDaltons
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
Log K_{ow}	n-octanol/water partition coefficient
MBq	megabecquerels
MDa	megadaltons
MW	molecular weight
MTD	maximum tolerable dose
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
ND	not detected
Nfr2	nuclear factor erythroid 2-related factor 2
NIBUT	non-invasive break-up time
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOAEL	no-observed-adverse-effect-level
NOEL	no-observed-effect-level
NR	not reported
OECD	Organisation for Economic Cooperation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate-buffered saline
PSL	photo-stimulated luminescence
RFI	relative fluorescence intensity
SDS	sodium dodecyl sulfate
SPECT	single photon emission computed tomography
TG	test guidelines
US	United States
UVB	ultraviolet light B (mid-wavelength)
VCRP	Voluntary Cosmetic Registration Program
wINCI; *Dictionary*	web-based *International Cosmetic Ingredient Dictionary and Handbook*

ABSTRACT

The Expert Panel for Cosmetic Safety (Panel) assessed the safety of 7 hyaluronate ingredients, of which 3 were previously reviewed, as used in cosmetic formulations. These ingredients are reported to function in cosmetics as skin and/or hair conditioning agents and humectants. The Panel noted that these ingredients may be derived from animal-sources (i.e., rooster combs), and stressed that such ingredients must be free of detectable pathogenic viruses, infectious agents, and/or biologically-derived impurities (e.g., nucleic acids, proteins, endotoxins). Industry should continue to use good manufacturing practices to limit impurities that could be present in these ingredients. The Panel reviewed all relevant data and concluded that these 7 ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This assessment reviews the safety of the following 7 ingredients as used in cosmetic formulations:

Hyaluronic Acid*
Hydrolyzed Calcium Hyaluronate
Hydrolyzed Hyaluronic Acid
Hydrolyzed Sodium Hyaluronate
Potassium Hyaluronate*
Sodium Acetylated Hyaluronate
Sodium Hyaluronate*

* previously reviewed by the Panel

Sodium Acetylated Hyaluronate and Hydrolyzed Hyaluronic Acid were included on the 2022 Priority List due to high reported frequencies of use in the US Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP). Three structurally-similar ingredients (i.e., Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate) have previously been reviewed by the Panel in a safety assessment that was published in 2009.[1] Accordingly, in that these ingredients would soon be considered for re-review, the Panel deemed it appropriate to include the 3 previously-reviewed ingredients in this safety assessment. Additionally, two hydrolyzed salts of Hyaluronic Acid are included in this grouping. Hence, all ingredients reviewed in this report are structurally similar as they are salts or acetylated esters derived from Hyaluronic Acid (Hydrolyzed Calcium Hyaluronate and Hydrolyzed Sodium Hyaluronate).

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), the majority of ingredients included in this assessment are reported to function in cosmetics as skin and/or hair conditioning agents (Table 1).[2] Sodium Acetylated Hyaluronate is reported to function in cosmetics only as a humectant.

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 the 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.

In its original 2009 review of Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate, the Panel concluded that these 3 ingredients are safe in the present practices of use and concentration, as described in the safety assessment.[1] Excerpts from the 2009 report are disseminated throughout the report, as appropriate, and are *identified by italicized text.* (This information is not included in the tables or the Summary section.) Accordingly, for these 3 ingredients, an exhaustive search of the world's literature was performed for studies dated 2004 forward (to February 2023), and relevant new data were included.

Information on cross-linked hyaluronic acid dermal fillers is available in the published literature. However, it should be noted that cross-linked hyaluronic acid ingredients are assigned separate INCI names, and these ingredients are not reviewed in this report. Accordingly, data on crosslinked hyaluronic acid ingredients are not included in this safety assessment. In addition, it should be noted that safety and efficacy data regarding Hyaluronic Acid (non-cross-linked and cross-linked) used as dermal fillers, as well in surgical procedures and arthritic therapy were found; however, with the exception of reference to studies regarding hypersensitivity reactions to injectable Hyaluronic Acid (which can be found in the Clinical Studies section), the other studies are not summarized in this report as no relevance to cosmetic use could be surmised, as exposure to Hyaluronic Acid and its derivatives would be topical when used in cosmetics.

CHEMISTRY

Definition and Structure

Hyaluronic Acid (CAS No. 9004-61-9; Figure 1) is a linear glycosaminoglycan composed of repeating disaccharides of β4-glucuronic acid-β3-*N*-acetylglucosamine.[3] The remaining ingredients in this report are derivatives of Hyaluronic Acid (e.g., Sodium Hyaluronate (CAS No. 9067-32-7) is a sodium salt of Hyaluronic Acid). The definitions of the ingredients included in this review are provided in Table 1.

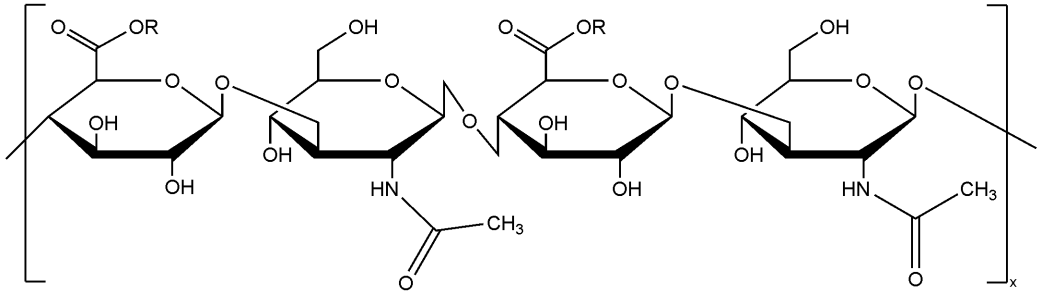

Figure 1. Hyaluronates (when R is hydrogen = Hyaluronic Acid; when R is sodium = Sodium Hyaluronate; etc.)

Chemical Properties

Hyaluronic Acid is a water-soluble substance that is available as a highly purified, freeze-dried powder or aqueous solution.[1] Hyaluronic Acid may also be presented as its potassium or sodium salt (i.e., Potassium Hyaluronate or Sodium Hyaluronate). The molecular weight (MW) of Hyaluronic Acid in cosmetics is highly variable and ranges from 5 – 1800 kiloDaltons (kDa), dependent upon manufacturing procedures. Hyaluronic Acid has a high capacity for water retention; 1 g of Hyaluronic Acid can hold up to 6 l of water.

These hyaluronates have a wide range of MW. For instance, according to the *Food Chemicals Codex* (*FCC*), the formula weight (FW) of Sodium Hyaluronate can vary from 80.2 to 4010 kDa. Other chemical properties of Hyaluronic Acid and Sodium Hyaluronate can be found in Table 2.

Method of Manufacture

Hyaluronic Acid is an ubiquitous substance that can be derived from several natural sources.[1] These sources can be found in the Natural Occurrence section of this report. According to unpublished data, Hyaluronic Acid obtained for cosmetic use is derived via either bacterial fermentation or extraction from rooster combs.[1]

Hyaluronic Acid

In order to manufacture Hyaluronic Acid from rooster combs, the frozen tissue is first thoroughly washed with water, acetone, ethanol, or a mixture of ethanol and chloroform.[4] The tissues are then grounded and extracted with a solvent. Examples of solvents include distilled water, salt solutions, and aqueous-organic mixtures. The substance then undergoes purification to remove potential impurities such as proteins, peptides, lipids, nucleic acids, mucopolysaccharides, and low MW precursors. Purification can be performed via extraction using ethanol, acetone, acetic acid, or a double volume of ethanol with sodium acetate. Proteins are typically removed using a water-chloroform or chloroform-iso-amyl alcohol extraction, followed by intensive stirring. In order to remove covalently bonded peptides and proteins, proteolytic enzymes such as pepsin, trypsin, papain, or pronase, may be used. A fractional precipitation with cetylpyridinium chloride followed by dissolution with sodium chloride may be performed to remove mucopolysaccharides from the final product. Polysaccharides can be removed with ion-exchange chromatography, cellulose, and gel-filtration. Other purification methods include ultrafiltration, sorption on the activated carbon, ion-exchange resin, electrodialysis, electrophoresis, and ultracentrifusion with cesium chloride.

Hyaluronic Acid derived from bacterial strains (e.g., *Streptococcus* sp.) involve the cultivation of these bacteria in conditions where the polysaccharide capsule containing Hyaluronic Acid is formed.[4] The cultural liquid containing accumulated Hyaluronic Acid is then ultrafiltrated, precipitated with an organic solvent, and purified using similar methods as described above for rooster comb-derived Hyaluronic Acid.

Hydrolyzed Hyaluronic Acid

Hydrolyzed Hyaluronic Acid (MW = 37 – 56 megaDaltons (MDa)) is manufactured via similar methods as stated below (see manufacturing process of Hydrolyzed Sodium Hyaluronate (FW = 5 - 10 kDa)).[5] However, when manufacturing Hydrolyzed Hyaluronic Acid, the Hyaluronic Acid product is mixed with ethanol and hydrochloric acid. When the Hyaluronic Acid is degraded to the set point, the pH is adjusted via a sodium hydroxide solution. Finally, the resulting solution is dehydrated and dried, yielding the final product.

Hydrolyzed Sodium Hyaluronate

Hydrolyzed Sodium Hyaluronate (formula weight (FW) = 5 - 10 kDa) manufactured for cosmetic use may be produced from the bacterial strain *Streptococcus equi* subsp. *zooepidemicus*.[6] The process begins with the preparation of a seed broth prepared from seed culture, which is transferred from a fermenter containing sterilized fermentation medium. After fermentation, the seed broth is mixed with ethanol. The crude Sodium Hyaluronate precipitate is dissolved in water and filtered to remove impurities and inactivated fragments. The resulting filtrate is precipitated, dehydrated, and dried, yielding

the Hyaluronic Acid product. This product is dissolved in purified water and combined with hyaluronidase to create a solution that is then degraded, heated, filtered, precipitated, dehydrated, and dried.

Low-FW Hydrolyzed Sodium Hyaluronate (FW = 1 - 5 kDa) is produced via the hydrolysis by hyaluronidase from low MW Sodium Hyaluronate.[6] This process includes dissolution, enzymatic hydrolysis, inactivation, filtration, spray drying, sieving, and packaging. In order to produce Hydrolyzed Sodium Hyaluronate of a very low FW (FW < 1 kDa), Sodium Hyaluronate (FW > 1 MDa) undergoes enzymatic hydrolysis via purified water and hyaluronidase.[7] The resulting solution is ultrafiltrated and heated to denature and remove the remaining hyaluronidase. Activated carbon is then used to absorb the denatured hyaluronidase, and the residual hyaluronidase is removed via the removal of activated carbon through multistage filtration. The resulting filtrate is dried, yielding the final product.

Sodium Hyaluronate

According to a supplier, Sodium Hyaluronate, is manufactured via a similar process to that stated above for Hydrolyzed Sodium Hyaluronate (FW = 5 - 10 kDa; omitting enzymatic hydrolysis) using the bacterial strain *Streptococcus equi* subsp. *zooepidemicus*.[8,9] The manufacturing process for low MW Sodium Hyaluronate is the same; however, when manufacturing low MW Sodium Hyaluronate, the seed broth is degraded prior to mixing with ethanol.[10]

Impurities

When derived from animal sources, Hyaluronic Acid may contain several impurities.[1] These impurities include proteins, DNA, and chondroitin sulfate.

Hyaluronic Acid

The impurities (nucleic acid, protein, endotoxins) of Hyaluronic Acid obtained from several sources (e.g., *Streptococcus zooepidemicus*, rooster comb, bovine vitreous, human umbilical cord) were evaluated.[11] Nucleic acid and protein impurities were highest in human umbilical cord- and bovine vitreous-derived Hyaluronic Acid, and were lowest in bacterial- and rooster comb-derived Hyaluronic Acid. Human umbilical cord-, bovine vitreous-, and rooster comb-derived Hyaluronic Acid preparations contained high levels of endotoxin contaminants. Bacterially-derived Hyaluronic Acid was nearly endotoxin-free. The specific levels of impurities evaluated in these samples can be found in Table 3.

Hydrolyzed Hyaluronic Acid

A supplier reported that Hydrolyzed Hyaluronic Acid (MW = 37 - 56 kDa) contained < 0.5 endotoxin units (EU)/mg, < 0.05% protein, < 0.5% chlorides, < 20 ppm total metals, and < 2 ppm arsenic.[12]

Hydrolyzed Sodium Hyaluronate

The same supplier as referenced above reported that several Hydrolyzed Sodium Hyaluronate ingredients of different FWs (<1 kDa, 1 - 5 kDa, and 5 - 10 kDa) contained < 0.5 EU/mg, < 0.05% protein, < 0.5% chlorides, and ≤ 10 - 20 ppm total metals.[13]

Sodium Hyaluronate

According to a manufacturer, Sodium Hyaluronate contained < 5000 ppm residual solvents (ethanol), < 20 ppm heavy metals, < 2 ppm arsenic, and < 0.1% protein.[14] A different manufacturer reported that both Sodium Hyaluronate (FW ≥ 1 MDa) and low FW Sodium Hyaluronate (FW = 100 kDa -1 MDa) contained < 0.5 EU/mg, < 0.05% protein, < 0.5% chlorides, and ≤ 20 ppm total metals.

The *FCC* states that Sodium Hyaluronate manufactured for use in foods may not contain more than 1 mg/kg lead, 2 mg/kg arsenic, or 0.5% chloride.[15] A manufacturer of food-use Sodium Hyaluronate states that potential contaminants of Sodium Hyaluronate include microbes and heavy metals.[16] This manufacturer requires a purity level of ≥ 93% Sodium Hyaluronate, and maximum lead and arsenic levels of 1 and 2 ppm, respectively. The same manufacturer also requires bacteria counts of ≤ 500 colony forming units (cfu)/g, yeast and mold counts of ≤ 100 cfu/g, and negative test readings for *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* sp.

Natural Occurrence

Hyaluronic Acid and its derivatives can be found distributed throughout vertebrate tissues such as the brain, vitreous humor, umbilical cord, synovial fluid, skin, rooster combs, neural tissues, and epithelium.[3,17] Hyaluronic Acid is also a signaling molecule involved in biological processes such as embryonic development, wound healing, inflammation, and cancer. In addition, Hyaluronic Acid can be found in the extracellular capsule formed by gram-positive microorganisms such as *Streptococcus* sp. and *Pasteurella* sp..[4]

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US FDA and the cosmetics industry on the expected use of these ingredients in cosmetics, and does not cover their use in

airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's VCRP database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2022 FDA VCRP data, Sodium Hyaluronate has the highest frequency of use (4048 total formulations; 3680 leave-on formulations, 366 rinse-off formulations, and 2 formulations diluted for bath use; Table 4).[18] This use of this ingredient has increased significantly since it was last reviewed; it was reported to be used in 601 formulations in 2005.[1] All other ingredients are reported to be used in 568 formulations or less. The results of the 2021 concentration of use survey conducted by the Council indicate Sodium Hyaluronate also has the highest concentration of use; it is used at up to 7.5% in face and neck products (not spray).[19] In 2005, Sodium Hyaluronate was reported to be used at up to 2%. Current FDA VCRP data on the four hyaluronate ingredients included in this report that have not been previously reviewed (i.e., Hydrolyzed Calcium Hyaluronate, Hydrolyzed Hyaluronic Acid, Hydrolyzed Sodium Hyaluronate, and Sodium Acetylated Hyaluronate) can be found in Table 5.

Incidental ingestion of several of these ingredients may occur as they are reported to be used in lipstick formulations (e.g., Sodium Hyaluronate is used in lipsticks at up to 0.39%). In addition, these ingredients are also reported to be used in products that are applied near the eye; for example, Sodium Hyaluronate is used in eye shadows at up to 0.96%. Sodium Hyaluronate is also used in baby products at up to 0.005%.

Some of these hyaluronate ingredients are used in cosmetic sprays and powders, and could possibly be inhaled; for example, Sodium Hyaluronate is reported to be used at up to 0.01% in other skin care preparations (spray) and at up to 0.099% in face powders. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. 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.

Although products containing some of these ingredients may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

All of the hyaluronate ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[20]

Non-Cosmetic

Hyaluronic Acid and Sodium Hyaluronate are reported to be used in FDA-approved medical devices as dermal fillers, surgical fluids, topical wound creams, osteoarthritis treatments, periodontitis treatments, and ophthalmic surgery aids.[21] In addition, Sodium Hyaluronate is used as in inactive ingredient in several FDA-approved medications.[22] These medications include injectable intra-articular, intramuscular, and intravitreal treatments containing Sodium Hyaluronate at up to 2.3% for various conditions such as arthritis and hypotony. Sodium Hyaluronate is also used in FDA-approved topical medications at up to 0.01% as a skin lubricant. In addition to human medicine, Sodium Hyaluronate has FDA-approved uses in veterinary medicine as an implantable or injectable treatment for joint ailments, as indicated in CFR 522.1145.[23]

Hyaluronic Acid is found as a natural component in foods as it is present in animal products.[16] Rooster combs, which are rich in Hyaluronic Acid, are eaten alone or in dishes such as chicken soups or stews in European countries. Sodium Hyaluronate is used as an ingredient in food (e.g., ready-to-eat cereal preparations and candies) and beverages including fruit drinks, soft drinks, milk, and milk products.[16] In addition, both Hyaluronic Acid and Sodium Hyaluronate are reported to be ingredients of dietary supplements on the market in the US.[24]

TOXICOKINETIC STUDIES

Dermal Penetration

Autoradiography was used to detect the dermal penetration of Hyaluronic Acid (in the form of [^{3}H]hyaluronan) in SKh/1 hairless mice (4 animals/group; sex not stated; one group treated with radioactive gel; one group treated without radioactive gel).[1] Mice were treated for either 3 or 12 total applications (12 h intervals). Twelve to 16 h after the last application, animals were examined. Radioactivity was found mainly in the dermis, from the outermost layer to the

lymphatic and blood vessels. In a second experiment using 10 mice (strain and sex not reported; performed according to similar procedures), radioactivity was found in the same distribution within the dermis. In both assays, grains were found in the keratinized layer of the skin and hair follicles. In a dermal penetration assay performed in 11 Sprague-Dawley rats, Hyaluronic Acid (1.35 – 4.5 kDa) was applied dermally, twice daily, for 5 d. The Hyaluronic Acid penetrated to a maximum depth of 136 µm beneath the epidermis. In a different assay, radiolabeled Hyaluronic Acid was placed on the back of one Sprague-Dawley rat (sex not stated; singular dose). After a 4-h absorption period, the test substance was found to penetrate the rat skin to a maximum depth of approximately 800 µm. Autoradiography was used to detect the dermal penetration of [^{3}H]Hyaluronic Acid gel (56.3 – 56.4 mg) in the forearm of one male subject (2 total applications 12 h apart; skin removed by biopsy 7 h after last treatment). The test substance was shown to disseminate through all layers of the skin.

Hyaluronic Acid

The dermal penetration of three Hyaluronic Acid solutions, with three different MW (20 - 50 kDa, 100 - 300 kDa, and 1000 - 1400 kDa), was evaluated via Raman microimaging.[25] Test solutions contained 1% Hyaluronic Acid in distilled water. The solution (300 µl) was placed on human dermatomed skin samples for 8 h. Control skin samples were treated with either water (negative control) or glycerin (positive control). After the diffusion period, the skin surface was cleaned, samples were frozen, 10 µm-thick transverse skin sections were obtained, and spectral images were recorded. Spectral images revealed that the Hyaluronic Acid solution with the lowest MW (20 – 50 kDa) was present in the skin section at around 100 µm (full epidermal depth). The Hyaluronic Acid solution with a MW range of 100 - 300 kDa was present at an epidermal depth of approximately 50 µm. Permeation did not exceed 25 µm for the 1 – 14 MDa Hyaluronic Acid solution. The majority of each of the Hyaluronic Acid solutions, regardless of MW, was found in the stratum corneum, around 25 µm from the skin surface.

Effect on Penetration of Other Chemicals

According to a review article evaluating Hyaluronic Acid's influence as a drug delivery system for diclofenac, it was observed that dermal penetration was dependent on animal species.[1,26] The drug reservoir was formed in the deeper layers of the skin (dermis) in mice, while the drug reservoir was formed in more shallow layers of the skin (epidermis) in humans. Hyaluronic Acid can moderate the penetration of other chemicals such as diclofenac, causing a slower absorption of the drug, and preferential accumulation in the epidermis.

Absorption, Distribution, Metabolism, and Excretion (ADME)

Details on the oral absorption, distribution, and excretion studies summarized below can be found in Table 6.

In an absorption assay in which male Sprague-Dawley rats (n = 3) were dosed with 25 mg/kg [^{14}C]Hyaluronic Acid (MW = 920 kDa) via gavage, the peak plasma radioactivity level was 7.6 µg eq/ml 8 h post-administration.[27] The highest amount of radioactivity was observed in intestinal contents 8 h post-administration. When evaluating excretion, the total excretion of radioactivity in the urine, feces, and expired air was 91.3% by 168 h post-administration. In a different assay, male Sprague-Dawley rats (6/group) were orally administered Hyaluronic Acid (MW = 300 kDa; 200 mg/kg bw), and blood, cecal content, and ventral skin were evaluated at different time intervals.[28] The recovery rate of unsaturated hyaluronic disaccharides and unsaturated hyaluronic tetrasaccharides in the serum and skin was approximately 25 and 70%, respectively. Male Sprague-Dawley rats (8/group) were given Hyaluronic Acid (MW = 300,000 Da) in distilled water (5 ml/kg bw/d; 1 and 5% concentrations; 5 d administration) via gavage, and excretion parameters were evaluated. Hyaluronic Acid was below the detection limit in the feces for all treated groups.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

No deaths were observed in an acute oral toxicity assay in which ICR mice were given > 1200 mg/kg Hyaluronic Acid.[1] No other details were provided.

Details on the acute oral toxicity studies summarized below can be found in Table 7.

Several acute oral toxicity assays were performed using Sodium Hyaluronate in mice (at up to 15,000 mg/kg bw) and rats (at up to 5280 mg/kg bw).[16] No signs of toxicity or deaths were reported in any of these assays.

Short-Term and Subchronic Toxicity Studies

No toxicity was observed in in a short-term inhalation toxicity assay performed in male Beagle dogs exposed to 10% Hyaluronic Acid formulations containing insulin. No other details were provided for this study.

Details on the short-term and subchronic oral toxicity assays summarized below can be found in Table 8.

No signs of toxicity were observed in short-term and subchronic oral toxicity assays of Sodium Hyaluronate.[16] These assays include a 30-d study in which Wistar rats (10/sex/group) were given up to 1500 mg/kg bw Sodium Hyaluronate via feed, a 90-d assay in which Sprague-Dawley rats (5 - 10/sex/group) were given up to 48 mg/kg bw/d of a 1% Sodium Hyaluronate ophthalmic solution via gavage, a 90-d assay in which Wistar rats (10/sex/group) were given up to 1000 mg/kg

bw/d Sodium Hyaluronate via feed, and a 90-d study using Wistar rats (12/sex/group) given up to 1333 mg/kg bw/d Sodium Hyaluronate in corn oil via gavage.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Several reproductive and developmental studies on subcutaneously injected Hyaluronic Acid (up to 60 mg/kg/d) and Sodium Hyaluronate (up to 50 mg/kg/d) were performed in rats and rabbits (majority of dosing occurred on days 7 to 17 of pregnancy or day 17 of pregnancy to day 20-21 after delivery in rats, and on days 6-18 of gestation in rabbits).[1] In most assays, treatment with the test substance had no effect on mortality, necropsy observations post-delivery, food or water consumption, or fertility in dams. However, in one assay in which rats were given up to 60 mg/kg bw of a 1% Hyaluronic Acid solution in physiological saline via subcutaneous injection, nodular hyperplasia of reticular zone cells was present in the adrenal glands of treated dams (treatment on day 17 of pregnancy to day 20 after parturition). No fetal abnormalities were observed in rats or rabbits.

Sodium Hyaluronate

A sperm malformation assay was performed in adult male mice (strain not stated; 10/group).[29] Sodium Hyaluronate (20 ml/kg bw), cyclophosphamide (40 mg/kg bw (positive control), and distilled water (negative control) was given to animals via gavage, once a day, for 5 d. Mice were killed 30 d after the last administration. No other details were provided. The test substance had no influence on sperm malformation rate.

A teratogenicity assay was performed in Wistar rats (15/group) given Sodium Hyaluronate (FW = 270 kDa) via gavage in doses of 0, 170, 330, or 670 mg/kg bw/d (administration during gestation days 7 - 16).[16] Dams were euthanized and evaluated on day 20 of gestation. No statistically significant differences ($P > 0.05$) were observed in the maternal, uterine, and ovary weights or in the number of corpus lutea and nidation between test and control groups. In addition, no statistically significant differences ($P > 0.05$) were observed between the control and treated groups regarding the weight, length, and number of living embryos. No evidence of maternal or embryo toxicity resulting from the test substance administration was observed.

In a different teratogenicity assay, Sprague Dawley rats (12/group) were given Sodium Hyaluronate, via gavage, in doses of 333, 667, or 1333 mg/kg bw/d, on gestation days 7 - 16.[16] A negative control group was given water and a positive control group was given aspirin on the same gestation days. Animals were euthanized and evaluated on gestation day 20. No statistical differences in maternal body weight, number of corpus lutea, implantations, uterine weight, placental weight, live fetus rate, fetal death rate, or absorbed fetus rate were observed between test and negative control treated groups. Fetal development and growth were similar between negative control and treated groups. No evidence of maternal toxicity or teratogenicity resulting from test substance administration was observed.

GENOTOXICITY STUDIES

No genotoxicity was observed in an Ames assays evaluating Sodium Hyaluronate (up to 1%; up to 5000 µg/plate) in Staphylococcus aureus, Salmonella typhimurium and Escherichia coli or in an in vitro chromosomal aberration assay evaluating the genotoxic potential of Sodium Hyaluronate (up to 1000 µg/ml) in Chinese hamster lung fibroblasts. Negative results were also observed in an in vivo micronucleus assay using 1% Sodium Hyaluronate (up to 400 mg/kg) in CD-1 (ICR) mice and in a micronucleus assay evaluating ICR (Crj: CD-1) mice treated with 360 mg/kg Sodium Hyaluronate for up to 4 d.

Details on the genotoxicity assays summarized below can be found in Table 9.

Hydrolyzed Sodium Hyaluronate (FW = 5-10 kDa), Hydrolyzed Sodium Hyaluronate (FW < 1 kDa) and Sodium Hyaluronate were determined to be non-genotoxic in several Ames assays when tested at up to 5 mg/plate, with and without metabolic activation (assays performed in *S. typhimurium* strains TA97a, TA98, TA100, TA102, TA1535, TA537 and *E. coli* WP2 *uvrA*).[16,29-31] In addition, Sodium Hyaluronate (up to 5000 mg/kg bw) was non-mutagenic in mouse micronucleus assays.[29]

CARCINOGENICITY STUDIES

No carcinogenicity data were found in the literature, and no unpublished data were submitted.

OTHER RELEVANT STUDIES

Immunogenicity

Multiple injections of Hyaluronic Acid derived from human umbilical cords or streptococcal fermentation did not result in sensitization in immunogenicity studies performed in rabbits (administration of test substance via either subcutaneous or intramuscular route). In an antigenicity assay using injected streptococcal-derived Hyaluronic Acid in rabbits, precipitating antibodies were observed. A similar assay was performed using purified Hyaluronic Acid derived from rooster combs and human umbilical cords in rabbits. No formations of passive cutaneous anaphylaxis reactive antibodies were observed.

Antibody response by rooster comb-derived Hyaluronic Acid caused an enhanced secondary antibody response to birch pollen, egg albumen (the protein in egg whites), and dog albumen in rats. Neither commercial Sodium Hyaluronate preparations nor a crude rooster comb Sodium Hyaluronate preparation elicited a Hyaluronic Acid-specific antibody response in rabbits.

Cancer Cell Biology

Mouse melanoma cell lines with high Hyaluronic Acid production had increased lung metastasis and lower survival than melanoma cell lines with lower Hyaluronic Acid production.[1] Aneuploid human breast adenocarcinoma cells modified with antisense inhibition of hyaluronan synthase 2 expression produced more high MW Hyaluronic Acid. Injection of these cells into mice did not result in primary tumors. In other studies, well-differentiated tumors (e.g., salivary gland, stomach, colon) had intense Hyaluronic Acid-staining in the tumor cells, intratumoral and associated surrounding stroma. Poorly differentiated tumor samples (e.g., astrocytomas, infiltrating breast, stomach, gallbladder) with carcinoma or sarcoma had almost no Hyaluronic Acid when stained. Enhanced motility of human pancreatic carcinoma cells was dependent on the cluster of differentiation 44 (CD44)-hyaluronic acid interaction where low MW Hyaluronic Acid induced angiogenesis, enhanced CD44 cleavage, and promoted the migration of the tumor cells in a CD44-dependent manner. In a different study, stromal Hyaluronic Acid was not related to survival or recurrence-free survival from cutaneous melanoma. Compared with normal epidermis, in situ carcinomas and well-differentiated squamous cell carcinomas showed an enhanced Hyaluronic Acid signal on carcinoma cells, while CD44 expression resembled normal skin. Less-differentiated squamous cell carcinoma samples had reduced and irregular expression of Hyaluronic Acid and CD44 on carcinoma cells. In basal cell carcinoma samples, Hyaluronic Acid was frequently present on cell nuclei but not in the other types of samples. Hyaluronidase applied to tumors or tumor cells injected into the footpads of mice reduced growth rates in human breast carcinoma. Hyaluronic Acid levels have been found to be increased in tissues surrounding some breast cancer, gastric cancer, poorly differentiated, serous histological type, advanced stage, and large primary tumor epithelial ovarian cancer, endometrial cancer, ganglioma, thyroid cancer, and salivary gland cancer. Normal and low levels of stromal Hyaluronic Acid were associated with early International Federation of Gynecology and Obstetrics (FIGO) stage, mucinous histological-type epithelial ovarian cancer, and murine astrocytoma. Increased Hyaluronic Acid intensity in breast cancer patients was related to axillary lymph node positivity and poor survival.

Ocular Toxicity

Owl monkey and rhesus monkeys had no ill effects from repeated injection of Hyaluronic Acid into the eyes.[1] Repeated injections of Sodium Hyaluronate into the eyes of owl monkeys increased the leukocyte count up to 2000 cells/mm^3 after 48 h. The severity of haze and flare in the eyes after the injections did not increase over time, and there was no immunogenic response. This experiment was continued for up to 9 yr in 2 eyes with no adverse effects.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Hyaluronic Acid was non-irritating in a single-stimulus skin test using Japanese rabbits and Hartley guinea pigs.[1] In addition, no irritation was observed in a human closed skin patch test using Hyaluronic Acid produced via fermentation. No details were provided for either study. A skin prick test was performed in 9 subjects. The forearm of each subject was pricked with Sodium Hyaluronate (10 mg/ml), and evaluated 15 min, and 2, 6, and 24 h after pricking. No skin reactions were observed.

Details on the dermal irritation and sensitization data summarized below can be found in Table 10.

In vitro dermal irritation assays performed on two trade name mixtures containing 1% Hyaluronic Acid (tested neat), a trade name mixture containing 3% Hyaluronic Acid (tested neat), Hydrolyzed Sodium Hyaluronate (FW < 1 kDa; tested at 1%), a trade name mixture containing 0.5% Sodium Hyaluronate (tested neat), and Sodium Hyaluronate (concentration not reported) yielded negative results.[29,31-35] No irritation was observed in human dermal irritation assays performed under occlusive conditions using Hydrolyzed Sodium Hyaluronate (several FW tested; 30 - 32 subjects; tested at up to 2%).[31] No responses predicting sensitization were noted in a direct peptide reactivity assay (DPRA) performed on a trade name mixture containing 1% Hyaluronic Acid (tested at up to 25 mM), in a KeratinoSens™ assay performed on a trade name mixture containing 1% Hyaluronic Acid (up to 2 mM), and in a human cell line activation test (h-CLAT) performed on Sodium Hyaluronate (tested at 1 mg/ml).[29,36,37] Similarly, no sensitization was observed in human repeat insult patch tests (HRIPTs) performed using a formula containing 0.2% Hyaluronic Acid (114 subjects; tested neat), a formula containing 0.2% Sodium Acetylated Hyaluronate (104 subjects; tested neat), Hydrolyzed Sodium Hyaluronate (FW = 5 - 10 kDa; 55 subjects; tested at 0.5%); Sodium Hyaluronate (50 - 100 subjects; tested at 0.2%), and a formula containing 1.5% Sodium Hyaluronate (198 subjects; tested neat).[29-31,38-40]

Phototoxicity

In Vitro

Summary data were provided from a supplier on 3T3 neutral red uptake phototoxicity assays performed on Hydrolyzed Sodium Hyaluronate (FW < 1 kDa; up to 128 mg/ml) and Sodium Hyaluronate (125 µg/ml).[29,31] Neither test substance was predicted to induce phototoxicity.

OCULAR IRRITATION STUDIES

Details on the ocular irritation summarized below can be found in Table 11.

No ocular irritation was observed in EpiOcular™ assays performed on 2 trade name mixtures containing 1% Hyaluronic Acid (tested neat), a trade name mixture containing 3% Hyaluronic Acid (tested neat), and a trade name mixture containing 0.5% Sodium Hyaluronate (tested neat).[32-35] Test substances were considered to be non-irritating/slightly irritating in chorioallantoic membrane vascular assays (CAMVA) performed using Hydrolyzed Sodium Hyaluronate (FW < 1 kDa; concentration tested not reported) and Sodium Hyaluronate (tested neat).[29,31] Similar results were observed in a bovine corneal opacity and permeability (BCOP) test performed using Sodium Hyaluronate (tested neat). In addition, a *Bacillus*-derived and *Streptococcus*-derived Hyaluronic Acid (up to 0.3%) was considered to be very well-tolerated when tested in the eyes of New Zealand white rabbits (3/group).[41]

CLINICAL STUDIES

Nebulized Nasal Hypertonic Solution in the Treatment of Chronic Rhinosinusitis

Hyaluronic Acid

Eighty patients with chronic rhinosinusitis were instructed to use a nasal spray containing high MW Hyaluronic Acid and sodium chloride, twice daily (2 puffs per nostril at each administration), for 20 d.[42] Patients were assessed at baseline, on day 10, and day 20. An endoscopic nasal examination and nasal obstruction symptom evaluation instrument (I-NOSE) questionnaire were performed during each visit, and safety parameters (adverse effects and local tolerability (burning, irritation, congestion) were assessed. Patients were instructed to keep diaries to log changes in symptoms. The improvement in chronic rhinosinusitis compared to baseline as measured by the I-NOSE score, was statistically significantly ($P < 0.001$). According to patient diaries, nasal blockage, congestion, drainage, and rhinorrhea was significantly improved between baseline and day 20 ($P < 0.01$). Fourteen patients experienced at least one adverse effect; however, these effects were not related to study treatment. No symptoms related to local tolerability at the site of administration were reported.

Immediate and Delayed Hypersensitivity to Intracutaneous Hyaluronic Acid

Hyaluronic Acid

Twelve patients with previously reported inflammatory responses to Hyaluronic Acid fillers were subjected to intracutaneous tests.[43] Approximately 0.1 ml of each filler was tested on the inner sides of the upper arms in a randomized manner. Tests were read after 15 min, and 2, 3, 4, and 7 d following application. Potential late reactions were monitored after 2 and 4 wk, and patients were instructed to contact study conductors in case at any later reaction. No positive reactions were observed for any of the tested Hyaluronic Acid fillers during the testing period, or during the 4 mo follow-up.

Treatment of Dry Eye

Sodium Hyaluronate

The effectiveness of Sodium Hyaluronate eye drops was evaluated in 13 patients with moderate dry eye.[44] Patients were treated with instillations of 40 µl of 0.1% Sodium Hyaluronate, 0.3% Sodium Hyaluronate, or 0.9% saline, in a randomized, double-masked manner (vehicles for treatment not stated). Symptom intensity and non-invasive break-up time (NIBUT) were evaluated at 5, 15, 30, 45, 60 min, and hourly, until 6 h after drop instillation. This process was repeated twice following an interval of approximately 7 d, but with a different treatment, so that at the end of the final visit, each subject had trialed all products. Symptoms and NIBUT improved with all treatments; however, improvement (reduction in eye irritation) was of a greater magnitude and longer duration with Sodium Hyaluronate drops. Eye drops containing 0.3% Sodium Hyaluronate performed better than 0.1% Sodium Hyaluronate ($P = 0.04$).

Treatment of Rosacea

Sodium Hyaluronate

The effect of a cream containing 0.2% Sodium Hyaluronate (containing low MW Hyaluronic Acid) was evaluated in 14 patients with mild to moderate facial rosacea.[45] Patients were instructed to apply the cream, following cleansing, on the whole face, twice daily, for 4 wk. After 4 wk, patients continued the cleansing regimen for an additional 4 wk, but discontinued the use of the cream containing Sodium Hyaluronate. Patients were evaluated for papules, pustules, erythema, edema, telangiectasia, burning, stinging, and/or dryness at baseline and at 2-wk intervals following administration. No patients experienced adverse effects throughout the study. The largest reduction in erythema was observed at the 2-wk visit (48.3% reduction). At the 4-wk visit, it was reported that treatment with 0.2% Sodium Hyaluronate cream resulted in a reduction of papules, erythema, burning/stinging, and dryness in all patients.

Use in Dissolving Microarray Patches

Hyaluronic Acid

Dissolving microarray patches containing 30% Hyaluronic Acid (in distilled water) were placed under and at the corner of the eyes of 30 female subjects aged 35 - 60.[46] Patches were applied 3x/wk for 4 wk. Safety was assessed by the degree of

adverse effects, including facial itching, prickling, burning, erythema, edema, and swelling. These parameters were evaluated by participant questionnaires. No adverse effects on the skin or eyes were reported throughout the study.

Case Reports

Numerous case reports were found in the literature regarding hypersensitivity/adverse reactions to Hyaluronic Acid used as an injectable dermal filler. A summary of these studies has been provided and can be found in Table 12. In addition, it should be noted that case reports were also found on the adverse effects of Hyaluronic Acid following other methods of administration (e.g., intra-articular injections for osteoarthritis, injection during surgical procedures). These studies were not summarized in the table, as their relevance to the cosmetic use of Hyaluronic Acid is not likely; however, two case report regarding photodermatitis following the intra-articular administration of Hyaluronic Acid in the knee has been summarized below, as it may have relevance in evaluating the photosensitivity-inducing potential of Hyaluronic Acid. In addition, a case report regarding an anaphylactic response in an elderly patient following oral exposure to Hyaluronic Acid has also been included.

A 71-yr-old man with a history of osteoarthritis reported previous treatment with three-series 2 ml Hyaluronic Acid injections in the knee for 5 yr with no adverse reactions.[47] The patient switched to a single 6 ml Hyaluronic Acid injection and immediately developed pain and swelling at injection site. These effects were also seen 5 mo later following a second injection of 6 ml Hyaluronic Acid. Six mo after the second injection, the patient received another 6 ml Hyaluronic Acid injection in the knee and developed a similar localized inflammatory reaction with chills. Eight days later, the patient developed erythematous, pruritic, scaly papules and plaques near the injection site. Several weeks later, he presented with photo-distributed scale and scattered excoriations on the bilateral cheeks and all four extremities. A similar reaction was observed in a 65-yr-old woman who also switched from three-series 2 ml Hyaluronic Acid injections in the knee, with no adverse effects for 13 yr, to a singular 6 ml Hyaluronic Acid injection. Following the singular injection, the patient displayed a localized inflammatory reaction at the injection site, followed by the development of photo-distributed erythematous macules and papules with pustules and scale on the face and all four extremities. Both patients recovered following treatment with triamcinolone and prednisone.

Upper airway angioedema was observed in a 100-yr-old woman following application of a spray containing xylitol and Hyaluronic Acid (0.01%) to the inner lower lip and gums to treat gingival sores for the third time in 2 d.[48] The previous two applications were much smaller in quantity. Following admission to the emergency department, the patient became dyspneic and hypoxemic, with edema of the lip, lower face, and epiglottis. The patient recovered following treatment with oxygen, epinephrine, methylprednisolone, diphenhydramine, ranitidine, and icatibant.

SUMMARY

The safety of Hyaluronic Acid and 6 hyaluronate ingredients are reviewed in this safety assessment. The majority of these ingredients are reported to function in cosmetics as skin and/or hair conditioning agents. Sodium Acetylated Hyaluronate is reported to function in cosmetics as a humectant. In cosmetics, these hyaluronates are derived from either bacterial fermentation or rooster combs. Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate have previously been reviewed by the Panel and were considered safe in the present practices of use and concentration as described in the safety assessment published in 2009.

According to 2022 VCRP survey data, Sodium Hyaluronate is reported to be used in 4048 formulations (3680 leave-on formulations, 366 rinse-off formulations, and 2 formulations diluted for bath use), and Hyaluronic Acid is reported to be used in 568 formulations (493 leave-on formulations, 72 rinse-off formulations, and 3 formulations diluted for bath use). All other ingredients are reported to be used in 378 formulations or less. The results of the 2021 concentration of use survey conducted by the Council indicate Sodium Hyaluronate also has the highest concentration of use in a leave-on formulation; it is used at up 7.5% in face and neck products (not spray).

A dermal penetration assay was performed in human dermatomed skin samples using Hyaluronic Acid solutions of three different MW (20 - 50 kDa, 100 - 300 kDa, and 1 – 1.4 MDa). Hyaluronic Acid solutions, from lowest to highest MW, were present at epidermal depths of 100 µm, 50 µm, and 25 µm, respectively. Regardless of the MW of the Hyaluronic Acid solution, the majority quantity of Hyaluronic Acid was found in the stratum corneum, approximately 25 µm from the skin surface.

In an absorption assay in which male Sprague-Dawley rats (n = 3) were dosed with 25 mg/kg [^{14}C]Hyaluronic Acid (MW = 920 kDa) via gavage, the peak plasma radioactivity level was 7.6 µg eq/ml 8 h post-administration. When evaluating excretion, the total excreted radioactivity in the urine, feces, and expired air was 91.3% by 168 h post-administration. In a different assay, male Sprague-Dawley rats were orally administered Hyaluronic Acid (MW = 300 kDa; 200 mg/kg bw), and blood, cecal content, and ventral skin were evaluated at different time intervals. The recovery rate of unsaturated hyaluronic disaccharides and unsaturated hyaluronic tetrasaccharides in the serum and skin was approximately 25 and 70%, respectively. Male Sprague-Dawley rats were given Hyaluronic Acid (MW = 300 kDa) in distilled water (5 ml/kg bw/d; 1 and 5% concentrations; 5 d administration) via gavage, and excretion parameters were evaluated. Hyaluronic Acid was below the detection limit in the feces for all treated groups.

No signs of toxicity were observed in several acute oral toxicity assays performed using Sodium Hyaluronate in mice (at up to 15,000 mg/kg bw) and rats (at up to 5280 mg/kg bw). Similarly, no signs of toxicity were observed in 30- and 90-d oral toxicity assays performed in rats given Sodium Hyaluronate (up to 1333 mg/kg bw/d).

Sodium Hyaluronate (20 ml/kg) did not have an influence on sperm malformation in adult male mice. No maternal toxicity or teratogenicity resulting from Sodium Hyaluronate (up to 1333 mg/kg bw/d) were observed in two developmental toxicity assays using rats. In both assays, animals were treated via gavage, on gestation days 7 - 16. All measured parameters (e.g., ovary weights, number of living embryos, implantations) were similar among control and treated groups.

Hydrolyzed Sodium Hyaluronate and Sodium Hyaluronate were determined to be non-genotoxic in several Ames assays performed using strains of *S. typhimurium*, at concentrations up to 5 mg/plate, with and without metabolic activation. Similarly, no mutagenicity was observed in micronucleus assays using mice given up to 5000 mg/kg bw Sodium Hyaluronate.

In vitro dermal irritation assays performed on two trade name mixtures containing 1% Hyaluronic Acid (tested neat), a trade name mixture containing 3% Hyaluronic Acid (tested neat), Hydrolyzed Sodium Hyaluronate (FW < 1 kDa; tested at 1%), a trade name mixture containing 0.5% Sodium Hyaluronate (tested neat), and Sodium Hyaluronate (concentration not reported) yielded negative results. No irritation was observed in human dermal irritation assays performed under occlusive conditions using Hydrolyzed Sodium Hyaluronate (tested at up to 2%). No responses predicting sensitization was noted in a DPRA performed on a trade name mixture containing 1% Hyaluronic Acid (tested at up to 25 mM), in a KeratinoSens™ assay performed on a trade name mixture containing 1% Hyaluronic Acid (up to 2 mM), or in an h-CLAT performed on Sodium Hyaluronate (tested at 1 mg/ml). Similarly, no sensitization was observed in HRIPTs performed using a formula containing 0.2% Hyaluronic Acid (tested neat), a formula containing 0.2% Sodium Acetylated Hyaluronate (tested neat), Hydrolyzed Sodium Hyaluronate (FW = 5 - 10 kDa; tested at 0.5%); Sodium Hyaluronate (tested at 0.2%), and a formula containing 1.5% Sodium Hyaluronate (tested neat). No potential for phototoxicity was observed in in vitro phototoxicity assays performed on Hydrolyzed Sodium Hyaluronate (FW < 1 kDa; up to 128 mg/ml) and Sodium Hyaluronate (125 µg/ml).

In vitro ocular irritation assays performed on several test substances (trade name mixtures containing 1% Hyaluronic Acid, trade mixture containing 3% Hyaluronic Acid, Hydrolyzed Sodium Hyaluronate, Sodium Hyaluronate (100%), and a trade name mixture containing 0.5% Sodium Hyaluronate) yielded slightly irritating/non-irritating or non-irritating results. In addition, a *Bacillus*-derived and *Streptococcus*-derived Hyaluronic Acid (up to 3%) was considered to be very well-tolerated when tested in the eyes of rabbits.

Eighty patients with chronic rhinosinusitis were treated with a nasal spray containing high MW Hyaluronic Acid and sodium chloride (2 puffs/nostril/d for 20 d). A statistically significant improvement in rhinosinusitis symptoms was observed at the end of treatment compared to baseline ($P < 0.01$). No study-related adverse effects were observed. The treatment was considered to be well-tolerated.

Twelve patients with previously-reported inflammatory responses to Hyaluronic Acid fillers were subjected to intracutaneous tests using 6 different types of Hyaluronic Acid fillers. No positive reactions were observed for any of the tested Hyaluronic Acid fillers during the testing period, or during the 4-mo follow-up

The effect of Sodium Hyaluronate eye drops was evaluated in 13 patients with dry eye. Patients were treated with instillations of 40 µl of 0.1% Sodium Hyaluronate, 0.3% Sodium Hyaluronate, or 0.9% saline, in a randomized, double-masked manner (vehicles for treatment not stated). Symptoms of dry eye improved with the use of all treatments; however, improvement was greatest with the use of 0.3% Sodium Hyaluronate drops.

The effect of a cream containing 0.2% Sodium Hyaluronate was evaluated in 14 patients with facial rosacea. Use of the cream (2x/d for 4 wk) resulted in a reduction in rosacea symptoms. No adverse effects were reported throughout the study.

The safety of dissolving microarray patches containing Hyaluronic Acid (30% in distilled water) placed under the eyes was evaluated in 30 subjects (patches applied 3x/wk for 4 wk). No adverse dermal or ocular effects were reported throughout the study.

Case reports were found in the literature regarding hypersensitivity/adverse reactions to Hyaluronic Acid used as an injectable dermal filler and injections used as treatments for osteoarthritis. Two case reports stated that treatment of osteoarthritis with three-series 2 ml Hyaluronic Acid injections was performed without adverse effects; however, switching to a single 6 ml Hyaluronic Acid injection did result in adverse effects. In another case report, upper airway angioedema was observed in a 100-yr-old woman after use of a spray containing xylitol and Hyaluronic Acid (0.01%) to the inner lower lips and gums; the patient recovered following treatment.

DISCUSSION

This report was precipitated by the high frequencies of use of Sodium Acetylated Hyaluronate and Hydrolyzed Hyaluronic Acid as indicated by 2022 FDA VCRP data. In 2009, the Panel published a final report on 3 structurally-similar ingredients (Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate) and concluded that these ingredients were safe as used in cosmetic products. As these ingredients would soon be considered for re-review, the Panel deemed it

appropriate to include the 3 previously-reviewed ingredients in this safety assessment. Also included in this ingredient group are Hydrolyzed Calcium Hyaluronate and Hydrolyzed Sodium Hyaluronate. Furthermore, the Panel reviewed the available data, and concluded that all 7 hyaluronate ingredients reviewed in this report are safe in cosmetics in the present practices of use and concentration. The safety of these ingredients was supported by available toxicity data, the presence of Hyaluronic Acid as an endogenous substance in the skin, and the extensive use of these ingredients with few reported adverse effects.

The Panel noted sensitization studies included in this report were not performed at maximum use concentrations. However, the Panel determined that additional studies were not needed to determine the safety of this ingredient group because these ingredients have large molecular weights (and as such, are not expected to absorb into the skin), and because although these ingredients are widely utilized, and there are a lack of case reports following topical application. The Panel did note case reports of hypersensitivity reactions following use of Hyaluronic Acid dermal fillers, but stated these effects would not be relevant to cosmetic safety as dermal fillers are administered via intradermal injection, and therefore bypass the stratum concern. Concern was further mitigated as the majority of Hyaluronic Acid filler contain cross-linked hyaluronates, which chemically differ from the non-cross-linked ingredients reviewed in this report.

The Panel was concerned with the risks inherent in using animal-derived ingredients (i.e., rooster combs), namely the transmission of infectious agents and biologically-derived impurities (e.g., nucleic acids, proteins, endotoxins). The Panel stressed that these ingredients must be free of detectible pathogenic viruses, infectious agents, and/or biologically-derived impurities. Suppliers and users of these ingredients must accept responsibility for assuring that these ingredients are risk-free. Tests to assure the absence of a pathogenic agent in the ingredients or controls to assure derivation from pathogen-free sources are two approaches that should be considered.

In addition, the Panel expressed concern regarding heavy metals that may be present in these ingredients. The Panel stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit these impurities in cosmetic formulations.

The Panel discussed the issue of incidental inhalation exposure resulting from these ingredients (e.g., Sodium Hyaluronate is reported to be used at up to 0.01% in other skin care preparations (spray)). Inhalation toxicity data were limited; however, the Panel noted that in aerosol products, the majority of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which the ingredients are used in potentially inhaled products, 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.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be assessed by the Panel. Therefore, the Panel has found the data insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel concluded that the 7 following hyaluronate ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment:

Hyaluronic Acid
Hydrolyzed Calcium Hyaluronate
Hydrolyzed Hyaluronic Acid
Hydrolyzed Sodium Hyaluronate
Potassium Hyaluronate
Sodium Acetylated Hyaluronate
Sodium Hyaluronate

TABLES

Table 1. Definitions and reported functions of the hyaluronate ingredients[2]

Ingredient	Definition	Function
Hyaluronic Acid (9004-61-9)	Hyaluronic Acid is the natural mucopolysaccharide formed by bonding *N*-acetyl-D-glucosamine with glucuronic acid. *See Figure 1, wherein "R" is hydrogen.*	Skin-Conditioning Agents - Miscellaneous; Viscosity Increasing Agents - Aqueous
Hydrolyzed Calcium Hyaluronate	Hydrolyzed Calcium Hyaluronate is the hydrolysate of the calcium salt of Hyaluronic Acid derived by acid, enzyme, or other method of hydrolysis. *See Figure 1, wherein 2 "R" are replaced by 1 calcium cation.*	Skin-Conditioning Agents - Miscellaneous
Hydrolyzed Hyaluronic Acid	Hydrolyzed Hyaluronic Acid is the hydrolysate of Hyaluronic Acid derived by acid, enzyme, or other method of hydrolysis.	Hair Conditioning Agents; Skin-Conditioning Agents - Humectant
Hydrolyzed Sodium Hyaluronate	Hydrolyzed Sodium Hyaluronate is the hydrolysate of Sodium Hyaluronate derived by acid, enzyme, or other method of hydrolysis.	Skin-Conditioning Agents - Miscellaneous
Potassium Hyaluronate (31799-91-4)	Potassium Hyaluronate is the potassium salt of Hyaluronic Acid. *See Figure 1, wherein R is potassium.*	Skin-Conditioning Agents - Miscellaneous
Sodium Acetylated Hyaluronate	Sodium Acetylated Hyaluronate is the acetyl ester of Sodium Hyaluronate. *See Figure 1, wherein "R" is sodium, and one or more hydroxyl groups are acetylated.*	Humectants
Sodium Hyaluronate (9067-32-7)	Sodium Hyaluronate is the sodium salt of Hyaluronic Acid. *See Figure 1, wherein "R" is sodium.*	Skin-Conditioning Agents - Miscellaneous

Table 2. Chemical properties of Hyaluronic Acid and Sodium Hyaluronate

Property	Value	Reference
Hyaluronic Acid		
Physical Form	powder	[1]
MW (kDa)	5 - 1800	[1]
Sodium Hyaluronate		
Physical Form	powder	[1]
Color	white	[1]
Odor	faint odor	[1]
FW (kDa)	80.2 - 4010	[15]

FW = formula weight; MW = molecular weight

Table 3. Molecular weight (MW) and impurities measurements of Hyaluronic Acid derived from different sources (per 1 mg Hyaluronic Acid)[11]

	Human umbilical cord	Bacterially-derived	Bacterially-derived*	Rooster comb	Bovine vitreous
MW (x 10^6 Da)	1.3 ± 0.1	1.6	1.4	1.4	0.4
Endotoxin (EU/mg HA)	> 100	< 0.02	0.022	23	> 100
Total Protein (µg/ml HA)	47.7 ± 3	1.1	ND	1.0	36.2
RNA (µg/mg HA)	6.7 ± 0.1	ND	ND	ND	1.9
DNA (µg/mg HA)	16.8 ± 4.5	ND	ND	ND	1.1

*two different bacterially-derived (*Streptococcus zooepidemicus*) samples were tested; EU = endotoxin units; HA = Hyaluronic Acid; ND = not detected

Table 4. 2022/2021 and historical frequency and concentration of use according to duration and exposure

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2022[18]	2005[1]	2021[19]	2005[1]	2022[18]	2005[1]	2022[18]	2005[1]	2022[18]	2005[1]	2022[18]	2005[1]
	Hyaluronic Acid				Potassium Hyaluronate				Sodium Hyaluronate			
Totals	**568**	**223**	**0.000002 – 0.83**	**0.00005 – 1**	**44**	**11**	**NR**	**NR**	**4048**	**601**	**0.00001 – 7.5**	**0.000001 – 2**
summarized by likely duration and exposure*												
Duration of Use												
Leave-On	*493*	*194*	*0.000002 – 0.3*	*0.00005 – 1*	*43*	*10*	*NR*	*NR*	*3680*	*552*	*0.00001 – 7.5*	*0.000001 – 2*
Rinse-Off	*72*	*29*	*0.002 – 0.83*	*0.001 – 0.3*	*1*	*1*	*NR*	*NR*	*366*	*49*	*0.0001 – 0.12*	*0.000001 – 0.5*
Diluted for (Bath) Use	*3*	*NR*	*0.0089*	*NR*	*NR*	*NR*	*NR*	*NR*	*2*	*NR*	*NR*	*0.001 – 0.5*
Exposure Type**												
Eye Area	45	33	0.001	0.001 – 0.07	NR	NR	NR	NR	259	49	0.0001 – 0.96	0.0001 – 0.7
Incidental Ingestion	3	NR	0.003 – 0.05	0.01	NR	NR	NR	NR	219	96	0.24 – 0.39	0.0002 – 0.5
Incidental Inhalation-Spray	3; 213[a]; 158[b]	57[a]; 21[b]	NR	0.001[a]; 0.001 – 1[b]	22[a]; 14[b]	4[a]; 6[b]	NR	NR	10; 1376[a]; 1250[b]	1; 180[a]; 73[b]	0.01; 2[a]	0.000001 – 1[a]; 0.0001 – 2[b]
Incidental Inhalation-Powder	4; 158[b]; 5[c]	6; 21[b]	0.003 – 0.3[c]	0.00005; 0.001 – 1[b]	14[b]	6[b]	NR	NR	34; 1250[b]; 9[c]	16; 73[b]	0.001 – 0.099; 0.00002 – 7.5[c]	0.0005 – 0.5; 0.0001 – 2[b]; 0.5[c]
Dermal Contact	542	216	0.000002 – 0.83	0.00005 – 1	44	11	NR	NR	3746	482	0.00001 – 7.5	0.000001 – 2
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	4[a]	NR	0.013	0.5[a]
Hair - Non-Coloring	22	4	0.0036	NR	NR	NR	NR	NR	62	12	0.005 – 2	0.001 – 0.5
Hair-Coloring	NR	1	0.002	NR	NR	NR	NR	NR	1	2	NR	0.5
Nail	NR	2	NR	0.001 – 0.01	NR	NR	NR	NR	1	NR	0.025	0.01 – 0.5
Mucous Membrane	27	1	0.003 – 0.05	0.01	NR	NR	NR	NR	251	97	0.01 – 0.39	0.0002 – 0.5
Baby Products	6	NR	NR	0.001	NR	NR	NR	NR	14	NR	0.005	0.5
as reported by product category												
Baby Products												
Baby Shampoos	1	NR	NR	NR					NR	NR	NR	0.5
Baby Lotions/Oils/Powders/Creams	5	NR	NR	NR					9	NR	NR	0.5
Other Baby Products	NR	NR	NR	0.001					5	NR	0.005	0.5
Bath Preparations (diluted for use)												
Bath Oils, Tablets, and Salts	1	NR	NR	NR					1	NR	NR	0.5
Bubble Baths	1	NR	NR	NR					NR	NR	NR	0.001 – 0.5
Bath Capsules									NR	NR	NR	0.5
Other Bath Preparations	1	NR	0.0089	NR					1	NR	NR	0.001 – 0.5
Eye Makeup Preparations												
Eyebrow Pencil	3	NR	NR	NR					1	3	NR	0.5
Eyeliner	1	NR	NR	NR					9	4	NR	0.001 – 0.5
Eye Shadow	1	15	NR	0.02					21	11	0.097 – 0.96	0.0001 – 0.5
Eye Lotion	16	5	0.001	NR					95	6	0.1	0.001 – 0.7
Eye Makeup Remover	1	2	NR	0.001					7	NR	0.12	NR
Mascara									19	9	0.0001 – 0.1	0.0001 – 0.5
Other Eye Makeup Preparations	23	11	NR	0.07					107	16	0.001 – 0.1	0.0001 – 0.5
Fragrance Preparations												
Cologne and Toilet Water									1	NR	NR	NR
Perfumes									NR	1	NR	0.5
Powders (dusting/talcum, excl aftershave talc)	NR	1	NR	NR					NR	1	NR	0.5
Sachets									ǂ	NR	ǂ	0.5
Other Fragrance Preparation	2	NR	NR	NR					8	NR	NR	0.0002
Hair Preparations (non-coloring)												
Hair Conditioner	9	2	0.0036	NR					15	5	NR	0.001 – 0.5
Hair Spray (aerosol fixatives)	1	NR	NR	NR					1	NR	NR	0.5

Table 4. 2022/2021 and historical frequency and concentration of use according to duration and exposure

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2022[18]	2005[1]	2021[19]	2005[1]	2022[18]	2005[1]	2022[18]	2005[1]	2022[18]	2005[1]	2022[18]	2005[1]
	Hyaluronic Acid				Potassium Hyaluronate				Sodium Hyaluronate			
Hair Straighteners									2	1	NR	0.5
Permanent Waves	NR	1	NR	NR								0.5
Rinses (non-coloring)									1	NR	NR	0.001 – 0.5
Shampoos (non-coloring)	5	NR	0.0036	NR					21	6	0.01	0.001 – 0.5
Tonics, Dressings, and Other Hair Grooming Aids	3	1	NR	NR					8	NR	2	0.02 – 0.5
Wave Sets									NR	NR	NR	0.5
Other Hair Preparations	3	NR	NR	NR					14	NR	0.005	0.5
Hair Coloring Preparations												
Hair Dyes and Colors (all types requiring caution statements and patch tests)									NR	NR	NR	0.5
Hair Tints	NR	NR	0.002	NR					NR	NR	NR	0.5
Hair Rinses (coloring)	NR	1	NR	NR					NR	NR	NR	0.5
Hair Shampoos (coloring)									1	NR	NR	NR
Hair Color Sprays (aerosol)									NR	NR	NR	0.5
Hair Lighteners with Color									NR	NR	NR	0.5
Hair Bleaches									NR	NR	NR	0.5
Other Hair Coloring Preparation									NR	2	NR	0.5
Makeup Preparations												
Blushers (all types)	NR	7	NR	0.02					11	20	0.05	0.001 – 0.5
Face Powders	4	5	NR	0.00005					34	15	0.001 – 0.099	0.0005 – 0.5
Foundations	4	24	0.000002 – 0.1	0.002					67	27	0.015 – 0.2	0.001 – 0.5
Leg and Body Paints									1	1	NR	0.001 – 0.5
Lipstick	3	NR	0.003 – 0.05	0.01					213	96	0.24 – 0.39	0.0002 – 0.5
Makeup Bases	4	22	0.1	NR	3	NR	NR	NR	29	15	NR	0.002 – 0.5
Rouges									1	10	0.001	0.0001 – 0.5
Makeup Fixatives									6	3	NR	0.05 – 0.5
Other Makeup Preparations	4	NR	NR	0.001					61	17	0.025 – 0.1	0.0001 – 0.5
Manicuring Preparations (Nail)												
Basecoats and Undercoats									NR	NR	NR	0.5
Cuticle Softeners	NR	1	NR	0.001					NR	NR	NR	0.01 – 0.5
Nail Creams and Lotions									NR	NR	NR	0.5
Nail Extenders									NR	NR	NR	0.5
Nail Polish and Enamel									NR	NR	NR	0.5
Nail Polish and Enamel Removers									NR	NR	NR	0.5
Other Manicuring Preparations	NR	1	NR	0.01					1	NR	0.025	0.5
Oral Hygiene Products												
Dentifrices									3	NR	NR	NR
Mouthwashes and Breath Fresheners									3	NR	NR	NR
Personal Cleanliness Products												
Bath Soaps and Detergents	15	NR	NR	NR					11	1	0.01	0.001 – 0.5
Deodorants (underarm)									4	NR	0.013 (not spray)	0.5
Douches	1	NR	NR	NR								
Feminine Deodorants									1	NR	NR	0.001
Other Personal Cleanliness Products	5	1	NR	NR					18	NR	NR	0.5

Table 4. 2022/2021 and historical frequency and concentration of use according to duration and exposure

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2022[18]	2005[1]	2021[19]	2005[1]	2022[18]	2005[1]	2022[18]	2005[1]	2022[18]	2005[1]	2022[18]	2005[1]
	Hyaluronic Acid				Potassium Hyaluronate				Sodium Hyaluronate			
Shaving Preparations												
Aftershave Lotion	1	1	NR	NR					10	6	0.1	0.001 – 0.5
Beard Softeners									NR	NR	NR	0.5
Mens Talcum									NR	NR	NR	0.5
Preshave Lotions (all types)									NR	NR	NR	0.5
Shaving Cream	NR	3	NR	0.3					7	NR	NR	0.001 – 0.5
Shaving Soap									NR	NR	NR	0.5
Other Shaving Preparations	3	NR	0.008	NR					5	2	0.01	0.5
Skin Care Preparations												
Cleansing	24	6	NR	0.001					146	20	0.0001 – 0.1	0.000001 – 0.5
Depilatories									1	NR	NR	0.5
Face and Neck (exc shave)	147	8	0.003 – 0.3 (not spray)	0.1	12	6	NR	NR	1104	48	0.005 – 7.5 (not spray)	0.005 – 1
Body and Hand (exc shave)	10	12	0.05 (not spray)	0.001 – 1	2	NR	NR	NR	145	25	0.00002 – 0.86 (not spray)	0.0001 – 2
Foot Powders and Sprays	1	1	NR	NR					NR	NR	NR	1
Moisturizing	179	37	0.08 – 0.2 (not spray)	0.001 – 0.1	22	4	NR	NR	1170	151	0.001 – 0.4 (not spray)	0.000001 - 1
Night	14	17	0.15 (not spray)	0.02					122	11	0.00001 – 0.3 (not spray)	0.0001 - 1
Paste Masks (mud packs)	8	13	0.83	0.001	1	1	NR	NR	125	12	0.024	0.005 – 0.5
Skin Fresheners	13	NR	NR						59	10	0.01	0.05 – 0.5
Other Skin Care Preparations	47	23	NR	0.001	4	NR	NR	NR	329	38	0.02 – 0.1	0.001 - 1
Suntan Preparations												
Suntan Gels, Creams, and Liquids	1	1	NR	NR					2	4	NR	0.000001 – 1
Indoor Tanning Preparations	3	1	NR	0.001[a]					5	1	NR	0.001 – 0.5
Other Suntan Preparations									7	3	NR	0.001 – 0.5

NR – not reported

*likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

**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 are sprays, but it is not specified whether the reported uses are sprays.

[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

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

ǂ Sachets are no longer listed as a product category in the VCRP.

Table 5. Frequency (2022) and concentration (2021) of use of ingredients not previously reviewed[18,49]

	# 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 Calcium Hyaluronate		Hydrolyzed Hyaluronic Acid		Hydrolyzed Sodium Hyaluronate		Sodium Acetylated Hyaluronate	
Totals	**2**	**NR**	**362**	**0.002 – 0.2**	**108**	**0.0015 – 0.15**	**378**	**0.002 – 0.1**
summarized by likely duration and exposure*								
Duration of Use								
Leave-On	*2*	*NR*	*320*	*0.01 – 0.2*	*105*	*0.0015 – 0.15*	*353*	*0.002 – 0.1*
Rinse-Off	*NR*	*NR*	*42*	*0.002 – 0.01*	*3*	*NR*	*25*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type*								
Eye Area	NR	NR	9	0.01	8	NR	43	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	68	NR
Incidental Inhalation-Spray	2[a]	NR	166[a]; 94[b]	0.02	49[a]; 29[b]	NR	107[a]; 50[b]	0.0085 – 0.1[a]
Incidental Inhalation-Powder	NR	NR	4; 94[b]; 4[c]	0.01[c]	29[b]	0.15[c]	11; 50[b]; 3[c]	0.1[c]
Dermal Contact	2	NR	352	0.01 – 0.2	108	NR	307	0.002 – 0.1
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	10	0.002 – 0.02	NR	NR	1	NR
Hair-Coloring	NR	NR	NR	0.002	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	7	NR	NR	NR	71	NR
Baby Products	NR	NR	7	NR	NR	NR	6	NR
as reported by product category								
Baby Products								
Baby Shampoos								
Baby Lotions/Oils/Powders/Creams			4	NR			3	NR
Other Baby Products			3	NR			3	NR
Bath Preparations (diluted for use)								
Bath Oils, Tablets, and Salts								
Bubble Baths								
Bath Capsules								
Other Bath Preparations								
Eye Makeup Preparations								
Eyebrow Pencil							1	NR
Eyeliner							8	NR
Eye Shadow							13	NR
Eye Lotion			2	NR	5	NR	12	NR
Eye Makeup Remover								
Mascara							2	NR
Other Eye Makeup Preparations			7	0.1	3	NR	7	NR
Fragrance Preparations								
Cologne and Toilet Water								
Perfumes								
Powders (dusting/talcum, excl aftershave talc)								
Other Fragrance Preparation								
Hair Preparations (non-coloring)								
Hair Conditioner			4	NR			1	NR
Hair Spray (aerosol fixatives)								
Hair Straighteners								
Permanent Waves								
Rinses (non-coloring)								
Shampoos (non-coloring)			3	0.002				

Table 5. Frequency (2022) and concentration (2021) of use of ingredients not previously reviewed[18,49]

	# 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 Calcium Hyaluronate		Hydrolyzed Hyaluronic Acid		Hydrolyzed Sodium Hyaluronate		Sodium Acetylated Hyaluronate	
Tonics, Dressings, and Other Hair Grooming Aids			1	0.02				
Wave Sets								
Other Hair Preparations			2	NR				
Hair Coloring Preparations								
Hair Dyes and Colors (all types requiring caution statements and patch tests)			NR	0.002				
Hair Tints								
Hair Rinses (coloring)								
Hair Shampoos (coloring)								
Hair Color Sprays (aerosol)								
Hair Lighteners with Color								
Hair Bleaches								
Other Hair Coloring Preparation								
Makeup Preparations								
Blushers (all types)							4	NR
Face Powders			4	NR			11	NR
Foundations			1	NR			42	0.002
Leg/Body Paints								
Lipstick							68	NR
Makeup Bases			3	NR	3	NR	4	NR
Rouges								
Makeup Fixatives			1	NR	1	NR	2	NR
Other Makeup Preparations			3	NR	1	NR	5	NR
Manicuring Preparations (Nail)								
Basecoats and Undercoats								
Cuticle Softeners								
Nail Creams and Lotions								
Nail Extenders								
Nail Polish and Enamel								
Nail Polish and Enamel Removers								
Other Manicuring Preparations								
Oral Hygiene Products								
Dentifrices								
Mouthwashes and Breath Fresheners								
Personal Cleanliness Products								
Bath Soaps and Detergents			4	NR			1	NR
Deodorants (underarm)								
Douches								
Feminine Deodorants								
Other Personal Cleanliness Products			3	NR			2	NR
Shaving Preparations								
Aftershave Lotion								
Beard Softeners								
Mens Talcum								
Preshave Lotions (all types)								
Shaving Cream								
Shaving Soap								
Other Shaving Preparations								

Table 5. Frequency (2022) and concentration (2021) of use of ingredients not previously reviewed[18,49]

	# 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 Calcium Hyaluronate		Hydrolyzed Hyaluronic Acid		Hydrolyzed Sodium Hyaluronate		Sodium Acetylated Hyaluronate	
Skin Care Preparations								
Cleansing			24	0.01	2	NR	18	NR
Depilatories								
Face and Neck (exc shave)			89	0.01 (not spray)	28	0.15 (not spray)	43	0.1 (not spray)
Body and Hand (exc shave)					1	NR	7	NR
Foot Powders and Sprays			5	NR				
Moisturizing	2	NR	145	0.1 – 0.2 (not spray)	4	0.0015 (not spray)	96	0.0085 – 0.1
Night			12	0.15 (not spray)	2	NR	7	NR
Paste Masks (mud packs)			4	NR	1	NR	3	NR
Skin Fresheners			8	NR	14	NR	4	NR
Other Skin Care Preparations			30	0.2			11	0.1
Suntan Preparations								
Suntan Gels, Creams, and Liquids								
Indoor Tanning Preparations								
Other Suntan Preparations								

NR – not reported

*likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

**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 are sprays, but it is not specified whether the reported uses are sprays.

[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

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

Table 6. Oral ADME studies

Parameter Measured	Test Substance	Animals	No./Group	Vehicle/Dose	Dose/Protocol	Results	References
Absorption	[^{14}C]Hyaluronic Acid (MW = 920 kDa)	Male Sprague-Dawley rats	3	Distilled water; 25 mg/kg	Animals were given a single oral dose of the test substance via gavage. Administered radioactivity was 2.04 megabecquerel (MBq)/kg bw. The transition of plasma ^{14}C radioactivity was evaluated by collecting blood from the tail vein of treated animals at 5, 15, and 30 min, and 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, and 168 h post-administration.	The peak plasma radioactivity level was 7.6 µg eq/ml, at 8 h. The half-life was approximately 1.9 d. The area under the concentration-time curves of plasma was determined to be 309 µg of eq/ml/h.	[27]
Distribution	[^{14}C]Hyaluronic Acid (MW = 920 kDa)	Male Sprague-Dawley rats	1/group	Distilled water; 25 mg/kg	Animals were given a single dose of the test substance via gavage, and killed under anesthesia at 8, 24, or 96 h after administration. Whole-body autoradiographs were prepared from radioactivity images recorded on imagine plates.	^{14}C was detected in the skin as follows: 2.36 PSL/mm^2 at 8 h, 3.81 PSL/mm^2 at 24 h, and 1.98 PSL/mm^2 at 96 h after administration. ^{14}C was detected in the blood as follows: 2.12 PSL/mm^2 at 8 h, 1.68 PSL/mm^2 at 24 h, and 0.84 PSL/mm^2 at 96 h after administration. Radioactivity was higher in the skin than in the blood at 24 and 96 h post-administration. In other tissues, the highest levels of radioactivity were observed in the intestinal contents 8 h post-administration (710 PSL/mm^2). Readings in the pancreas (17.45 PSL/mm^2), harderian gland (12.27 PSL/mm^2), liver (9.22 PSL/mm^2), and mandibular gland (7.49 PSL/mm^2) were also high 8 h post-administration. At 96 h post-administration, all radioactivity dropped.	[27]
Distribution	[^{14}C]Hyaluronic Acid (MW = 920,000 kDa)	Male Sprague-Dawley rats	3	Distilled water; 25 mg/kg	Animals were given a single oral dose of the test substance via gavage, and housed in metabolic cages. ^{14}C-excretion rates in the urine, feces, and expired air were evaluated at predetermined times (0-168 h post-administration). Animals were killed at the end of the study to measure the residual radioactivity in the body.	Radioactivity was excreted in the urine as follows: 2.5% of the dose by 24 h, 2.9% by 96 h, and 3% by 168 h. In feces, radioactivity was excreted as follows: 7.8% by 24 h, 11.6% by 96 h, and 11.9% by 168 h. In expired air, radioactivity was excreted as follows: 70.7% of the dose by 24 h, 75.4% by 96 h, and 76.5% by 168 h. The total excretion rate in the urine, feces, and expired air was 91.3% of the administered dose by 168 h post-administration. Approximately 8.8% of the dose remained in the carcass 168 post-administration.	[27]
Metabolism/ Distribution	Hyaluronic Acid (MW = 300 kDa)	Male Sprague-Dawley rats	6/group	Vehicle not stated; 200 mg/kg bw	After overnight fasting, rats were given a single dose of the test substance (method of oral administration not stated). Samples of cecal content, blood, and shaved ventral skin were collected 0, 2, 4, 6, and 8 h after administration. Unsaturated Hyaluronic Acid disaccharides (u-HA2) and tetrasaccharides (u-HA4) in the serum and the supernatant of homogenized skin were analyzed via liquid chromatography-tandem mass spectrometry (LC/MS/MS).	Hyaluronic Acid is degraded by intestinal bacteria and oligosaccharide Hyaluronic Acid is absorbed in the small intestines and widely distributed. Oligosaccharide (di-, tetra-, hexa-, octa-, and decasaccharides) Hyaluronic Acid was observed in cecal content 2 h after test substance administration. The recovery rate of u-HA2 and u-HA4 in the serum and skin was approximately 25 and 70%, respectively. U-HA2 was observed in the serum 2 h after test substance administration, and peaked in concentration after approximately 6-8 h post-administration. U-HA4 was observed in the serum 8 h after test substance administration. Both u-HA2 and u-HA4 were observed in the skin 6 h after test substance administration, and peaked after 8 h.	[28]

Table 6. Oral ADME studies

Parameter Measured	Test Substance	Animals	No./Group	Vehicle/Dose	Dose/Protocol	Results	References
Excretion	Hyaluronic Acid (MW = 300 kDa)	Male Sprague-Dawley rats	8/group	Distilled water; 5 ml/kg bw/d; 1 and 5%	Animals were orally administered the test substance (method of oral administration not stated) for 5 d. Control animals received distilled water only. On the last 3 d of treatment, feces were collected, freeze-dried, and ground for analysis. Hyaluronic Acid concentration was measured using a hyaluronan assay kit with a Hyaluronic Acid binding protein.	Hyaluronic Acid was below the detection limit (10 µg/3 d) in all groups.	[28]

kDa = kilodalton; MW = molecular weight; PSL = photo-stimulated luminescence; SPECT = single photon emission computed tomography

Table 7. Acute oral toxicity studies on Sodium Hyaluronate[16]

Test Article	Vehicle	Animals/Group	Concentration/Dose	Protocol	LD_{50}/Results
Sodium Hyaluronate (FW = 1800-2100 kDa)	NR	ICR mice (number of animals not stated)	500 mg/kg bw	single dose; method of oral administration not stated	LD_{50} > 500 mg/kg bw
Sodium Hyaluronate	peanut oil	Kunming mice (10/sex)	2000 mg/kg bw	single dose; gavage; 14-d evaluation	LD_{50} > 2000 mg/kg bw; no signs of toxicity or deaths
Sodium Hyaluronate	peanut oil	Kunming mice (20/sex)	1000, 2150, 4640, or 10,000 mg/kg bw	single dose; gavage; 14-d evaluation	LD_{50} > 10,000 mg/kg bw; no signs of toxicity or deaths
Sodium Hyaluronate	corn germ oil	Kunming mice (10/sex)	15,000 mg/kg bw	single dose; gavage; 14-d evaluation	LD_{50} > 15,000 mg/kg bw; no signs of toxicity or deaths
Sodium Hyaluronate (FW = 1800 – 2100 kDa)	NR	Rats (strain and number of animals not stated)	200 mg/kg bw	single dose; method of oral administration not stated	LD_{50} > 200 mg/kg bw; no signs of toxicity or deaths
Sodium Hyaluronate (FW = 270 kDa)	distilled water	Wistar rats (10/sex)	5280 mg/kg bw	single dose; gavage	MTD > 5280 mg/kg bw; no signs of toxicity or deaths

LD_{50} = median lethal dose; MTD = maximum tolerable dose; MW = molecular weight; FW = formula weight; NR = not reported

Table 8. Oral repeated dose toxicity studies on Sodium Hyaluronate[16]

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results
Sodium Hyaluronate	feed	Wistar rats (10/sex/group)	30 d	0, 167, 500, or 1500 mg/kg bw	rats given test substance via feed; body weight changes, hematological, biochemistry, and macroscopic parameters were evaluated	no deaths or changes in body weight, food consumption, or weight gain; all blood chemistry parameters were within normal ranges; no changes in organ weight/histopathological parameters; NOAEL = 1500 mg/kg bw/d
ophthalmic solution containing 1% Sodium Hyaluronate	NR	Sprague-Dawley rats (5-10/sex/group)	90 d	0, 3, 12, or 48 mg/kg bw/d	rats given test substance via gavage; body weight, food efficiency, urinalysis, and gross pathological and histopathological parameters evaluated	no dose-dependent changes in body weight, histopathological parameters, or hematological parameters were observed; NOAEL = 48 mg/kg bw/d
Sodium Hyaluronate (FW = 2270 kDa)	feed	Wistar rats (10/sex/group)	90 d	0, 330, 670, or 1000 mg/kg bw	rats given test substance via feed; evaluated for 28 d following treatment period; body weight changes, hematological, biochemistry, and macroscopic, and gross pathological parameters were evaluated	no deaths or changes in body weight, food consumption, weight gain, hematological parameters, or histopathological parameters; NOAEL = 1000 mg/kg bw/d
Sodium Hyaluronate	corn germ oil	Sprague-Dawley rats (12/sex/group)	90 d	0, 667, 100, or 1333 mg/kg bw/d	rats given test substance via gavage; body weight changes, hematological, biochemistry, and macroscopic, and gross pathological parameters were evaluated	no changes in behavior, feeding, body weight, food consumption, hematological parameters, organ weights, or macroscopic/histological parameters were observed; NOAEL = 1333 mg/kg bw/d

FW = formula weight; NOAEL = no-observed-adverse-effect-level; NR = not reported

Table 9. Genotoxicity studies[16,29-31]

Test Substance	Test Concentration/Dose	Vehicle	Test System	Procedure	Results
			IN VITRO		
Hydrolyzed Sodium Hyaluronate (FW = 5-10 kDa)	up to 5 mg/plate (specific concentrations not stated)	NR	*S. typhimurium* strains TA97a, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
Hydrolyzed Sodium Hyaluronate (FW < 1kDa)	up to 5 mg/plate (specific concentrations not stated)	NR	*S. typhimurium* strains TA97a, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	1 mg/plate	NR	*S. typhimurium* strains TA97a, TA98, TA100, TA102, and TA1535	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	up to 1 mg/plate (specific concentrations not stated)	NR	*S. typhimurium strains TA97a, TA98, TA100, TA102, and TA1535*	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	0.008, 0.04, 0.2, 1, and 5 mg/plate	NR	*S. typhimurium* strains TA97a, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	0.2, 0.5, 1, 2.5, and 5 mg/plate	NR	*S. typhimurium* strains TA97, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	up to 5 mg/plate (specific concentrations not stated)	NR	*S. typhimurium* strains TA97a, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	without metabolic activation/*S. typhimurium* and *E. coli*: 313, 625, 1250, 2500, 5000 µg/plate with metabolic activation/ *S. typhimurium*: 39.1, 78.1, 156, 313, 625, 1250 µg/plate with metabolic activation/*E. coli*: 313, 625, 1250, 2500, 5000 µg/plate	NR	*S. typhimurium strains TA98, TA100, TA1535, TA1537* and *E. coli* WP2 *uvrA*	Ames assay performed with and without metabolic activation	Non-genotoxic
			IN VIVO		
Sodium Hyaluronate	20 ml/kg bw	NR	mice (strain not reported; 5/sex/group)	Mouse micronucleus assay; cyclophosphamide used as positive control; distilled water used as negative control; administrations via gavage	non-mutagenic
Sodium Hyaluronate	440, 880, 1760 mg/kg bw	NR	KS mice (5/sex/group)	Mouse micronucleus assay; cyclophosphamide used as positive control; distilled water used as negative control; administrations via gavage	non-mutagenic
Sodium Hyaluronate	1250, 2500, and 5000 mg/kg bw	NR	KS mice (5/sex/group)	Mouse micronucleus assay; cyclophosphamide used as positive control; corn germ oil used as negative control; administrations via gavage	non-mutagenic

NR = not reported

Table 10. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/ Dose	Test Population	Procedure	Results	Reference
IRRITATION						
IN VITRO						
Trade name mixture containing 1% Hyaluronic Acid	NR	100%: 30 µl	3	EpiDerm™ assay; 60 min incubation period; sterile PBS and sterile deionized water used as negative controls; 5% SDS solution and methyl acetate used as positive control; cell viability evaluated via MTT assay	Non-irritating	[32]
Trade name mixture containing 1% Hyaluronic Acid	NR	100%: 30 µl	3	Same as above	Non-irritating	[33]
Trade name mixture containing 3% Hyaluronic Acid	NR	100%: 30 µl	3	Same as above	Non-irritating	[34]
Hydrolyzed Sodium Hyaluronate (FW < 1 kDa)	NR	1%	NR	OECD TG 439; reconstructed human epidermis assay	Non-irritating	[31]
Trade name mixture containing 0.5% Sodium Hyaluronate	NR	100%; 30 µl	3	EpiDerm™ assay; 60 min incubation period; sterile PBS and sterile deionized water used as negative controls; 5% SDS solution and methyl acetate used as positive control; cell viability evaluated via MTT assay	Non-irritating	[35]
Sodium Hyaluronate	NR	NR	NR	OECD TG 439; reconstructed human epidermis assay	Non-irritating	[29]
HUMAN						
Hydrolyzed Sodium Hyaluronate (FW = 1-5 kDa)	NR	0.5%	32	Human skin closed patch test; test substance applied to skin of individuals with sensitive skin for 24 g	Non-irritating	[31]
Hydrolyzed Sodium Hyaluronate (FW = 1-5 kDa)	NR	0.5 and 2%	30	Human skin closed patch test; applications on healthy skin; no other details provided	Non-irritating	[31]
Hydrolyzed Sodium Hyaluronate (FW = 5-10 kDa)	NR	1%	NR	Human skin closed patch test; no details provided	Non-irritating	[31]
Hydrolyzed Sodium Hyaluronate (FW < 1 kDa)	NR	1%	30	Human skin closed patch test; applications on healthy skin; no other details provided	Non-irritating	[31]
SENSITIZATION						
IN CHEMICO/IN VITRO						
Trade name mixture containing 1% Hyaluronic Acid	NR	5 (0.05 ml) and 25 mM (250 µl)	3/concentration tested	OECD TG 442C; DPRA; 24 h incubation period; mean percent depletion of cysteine and lysine evaluated; positive control: cinnamic aldehyde in acetonitrile; negative control: peptide in buffer	Mean percent depletion of cysteine and lysine was 3.11%; prediction of non-sensitizing	[36]
Trade name mixture containing 1% Hyaluronic Acid	NR	0.00098 – 2mM; 0.05 ml	3/concentration tested	OECD TG 442D: ARE-Nrf2 Luciferase Test Method; KeratinoSens™ cell line; positive control: cinnamic aldehyde; negative control: DMSO	I_{max} of 0.35 compared to I_{max} of 31.5 and 0.33 for positive and negative control, respectively; prediction of non-sensitizing	[37]
Sodium Hyaluronate	NR	1 mg/ml	NR	OECD TG 442E; h-CLAT	Mean RFI_{CD54} < 200 and mean RFI_{CD86} < 150; cell activity > 50%; prediction of non-sensitizing	[29]

Table 10. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/ Dose	Test Population	Procedure	Results	Reference
			HUMAN			
Formula containing 0.2% Hyaluronic Acid	NR	100%; 20 mg	115	HRIPT; occlusive conditions; 9 applications over a 3-wk period for induction period; challenge phase (48-h patch application) after a 10-15 d rest period; challenge patches evaluated immediately after removal, and 24, 48 and 72 h after patch removal	Non-irritating and non-sensitizing	[38]
Formula containing 0.2% Sodium Acetylated Hyaluronate		100%; 0.02 ml	104	HRIPT; occlusive conditions; 9 applications over a 3-wk period for induction period; challenge phase (48-h patch application) after a 14-d rest period; challenge patches evaluated 15 min and around 48 h after patch removal	Non-irritating and non-sensitizing	[39]
Hydrolyzed Sodium Hyaluronate (FW = 5-10 kDa)	NR	0.5%	55	HRIPT; no details provided	Non-irritating and non-sensitizing	[31]
Sodium Hyaluronate	NR	0.2%	50	HRIPT; no details provided	Non-sensitizing	[30]
Sodium Hyaluronate	NR	0.2%	100	HRIPT; no details provided	Non-irritating and non-sensitizing	[29]
Formula containing 1.5% Sodium Hyaluronate	NR	100%; 0.2 ml	198	HRIPT; occlusive conditions; 9 applications over a 3-wk period for induction period; challenge phase (48-h patch application) after a 10-15 d rest period; challenge patches evaluated immediately after removal, and 24, 48 and 72 h after patch removal	Non-irritating and non-sensitizing	[40]

ARE = antioxidant response element; DMSO = dimethyl sulfoxide; DPRA - direct peptide reactivity assay; h-CLAT - human cell line activation test; HRIPT = human repeated insult patch test; I_{max} = maximum response value; MTT = 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; NR = not reported; Nfr2 = nuclear factor erythroid 2-related factor 2; OECD TG = Organisation for Economic Cooperation and Development Test Guidelines; PBS = phosphate-buffered saline; RFI = relative fluorescence intensity; SDS = sodium dodecyl sulfate

Table 11. Ocular irritation studies

Test Article	Vehicle	Test Concentration/ Dose	Test Population	Procedure	Results	Reference
IN VITRO						
Trade name mixture containing 1% Hyaluronic Acid	NR	100%; 50 µl	2	EpiOcular™ assay; 90 min incubation period; sterile PBS and sterile deionized water used as negative controls; 5% SDS solution and methyl acetate used as positive control; cell viability evaluated via MTT assay	Non-irritating	[32]
Trade name mixture containing 1% Hyaluronic Acid	NR	100%; 50 µl	2	Same as above	Non-irritating	[33]
Trade name mixture containing 3% Hyaluronic Acid	NR	100%; 50 µl	2	Same as above	Non-irritating	[34]
Hydrolyzed Sodium Hyaluronate (FW < 1kDa)	NR	NR	NR	CAMVA (no details provided)	Non-irritating/slightly irritating	[31]
Trade name mixture containing 0.5% Sodium Hyaluronate	NR	100%; 50 µl	2	EpiOcular™ assay; 90 min incubation period; sterile PBS and sterile deionized water used as negative controls; 5% SDS solution and methyl acetate used as positive control; cell viability evaluated via MTT assay	Non-irritating	[35]
Sodium Hyaluronate	NR	100%	NR	CAMVA (no details provided)	Non-irritating/slightly irritating	[29]
Sodium Hyaluronate	NR	100%	NR	BCOP test (no details provided)	Non-irritating/slightly irritating	[29]
ANIMAL						
Bacillus-derived and *Streptococcus*-derived Hyaluronic Acid	NR	0.1 and 0.3%; 25 µl	New Zealand white rabbits (3/group)	Test substances were placed on the right eye, 4x/d, for 3 d. After the last instillation, rabbits were sedated, and eyes were evaluated via fluorescent imaging	The test substance was considered to be very well-tolerated	[41]

BCOP - bovine corneal opacity and permeability; CAMVA - chorioallantoic membrane vascular assay; MTT = 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; NR = not reported; PBS = phosphate-buffered saline; SDS = sodium dodecyl sulfate

Table 12. Case reports of hypersensitivity following injectable Hyaluronic Acid dermal fillers

Patient	Case report summary	Reference
50-yr-old female	-patient had previous Hyaluronic Acid injections in glabellar region and nasolabial fold with no adverse effects -1 yr later, patient received same treatment, and presented with erythematous, livedoid rash 3 d after injections -rash cleared within 10 d of treatment with antibiotics and steroids -patient repeated injections 3 yr later with no adverse effects	[50]
47-yr-old female	-facial Hyaluronic Acid injections at different locations of the face -12 mo later, patient complained of abdominal pain, asymmetric erythematous swelling of the lips, and pain and tingling of lips -angioedema resolved with injection of hyaluronidase	[51]
54-yr-old female	-facial injections of Hyaluronic Acid gel at multiple areas of face, once in April 1998 and again in November 1988, with no adverse effects -patient received Hyaluronic Acid injections in melolabial folds in June 1999, and developed indurated and erythematous papulocystic nodules 2 wk after injections -patient treated with corticosteroids and warm compresses, and nodules improved -2 wk later, patient returned with recurrent inflammation of the right mesolabial fold with tender nodules -patient again treated with corticosteroids and warm compresses, and experienced rapid resolution of symptoms	[52]
59-yr-old female	-injections of Hyaluronic Acid in melolabial folds, glabella, lips, and perioral rhytids -2 d after injections, patient noted significant swelling and pain at injection sites -5 d after injections, patient admitted to hospital for significant facial swelling -treated with corticosteroids and an immunosuppressant	[53]
52-yr-old female	-left and right upper lip injections of Hyaluronic Acid -5 min after injections, patient experienced worsening edema and erythema -patient treated with dexamethasone sodium phosphate, prednisone, and valacyclovir -the following day, the patient still had severe edema and fissures on lip mucosa -patient instructed to apply an emollient ointment, and reported improved symptoms	[54]
56-yr-old female	-melolabial fold injections of Hyaluronic Acid -27 d after injections, patient developed erythematous indurated papules at injection sites -treatment with steroids resolved symptoms	[55]
65-yr-old female	-prior to injections, skin prick tests of Hyaluronic Acid performed and yielded negative results -lip, nasolabial fold, and perioral rhytide injections of Hyaluronic Acid with no adverse effects -a second treatment was performed 3 mo later with no adverse effects -a third treatment was performed 6 mo later with no adverse effects -1 mo after the third treatment, patient re-treated, and presented with erythema, edema, and induration of injection regions 6 wk after 4th series of injections -symptoms improved with steroid treatment	[56]
54-yr-old female	-patient reported previous facial injections of Hyaluronic Acid (every 4 mo) with no adverse effects -10 d after an injection of Hyaluronic Acid to melolabial folds, patient reported granulomatous reaction -symptoms improved with betamethasone treatment	[57]
28-yr-old female	-swelling, pain, and tenderness at injection sites 3 mo after chin injections of Hyaluronic Acid -antibiotics did not resolve symptoms -treatment with corticosteroid, antihistamine, and clindamycin resolved symptoms	[58]
29-yr-old female	-asymmetry, edema, and inflammatory nodules seen at injection sites 112 d after facial injections of Hyaluronic Acid -treatment with steroids, antihistamines, and antibiotics resolved symptoms	[59]
49-yr-old female	-facial edema observed 28 d after glabella and eye area injections of Hyaluronic Acid -treatment with corticosteroids resolved symptoms	[59]
52-yr-old female	-inflammatory nodules, pustules, and fever observed 2 d after glabella injections of Hyaluronic Acid -treatment with steroids, antibiotics, and coloplast cream resolved symptoms	[59]
56-yr-old female	-pruritis and blisters 14 d after facial Hyaluronic Acid injections -treatment with steroids, antihistamines, saline dressings, and betamethasone resolved symptoms	[59]
42-yr-old-female	-inflammatory nodules 1 yr after facial Hyaluronic Acid injections -treatment with moxypen cefamezin resolved symptoms	[59]
60-yr-old female	-patient reported 2 previous series of Hyaluronic Acid injections with no adverse effects -erythema, pain, and edema observed 14 d after 3rd round of Hyaluronic Acid injections in the cheeks -patient treated with antibiotics, pulsed light therapy, and physical therapy	[60]
30-yr-old female	-patient reported previous facial Hyaluronic Acid injections with no adverse effects -5 yr after previous injections, patient was treated with Hyaluronic Acid injections in the cheeks, mandible, and chin -the following day, patient reported sore throat and treated with antibiotics -by day 10, patient presented with erythema and edema of lip and chin, treated with corticosteroids -by day 18, patient presented with painful, palpable, subcutaneous collections at the chin, cheekbone, and mandible -patient treated with antibiotics and incision/drainage of collections -patch and intradermal testing to evaluate the potential of a hypersensitivity reaction to Hyaluronic Acid was performed 3 mo later, and resulted in negative results	[60]
56-yr-old female	-swelling 4 mo after injections of Hyaluronic Acid to cheeks -1 yr later, patients re-treated with injections in the cheeks, and developed facial swelling 4 mo after treatment -patient treated with antibiotics and hyaluronidase	[61]

Table 12. Case reports of hypersensitivity following injectable Hyaluronic Acid dermal fillers

Patient	Case report summary	Reference
57-yr-old female	-patient reported previous treatment with Hyaluronic Acid to the perioral area, on 2 occasions, with no adverse effects -patient experienced erythema, warmth, and rigidity at injection sites 3 wk after 3rd series of facial Hyaluronic Acid injections -3 mo later, a nontender deep, firm, palpable thickening over both zygomatic arches was apparent -patient treated with antibiotics and hyaluronidase -a recurrent episode occurred 3 mo later, and was again treated with hyaluronidase	61
32-yr-old female	-patient reported previous Hyaluronic Acid injections lips with no adverse effects, and acute swelling after injection to hands -erythema and swelling at injection sites 6 mo after treatment with Hyaluronic Acid injections in cheeks -patient treated with hyaluronidase -recurrent redness and swelling occurred 2 mo later, and was again treated with hyaluronidase	61
48-yr-old female	-patient reported previous Hyaluronic Acid injection treatment in marionette lines with no adverse effects -swelling of cheeks 1 wk after Hyaluronic Acid injections to cheeks -treatment with corticosteroids -flare-ups occurred 3 and 4 mo post-injection, treated with corticosteroids	61
54-yr-old female	-redness and swelling of the nasolabial folds after Hyaluronic Acid injections -severe palpable and painful erythematous nodular papulocystic lesions 3 mo after injections -patient surgically treated	62
48-yr-old female	-blue/gray coloring of lips, cheek, and nose 8 h after Hyaluronic Acid injections -treatment with nitroglycerin and hyperbaric chamber	63
41-yr-old female	-erythematous nodules at injection sites 5 wk after melolabial, glabellar, and periorbital area injections of Hyaluronic Acid -treatment with antibiotics and steroids	64
49-yr-old female	-asymptomatic hard lesions along melomental folds 4 mo after lower facial injections of Hyaluronic Acid -treatment with corticosteroids and hyaluronidase -patch tests performed were negative at 48 and 96 h -intradermal injection into forearm was negative at 20 min and 96 h, but turned positive 2 mo later	65
72-yr-old female	-well-defined, millimetric, firm nodules on lips and oral mucosa 5 mo after Hyaluronic Acid injections -treatment with corticosteroids and hyaluronidase	66
45-yr-old female	-glabellar, neck, eyelid injections of Hyaluronic Acid and *Botulinum* toxin -1 mo after injection, patient developed facial pain, erythema, and edema -patient's symptoms improved following treatment with pain medication, antibiotics, and steroids	67
53-yr-old female	-patient reported previous Hyaluronic Acid injection to nasolabial folds and lips -asymmetry of nasolabial fold, palpable pea sized-lesions 1 yr after Hyaluronic Acid injections -patient treated with antibiotics and ibuprofen	68
40-yr-old female	-dusky, red, firm, linear rash 4 mo after injection of a mixture of Hyaluronic Acid gel and acrylic hydrogel to the nasolabial folds -treatment with betamethasone	69
66-yr-old female	-patient reported 12 treatments of facial Hyaluronic Acid injection over the course of 5 yr -patient reported an increasing number of hard lumps in areas that were repeatedly treated with Hyaluronic Acid gel and acrylic hydrogel -eventually developed into symmetrical linear purple plaques, nodules, induration of lips -treatment with steroids	70
65-yr-old female	-patient reported 3 treatments with a mixture of Hyaluronic Acid gel and acrylic hydrogel -hard subcutaneous nodules in nasolabial folds, upper lip, and glabella 2 yr after last treatment -treatment with steroids	70

*It should be noted that dermal fillers are derived from one of two methods: a non-animal method (bacterial fermentation using *Streptococcus*) or via extraction of chicken/rooster combs.[71]

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