Safety Assessment of Hyaluronates as Used in Cosmetics

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All interested persons are provided 60 days from the above release date December 4, 2022) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party, and may be cited in a peer-reviewed scientific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

BDDE	1,4-butanediol diglycidyl ether
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
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
Dictionary	*International Cosmetic Ingredient Dictionary and Handbook*
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
EU	endotoxin units
FCC	*Food Chemicals Codex*
FDA	Food and Drug Administration
FW	formula weight
HA	Hyaluronic Acid
HRIPT	human repeated insult patch test
I-NOSE	Nasal Obstruction Symptom Evaluation Instrument
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
MW	molecular weight
ND	not detected
NIBUT	non-invasive break-up time
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOEL	no-observed-effect-level
NOAEL	no-observed-adverse-effect-level
NR	not reported
OECD	Organisation for Economic Cooperation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PSL	photo-stimulated luminescence
SPECT	single photon emission computed tomography
^{99m}Tc	technetium-99m (a radionuclide nuclear agent)
TG	test guidelines
US	United States
UVB	ultraviolet light B (mid-wavelength)
VCRP	Voluntary Cosmetic Registration Program

INTRODUCTION

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

Hyaluronic Acid	Potassium Hyaluronate
Hydrolyzed Calcium Hyaluronate	Sodium Acetylated Hyaluronate
Hydrolyzed Hyaluronic Acid	Sodium Hyaluronate
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).[1] Sodium Acetylated Hyaluronate is reported to function in cosmetics as a humectant.

All ingredients in this report are structurally similar as they are salts or acetylated esters derived from Hyaluronic Acid. In 2009, the Expert Panel for Cosmetic Ingredient Safety (Panel) published a final report on the safety of Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate.[2] Based on the available data, the Panel concluded that these 3 ingredients are safe in the present practices of use and concentration, as described in the safety assessment. (The report is available on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/ingredients).) Because it has been almost 15 years since that report was published, and a re-review on Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate is imminent, these ingredients are being reviewed herein.

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 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.

Excerpts from the 2009 report of Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate 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, and relevant new data were included

Hyaluronic Acid injections are used as dermal fillers, in surgical procedures, and arthritic therapy. Subsequently, safety and efficacy data for these uses were found in the published literature. 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 and structures of the ingredients included in this review are provided in Table 1.

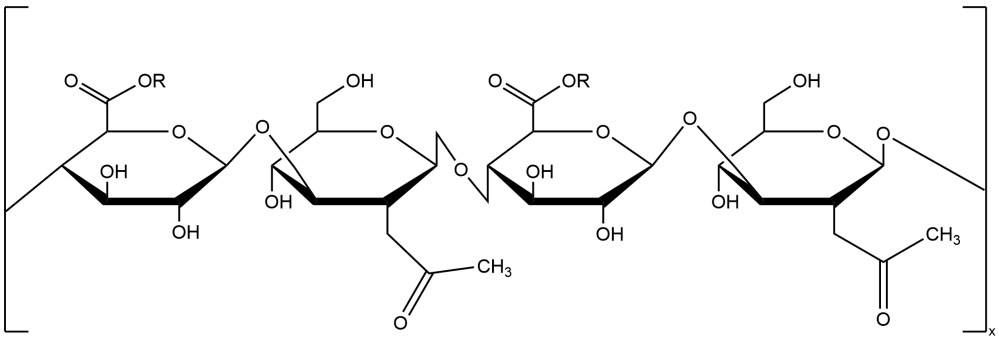

Figure 1. Hyaluronates (when R is hydrogen = Hyaluronic Acid; when R is sodium = Sodium Hyaluronate; etc.)

In order to improve the mechanical properties, degradation rate, and clearance of Hyaluronic Acid, Hyaluronic Acid can be cross-linked to form a hydrogel material.[4] According to the literature, the most commonly-used cross-linker in Hyaluronic Acid dermal fillers in the US is 1,4-butanediol diglycidyl ether (BDDE).[5] Unreacted cross-linking agents and their

byproducts are potentially toxic compounds, and should be removed from gels before use. The impurities of a BDDE-crosslinked Hyaluronic Acid dermal filler can be found in the Composition and Impurities section of this report

Chemical Properties

Hyaluronic Acid is a water-soluble substance that is in a highly purified, freeze-dried powder or aqueous solution, and as its potassium or sodium salt.[2] 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.[2] 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.[2]

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.[6] 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 caesium 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.[6] 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.

Sodium Hyaluronate

Like Hyaluronic Acid, Sodium Hyaluronate can be derived from animal tissues (e.g., rooster combs) or microbial fermentation.[7] Sodium Hyaluronate manufactured for food use was reported to be manufactured via fermentation using the bacterial strain *Streptococcus equi* subsp. *zooepidemicus*.[8,9] This process begins with the preparation of a seed broth prepared from seed culture, which is transferred to a fermenter containing sterilized fermentation medium. Following fermentation, the 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 final product.

Impurities

When derived from animal sources, Hyaluronic Acid may contain several impurities.[2] These impurities include proteins, DNA, and chondroitin sulfate.

Hyaluronic Acid

The impurities (nucleic acid, protein, endotoxins) of Hyaluronic Acid obtained from several sources (*Streptococcus zooepidemicus*, rooster comb, bovine vitreous, human umbilical cord) were evaluated.[10] Nucleic acid and protein impurities were highest in human umbilical cord- and bovine vitreous-derived Hyaluronic Acid, and was 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.

The type of Hyaluronic Acid cross-linkage may affect the safety and impurities profile of the ingredient.[5] The major impurity present in BDDE-cross-linked Hyaluronic Acid dermal fillers is 1,4-butanediol di-(propan-2,3-diolyl) ether.

Sodium Hyaluronate

According to the *FCC*, Sodium Hyaluronate manufactured for use in foods may not contain more than 1 mg/kg lead, 2 mg/kg arsenic, or 0.5% chloride.[9] A manufacturer for food-use Sodium Hyaluronate states that potential contaminants of

Sodium Hyaluronate include microbes and heavy metals.[8] 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.[3,7] 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..[6]

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics, and does not cover their use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration Program (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)). All other ingredients are reported to be used in 568 formulations or less. In addition, 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).[11] Current FDA VCRP data on the four hyaluronate ingredients included in this report that have not been previously reviewed (Hydrolyzed Calcium Hyaluronate, Hydrolyzed Hyaluronic Acid, Hydrolyzed Sodium Hyaluronate, and Sodium Acetylated Hyaluronate) can be found in Table 5. According to previous (2005) FDA VCRP data, Sodium Hyaluronate had the highest number of uses and concentration of use; this ingredient was used in 601 formulations at concentrations of up to 2% (Table 4).

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.[12]

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.[13] In

addition, Sodium Hyaluronate is used as in inactive ingredient in several FDA-approved medications.[14] 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.[15]

Hyaluronic Acid is found as a natural component in foods as it is present in animal products.[8] 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.[8] In addition, both Hyaluronic Acid and Sodium Hyaluronate are reported to be ingredients of dietary supplements on the market in the US.[16]

TOXICOKINETIC STUDIES

Dermal Penetration

Autoradiography was used to detect the dermal penetration of Hyaluronic Acid in SKh/1 hairless mice (4 animals/group; sex not stated; one group treated with radioactive gel; one group treated without radioactive gel).[2] Mice were treated every 12 h for 3 or 12 total applications. 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 performed according to similar procedures using 10 mice (strain and sex not reported), 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 (1350 – 4500 Da) 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.[17] 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, and 10 µm-thick transverse skin sections were realized 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 1000 – 14,000 kDa 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.

Penetration Enhancement

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.[2,18] 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.

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 = 920,000 Da) via gavage, the peak plasma radioactivity level was 7.6 µg eq/ml 8 h post-administration.[19] The highest amount of radioactivity was observed in intestinal contents 8 h post-administration. When evaluating excretion, the total excretion rate in the urine, feces, and expired air was 91.3% of the administered dose by 168 h post-administration. In a different assay, male Sprague-Dawley rats (6/group) were orally administered Hyaluronic Acid (MW = 300,000 Da; 200 mg/kg bw), and blood, cecal content, and ventral skin were evaluated at different time intervals.[20] 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. The distribution of [^{99m}Tc]Hyaluronic Acid (0.2 ml; single dose; MW = 1.1 – 1.5 MDa) was evaluated in Wistar rats (3/group).[21] A rapid uptake of radioactivity was observed in the bone, muscle, small intestine, and large intestine at the 5- and 15-min time points. Excretion assays were performed in Wistar rats (n = 5) and Beagle dogs (n = 2) using the same test substance ([^{99m}Tc]Hyaluronic Acid; 0.2 ml in

rats and 1.5 ml in dogs; single dose administration). In rats, the total urine and feces excretion of the ingested dose was 86.7 ± 8.0%. In dogs, urinary clearance of the test substance appeared after 30 min, and returned to background after 12 h.

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.[2] 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).[8] 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 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.[8] 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.[2] In the majority of 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 were present in the adrenal glands of treated animals. No severe fetal abnormalities were observed in rats or rabbits.

Sodium Hyaluronate

A teratogenicity assay was performed in Wistar rats (15/group) given Sodium Hyaluronate (MW = 270 kDa) via gavage in doses of 0, 170, 330, or 670 mg/kg bw/d (administration during gestation days 7 - 16).[8] 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.[8] 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 control and negative control treated groups. No evidence of maternal toxicity or teratogenicity resulting from test substance administration were observed.

GENOTOXICITY

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.

Sodium Hyaluronate was determined to be non-genotoxic in several Ames assays when tested at up to 5 mg, with and without metabolic activation (assays performed in *S. typhimurium* strands TA97a, TA98, TA100, TA102, and TA1535).[8] In

addition, Sodium Hyaluronate (up to 5000 mg/kg bw) was non-mutagenic in mouse micronucleus assays using KS mice (5/sex/group).

CARCINOGENICITY STUDIES

No carcinogenicity studies were found in the published literature, and unpublished data were not submitted.

OTHER RELEVANT STUDIES

Multiple injections of Hyaluronic Acid derived from human umbilical cords or streptococcal fermentation did not result in sensitization in immunogenicity studies performed in rabbits. 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, 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. Injections of cross-linked Hyaluronic Acid resulted in acute cutaneous anaphylaxis and delayed-type hypersensitivity in guinea pigs 48 post-administration.

Use in Dissolving Microarray Patches

Hyaluronic Acid

The following study is included in this report as it may be helpful in addressing cosmetic safety concerns regarding irritation following dermal exposure to 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.[22] 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.

DERMAL IRRITATION AND SENSITIZATION

Hyaluronic Acid was non-irritating in a single-stimulus skin test using Japanese rabbits and Hartley guinea pigs.[2] 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.

No additional irritation studies and no sensitization studies were found in the published literature, and unpublished data were not submitted.

OCULAR IRRITATION STUDIES

Hyaluronic Acid

An ocular irritation assay was performed in New Zealand white rabbits (n = 3) evaluating the tolerance of both *Bacillus*-derived and *Streptococcus*-derived Hyaluronic Acid (each test substance was tested at concentrations of 0.1 and 0.3%).[23] The test substances (25 µl) were placed on the right eye, 4 times per day, for 3 d. After the last instillation, rabbits were sedated, and 25 µl of sodium fluorescein (0.5%) was instilled in the right eye to selectively marked injured areas. After 2 min, eyes were rinsed with a 0.9% sodium chloride solution, the fluorescent image of the central corneal apex was taken, and eyes were evaluated. The test substances were considered to be very well-tolerated.

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.[24] Patients were assessed at baseline, on day 10, and day 20. An endoscopic nasal examination and I-NOSE (Nasal Obstruction Symptom Evaluation Instrument) 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 6 Hyaluronic Acid fillers were subjected to intracutaneous tests using 6 different types of Hyaluronic Acid dermal fillers.[25] 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.[26] 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 that 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.[27] 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.

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 10. 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.[28] 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.[29] 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 1000 - 1400 kDa). Hyaluronic Acid solutions, from lowest to highest MW, were present in the epidermis at 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 = 920,000 Da) via gavage, the peak plasma radioactivity level was 7.6 µg eq/ml 8 h post-administration. When evaluating excretion, the total excretion rate in the urine, feces, and expired air was 91.3% of the administered dose 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. The distribution and excretion of [^{99m}Tc]Hyaluronic Acid (MW = 1.1 – 1.5 MDa) was evaluated in Wistar rats and Beagle dogs. In rats, a rapid uptake of radioactivity was observed in the bone, muscle, small intestine, and large intestine at the 5- and 15-min time points. The total urine and feces excretion of the ingested dose was 86.7 ± 8.0%. In dogs, urinary clearance of the test substance appeared after 30 min, and returned to background after 12 h.

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).

No maternal toxicity or teratogenicity resulting from Sodium Hyaluronate (up to 1333 mg/kg bw/d) were observed in two reproductive 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.

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

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.

No irritation was noted in an ocular irritation assay performed in New Zealand white rabbits. Bacterially-derived Hyaluronic Acid (up to 0.3%) was instilled in the eyes of rabbits, 4x/d, for 3 d. The test substance was considered to be very well tolerated.

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.

Case reports were found in the literature regarding hypersensitivity/adverse reactions to Hyaluronic Acid used as an injectable dermal filler. 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.

INFORMATION SOUGHT

The following information on the hyaluronates reviewed in this report is being sought for use in the resulting safety assessment:

- Composition and impurities data
- Method of manufacturing data, specific to use in cosmetic formulations
- Carcinogenicity data
- Dermal irritation/sensitization data

TABLES

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

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	[2]
Molecular Weight (kDa)	5 - 1800	[2]
Sodium Hyaluronate		
Physical Form	powder	[2]
Color	white	[2]
Odor	faint odor	[2]
Formula Weight (kDa)	80.2 - 4010	[9]

Table 3. Impurities of Hyaluronic Acid derived from different sources (per 1 mg Hyaluronic Acid)[10]

	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 samples were tested; EU = endotoxin units; HA = Hyaluronic Acid; MW = molecular weight; ND = not detected

Table 4. Current and historical frequency and concentration of use of the previously-reviewed ingredients according to duration and exposure

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	Hyaluronic Acid				Potassium Hyaluronate			
	2022[30]	2005[2]	2021[11]	2005[2]	2022[30]	2005[2]	2021[11]	2005[2]
Totals*	**568**	**223**	**0.000002 – 0.83**	**0.00005 - 1**	**44**	**11**	**NR**	**NR**
Duration of Use								
Leave-On	*493*	*194*	*0.000002 – 0.3*	*0.00005 – 1*	*43*	*10*	*NR*	*NR*
Rinse-Off	*72*	*29*	*0.002 – 0.83*	*0.001 – 0.3*	*1*	*1*	*NR*	*NR*
Diluted for (Bath) Use	*3*	*NR*	*0.0089*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	45	33	0.001	0.001 – 0.07	NR	NR	NR	NR
Incidental Ingestion	3	NR	0.003 – 0.05	0.01	NR	NR	NR	NR
Incidental Inhalation-Spray	3; 213[a]; 158[b]	57[a]; 21[b]	NR	0.001 – 0.1[a]; 0.001 – 1[b]	22[a]; 14[b]	4[a]; 6[b]	NR	NR
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
Dermal Contact	542	216	0.000002 – 0.83	0.00005 – 1	44	11	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	22	4	0.0036	NR	NR	NR	NR	NR
Hair-Coloring	NR	1	0.002	NR	NR	NR	NR	NR
Nail	NR	2	NR	0.001 – 0.01	NR	NR	NR	NR
Mucous Membrane	27	1	0.003 – 0.05	0.01	NR	NR	NR	NR
Baby Products	6	NR	NR	0.001	NR	NR	NR	NR

	Sodium Hyaluronate			
	2022[30]	2005[2]	2021[11]	2005[2]
Totals*	**4048**	**601**	**0.00001 – 7.5**	**0.000001 – 2**
Duration of Use				
Leave-On	*3680*	*552*	*0.00001 – 7.5*	*0.000001 – 2*
Rinse-Off	*366*	*49*	*0.0001 – 0.12*	*0.000001 – 0.5*
Diluted for (Bath) Use	*2*	*NR*	*NR*	*0.001 – 0.5*
Exposure Type				
Eye Area	259	49	0.0001 – 0.96	0.0001 – 0.7
Incidental Ingestion	219	96	0.24 – 0.39	0.0002 – 0.5
Incidental Inhalation-Spray	10; 1376[a]; 1250[b]	1; 180[a]; 73[b]	0.01; 2[a]	0.0002 – 0.5; 0.000001 – 1[a]; 0.0001 – 2[b]
Incidental Inhalation-Powder	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	3746	482	0.00001 – 7.5	0.000001 – 2
Deodorant (underarm)	4[a]	NR	0.013	0.5[a]
Hair - Non-Coloring	62	12	0.005 – 2	0.001 – 0.5
Hair-Coloring	1	2	NR	0.5
Nail	1	NR	0.025	0.01 – 0.5
Mucous Membrane	251	97	0.01 – 0.39	0.0002 – 0.5
Baby Products	14	NR	0.005	0.5

*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

NR – no reported use

Table 5. Frequency (2022) and concentration (2021) of use of ingredients not previously reviewed[11,30,31]

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Hydrolyzed Calcium Hyaluronate		Hydrolyzed Hyaluronic Acid	
Totals*	**2**	**NR**	**362**	**0.002 – 0.2**
Duration of Use				
Leave-On	*2*	*NR*	*320*	*0.01- 0.2*
Rinse-Off	*NR*	*NR*	*42*	*0.002 – 0.01*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	NR	NR	9	0.01
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	2[a]	NR	89; 166[a]; 5[b]	0.02
Incidental Inhalation-Powder	NR	NR	4; 5[b]; 4[c]	0.01[c]
Dermal Contact	2	NR	352	0.01 – 0.2
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	10	0.002 – 0.02
Hair-Coloring	NR	NR	NR	0.002
Nail	NR	NR	NR	NR
Mucous Membrane	NR	NR	7	NR
Baby Products	NR	NR	7	NR

	Hydrolyzed Sodium Hyaluronate		Sodium Acetylated Hyaluronate	
Totals*	**108**	**0.0015 – 0.15**	**378**	**0.002 – 0.1**
Duration of Use				
Leave-On	*105*	*0.0015 – 0.15*	*353*	*0.002 – 0.1*
Rinse Off	*3*	*NR*	*25*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	8	NR	43	NR
Incidental Ingestion	NR	NR	68	NR
Incidental Inhalation-Spray	49[a]; 29[b]	NR	107[a]; 50[b]	0.0085 – 0.1[a]
Incidental Inhalation-Powder	29[b]	0.15[c]	11; 50[b]; 3[c]	0.1[c]
Dermal Contact	108	0.0015 – 0.15	307	0.002 – 0.1
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	1	NR
Hair-Coloring	NR	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	NR	NR	71	NR
Baby Products	NR	NR	6	NR

*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

NR – no reported use

Table 6. Oral ADME studies

Parameter Measured	Test Substance	Animals	No./Group	Vehicle/Dose	Dose/Protocol	Results	References
Absorption	[^{14}C]Hyaluronic Acid (MW = 920,000 (units not specified)	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.	[19]
Distribution	[^{14}C]Hyaluronic Acid (MW = 920,000 (units not specified)	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 $_{\text{radioactivity}}$ dropped.	[19]
Distribution	[^{14}C]Hyaluronic Acid (MW = 920,000 (units not specified)	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 rate 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 cadaver 168 post-administration.	[19]
Distribution	[^{99m}Tc]Hyaluronic Acid (MW = 1.1 – 1.5 MDa)	Wistar rats (sex not stated)	3/group	0.2 ml (vehicle not stated)	A single dose of the test substance was given to each animal via gavage. Animals were killed at each of the following times post-administration: 5, 15, 30 min, 1, 2, 4, 6, 12, and 24 h. Radioactivity was examined in organs, tissues, and carcasses. Tissues measured include blood, bone, brain, heart, kidney, knee joints, large intestine, liver, lung, muscle, small intestine, skin, spleen, thyroid, and bladder. In addition, whole-body scintigraphs SPECT scans were performed in order to further evaluate biodistribution of radioactivity.	Radioactivity appeared to remain in the GI tract, keeping with normal gut transition times. A very rapid uptake of radioactivity in bone, muscle, small intestine, and large intestine was seen at the 5- and 15-min time points. Tissue uptake of radioactivity into blood and peripheral tissue coincided with the presence of the test substance in absorptive sections of the GI tract. Whole-body scintigraphs revealed non-alimentary radioactivity concentrated in the joints, vertebrae, and salivary glands 4 h post-administration. Approximately 10% of the ingested radioactivity remained in the tissues 72 h post-administration.	[21]

Table 6. Oral ADME studies

Parameter Measured	Test Substance	Animals	No./Group	Vehicle/Dose	Dose/Protocol	Results	References
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).	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.	[20]
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 was below the detection limit (10 µg/3 d) in all groups.	[20]
Excretion	[^{99m}Tc]Hyaluronic Acid (MW = 1.1 – 1.5 MDa)	Wistar rats (sex not stated)	5 rats total	0.2 ml (vehicle not stated)	A single dose of the test substance was administered to the animals via gavage. Urine and feces were collected 12, 24, 48, and 72 h post-administration.	The average total excretions of the test substance over the 72-h period in feces and urine were 84.6 ± 7.8% and 2.0 ± 0.63% of the ingested dose, respectively. Almost all radioactivity was excreted within 24 h.	[21]
Excretion	[^{99m}Tc]Hyaluronic Acid (MW = 1.1 – 1.5 MDa)	Beagle dogs (sex not stated)	2 dogs total	1.5 ml (vehicle not stated)	A single dose of the test substance was administered to the animals via gavage. Blood and urine samples were collected 2, 5, 15, 30 min, 1, 2, 4, 6, 12, 24, 48, and 72 h post-administration. Results were reported as the percent of administered dose/gram of whole blood or urine.	After administration, blood clearance rose immediately, and returned to background by 6 h. Urinary clearance of the test substance appeared after 30 min, and returned to background after 12 h.	[21]

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

Table 7. Acute oral toxicity studies on Sodium Hyaluronate[8]

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[8]

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 on Sodium Hyaluronate[8]

Concentration/Dose	Vehicle	Test System	Procedure	Results
		IN VITRO		
1 mg	NR	*S. typhimurium* strains TA97a, TA98, TA100, TA102, and TA1535	Ames assay performed with and without metabolic activation	Non-genotoxic
0.008, 0.04, 0.2, 1, and 5 mg	NR	*S. typhimurium* strains TA97a, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
0.2, 0.5, 1, 2.5, and 5 mg	NR	*S. typhimurium* strains TA97, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
		IN VIVO		
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
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. 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	[32]
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	[33]
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	[34]
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	[35]
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	[36]
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	[37]
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	[38]
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	[39]
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	[40]
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	[41]
49-yr-old female	-facial edema observed 28 d after glabella and eye area injections of Hyaluronic Acid -treatment with corticosteroids resolved symptoms	[41]
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	[41]
56-yr-old female	-pruritis and blisters 14 d after facial Hyaluronic Acid injections -treatment with steroids, antihistamines, saline dressings, and betamethasone resolved symptoms	[41]
42-yr-old-female	-inflammatory nodules 1 yr after facial Hyaluronic Acid injections -treatment with moxypen cefamezin resolved symptoms	[41]
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	[42]
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	[42]
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	[43]

Table 10. 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	[43]
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	[43]
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	[43]
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	[44]
48-yr-old female	-blue/gray coloring of lips, cheek, and nose 8 h after Hyaluronic Acid injections -treatment with nitroglycerin and hyperbaric chamber	[45]
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	[46]
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	[47]
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	[48]
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	[49]
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	[50]
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	[51]
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	[52]
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	[52]

*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.[53]

REFERENCES

1. Nikitakis J (ed), Kowcz A (ed). wINCI: *International Cosmetic Ingredient Dictionary and Handbook.* http://webdictionary.personalcarecouncil.org/jsp/Home.jsp. Washington, DC: Personal Care Products Council. Last Updated: 2022. Accessed: May 20, 2022.

2. Becker LC, Bergfeld WF, Belsito DV, et al. Final report of the safety assessment of hyaluronic acid, potassium hyaluronate, and sodium hyaluronate. *Int J Toxicol.* 2009;28(4 Suppl):5-67.

3. Sze JH, Brownlie JC, Love CA. Biotechnological production of hyaluronic acid: a mini review. *3 Biotech.* 2016;6(1):67.

4. Segura T, Anderson BC, Chung PH, Webber RE, Shull KR, Shea LD. Crosslinked hyaluronic acid hydrogels: a strategy to functionalize and pattern. *Biomaterials.* 2005;26(4):359-371.

5. Guarise C, Barbera C, Pavan M, Panfilo S, Beninatto R, Galesso D. HA-based dermal filler: downstream process comparison, impurity quantitation by validated HPLC-MS analysis, and in vivo residence time study. *J Appl Biomater Funct Mater.* 2019;17(3):1-9.

6. Boykov PY, Khabarov VN, Polyak F, Selyanin MA. Methods of Hyaluronic Acid Production. In: *Hyaluronic Acid.* Chichester, UK: John Wiley & Sons, Ltd; 2015:77-95.

7. Xin Y, Jianhao L, Tiansheng S, et al. The efficacy and safety of sodium hyaluronate injection (Adant®) in treating degenerative osteoarthritis: a multi-center, randomized, double-blind, positive-drug parallel-controlled and non-inferiority clinical study. *Int J Rheum Dis.* 2016;19(3):271-278.

8. Bloomage Biotechnology Corp Ltd. Conclusion of GRAS Status of Sodium Hyaluronate. GRAS Associates, LLC;2020. https://www.fda.gov/media/152869/download. Accessed June 6, 2022.

9. United States Pharmacopeial Convention. Food Chemicals Codex *9th ed*. In: (USP) USP, ed. Rockville, MD 2014. Accessed June 6, 2022.

10. Shiedlin A, Bigelow R, Christopher W, et al. Evaluation of hyaluronan from different sources: Streptococcus zooepidemicus, rooster comb, bovine vitreous, and human umbilical cord. *Biomacromolecules.* 2004;5(6):2122-2127.

11. Personal Care Products Council. 2021. Concentration of Use by FDA Product Category: Hyaluronic acid and its salts. (Unpublished data submitted by Personal Care Products Council on January 10, 2022.)

12. European Commission. CosIng database; following Cosmetic Regulation No. 1223/2009. http://ec.europa.eu/growth/tools-databases/cosing/. Last Updated: 2020. Accessed: May 23, 2022.

13. U.S. Food and Drug Administration. Devices @ FDA. https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm. Last Updated: 2022. Accessed: June 8, 2022.

14. U.S. Food and Drug Administration. Inactive Ingredient Search for Approved Drug Products. https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm. Last Updated: 2022. Accessed: June 8, 2022.

15. U.S. Food and Drug Administration. FDA Approved Animal Drug Products. https://animaldrugsatfda.fda.gov/adafda/views/#/search. Last Updated: 2022. Accessed: June 8, 2022.

16. National Institutes of Health Office of Dietary Supplements. Dietary Supplement Label Database. https://dsld.od.nih.gov/. Last Updated: 2022. Accessed: June 9, 2022.

17. Essendoubi M, Gobinet C, Reynaud R, Angiboust JF, Manfait M, Piot O. Human skin penetration of hyaluronic acid of different molecular weights as probed by Raman spectroscopy. *Skin Res Technol.* 2016;22(1):55-62.

18. Brown MB, Jones SA. Hyaluronic acid: a unique topical vehicle for the localized delivery of drugs to the skin. *J Eur Acad Dermatol Venereol.* 2005;19(3):308-318.

19. Oe M, Mitsugi K, Odanaka W, et al. Dietary hyaluronic acid migrates into the skin of rats. *Scientific World Journal.* 2014;2014:378024.

20. Kimura M, Maeshima T, Kubota T, Kurihara H, Masuda Y, Nomura Y. Absorption of Orally Administered Hyaluronan. *J Med Food.* 2016;19(12):1172-1179.

21. Balogh L, Polyak A, Mathe D, et al. Absorption, uptake and tissue affinity of high-molecular-weight hyaluronan after oral administration in rats and dogs. *J Agric Food Chem.* 2008;56(22):10582-10593.

22. Ma Y, Li C, Mai Z, Yang J, Tai M, Leng G. Efficacy and safety testing of dissolving microarray patches in Chinese subjects. *J Cosmet Dermatol.* 2021.

23. Guillaumie F, Furrer P, Felt-Baeyens O, et al. Comparative studies of various hyaluronic acids produced by microbial fermentation for potential topical ophthalmic applications. *J Biomed Mater Res A.* 2010;92(4):1421-1430.

24. Monzani D, Molinari G, Gherpelli C, Michellini L, Alicandri-Ciufelli M. Evaluation of Performance and Tolerability of Nebulized Hyaluronic Acid Nasal Hypertonic Solution in the Treatment of Chronic Rhinosinusitis. *Am J Rhinol Allergy.* 2020;34(6):725-733.

25. Decates T, Kadouch J, Velthuis P, Rustemeyer T. Immediate nor Delayed Type Hypersensitivity Plays a Role in Late Inflammatory Reactions After Hyaluronic Acid Filler Injections. *Clin Cosmet Investig Dermatol.* 2021;14:581-589.

26. Johnson ME, Murphy PJ, Boulton M. Effectiveness of sodium hyaluronate eyedrops in the treatment of dry eye. *Graefe's Arch Clin Exp Ophthalmol.* 2006;244(1):109-112.

27. Schlesinger TE, Powell CR. Efficacy and tolerability of low molecular weight hyaluronic acid sodium salt 0.2% cream in rosacea. *J Drugs Dermatol.* 2013;12(6):664-667.

28. Sullivan DY, Hawkes JE. Photodermatitis following knee intra-articular hylan G-F 20 injection for osteoarthritis: two cases. *Dermatol Online J.* 2021;27(12).

29. Schattner A, Haj-Yahya A. Upper airway angioedema after topical hyaluronic acid in a patient treated with bisoprolol. *J Postgrad Med.* 2018;64(2):128.

30. US Food and Drug Administration (FDA) Center for Food Safety & Applied Nutrition (CFSAN). 2022. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients. (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2022; received January 11, 2022). College Park, MD.

31. Personal Care Products Council. 2021. Concentration of Use by FDA Product Category: Concentration of use by FDA Product Category – Hyaluronates Not Previously Reviewed by CIR. (Unpublished data submitted to Personal Care Products Council on July 6, 2021.)

32. Vidič M, Bartenjev I. An adverse reaction after hyaluronic acid filler application: a case report. *Acta Dermatovenerol Alp Pannonica Adriat.* 2018;27(3):165-167.

33. Alawami AZ, Tannous Z. Late onset hypersensitivity reaction to hyaluronic acid dermal fillers manifesting as cutaneous and visceral angioedema. *J Cosmet Dermatol.* 2021;20(5):1483-1485.

34. Lupton JR, Alster TS. Cutaneous hypersensitivity reaction to injectable hyaluronic acid gel. *Dermatol Surg.* 2000;26(2):135-137.

35. Arron ST, Neuhaus IM. Persistent delayed-type hypersensitivity reaction to injectable non-animal-stabilized hyaluronic acid. *J Cosmet Dermatol.* 2007;6(3):167-171.

36. Leonhardt JM, Lawrence N, Narins RS. Angioedema acute hypersensitivity reaction to injectable hyaluronic acid. *Dermatol Surg.* 2005;31(5):577-579.

37. Bisaccia E, Lugo A, Torres O, Johnson B, Scarborough D. Persistent inflammatory reaction to hyaluronic acid gel: a case report. *Cutis.* 2007;79(5):388-389.

38. Patel VJ, Bruck MC, Katz BE. Hypersensitivity reaction to hyaluronic acid with negative skin testing. *Plast Reconstr Surg.* 2006;117(6):92e-94e.

39. Bardazzi F, Ruffato A, Antonucci A, Balestri R, Tabanelli M. Cutaneous granulomatous reaction to injectable hyaluronic acid gel: another case. *J Dermatolog Treat.* 2007;18(1):59-62.

40. Wang C, Sun T, Li H, Li Z, Wang X. Hypersensitivity Caused by Cosmetic Injection: Systematic Review and Case Report. *Aesthetic Plast Surg.* 2021;45(1):263-272.

41. Bitterman-Deutsch O, Kogan L, Nasser F. Delayed immune mediated adverse effects to hyaluronic Acid fillers: report of five cases and review of the literature. *Dermatol Reports.* 2015;7(1):5851.

42. Homsy A, Rüegg EM, Jandus P, Pittet-Cuénod B, Modarressi A. Immunological reaction after facial hyaluronic acid injection. *Case Reports Plast Surg Hand Surg.* 2017;4(1):68-72.

43. O'Reilly P, Malhotra R. Delayed hypersensitivity reaction to Restylane ® SubQ. *Orbit.* 2011;30(1):54-57.

44. Hönig JF, Brink U, Korabiowska M. Severe granulomatous allergic tissue reaction after hyaluronic acid injection in the treatment of facial lines and its surgical correction. *J Craniofac Surg.* 2003;14(2):197-200.

45. Banh K. Facial ischemia after hyaluronic acid injection. *J Emerg Med.* 2013;44(1):169-170.

46. Ghislanzoni M, Bianchi F, Barbareschi M, Alessi E. Cutaneous granulomatous reaction to injectable hyaluronic acid gel. *Br J Dermatol.* 2006;154(4):755-758.

47. Pérez-Pérez L, García-Gavín J, Wortsman X, Santos-Briz Á. Delayed Adverse Subcutaneous Reaction to a New Family of Hyaluronic Acid Dermal Fillers With Clinical, Ultrasound, and Histologic Correlation. *Dermatol Surg.* 2017;43(4):605-608.

48. Caldas Pozuelo C, Domínguez De Dios J, Mota Rojas X. Multiple oral granulomatous nodules to hyaluronic acid filler. *J Cosmet Dermatol.* 2020;19(12):3453-3455.

49. Ross CL, Heitmiller K, Saedi N. Hypersensitivity reaction to RHA filler. *J Cosmet Dermatol.* 2022;21(1):412-413.

50. Pezier T, Morand G, Tischler V, Kleinjung T. Delayed granulomatous reaction to hyaluronic acid gel injection. *Eur J Plast Surg.* 2014;37(1):45-48.

51. Sidwell RU, Dhillon AP, Butler PE, Rustin MH. Localized granulomatous reaction to a semi-permanent hyaluronic acid and acrylic hydrogel cosmetic filler. *Clin Exp Dermatol.* 2004;29(6):630-632.

52. Angus JE, Affleck AG, Leach IH, Millard LG. Two cases of delayed granulomatous reactions to the cosmetic filler Dermalive, a hyaluronic acid and acrylic hydrogel. *Br J Dermatol.* 2006;155(5):1077-1078.

53. Matarasso SL, Herwick R. Hypersensitivity reaction to nonanimal stabilized hyaluronic acid. *J Am Acad Dermatol.* 2006;55(1):128-131.