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## Safety Assessment of Hyaluronates as Used in Cosmetics

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Status: Final Report  
Release Date: July 7, 2023  
Panel Meeting Date: June 12-13, 2023

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Priya Cherian, M.S., Senior Scientific Analyst/Writer, CIR.

## 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	<i>Food Chemicals Codex</i>
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
HR IPT	human repeated insult patch test
I-NOSE	Nasal Obstruction Symptom Evaluation Instrument
I <sub>max</sub>	maximum response value
K <sub>ow</sub>	n-octanol/water partition coefficient
kDa	kiloDaltons
LC <sub>50</sub>	median lethal concentration
LD <sub>50</sub>	median lethal dose
Log K <sub>ow</sub>	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 <i>International Cosmetic Ingredient Dictionary and Handbook</i>

## **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 the cosmetics industry should continue to use necessary procedures to limit infectious agents, and/or biologically-derived impurities (e.g., nucleic acids, proteins, endotoxins). Additionally, industry should minimize impurities, such as heavy metals, according to limits set by the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA). 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*	Potassium Hyaluronate*
Hydrolyzed Calcium Hyaluronate	Sodium Acetylated Hyaluronate
Hydrolyzed Hyaluronic Acid	Sodium Hyaluronate*
Hydrolyzed 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 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.<sup>1</sup> 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 (Hydrolyzed Calcium Hyaluronate and Hydrolyzed Sodium Hyaluronate). Hence, all ingredients reviewed in this report are structurally similar as they are salts or acetylated esters derived from Hyaluronic Acid.

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).<sup>2</sup> 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 search was last conducted May 2023. 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.

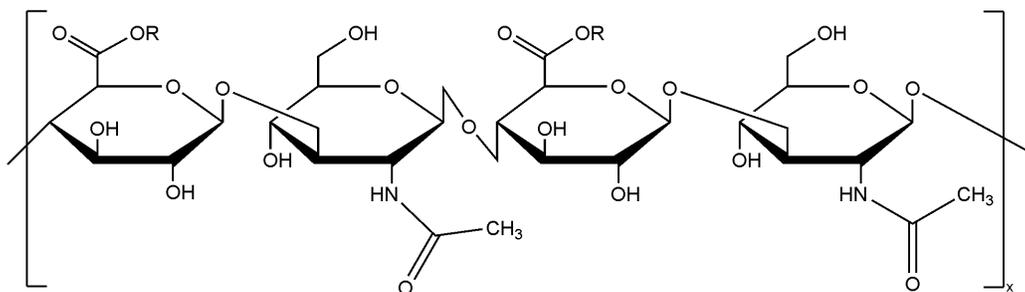
In its original 2009 review of Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate, the Panel concluded that these 3 ingredients are safe in the present practices of use and concentration, as described in the safety assessment.<sup>1</sup> Excerpts from the 2009 report are disseminated throughout 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, these 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  $\beta$ -D-glucuronic acid- $\beta$ 3-*N*-acetylglucosamine.<sup>3</sup> 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.<sup>1</sup> *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.<sup>1</sup> 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.<sup>1</sup>

#### 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.<sup>4</sup> The tissues are then ground 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 ultracentrifugation 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.<sup>4</sup> 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)).<sup>5</sup> 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. *zoepidemicus*.<sup>6</sup> 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.<sup>6</sup> 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.<sup>7</sup> 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*.<sup>8,9</sup> 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.<sup>10</sup>

### **Impurities**

*When derived from animal sources, Hyaluronic Acid may contain several impurities.<sup>1</sup> 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.<sup>11</sup> 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.<sup>12</sup>

### 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.<sup>13</sup>

### 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.<sup>14</sup> 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.<sup>15</sup> A manufacturer of food-use Sodium Hyaluronate states that potential contaminants of Sodium Hyaluronate include microbes and heavy metals.<sup>16</sup> 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..<sup>4</sup>

### **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.<sup>3,17</sup> 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..<sup>4</sup>

### **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 2023 FDA VCRP data, Sodium Hyaluronate has the highest frequency of use (4713 total formulations; 4317 leave-on formulations, 394 rinse-off formulations, and 2 formulations diluted for bath use; Table 4).<sup>18</sup> This use of this ingredient has increased significantly since it was last reviewed; it was reported to be used in 601 formulations in 2005.<sup>1</sup> Hyaluronic Acid is reported to be used in 663 formulations, Hydrolyzed Hyaluronic Acid is reported to be used in 476 formulations, and Sodium Acetylated Hyaluronate in 455 formulations. All other ingredients are reported to be used in 204 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).<sup>19</sup> In 2005, Sodium Hyaluronate was reported to be used at up to 2%. Current FDA VCRP data on the 4 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.<sup>20</sup>

### **Non-Cosmetic**

Hyaluronic Acid and Sodium Hyaluronate are reported to be used in FDA-approved medical devices as surgical fluids, topical wound creams, osteoarthritis treatments, periodontitis treatments, and ophthalmic surgery aids; crosslinked Hyaluronic Acid is FDA-approved as a dermal filler.<sup>21</sup> In addition, Sodium Hyaluronate is used as an inactive ingredient in several FDA-approved medications.<sup>22</sup> 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 21CFR 522.1145.<sup>23</sup>

Hyaluronic Acid is found as a natural component in foods as it is present in animal products.<sup>16</sup> 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.<sup>16</sup> In addition, both Hyaluronic Acid and Sodium Hyaluronate are reported to be ingredients of dietary supplements on the market in the US.<sup>24</sup>

## **TOXICOKINETIC STUDIES**

### **Dermal Penetration**

*Autoradiography was used to detect the dermal penetration of Hyaluronic Acid (in the form of [<sup>3</sup>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).<sup>1</sup> 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  $\mu\text{m}$  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  $\mu\text{m}$ . Autoradiography was used to detect the dermal penetration of [<sup>3</sup>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.<sup>25</sup> Test solutions contained 1% Hyaluronic Acid in distilled water. The solution (300  $\mu\text{l}$ ) 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  $\mu\text{m}$ -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  $\mu\text{m}$  (full epidermal depth). The Hyaluronic Acid solution with a MW range of 100 - 300 kDa was present at an epidermal depth of approximately 50  $\mu\text{m}$ . Permeation did not exceed 25  $\mu\text{m}$  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  $\mu\text{m}$  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.<sup>1,26</sup> 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 [<sup>14</sup>C]Hyaluronic Acid (MW = 920 kDa) via gavage, the peak plasma radioactivity level was 7.6  $\mu\text{g}$  eq/ml 8 h post-administration.<sup>27</sup> 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.<sup>28</sup> 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.<sup>1</sup> 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).<sup>16</sup> No signs of toxicity or deaths were reported in any of these assays.

### **Short-Term and Subchronic Toxicity Studies**

*No toxicity was observed 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.<sup>16</sup> 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 Sodium Hyaluronate

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).<sup>1</sup> 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).<sup>29</sup> 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).<sup>16</sup> 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.<sup>16</sup> 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*).<sup>16,29-31</sup> In addition, Sodium Hyaluronate (up to 5000 mg/kg bw via gavage) was non-mutagenic in mouse micronucleus assays.<sup>29</sup>

### **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 albumin (the protein in egg whites), and dog albumin 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.<sup>1</sup> 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.<sup>1</sup> Repeated injections of Sodium Hyaluronate into the eyes of owl monkeys increased the leukocyte count up to 2000 cells/mm<sup>3</sup> 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.<sup>1</sup> 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 (EpiDerm™ and reconstructed human epidermis 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.<sup>29,31-35</sup> 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%).<sup>31</sup> 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).<sup>29,36,37</sup> 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).<sup>29-31,38-40</sup>

## 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).<sup>29,31</sup> 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).<sup>32-35</sup> 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).<sup>29,31</sup> 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).<sup>41</sup>

### 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.<sup>42</sup> 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.

#### **Treatment of Dry Eye**

##### Sodium Hyaluronate

The effectiveness of Sodium Hyaluronate eye drops was evaluated in 13 patients with moderate dry eye.<sup>43</sup> 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.<sup>44</sup> 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.<sup>45</sup> 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

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.<sup>46</sup> 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 2023 VCRP survey data, Sodium Hyaluronate is reported to be used in 4713 formulations (4317 leave-on formulations, 394 rinse-off formulations, and 2 formulations diluted for bath use), and Hyaluronic Acid is reported to be used in 663 formulations (571 leave-on formulations, 89 rinse-off formulations, and 3 formulations diluted for bath use). Additionally, Hydrolyzed Hyaluronic Acid is reported to be used in 476 formulations and Sodium Acetylated Hyaluronate in 455 formulations. All other ingredients are reported to be used in 204 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 to 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  $\mu\text{m}$ , 50  $\mu\text{m}$ , and 25  $\mu\text{m}$ , respectively. Regardless of the MW of the Hyaluronic Acid solution, the majority quantity of Hyaluronic Acid was found in the stratum corneum, approximately 25  $\mu\text{m}$  from the skin surface.

In an absorption assay in which male Sprague-Dawley rats ( $n = 3$ ) were dosed with 25 mg/kg [<sup>14</sup>C]Hyaluronic Acid (MW = 920 kDa) via gavage, the peak plasma radioactivity level was 7.6  $\mu\text{g}$  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 via gavage.

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<sup>TM</sup> 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.

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.

In a 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**

Sodium Acetylated Hyaluronate and Hydrolyzed Hyaluronic Acid were included on the 2022 Priority List due to high reported frequencies of use in FDA VCRP. 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 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 the cosmetics industry should continue to use the necessary procedures to limit detectable pathogenic viruses, infectious agents, and/or biologically-derived impurities. 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. They stressed that the cosmetics industry should continue to use the necessary procedures to minimize impurities in cosmetic formulations according to limits set by the FDA and EPA.

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 spray other skin care preparations). 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<sup>2</sup>**

<b>Ingredient</b>	<b>Definition</b>	<b>Function</b>
Hyaluronic Acid (9004-61-9)	Hyaluronic Acid is the natural mucopolysaccharide formed by bonding <i>N</i> -acetyl-D-glucosamine with glucuronic acid. <i>See Figure 1, wherein "R" is hydrogen.</i>	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. <i>See Figure 1, wherein 2 "R" are replaced by 1 calcium cation.</i>	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. <i>See Figure 1, wherein R is potassium.</i>	Skin-Conditioning Agents - Miscellaneous
Sodium Acetylated Hyaluronate	Sodium Acetylated Hyaluronate is the acetyl ester of Sodium Hyaluronate. <i>See Figure 1, wherein "R" is sodium, and one or more hydroxyl groups are acetylated.</i>	Humectants
Sodium Hyaluronate (9067-32-7)	Sodium Hyaluronate is the sodium salt of Hyaluronic Acid. <i>See Figure 1, wherein "R" is sodium.</i>	Skin-Conditioning Agents - Miscellaneous

**Table 2. Chemical properties of Hyaluronic Acid and Sodium Hyaluronate**

<b>Property</b>	<b>Value</b>	<b>Reference</b>
<b>Hyaluronic Acid</b>		
Physical Form	powder	1
MW (kDa)	5 - 1800	1
<b>Sodium Hyaluronate</b>		
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)<sup>11</sup>**

	<b>Human umbilical cord</b>	<b>Bacterially-derived</b>	<b>Bacterially-derived*</b>	<b>Rooster comb</b>	<b>Bovine vitreous</b>
MW ( $\times 10^6$ Da)	1.3 $\pm$ 0.1	1.6	1.4	1.4	0.4
Endotoxin (EU/mg HA)	> 100	< 0.02	0.022	23	> 100
Total Protein ( $\mu$ g/ml HA)	47.7 $\pm$ 3	1.1	ND	1.0	36.2
RNA ( $\mu$ g/mg HA)	6.7 $\pm$ 0.1	ND	ND	ND	1.9
DNA ( $\mu$ g/mg HA)	16.8 $\pm$ 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. Frequency (2023; 2005) and concentration (2021; 2005) of use according to likely duration and exposure and by product category**

	Hyaluronic Acid				Potassium Hyaluronate				Sodium Hyaluronate			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023 <sup>18</sup>	2005 <sup>1</sup>	2021 <sup>19</sup>	2005 <sup>1</sup>	2023 <sup>18</sup>	2005 <sup>1</sup>	2022 <sup>47</sup>	2005 <sup>1</sup>	2023 <sup>18</sup>	2005 <sup>1</sup>	2022 <sup>47</sup>	2005 <sup>1</sup>
<b>Totals*</b>	663	223	0.000002 – 0.83	0.00005 – 1	68	11	NR	NR	4713	601	0.00001 – 7.5	0.000001 – 2
<b>summarized by likely duration and exposure**</b>												
<b>Duration of Use</b>												
<i>Leave-On</i>	571	194	0.000002 – 0.3	0.00005 – 1	60	10	NR	NR	4317	552	0.00001 – 7.5	0.000001 – 2
<i>Rinse-Off</i>	89	29	0.002 – 0.83	0.001 – 0.3	8	1	NR	NR	394	49	0.0001 – 0.12	0.000001 – 0.5
<i>Diluted for (Bath) Use</i>	3	NR	0.0089	NR	NR	NR	NR	NR	2	NR	NR	0.001 – 0.5
<b>Exposure Type**</b>												
Eye Area	47	33	0.001	0.001 – 0.07	NR	NR	NR	NR	278	49	0.0001 – 0.96	0.0001 – 0.7
Incidental Ingestion	1	NR	0.003 – 0.05	0.01	NR	NR	NR	NR	237	96	0.24 – 0.39	0.0002 – 0.5
Incidental Inhalation-Spray	3; 260 <sup>a</sup> ; 191 <sup>b</sup>	57 <sup>a</sup> ; 21 <sup>b</sup>	NR	0.001 <sup>a</sup> ; 0.001 – 1 <sup>b</sup>	30 <sup>a</sup> ; 22 <sup>b</sup>	4 <sup>a</sup> ; 6 <sup>b</sup>	NR	NR	9; 1707 <sup>a</sup> ; 1454 <sup>b</sup>	1; 180 <sup>a</sup> ; 73 <sup>b</sup>	0.01; 2 <sup>a</sup>	0.000001 – 1 <sup>a</sup> ; 0.0001 – 2 <sup>b</sup>
Incidental Inhalation-Powder	3; 191 <sup>b</sup> ; 4 <sup>c</sup>	6; 21 <sup>b</sup>	0.003 – 0.3 <sup>c</sup>	0.00005; 0.001 – 1 <sup>b</sup>	22 <sup>b</sup>	6 <sup>b</sup>	NR	NR	32; 1454 <sup>b</sup> ; 9 <sup>c</sup>	16; 73 <sup>b</sup>	0.001 – 0.099; 0.00002 – 7.5 <sup>c</sup>	0.0005 – 0.5; 0.0001 – 2 <sup>b</sup> ; 0.5 <sup>c</sup>
Dermal Contact	635	216	0.000002 – 0.83	0.00005 – 1	68	11	NR	NR	4371	482	0.00001 – 7.5	0.000001 – 2
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	6 <sup>a</sup>	NR	0.013	0.5 <sup>a</sup>
Hair - Non-Coloring	26	4	0.0036	NR	NR	NR	NR	NR	83	12	0.005 – 2	0.001 – 0.5
Hair-Coloring	NR	1	0.002	NR	NR	NR	NR	NR	2	2	NR	0.5
Nail	NR	2	NR	0.001 – 0.01	NR	NR	NR	NR	2	NR	0.025	0.01 – 0.5
Mucous Membrane	27	1	0.003 – 0.05	0.01	3	NR	NR	NR	275	97	0.01 – 0.39	0.0002 – 0.5
Baby Products	5	NR	NR	0.001	NR	NR	NR	NR	14	NR	0.005	0.5
<b>as reported by product category</b>												
<b>Baby Products</b>												
Baby Shampoos	1	NR	NR	NR					NR	NR	NR	0.5
Baby Lotions/Oils/Powders/Creams	4	NR	NR	NR					9	NR	NR	0.5
Other Baby Products	NR	NR	NR	0.001					5	NR	0.005	0.5
<b>Bath Preparations (diluted for use)</b>												
Bath Oils, Tablets, and Salts	1	NR	NR	NR					NR	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					2	NR	NR	0.001 – 0.5
<b>Eye Makeup Preparations</b>												
Eyebrow Pencil	2	NR	NR	NR					1	3	NR	0.5
Eyeliner	1	NR	NR	NR					12	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	15	5	0.001	NR					106	6	0.1	0.001 – 0.7
Eye Makeup Remover	1	2	NR	0.001					8	NR	0.12	NR
Mascara									18	9	0.0001 – 0.1	0.0001 – 0.5
Other Eye Makeup Preparations	28	11	NR	0.07					112	16	0.001 – 0.1	0.0001 – 0.5
<b>Fragrance Preparations</b>												
Cologne and Toilet Water									2	NR	NR	NR
Perfumes									1	1	NR	0.5
Powders (dusting/talcum, excl aftershave talc)	NR	1	NR	NR					NR	1	NR	0.5
Sachets									‡	NR	‡	0.5
Other Fragrance Preparation	2	NR	NR	NR					5	NR	NR	0.0002
<b>Hair Preparations (non-coloring)</b>												
Hair Conditioner	11	2	0.0036	NR					21	5	NR	0.001 – 0.5

**Table 4. Frequency (2023; 2005) and concentration (2021; 2005) of use according to likely duration and exposure and by product category**

	Hyaluronic Acid				Potassium Hyaluronate				Sodium Hyaluronate			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023 <sup>18</sup>	2005 <sup>1</sup>	2021 <sup>19</sup>	2005 <sup>1</sup>	2023 <sup>18</sup>	2005 <sup>1</sup>	2022 <sup>47</sup>	2005 <sup>1</sup>	2023 <sup>18</sup>	2005 <sup>1</sup>	2022 <sup>47</sup>	2005 <sup>1</sup>
Hair Spray (aerosol fixatives)	1	NR	NR	NR					1	NR	NR	0.5
Hair Straighteners									2	1	NR	0.5
Permanent Waves	NR	1	NR	NR					NR	NR	NR	0.5
Rinses (non-coloring)									2	NR	NR	0.001 – 0.5
Shampoos (non-coloring)	6	NR	0.0036	NR					25	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	4	NR	NR	NR					24	NR	0.005	0.5
<b>Hair Coloring Preparations</b>												
Hair Dyes and Colors (all types requiring caution statements and patch tests)									1	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
<b>Makeup Preparations</b>												
Blushers (all types)	NR	7	NR	0.02					13	20	0.05	0.001 – 0.5
Face Powders	3	5	NR	0.00005					32	15	0.001 – 0.099	0.0005 – 0.5
Foundations	2	24	0.000002 – 0.1	0.002					81	27	0.015 – 0.2	0.001 – 0.5
Leg and Body Paints									NR	1	NR	0.001 – 0.5
Lipstick	1	NR	0.003 – 0.05	0.01					229	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					76	17	0.025 – 0.1	0.0001 – 0.5
<b>Manicuring Preparations (Nail)</b>												
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									1	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
<b>Oral Hygiene Products</b>												
Dentifrices									3	NR	NR	NR
Mouthwashes and Breath Fresheners									4	NR	NR	NR
Other Oral Hygiene Products									1	NR	NR	NR
<b>Personal Cleanliness Products</b>												
Bath Soaps and Detergents	16	NR	NR	NR	3	NR	NR	NR	17	1	0.01	0.001 – 0.5
Deodorants (underarm)									6	NR	0.013 (not spray)	0.5
Douches	1	NR	NR	NR								
Feminine Deodorants									2	NR	NR	0.001

**Table 4. Frequency (2023; 2005) and concentration (2021; 2005) of use according to likely duration and exposure and by product category**

	Hyaluronic Acid				Potassium Hyaluronate				Sodium Hyaluronate			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023 <sup>18</sup>	2005 <sup>1</sup>	2021 <sup>19</sup>	2005 <sup>1</sup>	2023 <sup>18</sup>	2005 <sup>1</sup>	2022 <sup>47</sup>	2005 <sup>1</sup>	2023 <sup>18</sup>	2005 <sup>1</sup>	2022 <sup>47</sup>	2005 <sup>1</sup>
Other Personal Cleanliness Products	6	1	NR	NR					17	NR	NR	0.5
<b>Shaving Preparations</b>												
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					6	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 (razor lube strip)	0.5
<b>Skin Care Preparations</b>												
Cleansing	34	6	NR	0.001	5	NR	NR	NR	173	20	0.0001 – 0.1	0.000001 – 0.5
Depilatories									1	NR	NR	0.5
Face and Neck (exc shave)	175	8	0.003 – 0.3 (not spray)	0.1	15	6	NR	NR	1246	48	0.005 – 7.5 (not spray)	0.005 – 1
Body and Hand (exc shave)	16	12	0.05 (not spray)	0.001 – 1	7	NR	NR	NR	206	25	0.00002 – 0.86 (not spray)	0.0001 – 2
Foot Powders and Sprays	NR	1	NR	NR					NR	NR	NR	1
Moisturizing	212	37	0.08 – 0.2 (not spray)	0.001 – 0.1	27	4	NR	NR	1482	151	0.001 – 0.4 (not spray)	0.000001 - 1
Night	19	17	0.15 (not spray)	0.02	2	NR	NR	NR	131	11	0.00001 – 0.3 (not spray)	0.0001 - 1
Paste Masks (mud packs)	11	13	0.83	0.001	NR	1	NR	NR	107	12	0.024	0.005 – 0.5
Skin Fresheners	20	NR	NR						67	10	0.01	0.05 – 0.5
Other Skin Care Preparations	47	23	NR	0.001	5	NR	NR	NR	358	38	0.02 – 0.1 (spray)	0.001 - 1
<b>Suntan Preparations</b>												
Suntan Gels, Creams, and Liquids	1	1	NR	NR					5	4	NR	0.000001 – 1
Indoor Tanning Preparations	3	1	NR	0.001 <sup>a</sup>					5	1	NR	0.001 – 0.5
Other Suntan Preparations	2	NR	NR	NR					5	3	NR	0.001 – 0.5

NR – not reported

\*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

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

<sup>a</sup> It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

<sup>b</sup> 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

<sup>c</sup> It is possible these products are powders, but it is not specified whether the reported uses are powders.

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





**Table 5. Frequency (2023) and concentration (2021) of use of ingredients not previously reviewed according to likely duration and exposure and by product category**

	Hydrolyzed Calcium Hyaluronate		Hydrolyzed Hyaluronic Acid		Hydrolyzed Sodium Hyaluronate		Sodium Acetylated Hyaluronate	
	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Shaving Soap								
Other Shaving Preparations								
<b><i>Skin Care Preparations</i></b>								
Cleansing			41	0.01	9	NR	30	NR
Depilatories								
Face and Neck (exc shave)			114	0.01 (not spray)	63	0.15 (not spray)	63	0.1 (not spray)
Body and Hand (exc shave)			14	NR	11	NR	16	NR
Foot Powders and Sprays								
Moisturizing	2	NR	187	0.1 – 0.2 (not spray)	74	0.0015 (not spray)	122	0.0085 – 0.1 (not spray)
Night			14	0.15 (not spray)	7	NR	9	NR
Paste Masks (mud packs)			6	NR	1	NR	4	NR
Skin Fresheners			14	NR	15	NR	5	NR
Other Skin Care Preparations			31	0.2			12	0.1
<b><i>Suntan Preparations</i></b>								
Suntan Gels, Creams, and Liquids								
Indoor Tanning Preparations								
Other Suntan Preparations								

NR – not reported

\*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

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

<sup>a</sup> It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

<sup>b</sup> 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

<sup>c</sup> 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	[ <sup>14</sup> 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 <sup>14</sup> 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.	<sup>27</sup>
Distribution	[ <sup>14</sup> 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 imaging plates.	<sup>14</sup> C was detected in the skin as follows: 2.36 PSL/mm <sup>2</sup> at 8 h, 3.81 PSL/mm <sup>2</sup> at 24 h, and 1.98 PSL/mm <sup>2</sup> at 96 h after administration. <sup>14</sup> C was detected in the blood as follows: 2.12 PSL/mm <sup>2</sup> at 8 h, 1.68 PSL/mm <sup>2</sup> at 24 h, and 0.84 PSL/mm <sup>2</sup> 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 <sup>2</sup> ). Readings in the pancreas (17.45 PSL/mm <sup>2</sup> ), harderian gland (12.27 PSL/mm <sup>2</sup> ), liver (9.22 PSL/mm <sup>2</sup> ), and mandibular gland (7.49 PSL/mm <sup>2</sup> ) were also high 8 h post-administration. At 96 h post-administration, all radioactivity dropped.	<sup>27</sup>
Distribution	[ <sup>14</sup> 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. <sup>14</sup> 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 h post-administration.	<sup>27</sup>
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.	<sup>28</sup>

**Table 6. Oral ADME studies**

<b>Parameter Measured</b>	<b>Test Substance</b>	<b>Animals</b>	<b>No./Group</b>	<b>Vehicle/Dose</b>	<b>Dose/Protocol</b>	<b>Results</b>	<b>References</b>
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.	<sup>28</sup>

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

**Table 7. Acute oral toxicity studies on Sodium Hyaluronate<sup>16</sup>**

Test Article	Vehicle	Animals/Group	Concentration/Dose	Protocol	LD <sub>50</sub> /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 <sub>50</sub> > 500 mg/kg bw
Sodium Hyaluronate	peanut oil	Kunming mice (10/sex)	2000 mg/kg bw	single dose; gavage; 14-d evaluation	LD <sub>50</sub> > 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 <sub>50</sub> > 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 <sub>50</sub> > 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 <sub>50</sub> > 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<sub>50</sub> = 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<sup>16</sup>**

Test Article	Vehicle	Animals/Group	Study Duration	Dose*	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
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; satellite group consisted of 5 rats/sex given 48 mg/kg bw/d for 90 d, followed by a 28 d recovery period	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**<sup>16,29-31</sup>

Test Substance	Test Concentration/Dose	Vehicle	Test System	Procedure	Results
<b>IN VITRO</b>					
Hydrolyzed Sodium Hyaluronate (FW = 5-10 kDa)	up to 5 mg/plate (specific concentrations not stated)	NR	<i>S. typhimurium</i> 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	<i>S. typhimurium</i> strains TA97a, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	1 mg/plate	NR	<i>S. typhimurium</i> 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	<i>S. typhimurium</i> 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	<i>S. typhimurium</i> 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	<i>S. typhimurium</i> 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	<i>S. typhimurium</i> strains TA97a, TA98, TA100, and TA102	Ames assay performed with and without metabolic activation	Non-genotoxic
Sodium Hyaluronate	without metabolic activation/ <i>S. typhimurium</i> and <i>E. coli</i> : 313, 625, 1250, 2500, 5000 µg/plate  with metabolic activation/ <i>S. typhimurium</i> : 39.1, 78.1, 156, 313, 625, 1250 µg/plate  with metabolic activation/ <i>E. coli</i> : 313, 625, 1250, 2500, 5000 µg/plate	NR	<i>S. typhimurium</i> strains TA98, TA100, TA1535, TA1537 and <i>E. coli</i> WP2 <i>uvrA</i>	Ames assay performed with and without metabolic activation	Non-genotoxic
<b>IN VIVO</b>					
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
<b>IRRITATION</b>						
<b>IN VITRO</b>						
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
<b>HUMAN</b>						
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
<b>SENSITIZATION</b>						
<b>IN CHEMICO/IN VITRO</b>						
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 <sub>max</sub> of 0.35 compared to I <sub>max</sub> 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 <sub>D54</sub> < 200 and mean RFI <sub>CD86</sub> < 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
<b>HUMAN</b>						
Formula containing 0.2% Hyaluronic Acid	NR	100%; 20 mg	115	HR IPT; 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	HR IPT; 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	HR IPT; no details provided	Non-irritating and non-sensitizing	31
Sodium Hyaluronate	NR	0.2%	50	HR IPT; no details provided	Non-sensitizing	30
Sodium Hyaluronate	NR	0.2%	100	HR IPT; no details provided	Non-irritating and non-sensitizing	29
Formula containing 1.5% Sodium Hyaluronate	NR	100%; 0.2 ml	198	HR IPT; 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; HR IPT = human repeated insult patch test; I<sub>max</sub> = 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
<b>IN VITRO</b>						
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
<b>ANIMAL</b>						
<i>Bacillus</i> -derived and <i>Streptococcus</i> -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

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