Safety Assessment of Glucosamine Ingredients as Used in Cosmetics

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All interested persons are provided 60 days from the above release date (i.e., May 10, 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to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to the Cosmetic Ingredient Review (CIR) will be discussed in open meetings, will be available for review by any interested party, and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth

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ABBREVIATIONS

AUC_{ss}	area under the curve; extent of exposure
BAL	bronchoalveolar lavage
BCOP	bovine corneal opacity and permeability
BUN	blood urea nitrogen
C_{max}	peak serum concentration
C_{ss}	peak concentration
CAS	Chemical Abstracts Service
CI	confidence interval
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
DART	Developmental and Reproductive Toxicity
Dictionary	*International Cosmetic Ingredient Dictionary and Handbook*
DNFB	dinitrofluorobenzene
DPRA	Direct Peptide Reactivity Assay
ECHA	European Chemicals Agency
ET_{50}	Effective time causing 50% reduction in tissue viability
FDA	Food and Drug Administration
FITC	fluorescein isothiocyanate
FW	formula weight
GFR	glomerular filtration rate
h-CLAT	human cell line activation test
HPLC	high performance liquid chromatography
HR	hazard ratio
HRIPT	human repeated insult patch test
IC_{50}	half maximal inhibitory concentration
IgE	immunoglobulin E
IGF-1	insulin-like growth factor 1
IL	interleukin
K_{ow}	n-octanol/water partition coefficient
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LD_{50}	median lethal dose
ME	microemulsion
MnNCE	micronucleated normochromatic erythrocytes
MnPCE	micronucleated polychromatic erythrocytes
MoS	margin of safety
MW	molecular weight
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCE	normochromatic erythrocytes
NOAEL	no-observable-adverse-effect-level
NR	not reported
OECD	Organisation for Economic Cooperation and Development
OVA	ovalbumin
Panel	Expert Panel for Cosmetic Ingredient Safety
PCE	polychromatic erythrocytes
PBS	phosphate-buffered saline
SBP	systolic blood pressure
SHR	spontaneously hypertensive rats
SLS	sodium lauryl sulfate
SIAscopy™	noncontact spectrophotometric intracutaneous analysis
SIDS	screening information dataset
SPF	sun protection factor
$T_{1/2}$	elimination half life
TG	test guidelines
THP-1	human monocytic cell line
T_{max}	time to reach serum concentration
US	United States
UV	ultraviolet
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Acetyl Glucosamine, Glucosamine, Glucosamine HCl, and Glucosamine Sulfate. Two of these ingredients are reported to function in cosmetics as skin-conditioning agents, one is reported to function as a pH adjuster, and the function of Glucosamine is not reported. The Panel reviewed the available data and concluded that these glucosamine ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-irritating.

INTRODUCTION

This assessment reviews the safety of the following 4 ingredients as used in cosmetic formulations:

Acetyl Glucosamine	Glucosamine HCl
Glucosamine	Glucosamine Sulfate

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), Acetyl Glucosamine and Glucosamine Sulfate are reported to function in cosmetics as skin-conditioning agents – miscellaneous, Glucosamine HCl is reported to function as a pH adjuster, and the function of Glucosamine is not reported (Table 1).[1] These glucosamine ingredients are being reviewed together due to structural similarities, sharing an aminomonosaccharide core group in common.

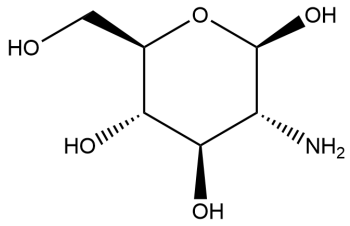
This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are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

Some of the data included in this safety assessment were found on the European Chemicals Agency (ECHA) website.[2,3] Please note that the ECHA website provides summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when ECHA is cited. Some types of data were found but not included, as no relevance to cosmetic use could be surmised (e.g., studies on the efficacy of Glucosamine for the treatment of arthritis).

CHEMISTRY

Definition and Structure

The definitions and structures of the ingredients included in this review are provided in Table 1. All of these ingredients share the ubiquitous aminomonosaccharide, Glucosamine (CAS No. 3416-24-8; Figure 1), as the core structure. Structurally, Glucosamine is modified glucose with an amine group replacing the hydroxyl group found on carbon two (C2).[4] Glucosamine and its salt forms, i.e., Glucosamine HCl (CAS No. 66-84-2) and Glucosamine Sulfate (CAS No. 29031-19-4), are metabolized to Acetyl Glucosamine (CAS Nos. 10036-64-3, 72-87-7, 7512-17-6) via the hexosamine pathway.[5]



Chemical Properties

Glucosamine HCl (formula weight (FW) = 215.63 g/mol; log K_{ow} = -1.91) and Glucosamine Sulfate (FW = 277.25 g/mol) are charged, highly polar, and water-soluble salts.[5] The acetylated glucosamine metabolite, Acetyl Glucosamine (MW = 222.21 g/mol; log K_{ow} = -2.2), is less polar and neutral. Available information on the chemical properties of the glucosamine ingredients are presented in Table 2.

Method of Manufacture

The methods described below are general to the processing of commercial forms of glucosamine ingredients. It is unknown if they apply to cosmetic ingredient manufacturing.

Acetyl Glucosamine

Acetyl Glucosamine may be prepared using chitin as a substrate via chemical, enzymatic, and biotransformation methods.[6] Chemical production of Acetyl Glucosamine involves the chemical degradation or dissolving of chitin with a strong acid, such as hydrochloric acid. Another method of chemical production of Acetyl Glucosamine involves the acetylation of Glucosamine using pyridine as a solvent, in the presence of tributylamine and acetic anhydride. In addition, enzymatic hydrolysis may be performed to produce Acetyl Glucosamine. Several of these enzymes include derivatives of *Trichoderma viride*, *Aspergillus niger*, *Carica papaya* L., and *Aeronomium*. Examples of commercial crude enzymes that degrade chitin include cellulose, lysozyme, papain, and lipase. Production of Acetyl Glucosamine via biotransformation involves the degradation of chitin using whole microbes (e.g., *Aeromonas caviae*, *Chitinibacter tainanensis*). Genetically modified microorganisms (e.g., *Escherichia coli*) may also be used to produce Acetyl Glucosamine, using glucose as a substrate.

Glucosamine, Glucosamine HCl, and Glucosamine Sulfate

Commercial forms of Glucosamine are prepared mainly from the hydrolysis of chitin, which is the main component of shells from crustaceans (crab, lobster, and shrimp).[7] The produced Glucosamine can then be transformed into Glucosamine Sulfate or Glucosamine HCl. Glucosamine Sulfate is typically stabilized by co-crystallization or co-precipitation with sodium chloride. Commercial forms of Glucosamine can also be prepared from the hydrolysis of chitin with *Aspergillus niger* biomass.[8] In order to derive Glucosamine HCl, the hydrolysate is acidulated with hydrochloric acid for several hours at 100 °C. The product is then filtered to remove solid impurities. Crystals are separated and purified by centrifugation and washing with water.

Impurities

Acetyl Glucosamine

Impurities following chemical and enzymatic synthesis of β-*N*-Acetyl Glucosamine were evaluated via high resolution mass spectrometry, nuclear magnetic resonance spectroscopy, and liquid chromatograph-tandem mass spectrometry.[9] The impurities α-*N*,6-diacetylglucosamine and α-*N*-acetylglucosamine were observed to be present. β-*N*-Acetyl Glucosamine prepared via chemical and enzymatic methods contained a concentration of 146 ± 0.15 and 10.90 ± 0.02 µg/kg α-*N*,6-diacetylglucosamine, respectively. Quantification of α-*N*-acetylglucosamine was not performed, as the recovery value was too low.

Glucosamine HCl

The *United States Pharmacopeia* states that Glucosamine HCl must have a minimum of 98% purity and contain ≤ 3 ppm arsenic and ≤ 0.001 % heavy metals.[10] The purity of Glucosamine HCl sourced from *Aspergillus niger* is reported to be 83.1% free-base glucosamine.[8]

Natural Occurrence

Glucosamine is a monosaccharide that is synthesized from glucose by the hexosamine biosynthetic pathway in nearly all types of human body cells.[11] This natural compound is a constituent of mucosal secretions, skin, tendons, ligaments, and cartilage.[7] In mammals, Acetyl Glucosamine may be found as a component of glycoproteins, proteoglycans, glycosaminoglycans, and other connective tissue building blocks.[6] Acetyl Glucosamine may also be found in human milk at levels of 600 - 1500 mg/ml. Acetyl Glucosamine is the monomeric unit of chitin, which is found in arachnids, most fungal cell walls, insect exoskeletons, the shells of crustaceans, and parts of invertebrates. It may also be present as an extracellular polymer of some microbes.

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. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. The cosmetic product categories named in the VCRP database indicate the intended uses of cosmetic ingredients, and are identified in 21 CFR Part 720. Data are submitted by the cosmetic industry in response to a survey conducted by the Personal Care Products Council (Council) of maximum reported use concentrations, also by product category. Neither the categories provided by the VCRP, nor those provided by the Council survey, include a designation for use via airbrush application. Airbrush devices, alone, are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients used in airbrush devices are within the jurisdiction of the FDA. As airbrush technology use for cosmetics has neither been evaluated by the CPSC, nor the use of cosmetic ingredients in airbrush technology by the FDA, no US regulatory authority has evaluated the safety of this delivery methodology for cosmetic ingredients. Moreover, no consumer habits and practices data are available to evaluate the risks associated with this use type.

According to 2022 VCRP survey data, Acetyl Glucosamine is reported to be used in 198 formulations (185 leave-on formulations and 13 rinse-off formulations; Table 3), and Glucosamine HCl is reported to be used in 77 formulations (64 leave-on formulations and 13 rinse-off formulations).[12] Glucosamine is reported to be used in 2 leave-on formulations. The results of the concentration of use survey reported by the Council in 2020 indicate Acetyl Glucosamine also has the highest concentration of use in a leave-on formulation; it is used at up to 5% in face and neck products (not spray).[13] Glucosamine Sulfate is not reported to be in use according to 2022 VCRP and 2020 concentration of use data, as indicated in Table 4.

Incidental ingestion of Acetyl Glucosamine may occur, as it is used in lipstick formulations at concentrations up to 2%. In addition, Acetyl Glucosamine and Glucosamine HCl are used in formulations applied near the eye; for example, Acetyl Glucosamine is reported to be used at concentrations up to 2% in eye lotions.

Some of these glucosamine ingredients are used in formulations that could possibly be inhaled. For example, Acetyl Glucosamine is reported to be used at 0.1% in pump hair sprays and at up to 0.07% in face powders. In practice, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[14,15] 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.[16-18]

Additionally, although products containing some of these ingredients may be marketed for use with airbrush technology, 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 related to this use technology), the data are insufficient to evaluate the safety thereof in airbrush applications.

All of the glucosamine ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[19]

Non-Cosmetic

In the US, Glucosamine (up to 1500 mg/d) and its metabolites are not classified as drugs, but as dietary supplements, under the US FDA Dietary Supplement Health and Education Act of 1994.[5,20] Acetyl Glucosamine and Glucosamine salts (Glucosamine Sulfate and Glucosamine HCl) are commercially available as dietary supplements, and are commonly administered in conjunction with chondroitin sulfate. According to 21 CFR 216.23, *N*-acetyl-D-glucosamine [Acetyl Glucosamine] is a bulk drug substance that may be used to compound topical drug products, in accordance with section 502A of the Federal Food, Drug, and Cosmetic Act.

In most European countries, Glucosamine is marketed as both a medicinal product and a food supplement.[7] In France, Glucosamine (in the form of the sulfate or HCl salt) is used in orally-ingested medicinal products as the only active ingredient (up to 1250 mg/d). In veterinary medicine, Glucosamine HCl is commonly used for treating osteoarthritis in dogs.[21]

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

Acetyl Glucosamine

The skin penetration of ^{14}C-*N*-Acetyl-D-Glucosamine was evaluated in split-thickness Caucasian cadaver skin.[22] The skin was cut and mounted in standard Franz-type diffusion cells (exposed skin surface area of 0.79 cm^2) maintained at 34 °C. The receptors were filled with phosphate-buffered saline (PBS) incorporating 1% polysorbate-20 and 0.02% sodium azide, and skin was allowed to equilibrate for 2 h. Aliquots of the test formulations were spiked with ^{14}C-niacinamide and assayed for total radiolabel in triplicate. Approximately 5 µl of the test formulations (2% Acetyl Glucosamine alone in an unknown vehicle, or a combination of 4% niacinamide and 2% Acetyl Glucosamine with an unknown vehicle) was applied to the cells using a positive displacement pipette (n = 8). The receptor solution was collected and replaced at 2, 4, and 6 h (termination) of study. Solutions were assayed for total radiolabel via liquid scintillation. Approximately 7% of the applied dose permeated the skin when the test substance containing Acetyl Glucosamine alone was applied. Approximately 6.5% of the applied dose permeated the skin when the test substance containing both Acetyl Glucosamine and niacinamide was applied. The test substances were found to readily penetrate into and through human skin.

Glucosamine HCl

Using a saturated aqueous solution of Glucosamine HCl, in vitro permeation studies were performed on human epidermal membranes prepared by a heat separation method and mounted in Franz-type diffusion cells with a diffusional area of 2.15 ± 0.1 cm^2.[23] Studies were performed over a 48 h period by loading donor compartments with 2 ml of the Glucosamine HCl solution of each diffusion cell (n = 5), and evaluating receptor solutions for permeation. Glucosamine HCl permeated through the skin with a flux of 1.497 ± 0.42 µg cm^2/h, a permeability coefficient of 5.66 ± 1.6 x 10^{-6} cm/h, and a lag time of 10.9 ± 4.6 h.

The transdermal penetration of 5% Glucosamine HCl in different vehicles (aqueous, oil-in-water cream, liposomal suspension, liposomal gel, cubic liquid crystalline bulk phase) was evaluated in the dorsal skin of Sprague-Dawley rats mounted in Franz diffusion cells (diffusional surface area of 2.14 cm^3).[24] Epidermal sides of the skin were exposed to the various formulations of Glucosamine HCl (100 mg). Aliquots (0.5 ml) were withdrawn from the receptor compartment over a period of 12 h and evaluated for Glucosamine HCl via high-performance-liquid-chromatography (HPLC). The steady state flux of the drug through the skin for the aqueous solution, cream, liposomal suspension, liposomal gel, and cubic phase was calculated to be 56.89 ± 23.76, 58.24 ± 29.46, 57.61 ± 26.72, 57.27 ± 4.35, and 248.89 ± 64.57 µg/h/cm^2, respectively. According to study authors, the reason for the enhanced permeation of Glucosamine HCl caused by the cubic phase was likely due to the structural similarity between the cubic phase and biomembrane.

Glucosamine Sulfate

Skin permeation of Glucosamine Sulfate was evaluated in Sprague-Dawley full-thickness rat skin.[25] Freshly excised rat skin was mounted between the donor and receptor cell (area of diffusion was 2.14 cm^2). Donor cells, facing the stratum corneum surface, contained 5% Glucosamine Sulfate aqueous solution (3 ml). Receptor cells, which faced the dermis side, were filled with normal saline solution (12 ml). At predetermined time intervals, 0.5 mL of the receptor solution was withdrawn and refilled with the same volume of fresh receptor solution. Samples were analyzed by HPLC. The skin permeation rate (amount recovered in receptor fluid) was determined to be 13.27 µg/cm^2/h.

Human

Glucosamine Sulfate

The penetration of a 10% Glucosamine Sulfate cream into the synovial fluid of patients with knee osteoarthritis (134 subjects/group) was evalauted.[26] For treated groups, cream (2 g) was placed on the knee, for 1-3 h, followed by synovial fluid collection. A control group was not subjected to any treatment, but their synovial fluid was collected. Synovial fluid from both treated and control groups was evaluated for Glucosamine concentrations via HPLC. The mean Glucosamine concentrations in treated and control patients were 100.56 ng/ml and 17.83 ng/ml, respectively ($p < 0.0001$).

Absorption, Distribution, Metabolism, and Excretion (ADME)

Animal

Oral

Glucosamine HCl

A pharmacokinetic analysis was performed via liquid chromatography-tandem mass spectrometry (LC-MS/MS) in 4 female Beagle dogs.[27] Animals were given a single oral dose of a dietary supplement containing 450 mg Glucosamine HCl. Blood samples from dogs were collected and analyzed 0, 1, 2, 4, 6, 8, 12, and 24 h post-administration. Glucosamine was detected up to 8 h post-dose, with a time to reach serum concentration (T_{max}) of 2 h and a peak serum concentration (C_{max}) of 9.69 µg/ml. The elimination half-life ($t_{1/2}$) of Glucosamine after administration of the test substance was approximately 35 min.

Glucosamine HCl and Glucosamine Sulfate

Blood levels, tissue distribution, and excretion patterns of radioactivity were studied in Sprague-Dawley rats (44 rats/ sex) after oral administration of [^{14}C]Glucosamine HCl diluted with unlabeled Glucosamine Sulfate (dose not reported).[28] Plasma, urine, feces, blood, and organs/tissues were evaluated for radiolabel concentrations. At 1 - 2 h after administration, Glucosamine radioactivity was bound to or incorporated into plasma proteins. After peaking at 2 - 4 h, radioactivity declined from plasma at a slower rate ($t_{1/2}$ = 46 h). Approximately half of the radioactivity was excreted as [^{14}C]carbon dioxide, and 40% of the radioactivity was excreted in the urine. Only 2% of the administered dose was excreted in feces. Radioactivity analysis in tissues and organs revealed that the [^{14}C] from the labeled Glucosamine quickly entered into all tissues, included cartilage, reaching a maximum at 8 h.

Human

Oral

Glucosamine HCl

Glucosamine HCl bioavailability from two different orally-administered formulations was evaluated in healthy adult males (9/group) under fasting conditions.[29] A single dose of Glucosamine HCl was administered to the volunteers via a dispersible tablet (240 mg Glucosamine HCl/tablet) or capsule (240 mg Glucosamine HCl/capsule). Subjects received either 2 Glucosamine HCl tablets or capsules with 250 ml water. Blood samples were collected before test substance administration, and at various intervals up to 12 h after administration. Plasma Glucosamine concentration was evaluated via the LC-MS/MS method. The mean C_{max}, T_{max}, and $T_{1/2}$ values were reported to be 907.1 ng/ml, 3.03 h, and 1.10 h, respectively, for the dispersible tablet formulation. For the capsule formulation, mean C_{max}, T_{max}, and $T_{1/2}$ values were reported to be 944.40 ng/ml, 3.30 h, and 1.50 h, respectively.

Glucosamine HCl and Glucosamine Sulfate

The pharmacokinetics of Glucosamine after oral administration of crystalline Glucosamine Sulfate and Glucosamine HCl were evaluated in 12 healthy volunteers (5 male and 7 female).[30] Volunteers received once-daily, oral administrations of crystalline Glucosamine Sulfate soluble powder at a dose of 1500 mg, or Glucosamine HCl capsules at a dose of 500 mg, for 3 consecutive days, alone, or in combination with chondroitin sulfate (400 mg). Glucosamine was determined at steady state in plasma collected up to 48 h after the last dose by a validated LC-MS/MS method. After Glucosamine Sulfate administration, peak concentrations (C_{ss}, max) and extent of exposure (AUC_{ss}) averaged 9.1 ± 6.3 µM and 76.5 ± 23.0 µM/h, respectively. Significantly lower plasma concentrations ($p \leq 0.005$) were determined after the administration of Glucosamine HCl alone ($C_{ss,\ max}$ and AUC_{ss} averaged 4.5 ± 1.8 µM and 21.4 ± 7.6 µM/h, respectively), or in combination with chondroitin sulfate ($C_{ss,\ max}$ and AUC_{ss} averaged 3.3 ± 1.0 µM and 13.8 ± 5.4 µM/h, respectively).

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

Details regarding the acute oral toxicity studies summarized below can be found in Table 5.

The reported median lethal dose (LD_{50}) values for Glucosamine were higher than the doses tested (> 15,000 mg/kg in mice and > 8000 mg/kg in rats and rabbits).[31] According to an ECHA dossier, the acute oral LD_{50} for Glucosamine HCl was reported to be 15,000 mg/kg bw in mice.[2]

Short-Term Toxicity Studies

Oral

Glucosamine HCl

The effect of oral Glucosamine was evaluated in male Sprague-Dawley and male spontaneously hypertensive rats (SHR; 8 rats/strain/group).[32] Four groups of both rat strains received either no treatment (control), Glucosamine (0.5%), chondroitin sulfate (0.4%), or a combination of both, for 9 wk, via diet. A concentration of 0.5% or 0.4% of Glucosamine and chondroitin sulfate roughly calculates to 1500 and 1200 mg/d, respectively. Systolic blood pressure (SBP) and body weight were evaluated weekly. Hematological and histological evaluations were performed. No statistically significant differences in body weight were observed in any of the four dietary groups. SBP of both strains consuming the two ingredients alone and in combination was statistically significantly lower than the SBP in control animals. No statistically significant histological differences were found in the hearts, kidneys, or livers among the treated and control groups. In Sprague-Dawley rats, there were no relevant trends in blood chemistries among the four groups, however BUN levels were significantly lower ($p < 0.03$) in the control group compared to the other three groups. In SHR, no hematological differences between groups were observed.

Subchronic Toxicity Studies

Animal

Oral

Acetyl Glucosamine

Acetyl Glucosamine was fed to F344 rats (10 rats/sex/group) via pelleted diets containing 0, 0.625, 1.25, 2.5 or 5% Acetyl Glucosamine for 13 wk.[33] Clinical signs, food intake, hematology, serum biochemistry, and histopathology were evaluated in all animals. All animals survived until the end of the experiment. A slight, non-significant increase in body weights was observed in males receiving 0.625, 1.25, and 2.5% Acetyl Glucosamine from wk 4 until the end of the experiment. Statistically significant elevation of weight gain was observed in males receiving 0.625, 1.25 and 2.5% Acetyl Glucosamine at the terminal sacrifice, which resulted in decreased relative weights in many organs. However, no obvious indications of toxicity were observed in any of the parameters evaluated. The no-observed-adverse-effect-level (NOAEL) was determined to be > 5%.

Human

Oral

Acetyl Glucosamine

The effect of orally ingested Acetyl Glucosamine was evaluated in healthy adult humans.[34] Safety assessments were performed via physical parameters, hematology, blood biochemistry, and urinalysis. The test supplement contained green tea extract powder and either 500 (n = 22) or 1000 (n = 22) mg of Acetyl Glucosamine. The placebo supplement contained green tea extract powder without Acetyl Glucosamine (n = 24). All subjects were instructed to take the supplements, dissolved in a cup of water, once a day for 16 wk. A total of 66 adverse events occurred in 12, 10, and 9 subjects receiving placebo, 500 mg/d Acetyl Glucosamine, and 1000 mg/d Acetyl Glucosamine, respectively, and there was no significant difference in the frequency among the 3 groups. Relatively frequent adverse symptoms included cold symptoms, gastric distress, and pain. These effects were generally mild. Routine physical and cardiovascular characteristics, hematology, and blood chemistry, did not show any significant abnormalities in all three groups.

Glucosamine HCl

A 16-wk, randomized, double-blind, placebo-controlled crossover trial of a combination of Glucosamine HCl (1500 mg/d), chondroitin sulfate (1200 mg/d), and manganese ascorbate (228 mg/d) was conducted in degenerative joint disease patients.[35] Thirty-four male patients were randomized and given either the test substance (a tablet containing a combination of Glucosamine HCl, chondroitin sulfate, and manganese ascorbate), or a placebo for 8 wk. For an additional 8-wk period, the patients crossed over to the regimen not followed previously. Patients were asked to complete a survey of symptoms consistent with toxicity and to return cards for fecal occult blood testing at the end of each protocol phase. No patients reported symptoms requiring termination of study, and symptom frequency on medication was similar to that at baseline. Vital signs, occult blood testing, and hematologic parameters were similar among the placebo and medicated groups.

Chronic Toxicity Studies

Oral

Acetyl Glucosamine

The chronic toxicity potential of Acetyl Glucosamine was evaluated in F344 rats (10 rats/sex/group).[36] Acetyl Glucosamine was administered via the diet at levels of 0, 1.25, 2.5 or 5%, for 52 wk. Clinical effects, mortality, hematology, serum biochemistry, and histopathology were evaluated. After gross examination, the brain, heart, lungs, liver, spleen, adrenals, kidneys, and testes were weighed. No toxic effects were observed in any parameter evaluated; however, slight suppression of body weight gain was observed in animals dosed with concentrations of greater than 2.5%. This effect appeared to be due to a slight reduction of caloric intake with the high concentration of test compound.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

Glucosamine

The effects of premating Glucosamine supplementation via drinking water on Sprague-Dawley rat litter homogeneity, uterine receptivity, and maternal hormones levels, were evaluated.[37] Female rats (29 animals/group) were given either normal drinking water, or drinking water supplemented with 0.5 mM Glucosamine, from 6 to 8 wk old. After a 2-wk administration, the rats were mated. Ovaries, uteri, implantation sites, pup birth weight, maternal placental efficiency, and plasma of dams were evaluated. Variation of within-litter birth weight in the Glucosamine-treated group was 5.55%, a significantly lower variation than that of the control group (8.17%). Birth weights and absolute and relative ovary weights were statistically significantly greater in the Glucosamine-treated group compared to the control group ($p < 0.05$). In the Glucosamine-treated group, there were more successfully implanted blastocysts (13.38 ± 0.63 and 15.75 ± 0.59 in the control and treated group, respectively), with more uniform distribution along the two uterine horns compared with the control group. Maternal progesterone, estradiol, and insulin-like growth factor 1 (IGF-1) concentrations on day 19.5 of pregnancy were significantly increased in treated rats, while insulin and total cholesterol levels were significantly decreased compared with control rats.

Intraperitoneal

Glucosamine

The effects of pre-conception Glucosamine administration on reproductive outcomes was evaluated in 8-wk-old and 16-wk-old adult female C57B1/6 mice (24 mice/group).[38] Animals were given either 0, 20 mg/kg Glucosamine per day (in phosphate-buffered saline (PBS))), via intraperitoneal injection, for 3 d. Mice that received no Glucosamine treatment during the feeding period received injections of PBS only. On day 3, females were mated with a male, and mating success was determined. On day 4, animals were once again treated with Glucosamine (same dose as previously given). For successfully-mated females, this was the final injection; females that did not mate were re-introduced to males and given daily injections until mating was successful (for a maximum of 4 nights). On day 18 of gestation, animals were killed, examined, and all fetal parameters were assessed. The total number of implantations ($p < 0.0001$) and viable fetuses ($p < 0.0001$) was lowest in the 8-wk-old, Glucosamine-treated group. The number of implantations and viable fetuses among the 16-wk-old Glucosamine-treated mice and control mice did not differ significantly. Fetal weight was reduced by Glucosamine treatment in 16-wk-old mice ($p < 0.05$), whereas the same treatment did not affect 8-wk old mice. Glucosamine also reduced fetal length in pups derived from 16-wk-old Glucosamine-treated mice ($p < 0.05$). In addition, a significantly higher number of abnormal fetuses was present in litters of 16-wk-old Glucosamine-treated mice, compared with all other groups ($p < 0.05$).

Intrauterine

Glucosamine

The effects of intrauterine Glucosamine were evaluated in female ICR mice (3 mice/group).[39] A hysterectomy of one uterine horn was performed according to standard surgical procedures. A 60-d sustained-release Glucosamine pellet (15, 150, or 1500 μg) or placebo pellet was implanted into the top of the remaining uterine horn. Females recovered independently for 10 d, and then mated with ICR male mice. The number of pups/litter was recorded until two litters after

the 60-d pellet release period. After hysterectomy and implantation of placebo pellets, litters were approximately half the size that they were before surgery (5.6 and 12.7 pups/litter, respectively). Mice that received Glucosamine pellets delivered significantly fewer live pups/litter over a 60-d pellet active period than those that received placebo pellets (15 µg Glucosamine, 2.75 ± 0.73 pups/litter; 150 µg Glucosamine, 2.13 ± 0.85 pups/litter; 1500 µg Glucosamine, 0.25 ± 0.25 pups/litter; placebo, 5.61 ± 0.66 pups). The gross morphological appearance of the pups from placebo and Glucosamine-treated mice were normal post-birth. Serum glucosamine levels were similar among placebo and treated groups. After the 60-d pellet release period, there was no statistically significant difference in litter sizes delivered by Glucosamine-treated and placebo-treated mice, except at the highest dose level.

GENOTOXICITY STUDIES

In Vitro

Acetyl Glucosamine

An Ames assay was performed according to Organization for Economic Co-Operation and Development test guideline (OECD TG) 471.[3] *Salmonella typhimurium* strains TA 1537, TA 1535, TA 98, TA 100, and TA 102 were exposed to Acetyl Glucosamine at concentrations of 156.25, 312.5, 625, 1250, 2500, and 5000 µg/plate, with and without metabolic activation. Plates were maintained in triplicate, and the number of revertant colonies were recorded after the 48-h incubation period. The test substance was non-mutagenic to any strain of *S. typhimurium* when tested under specified experimental conditions.

Glucosamine HCl

The potential genotoxicity of Glucosamine HCl derived from *Aspergillus niger* was evaluated in an Ames assay.[8] The tester strains (*S. typhimurium* TA 98, TA 100, TA 1535, and TA 1537, and *E.coli* WP2 uvrA) were exposed to Glucosamine HCl at concentrations of 100, 333, 1000, 3300, and 5000 µg/plate, with and without metabolic activation. The test substance was considered to be non-mutagenic.

In Vivo

Glucosamine HCl

An in vivo micronucleus assay was performed in accordance with OECD TG 474.[8] Mice (number of animals and strain not reported) were dosed with *Aspergillus niger*-derived Glucosamine HCl mixed with water, via gavage. The test substance was administered in doses of 500, 1000, or 2000 mg/kg bw. There was no statistically significant increase in micronucleated polychromatic erythrocytes (PCE) or decrease in the ratios of polychromatic PCEs and normochromatic erythrocytes (NCE) at any dose level. The test substance was considered to be non-toxic to bone marrow.

ANTI-GENOTOXICITY STUDIES

In Vitro

Acetyl Glucosamine and Glucosamine

The anti-genotoxic effect of Glucosamine and Acetyl Glucosamine in human peripheral lymphocytes exposed to oxidative stress was evaluated.[40] Lymphocytes were treated with Acetyl Glucosamine or Glucosamine at concentrations of 0, 2.5, 5, 10, 20, or 50 mM. Cells were also treated with 25 µM hydrogen peroxide to induce DNA damage. Control cells were treated with the vehicle (PBS) and hydrogen peroxide. Cells were analyzed and data were presented as % DNA in tail. Acetyl Glucosamine only indicated a slight DNA protection at a concentration of 50 mM ($p < 0.01$). Glucosamine, at all concentrations, showed a significant protective activity ($p < 0.001$) against hydrogen peroxide-induced DNA damage.

In Vivo

Glucosamine

The chemoprotective ability of Glucosamine against cisplatin-induced genotoxicity was evaluated in rat bone marrow cells.[41] Male Wistar rats (5/group) were fed diets containing either 75 or 150 mg/kg Glucosamine, for 7 consecutive d. On the 7th d, 1 h after Glucosamine treatment, a single intraperitoneal dose of cisplatin (5 mg/kg) was administered. Three control groups were used, a normal control group (oral PBS treatment and injection with saline), a Glucosamine control group (oral 150 mg/kg Glucosamine treatment and injection of PBS), and a cisplatin control group (oral PBS treatment and injection of cisplatin). All animals were killed 24-h post-treatment with cisplatin, and rat bone marrow cells were collected. For each experimental group, a total of 5000 PCE and corresponding NCE were scored to determine the number of micronucleated polychromatic erythrocytes (MnPCE) and micronucleated normochromatic erythrocytes (MnNCE). Pretreatment with 75 and 150 mg/kg Glucosamine prior to cisplatin injection significantly reduced the frequency of MnPCE and MnNCE ($p < 0.05$). Treatment with Glucosamine also prevented the fall in the PCE/(PCE + NCE) ratio as compared with the cisplatin control group ($p < 0.001$). The test substance was considered to be an effective chemoprotector against cisplatin-induced DNA damage.

CARCINOGENICITY STUDIES

Acetyl Glucosamine

The carcinogenic potential of Acetyl Glucosamine was evaluated in F344 rats (50 rats/sex/group).[36] Animals were given Acetyl Glucosamine in the diet at levels of 0, 2.5, or 5%, for 104 wk. Many tumors were found in males and females in all groups; however, all tumors observed were well-known to occur spontaneously in F344 rats. No significant intergroup differences in tumor frequency or histological types were apparent. Additionally, the number of neoplastic lesions observed in animals was similar among control and treated groups. The test substance was considered to be non-carcinogenic.

ANTI-CARCINOGENICITY STUDIES

In Vitro

Glucosamine

The anti-proliferative potential of Glucosamine in human renal cancer cell lines (786-O and caki-1) was studied via an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and annexin V-fluorescein isothiocyanate (FITC) assay.[42] To evaluate cell proliferation, renal cancer cells were treated with either 0, 1, 5, or 10 mM Glucosamine, and incubated. After incubation, MTT solution was added, cells were again incubated, followed by addition of dimethyl sulfoxide and the evaluation of optical density. Glucosamine inhibited the proliferation of renal cancer cells in a dose-dependent manner ($p < 0.05$) as compared with the control group. In order to evaluate cell apoptosis, cancer cells were serum-starved for 24 h, and treated with various doses of Glucosamine (0, 1, 5, or 10 mM) for 24 h. Cells were then collected and washed twice with PBS. Then, cells were re-suspended, stained with FITC-annexin V/PI and analyzed by flow cytometry. The apoptosis rate of both cell lines was up-regulated by the high concentration of Glucosamine (10 mM), but down-regulated by low concentrations of Glucosamine (1 and 5 mM), as compared with the control groups.

Acetyl Glucosamine, Glucosamine, and Glucosamine HCl

The growth inhibitory effects of Glucosamine, Glucosamine HCl, and Acetyl Glucosamine on human hematoma SMMC-721 cells were evaluated in vitro.[43] Tumor cells were cultured in a growth medium supplemented with 15% bovine calf serum, 100 U/ml penicillin, and 100 U/ml streptomycin at 37° C, seeded in 96-well plates, and incubated for 24 h. After incubation, cells were treated with Glucosamine, Glucosamine HCl, or Acetyl Glucosamine (10 - 1000 µg/ml), and again incubated for 24 – 120 h. Untreated cells were used as controls. Results measured by an MTT assay showed that Glucosamine HCl and Glucosamine caused a concentration-dependent reduction in hepatoma cell growth. In addition, human hepatoma cells treated with Glucosamine HCl resulted in the induction of apoptosis as assayed qualitatively by agarose gel electrophoresis. Acetyl Glucosamine did not inhibit the proliferation of SMMC-7721 cells.

Animal

Glucosamine HCl

Sarcoma 180 tumor ascites cells were subcutaneously inoculated (0.2 ml/mouse) into 8-wk-old Kunming male mice (number of animals not stated).[43] Mice were divided and given an oral dose of either saline (control group) or Glucosamine HCl dissolved in saline (125, 250, or 500 mg/kg/d). The method of oral administration was not stated. Administrations occurred once daily for 10 d. The tumor was allowed to grow on mice for 10 d before it was removed from the animal and evaluated. The anti-tumor activity of Glucosamine HCl was expressed as an inhibition ratio calculated as [(average tumor weight of control – average tumor weight of treated group)/average tumor weight of control] x 100%. Glucosamine HCl, at the intermediate dose (250 mg/kg/d), had the highest inhibition ratio (34.02%) on sarcoma 180 tumor growth. Inhibition ratios at the 125 and 500 mg/kg/d dose levels were reported to be 27.84 and 29.33%, respectively.

OTHER RELEVANT STUDIES

Effects on Pigmentation

The following studies are included in this report as they may be relevant to concerns regarding depigmentation, skin whitening, and anti-melanogenesis.

In Vitro

Acetyl Glucosamine

The effect of Acetyl Glucosamine on melanin production was evaluated in an in vitro assay using reconstituted human tanned epidermis.[44] Skin cultures were placed in 6-well tissue culture plates containing 2 ml/well of a growth medium. Administrations of either Acetyl Glucosamine (1, 3, or 5% in water) or water alone (30 µl) were applied topically, for 10 d. Culture medium and treatment was replenished daily. Skin equivalent cell cultures treated topically with 1, 3, or 5% Acetyl Glucosamine produced dose-dependent decreases in melanin content. According to the study authors, Acetyl Glucosamine can inhibit the enzymatic glycosylation of tyrosinase, resulting in pigmentation effects. In addition, pigmentation effects following Acetyl Glucosamine exposure may occur due to its effect on the expression of several pigmentation-relevant genes.

The anti-melanogenic effect of an Acetyl Glucosamine-loaded microemulsion was evaluated in B16 melanoma cells.[45] The microemulsion contained 1% Acetyl Glucosamine, 9% water, and 10% propylene glycol, 20% palm oil, and 60% of a surfactant mixture. A control solution was prepared using the same components as the test microemulsion, excluding Acetyl Glucosamine. In addition, an aqueous solution containing 1% Acetyl Glucosamine was also evaluated (untreated B16 cells used for control). B16 cells were first plated with 1 µmol/l of α-melanin stimulating hormone for 3 d, followed by incubation with microemulsions, at a 1:2000 dilution, for 24 h. Melanin content in B16 melanoma cells decreased by 21% and 44% after treatment with the microemulsion and the microemulsion control, respectively. Slight melanin reduction was noted in B16 cells treated with the aqueous Acetyl Glucosamine solution (7% reduction), compared to the untreated control.

Animal and Human

Acetyl Glucosamine

The whitening effect of Acetyl Glucosamine in skin was examined in humans (number of subjects not specified) and brown guinea pigs (strain and number of animals not specified) that were subjected to ultraviolet (UV; wavelength not provided)-induced pigmentation.[46] The 5% Acetyl Glucosamine (information regarding solution not provided) was applied to the dorsal skin of brown guinea pigs and the inner side of human forearm skin for 8 wk, twice a day. In humans, a visual reduction in hyperpigmentation was observed 2 wk after treatment with the Acetyl Glucosamine solution, compared to the vehicle-treated group, and a strong decrease in visible pigmentation was observed after 8 wk of Acetyl Glucosamine treatment. The degree of hypopigmentation at each time point measured after the application of Acetyl Glucosamine was higher than the vehicle control group. In guinea pigs, biopsy specimens were obtained from both the treated and control groups 4 wk after topical application. Acetyl Glucosamine-treated skin had decreased levels of melanin without affecting the number of melanocytes, compared to vehicle-treated skin.

Human

Acetyl Glucosamine

The reduction of facial hyperpigmentation after use of a moisturizer containing Acetyl Glucosamine and niacinamide was evaluated in a 10-wk, randomized, double-blind, vehicle-controlled trial.[47] During a 2-wk preconditioning period, the test subjects (101 women/group) used the same commercial facial cleanser, nighttime moisturizer, and daytime moisturizing lotion. After the 2-wk period, subjects used a daily regimen of either a morning sun protection factor (SPF) 15 sunscreen moisturizing lotion and evening moisturizing cream containing 4% niacinamide and 2% Acetyl Glucosamine, or the SPF 15 lotion and cream vehicles. Product-induced changes in apparent pigmentation were assessed by capturing digital photographic images of the women after 0, 4, 6, and 8 wk of product use. Images were evaluated by algorithm-based computer image analysis for colored spot area fraction, by expert visual grading, and by chromophore-specific image analysis based on noncontact spectrophotometric intracutaneous analysis (SIAscopy™) for melanin spot area fraction, and melanin chromophore evenness. By all parameters measured, the Acetyl Glucosamine and niacinamide formulation regimen caused a more pronounced decrease in detectable areas of facial spots and the appearance of pigmentation, compared to those that used the control formulation ($p < 0.05$).

A similar study, from Japan, was performed in healthy women (n = 25 women/group).[22] Volunteers were instructed to apply a formulation (0.3 g) containing either the placebo control or 2% Acetyl Glucosamine, on the side of the face, twice daily, for 8 wk. Digital images of each side of the face of all subjects were captured at baseline, and at week 4 and 8. Topical 2% Acetyl Glucosamine was effective in improving the appearance of facial hyperpigmentation based on computer image analysis, with an overall directional ($p = 0.089$) spot area fraction change across the entire study.

Forty-five Caucasian women (Fitzpatrick skin types I, II, and III), aged 40 - 65 yr, with moderate skin texture and the presence of at least mild to moderate-severe hyperpigmentation on the décolletage, were used in this study.[48] Volunteers were instructed to apply a neck cream containing 8% Acetyl Glucosamine and 4% triethyl citrate, each day, for 16 wk. Skin pigmentation and texture were graded using a 0 – 5 scale with half-point increments. Irritation/tolerability parameters (dryness, itching, stinging/burning) were measured at week 0, 8, 12, and 16 using a 0 - 3 scale (none, mild, moderate, severe). Colorimetric measurements were also made at week 0, 8, and 16. A significant reduction of skin pigmentation was observed at each time point ($p < 0.001$). After 16 wk, skin pigmentation was reduced by 23%. Chromameter measurements revealed significant improvement at week 8 and 16 in brightness ($p < 0.001$) and erythema ($p < 0.05$). The test cream was well-tolerated with no signs of irritation. One subject experienced an adverse event of contact dermatitis on two separate occasions. No other adverse events were reported.

Reduction of IgE-Mediated Hypersensitivity

The following studies are included in this report as they may be helpful in addressing cosmetic safety concerns regarding immunoglobulin E (IgE)-mediated hypersensitivity.

Glucosamine

The effect of Glucosamine on ovalbumin (OVA)-induced atopic dermatitis was evaluated in female BALB/c mice (5 mice/group).[49] Approximately 1.5 ml of OVA and 3 ml of aluminum hydroxide gel were mixed, and 150 µl of the mixture was intraperitoneally injected into mice 3 times a week, for 3 wk. After the first week of OVA injection, mice were

epicutaneously sensitized with OVA patches (1 cm x 1 cm patch containing 50 µl OVA). Patches were applied 3 times a week, for 2 wk. After atopic dermatitis was induced, mice were given 100 µl Glucosamine injections at concentrations of 1 mg/10 µl, 1 mg/5 µl, and 1 mg/2.5 µl. After a week of Glucosamine administration, 3 OVA patches were again attached during the next week. In addition, two control groups were used. One group received a PBS injection without OVA induction, and a second group received a PBS injection with OVA induction. Clinical dermatitis scores decreased with increasing Glucosamine dose ($p < 0.001$). Concentrations of tissue interleukin (IL)-13 and IL-17 decreased after Glucosamine administration (each group: $p = 0.002$ and $p < 0.001$, respectively), but the concentrations of tissue IL-4 did not show differences across groups. Serum IgE levels tended to be lower after Glucosamine administration ($p = 0.004$).

The anti-allergic effect of Glucosamine in female BALB/c mice with allergic rhinitis and asthma was studied.[11] Mice (8/group) were given an OVA intraperitoneal/intranasal challenge to induce allergic asthma and rhinitis. Thirty min prior to sensitization induction, animals were administered Glucosamine treatment, via intraperitoneal injection, at concentrations of either 1 or 5%. A negative control group received an intranasal/intraperitoneal challenge using sterile saline, and did not receive Glucosamine treatment. A positive control group received an OVA intranasal/intraperitoneal challenge, and no treatment with Glucosamine. Serum total and OVA-specific IgE, cytokine titers, and the number of inflammatory cells in bronchoalveolar lavage (BAL) fluid were evaluated. A histopathologic examination of the lung and nasal cavity was also performed. OVA-specific IgE and eosinophils in BAL fluid were significantly decreased after 5% Glucosamine treatment compared with the positive control group ($P < 0.05$). In addition, significant improvement of inflammation was apparent in groups treated with 1 and 5% Glucosamine when compared to the positive control group.

Acetyl Glucosamine and Glucosamine HCl

The anti-allergic effect of orally ingested Acetyl Glucosamine and Glucosamine HCl was evaluated in female BALB/c mice (3 animals/group).[50] The dorsal skin of each mouse was shaved and 100 µL 0.5% dinitrofluorobenzene (DNFB) in acetone-soybean oil was applied to induce sensitization. After induction, Acetyl Glucosamine or Glucosamine HCl (0.1 or 1 mg/mouse) was administered orally, once per day, for 6 d. The method of oral administration was not specified. One h after the final administration, both right and left ears were challenged with 20 µl 0.5% DNFB in acetone-soybean oil. The thickness of the right ear was measured with a dial thickness gauge 0, 6, and 24 h after DNFB challenge. In addition, the amount of histamine in the plasma of the right ear was measured. Oral administration of Acetyl Glucosamine or Glucosamine HCl significantly inhibited DNFB-induced ear swelling in mice at both 6 h and 24 h after DNFB challenge ($P < 0.05$), and reduced the concentration of histamine in both the ear and plasma of DNFB-treated mice ($P < 0.05$).

Effect of Oral Administration on Atopic Dermatitis

Glucosamine

The effect of orally-administered Glucosamine in the treatment of atopic dermatitis was evaluated in a placebo-controlled, double-blind, clinical trial. [51] Patients with atopic dermatitis received either a combination of 2 mg/kg cyclosporine and 25 mg/kg Glucosamine ($n = 16$; Group A), or a combination of 2 mg/kg cyclosporine and placebo ($n = 17$; Group B), for 8 wk. Among the 16 patients receiving Glucosamine treatment, 15 patients reported clinical improvement of atopic dermatitis symptoms. Clinical improvement was noted in 10 of 17 patients treated with the placebo. Among the 19 intention-to-treat patients in each group, three from group A and 4 from group B experienced adverse effects, with abdominal pain being the common adverse effect.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Details regarding the irritation and sensitization studies summarized below can be found in Table 6.

No irritation was noted in an vitro reconstructed human epidermis assay performed using Acetyl Glucosamine (99.42% purity).[3] Multiple in chemico/in vitro sensitization assays (direct peptide reactivity assay (DPRA), KeratinoSens™ assay, human cell line activation test (h-CLAT)) performed using Acetyl Glucosamine yielded negative results.[3] Very mild cumulative irritation was noted in a 21-d cumulative patch human dermal irritation using an eye cream containing 2% Acetyl Glucosamine (12 subjects; occlusive conditions).[52] HRIPTs performed using a mask containing 0.005% Acetyl Glucosamine (108 subjects), a liquid foundation containing 2% Acetyl Glucosamine (105 subjects), and a leave-on product containing 0.005% Glucosamine HCl (51 subjects) yielded negative results.[53-55] Similarly, no sensitization was noted in maximization assays performed, each in 25 subjects, using a product containing 0.01% Glucosamine and a product containing 0.25% Glucosamine HCl.[56,57]

OCULAR IRRITATION STUDIES

In Vitro

Acetyl Glucosamine

An EpiOcular™ 3-[4,5,-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) conversion assay was performed to determine the ocular irritation potential of a face serum containing 2% Acetyl Glucosamine.[58] Stratified human

keratinocytes were exposed to the neat test article for 8, 16, 20, and 24 h. The effective time (ET_{50}) at which the test substance caused a 50% reduction in tissue viability was 17.2 h. The ET_{50} of the positive control was 16.3 min.

A bovine corneal opacity and permeability (BCOP) test assay was performed according to OECD TG 437.[3] Bovine corneas (3/group) were treated with either 750 µl of a saline solution containing 20% Acetyl Glucosamine, 750 µl of saline alone (negative control), or 750 µl of a saline solution containing 20% imidazole (positive control). Corneas were exposed for 4 h ± 5 min at 32 ± 1 °C. The mean in vitro irritancy scores for the test substance, negative control, and positive control were 0.42, 0.70, and 105.42, respectively.

CLINICAL STUDIES

Lack of Hypersensitivity to Shrimp-Derived Glucosamine

Glucosamine

The tolerability of shrimp-derived Glucosamine was evaluated in shrimp-allergic individuals.[59] Subjects with a history of shrimp allergy were recruited and tested for both shrimp reactivity and shrimp-specific IgE by an ImmunoCAP™ assay. Fifteen individuals with a positive skin prick test to shrimp and an ImmunoCAP™ class level of two or greater were selected for a double-blind placebo-controlled food challenge using Glucosamine-chondroitin tablets containing 1500 mg of synthetically-produced (control) or shrimp-derived Glucosamine. Immediate and delayed reactions (up to 24 h post-challenge) were evaluated via a questionnaire. All subjects tolerated the 1500 mg Glucosamine administration from the shrimp-derived and synthetic sources, without any incidences of hypersensitivity.

Case Reports

Glucosamine

A 52-yr-old with a history of long-standing intermittent asthma complained of exacerbation of underlying asthma.[60] Exacerbation was characterized by shortness of breath and wheezing. Inhaled albuterol was not sufficient to extinguish or diminish symptoms. Aside from osteoarthritis of the knees and hips, mild stage 1 hypertension, and obesity, the patient was in reasonably stable health. During the course of 3 wk, the patient's condition waxed and waned despite an increased albuterol dose. The patient mentioned that her symptoms began after beginning a Glucosamine-chondroitin sulfate preparation 3 times per day for arthritis treatment. This preparation contained 500 mg Glucosamine and 400 mg chondroitin sulfate. Within 24 h of discontinuing Glucosamine and chondroitin treatment, the patient's asthma symptoms completely subsided.

A 67-yr-old male with type-2 diabetes was given oral antidiabetic medication (500 mg metformin, twice daily).[61] The patient had also been previously taking angiotensin-converting-enzyme inhibitors for hypertension for 5 yr, and Glucosamine (1200 mg), once daily, for 3 yr, to relieve osteoarthritic knee pain. Fourteen yr after starting the diabetic medication, the patient was referred to a nephrology consultant due to non-proteinuric renal insufficiency and a reduction of the glomerular filtration rate GFR), from 86 to 46 ml/min, within 3 mo. A kidney biopsy revealed non-inflammatory, 40 – 50% fibrosis of the renal cortex associated with acute tubular necrosis. The etiological investigation was negative apart from the daily ingestion of 1200 mg Glucosamine. After stopping Glucosamine for 3 wk, GFR increased from 47.5 to 60 ml/min. Reintroduction of Glucosamine resulted in loss of kidney function after 3 wk, with GFR reduced from 60 to 53 ml/min.

Glucosamine Sulfate

A 76-yr-old woman with arterial hypertension and osteoarthritis was referred for evaluation after an episode of urticaria after drug intake.[62] The patient was prescribed Glucosamine Sulfate for osteoarthritis, and suffered from erythematous lesions and facial swelling within several hours after Glucosamine Sulfate intake. The following day, 5 min after a new dose, the patient developed tongue, facial, and throat swelling with facial erythema. She was treated in the emergency department with antihistamines and corticosteroids. Symptoms resolved within 4 h. After a washout period, a skin prick test and intradermal test with Glucosamine Sulfate was performed. The skin prick test yielded negative results, however, the intradermal test (concentration of 1.5 mg/ml) yielded positive results with a papule of 35 mm^2. The intradermal test in 10 healthy volunteers was negative.

EPIDEMIOLOGICAL STUDIES

Cancer Endpoints

Glucosamine

The association between Glucosamine use and colorectal cancer risk was examined among 113,067 volunteers in the Cancer Prevention Study II Nutrition Cohort.[63] Those with a history of colorectal cancer prior to 2001, those with inflammatory conditions, and those without sufficient information to determine exposure category for the Glucosamine variable, were excluded from this study. Participants were first asked about Glucosamine intake in 2001 (baseline). Those who reported current use were then asked to report this frequency and duration of use. At baseline, 10.7% of participants (12,060), reported current Glucosamine use on ≥ 4 d/wk for ≤ 2 yr, and 5.6% of participants (6729), reported current use on ≥ 4 d/wk for ≥ 3 yr. Glucosamine intake was surveyed and updated every 2 yr until 2011. Current use of Glucosamine,

modeled using a time-varying exposure, was associate with a lower risk of colon cancer (hazard ratio (HR): 0.83, 95% confidence interval (CI): 0.71 - 0.97), compared to those who reported no ingestion of Glucosamine. This reduction in risk, however, was only observed for shorter duration use of Glucosamine (HR: 0.68, 95%, CI: 0.52 - 0.87), rather than the longer duration of use (HR: 0.99, 95% CI: 0.76 - 1.29).

Similarly, the association between lung cancer and Glucosamine was evaluated in 76,904 volunteers with no prior history of lung cancer.[64] The participants were queried on their use of Glucosamine from the years 2000 - 2010. Low use participants were considered to be volunteers who ingested Glucosamine < 4 d/wk or < 3 yr, and high use was considered to be ingestion of Glucosamine for ≥ 4 d/wk and ≥ 3 yr. Compared to non-use, use of Glucosamine was associated with a 20% reduction in lung cancer risk (HR: 0.80, 95% CI: 0.65 - 0.99) after multivariable adjustment. High 10-yr use of Glucosamine (HR: 0.77, 95% CI: 0.56 – 1.05; P-trend = 0.04) was associated with a linear 23% reduction in lung cancer risk. A large proportion of volunteers who reported Glucosamine use also used chondroitin. When the analysis of Glucosamine was restricted to non-users of chondroitin (Glucosamine-only) an inverse association with lung cancer was apparent (HR: 0.84, 95% CI: 0.61 – 1.17), and high 10-yr use of Glucosamine alone was associated with a 61% reduction in lung cancer risk (HR 0.39, 95% CI: 0.17- 0.86).

RISK ASSESSMENT

Glucosamine Sulfate

The Norwegian Food Safety Authority calculated margin of safety (MoS) values for the use of 10% Glucosamine Sulfate in a body lotion (35.0), leg cream (99.0), and face cream (178.0), and from overall exposure from cosmetics (29.2).[65] These values were calculated assuming 100% dermal absorption, a NOAEL value of 430 mg/kg/d (obtained from a repeated oral dose toxicity assay performed in dogs with a bioavailability of 20%), and a calculated relative daily exposure of 123.20, 43.50, and 24.13 mg/kg bw/d for the body lotion, leg cream, and face cream, respectively. According to this assessment, maximum use levels were reported to be 18, 10, and 3.5% in face, leg and body lotion, respectively.

SUMMARY

The safety of Acetyl Glucosamine, Glucosamine, Glucosamine HCl, and Glucosamine Sulfate as used in cosmetics is reviewed in this assessment. According to the *Dictionary*, Acetyl Glucosamine and Glucosamine Sulfate are reported to function in cosmetics as skin-conditioning agents – miscellaneous, and Glucosamine HCl is reported to function as a pH adjuster. The function of Glucosamine is not reported

According to 2022 VCRP survey data, Acetyl Glucosamine, Glucosamine HCl, and Glucosamine are reported to be used in 198, 77, and 2 formulations, respectively. The results of the concentration of use survey conducted by Council indicate that Acetyl Glucosamine has the highest concentration of use in a leave-on formulation; it is used at up to 5% in face and neck products (not spray). Glucosamine Sulfate is not reported to be in use.

The skin penetration of Acetyl Glucosamine was evaluated in split-thickness Caucasian cadaver skin. Approximately 7% of the applied test substance (which contained 2% Acetyl Glucosamine) permeated the skin after 6 h. An in vitro permeation assay was also performed with Glucosamine HCl in human epidermal membranes. Over a 48-h period, Glucosamine HCl permeated through the skin with a flux of 1.497 ± 0.42 μg/cm^2/h, a permeability coefficient of 5.66 ± 1.6 x 10^{-6} cm/h, and a lag time of 10.9 ± 4.6 h. The dermal penetration of 5% Glucosamine HCl in different vehicles was evaluated in rat skin. Transdermal flux of Glucosamine HCl was greatest in the cubic liquid crystalline formulation (248.89 ± 64.57 μg/h/cm^2). The skin permeation rate of Glucosamine Sulfate was determined to be 13.27 μg/cm^2/h when evaluated in Sprague-Dawley full-thickness rat skin. The amount of Glucosamine in synovial fluid was measured in osteoarthritis patients following an application of 10% Glucosamine Sulfate cream. A mean Glucosamine concentration of 100.56 ng/ml was observed in the synovial fluid of treated patients.

Female Beagle dogs were given a single dose of 450 mg Glucosamine HCl, and a pharmacokinetic analysis was performed. Glucosamine was detected in the blood up to 8 h post-dose, with a T_{max} of 2 h and a C_{max} of 9.69 μg/ml. [^{14}C]Glucosamine HCl diluted with unlabeled Glucosamine Sulfate was given to Sprague-Dawley rats to examine excretion patterns of radioactivity. Radioactivity analysis in tissues and organs revealed that the [^{14}C] from the labeled Glucosamine quickly entered into all tissues, included cartilage, reaching a maximum at 8 h. Bioavailability was also evaluated in humans. Healthy adult males, under fasting conditions, were given a single oral dose of 480 mg Glucosamine HCl in a dispersible tablet or capsule form. The mean C_{max}, T_{max}, and $T_{1/2}$ values were reported to be 907.1 ng/ml, 3.03 h, and 1.10 h, respectively, for the dispersible tablet form, and 944.40 ng/ml, 3.30 h, and 1.50 h, respectively, for the capsule form. The pharmacokinetics of Glucosamine after a single oral administration of Glucosamine Sulfate and Glucosamine HCl were evaluated in 12 healthy volunteers. Glucosamine was determined at steady state in plasma collected up to 48 h after the last dose by a validated LC-MS/MS method. After Glucosamine Sulfate administration, peak concentrations and extent of exposure averaged 9.1 ± 6.3 μM and 76.5 ± 23.0 μM/h, respectively. Significantly lower plasma concentrations ($p \leq 0.005$) were determined after the administration of Glucosamine HCl.

The reported LD_{50} values for Glucosamine were higher than the doses tested (> 15,000 mg/kg in mice and > 8000 mg/kg in rats and rabbits). According to an ECHA dossier, the acute oral LD_{50} for Glucosamine HCl was reported to be 15,000 mg/kg bw in mice. In a 9-wk study, Glucosamine (0.5%) was fed to male Sprague-Dawley and SHR rats. The systolic blood pressure in treated rats was statistically significantly lower than control animals. No statistically significant histological differences were found in the hearts, kidneys, and livers, among the treated and control groups. Acetyl Glucosamine (up to 5%) was fed to F344 rats for 13 wk. No obvious indications of toxicity were observed in any of the parameters evaluated. The NOAEL was determined to be > 5%. The effect of orally-ingested Acetyl Glucosamine (1000 mg) was evaluated in healthy adults. Volunteers ingested the dissolved Acetyl Glucosamine in water, once a day, for 16 wk. A control group received green tea extract powder. Routine physical and cardiovascular characteristics, hematology, and blood chemistry, did not show any significant abnormalities between control and treated groups. The potential toxic effects of a tablet containing Glucosamine HCl (1500 mg/d), chondroitin sulfate (1200 mg/d), and manganese ascorbate (228 mg/d) in degenerative disease patients was evaluated in a 16-wk crossover study. No patients reported symptoms requiring termination of study, and symptom frequency on medication was similar to that at baseline. Vital signs, occult blood testing, and hematologic parameters were similar among the placebo and medicated groups. The chronic toxicity potential of Acetyl Glucosamine (up to 5%) given in the diet for 52 wk was evaluated in F344 rats. No toxic effects were observed in any parameter evaluated, however, slight suppression of body weight gain was observed in animals dosed with concentrations of greater than 2.5%.

The effects of premating Glucosamine supplementation via drinking water on Sprague-Dawley rat litter homogeneity, uterine receptivity, and maternal hormones levels were evaluated. Female rats were given 0.5 mM Glucosamine via drinking water for 2 wk, and then mated. Birth weights and absolute and relative ovary weights were statistically significantly greater in the Glucosamine-treated group compared to the control group ($p < 0.05$). Maternal progesterone, estradiol, and IGF-1 concentrations on day 19.5 of pregnancy were significantly increased in treated rats, while insulin and total cholesterol levels were significantly decreased compared with control rats. The reproductive effects of intraperitoneal injections of Glucosamine (0 and 20 mg/kg) was evaluated in 8-wk old and 16-wk old adult female mice. Fetal weight and length were reduced in Glucosamine-treated 16-wk old mice, compared to control animals. In addition, a significantly higher number of abnormal fetuses was present in litters of 16-wk old Glucosamine-treated mice compared with all other groups ($p < 0.05$). The effects of intrauterine Glucosamine (up to 1500 µg) were evaluated in female ICR mice. Ten d after implantation of the Glucosamine pellet, mice were mated. Mice that received Glucosamine pellets delivered significantly fewer live pups/litter over a 60-d pellet active period than those that received placebo pellets. However, after the 60-d pellet active period, there was no statistically significant difference in litter sizes delivered by Glucosamine-treated and placebo-treated mice, except at the highest dose level.

Acetyl Glucosamine (up to 5000 µg/plate) was considered to be non-mutagenic in an Ames assay using *S. typhimurium* strains TA 1537, TA 1535, TA 98, TA 100, and TA 102, with and without metabolic activation. Similarly, an Ames assay was performed on Glucosamine HCl derived from *Aspergillus niger*. Tester strains (*S. typhimurium* and *E. coli* WP2 uvrA) were exposed to up to 5000 µg/plate of the test substance, with and without metabolic activation. No mutagenicity was observed. In an in vivo micronucleus assay, mice (strain not reported) were administered *Aspergillus niger*-derived Glucosamine HCl (up to 2000 mg/kg bw) in water, via gavage. There was no statistically significant decrease in the ratios of PCE and NCE at any dose level.

In an in vitro anti-genotoxicity assay, human peripheral lymphocytes were exposed to Glucosamine or Acetyl Glucosamine at concentrations up to 50 mM. DNA damage was induced with hydrogen peroxide. Glucosamine, at all concentrations, showed a significant protective activity ($p < 0.001$) against hydrogen peroxide-induced DNA damage. Acetyl Glucosamine only indicated a slight DNA protection at the highest test concentration. The chemoprotective ability of Glucosamine (diets containing up to 150 mg/kg Glucosamine; 7 d exposure) against cisplatin-induced genotoxicity was evaluated in male Wistar rats. The test substance was considered to be an effective chemoprotector against cisplatin-induced DNA damage.

The carcinogenic potential of Acetyl Glucosamine (up to 5% in the diet; 104-wk treatment) was evaluated in F344 rats. The test substance was considered to be non-carcinogenic. The anti-proliferative potential of Glucosamine (10 mM) was evaluated in human renal cancer cell lines (786-O and caki-1) via an MTT and FITC-annexin V/PI assay. The apoptosis rate of both cell lines was up-regulated by the high concentration of Glucosamine (10 mM), but down-regulated by low concentrations of Glucosamine (1 and 5 mM), as compared with the control groups. The growth inhibitory effects of Glucosamine, Glucosamine HCl, and Acetyl Glucosamine on human hematoma SMMC-721 cells was evaluated in vitro. Tumor cells were exposed to Glucosamine, Glucosamine HCl, or Acetyl Glucosamine, at concentrations of up to 1000 µg/ml. Results measured by an MTT assay showed that Glucosamine HCl and Glucosamine caused a concentration-dependent reduction in hepatoma cell growth. In an animal anti-carcinogenicity assay, Kunming male mice were inoculated with sarcoma 180 tumor cells. Mice were orally treated for 10 d with up to 500 mg/kg Glucosamine HCl dissolved in saline... Glucosamine HCl, at the intermediate dose (250 mg/kg/d), had the highest inhibition ratio (34.02%) on sarcoma 180 tumor growth.

The effect of Acetyl Glucosamine on melanin production was evaluated in an in vitro assay. Reconstituted human tanned epidermis cells were exposed to up to 5% Acetyl Glucosamine in water for 10 d. Dose-dependent decreases in

melanin content were observed. The whitening effect of Acetyl Glucosamine (5%) was evaluated in human and brown guinea pig skin subjected to UV-induced pigmentation. A visual reduction in hyperpigmentation was observed 2 wk after treatment with the Acetyl Glucosamine solution, in humans, compared to the vehicle-treated group. Acetyl Glucosamine-treated guinea pig skin had decreased levels of melanin without affecting the number of melanocytes, compared to vehicle-treated skin. Anti-melanogenic activity was evaluated using an Acetyl Glucosamine-loaded microemulsion and an aqueous solution containing 1% Acetyl Glucosamine in B16 melanoma cells. Melanin content decreased by 22% and 7%, after treatment with the microemulsion and the aqueous solution, respectively.

The reduction of facial hyperpigmentation after topical treatment on Acetyl Glucosamine was evaluated in a 10-wk trial. Volunteers (101 women/group) were instructed to apply a facial lotion containing 4% niacinamide and 2% Acetyl Glucosamine twice a day for 8 wk. A control group applied the lotion vehicle without 4% and 2% Acetyl Glucosamine. By all parameters measured, the niacinamide and Acetyl Glucosamine formulation regimen caused a significant reduction in the detectable area of facial spots and appearance of pigmentation compared to the controls ($p < 0.05$). In a similar study, from Japan, healthy women ($n = 25$ women/group) were instructed to apply a facial lotion containing 2% Acetyl Glucosamine on the side of the face, twice daily, for 8 wk. A control group applied the vehicle lotion that did not contain Acetyl Glucosamine. Topical 2% Acetyl Glucosamine reduced the appearance of facial hyperpigmentation, with an overall directional ($p = 0.089$) spot area fraction change across the entire study.

The effects of a neck cream formulation containing 8% Acetyl Glucosamine was evaluated in 45 Caucasian women. Applications of the cream occurred once a day, for 16 wk. The test cream was well-tolerated with no signs of irritation. One subject experienced an adverse event of contact dermatitis on two separate occasions. No other adverse events were reported.

The effect of Glucosamine injections (concentrations up to 1 mg/2.5 µl) on OVA-induced atopic dermatitis was evaluated in female BALB/c mice. Clinical dermatitis scores decreased with increasing Glucosamine dose ($p < 0.001$). Concentrations of tissue IL-13 and IL-17 decreased after Glucosamine administration (each group: $p = 0.002$ and $p < 0.001$, respectively), but the concentrations of tissue IL-4 did not show differences across groups. The anti-allergic effect of Glucosamine (concentrations up to 5%) in female BALB/c mice with allergic rhinitis was evaluated. OVA-specific IgE and eosinophils in BAL fluid were significantly decreased after 5% oral Glucosamine treatment compared with the positive control group. In addition, significant improvement of inflammation was apparent in groups treated with Glucosamine when compared to the positive control group. The anti-allergic effects of orally-ingested Acetyl Glucosamine and Glucosamine HCl (up to 1 mg/mouse; 6 d treatment) was also evaluated in BALB/c mice with DNFB-induced skin sensitization. Oral administration of Acetyl Glucosamine or Glucosamine HCl significantly inhibited DNFB-induced ear swelling in mice at both 6 h and 24 h after DNFB challenge ($p < 0.05$), and reduced the concentration of histamine in both the ear and plasma of DNFB-treated mice ($p < 0.05$). In vivo sensitization assays performed on humans using various test substances (a mask containing 0.005% Acetyl Glucosamine, a product containing 0.01% Glucosamine, a leave-on product containing 0.005% Glucosamine HCl, and a product containing 0.25% Glucosamine HCl) yielded negative results.

The effect of orally-administered Glucosamine (25 mg/kg) in the treatment of atopic dermatitis was evaluated in an 8-wk, placebo-controlled, double-blind, clinical trial. Among the 16 patients receiving Glucosamine treatment, 15 patients reported clinical improvement of atopic dermatitis symptoms. Three Glucosamine-treated patients reported adverse effects, with abdominal pain being the most common adverse effect.

Potential skin irritation of Acetyl Glucosamine was evaluated in an in vitro assay using 3 reconstructed human epidermis samples. Reduction of cell viability was similar in the negative control and treated groups; therefore, the substance was considered to be non-irritating. Acetyl Glucosamine was predicted to be non-sensitizing in a DPRA, KeratinoSens™ assay, and h-CLAT. Very mild cumulative irritation was observed in a 21-d cumulative patch irritation assay performed using an eye cream containing 2% Acetyl Glucosamine (12 subjects). HRIPTs performed using a mask containing 0.005% Acetyl Glucosamine (108 subjects), a liquid foundation containing 2% Acetyl Glucosamine (105 subjects), and a leave-on product containing 0.005% Glucosamine HCl (51 subjects) yielded negative results. Similarly, no sensitization was in maximization assays performed, each in 25 subjects, using a product containing 0.01% Glucosamine and a product containing 0.25% Glucosamine HCl.

In vitro ocular irritation assays were performed using a face serum containing 2% Acetyl Glucosamine and a saline solution containing 20% Acetyl Glucosamine. Neither test substance was considered to be irritating when compared to positive controls.

The tolerability of orally-ingested, shrimp-derived Glucosamine was evaluated in 15 shrimp-allergic individuals. Subjects were given either 1500 mg of synthetically-derived or shrimp-derived Glucosamine. All subjects tolerated the 1500 mg Glucosamine administration from the shrimp-derived and synthetic sources, without any incidences of hypersensitivity.

A 52-yr old complained of exacerbation of underlying asthma after beginning treatment with a Glucosamine-chondroitin sulfate preparation containing 500 mg Glucosamine. Within 24 h of discontinuing Glucosamine and chondroitin treatment, the patient's asthma symptoms completely resolved.

A 67-yr-old male with type-2 diabetes was referred to a nephrology consultant due to non-proteinuric renal insufficiency and a reduction in GFR supposedly due to Glucosamine intake for the past 3 yr. After stopping Glucosamine

for 3 wk, GFR increased from 47.5 to 60 ml/min. A 76-yr-old woman with arterial hypertension and osteoarthritis was referred for evaluation after an episode of urticaria after Glucosamine Sulfate intake. After treatment with antihistamines and corticosteroids, symptoms resolved within 4 h.

The association between Glucosamine use and colorectal cancer risk was examined among 113,067 volunteers. Participants were asked to log their Glucosamine intake from 2001 - 2011. Current use of Glucosamine, modeled using a time-varying exposure, was associated with a lower risk of colon cancer, for those using Glucosamine for a short duration (HR: 0.68, 95% CI: 0.52 - 0.87). Similarly, the association between lung cancer and Glucosamine was evaluated in 76,904 volunteers with no prior history of lung cancer. The participants were queried on their use of Glucosamine from the years 2000 - 2010. Compared to non-use, use of Glucosamine was associated with a 20% reduction in lung cancer risk (HR: 0.80, 95% CI: 0.65 - 0.99) after multivariable adjustment.

The Norwegian Food Safety Authority calculated MoS values for the use of 10% Glucosamine Sulfate in a body lotion, leg cream, face cream, and from overall exposure from cosmetics. The MoS for each of these formulation types were 35.0, 99.0, 178.0, and 29.2, respectively.

DISCUSSION

This assessment reviews the safety of Acetyl Glucosamine, Glucosamine, Glucosamine HCl, and Glucosamine Sulfate as used in cosmetic formulations. The Panel concluded that these ingredients are safe in the present practices of use and concentration as described in this safety assessment, when formulated to be non-irritating.

The Panel noted the mild cumulative irritation that was observed during the 21-d cumulative irritation patch test performed on 12 subjects using an eye lotion containing 2% Acetyl Glucosamine. Because this irritation was observed at a concentration of 2%, and the maximum concentration of use for Acetyl Glucosamine in cosmetics is reported to be 5%, the Panel was concerned that the potential exists for dermal irritation with the use of products formulated using glucosamine ingredients. Therefore, the Panel specified that products containing glucosamine ingredients must be formulated to be non-irritating.

In addition, the Panel considered the lack of human sensitization data at the maximum use concentration of 5%. However, the available in chemico/in vitro sensitization data (i.e., DPRA, KeratinoSens™ assay, and h-CLAT, all performed using Acetyl Glucosamine (99.42% purity)), clinical sensitization data s at up to 2% Acetyl Glucosamine, and a lack of case reports, as well as the Panel's clinical experience with these ingredients, mitigated any concern.

Reproductive effects were observed in mice and rats following oral ingestion and intraperitoneal injections of Glucosamine. The Panel determined that these effects would not be relevant to cosmetic exposure as administration in these studies resulted in a much higher systemic concentration of Glucosamine than would be expected with cosmetic use. The systemic safety of these ingredients is further supported by their use as dietary supplements/debulking agents, and available systemic toxicity data.

In addition, data included in this report indicate that Acetyl Glucosamine may have a skin lightening effect. The Panel noted that skin lightening is considered a drug effect, and should not occur during the use of cosmetic products. Because of that caveat, the Panel's knowledge of the mechanism of action (i.e., inhibition of tyrosinase activity resulting in reduced melanin synthesis), and clinical experience, concern for this effect in cosmetics was mitigated. Nevertheless, cosmetic formulators should only use this ingredient in products in a manner that does not cause depigmentation.

The Panel discussed the fact that some of these ingredients are used in formulations that could result in incidental inhalation (e.g., Acetyl Glucosamine is used at up to 0.1% in pump hair sprays). Inhalation toxicity data were not available; however, the oral toxicity data that were available did not report adverse effects. Additionally, 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 bronchial 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, the concentrations at which the ingredients are used, and a lack of systemic toxicity, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that of Acetyl Glucosamine, Glucosamine, Glucosamine HCl, and Glucosamine Sulfate* are safe in the present practices of use and concentration as described in this safety assessment when formulated to be non-irritating.

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

TABLES

Table 1. Definitions, structures, and functions of glucosamine ingredients.[1], CIR Staff

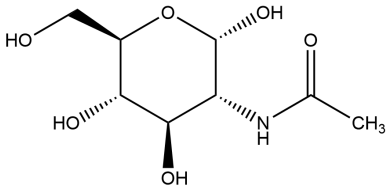
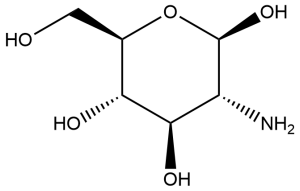
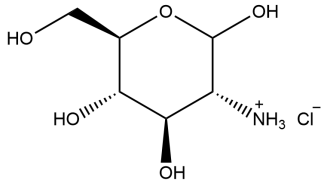
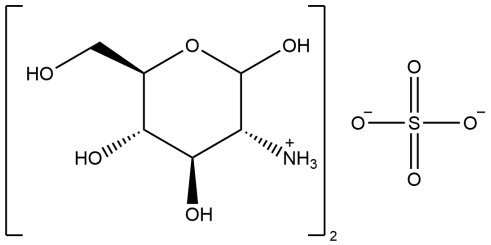
Ingredient	Definition	Function
Acetyl Glucosamine (10036-64-3; 72-87-7; 7512-17-6)	Acetyl Glucosamine is the organic compound that conforms to the structure: 	Skin-Conditioning Agents – Miscellaneous
Glucosamine (3416-24-8)	Glucosamine is the organic compound that conforms to the structure: 	Not Reported
Glucosamine HCl (66-84-2)	Glucosamine HCl is the amine salt that conforms to the structure: 	pH Adjusters
Glucosamine Sulfate (29031-19-4)	Glucosamine Sulfate is the amine salt that conforms to the structure: 	Skin-Conditioning Agents – Miscellaneous

Table 2. Chemical properties

Property	Value	Reference
Acetyl Glucosamine		
Physical Form	Solid	[3]
Color	White	[3]
Molecular Weight (g/mol)	221.21	[3]
Density (g/ml @ 20 ºC)	1.234	[3]
Vapor pressure (mmHg @ 20 ºC)	0.06	[3]
Melting Point (ºC)	162.7	[3]
Water Solubility (g/l @ 20 ºC)	256.8	[3]
log K_{ow} (@ 23.7 ºC)	-2.2	[3]
Glucosamine		
Physical Form	Solid	[66]
Molecular Weight (g/mol)	179.17	[66]
Vapor pressure (mmHg @ 25ºC)	0.0000000902	[67]
Melting Point (ºC)	88	[66]
Water Solubility (g/L)	551	[66]
log K_{ow}	-4.2	[67]
Disassociation constants (pKa)	7.58	[68]

Table 2. Chemical properties

Property	Value	Reference
Glucosamine HCl		
Physical Form	Crystalline	69
Formula Weight (g/mol)	215.63	70
Color	Off-White	69
Odor	Odorless	2
Specific Gravity (@ 38 ºC)	1.42	69
Melting Point (ºC)	190 - 194	69
Water Solubility	Soluble	2
log K_{ow}	-1.91	23
Disassociation constant (pKa) (@ 37 ºC)	7.75	23
Glucosamine Sulfate		
Physical Form	Solid	71
Color	Off-White	71
Formula Weight (g/mol)	277.25	71
Density(g/ml)	1.56	72
Boiling Point (ºC)	449.9	72
Water Solubility (g/l)	Freely soluble	72
Disassociation constants (pKa)	12.51 (estimated)	73

Table 3. Frequency (2022)[12] and concentration (2020)[13] of use

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Acetyl Glucosamine		Glucosamine		Glucosamine HCl	
Totals*	**198**	**0.001 – 5**	**2**	**0.04**	**77**	**0.0001 – 5**
Duration of Use						
Leave-On	*185*	*0.002 – 5*	*2*	*0.04*	64	*0.0001 – 0.9*
Rinse-Off	*13*	*0.001 – 5*	*NR*	*NR*	13	*0.07 – 5*
5Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	NR	*NR*
Exposure Type						
Eye Area	12	0.2 – 2	NR	NR	6	0.0001 – 0.2
Incidental Ingestion	3	0.002 – 2	NR	NR	NR	NR
Incidental Inhalation-Spray	75[a]; 72[b]	0.1; 0.005 – 0.07[b]	1[a]	NR	20[a]; 30[b]	NR
Incidental Inhalation-Powder	75[a]	0.07; 0.12 – 5[c]	1[a]	0.04[c]	20[a]	0.0006 – 0.38[c]
Dermal Contact	194	0.01 – 5	1	0.04	67	0.0001 – 5
Deodorant (underarm	NR	0.01	NR	NR	NR	NR
Hair - Non-Coloring	1	0.001 – 0.55	1	NR	10	0.55
Hair-Coloring	NR	0.01	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	4	0.002 – 2	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	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] 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
[b] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[c] It is possible these products are powders, but it is not specified whether the reported uses are powders
NR – not reported

Table 4. Ingredient not reported to be in use according to 2022 FDA VCRP and 2020 concentration of use data[12,13]

Glucosamine Sulfate

Table 5. Acute oral toxicity studies

Ingredient	Animals	No. /group	Dose/Route of Administration	LD_{50}/Results	Reference
Glucosamine	Mice (strain unspecified)	NR	5000 mg/kg; gavage	LD_{50} > 5000 mg/kg	31
Glucosamine	CD-1 Mice	NR	8000 mg/kg; gavage	LD_{50} > 8000 mg/kg	31
Glucosamine	Mice (strain unspecified)	NR	15,000 mg/kg; gavage	LD_{50} > 15,000 mg/kg	31
Glucosamine	Sprague-Dawley Rat	NR	8000 mg/kg; gavage	LD_{50} > 8000 mg/kg; no adverse effects reported	31
Glucosamine	Rabbit (strain unspecified)	NR	8000 mg/kg; gavage	LD_{50} > 8000 mg/kg	31
Glucosamine HCl	Mice (strain unspecified)	NR	15,000 mg/kg (method of oral administration not specified)	LD_{50} = 15,000 mg/kg	2

NR = Not reported

Table 6. Dermal irritation and sensitization studies

Ingredient	Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION						
In Vitro						
Acetyl Glucosamine	Acetyl Glucosamine (99.42% purity)	tested neat; 16 mg	3	reconstructed human epidermis; OECD TG 439; positive control: 5% sodium dodecyl sulfate; negative control: PBS; 42 min incubation	Non-irritating	[3]
Human						
Acetyl Glucosamine	Eye cream containing 2% Acetyl Glucosamine	tested neat; 0.2 g	12	21-d cumulative patch test; patches removed and re-applied each day for 21 days (excluding weekends); occlusive conditions	Average irritation score of 0.34/4; very mild cumulative irritation	[52]
SENSITIZATION						
In Chemico/In Vitro						
Acetyl Glucosamine	Acetyl Glucosamine (99.42% purity)	tested neat	NR	DPRA; OECD TG 442C; test material exposed to model synthetic peptides containing cysteine and lysine; mean percent depletion of cysteine and lysine calculated	Non-sensitizing; mean percent depletion of cysteine and lysine was 1%	[3]
Acetyl Glucosamine	Acetyl Glucosamine (99.42% purity)	0.98 to 2000 µM	3	KeratinoSens™ assay; OECD TG 442D; human epidermal keratinocytes exposed to test substance; cells analyzed for luciferase activity after 48 ± 2 h incubation period	Non-sensitizing; IC_{50} = > 2000 µM	[3]
Acetyl Glucosamine	Acetyl Glucosamine (99.42% purity)	1395 - 5000 µg/ml	NR	h-CLAT; OECD TG 442E; THP-1 cells incubated with test substance for 24 h and analyzed via flow cytometry	Non-sensitizing; cell viability > 50% at all tested concentrations	[3]
Human						
Acetyl Glucosamine	Mask containing 0.005% Acetyl Glucosamine	tested neat; 2cm x 2 cm; est. exposure under patch = 2.5 µg/cm^2	108	HRIPT; occlusive conditions	Non-sensitizing	[53,74]
Acetyl Glucosamine	Liquid foundation containing 2% Acetyl Glucosamine	tested neat; 2 cm x 2 cm; est. exposure under patch = 1000 µg/cm^2	105	HRIPT; occlusive conditions	Non-sensitizing	[55,74]
Glucosamine	Leave-on product containing 0.005% Glucosamine HCl	tested neat; 25-38 mg/cm^2; est. exposure under patch = 1.25 – 1.90 µg/cm^2	51	HRIPT; occlusive conditions	Non-irritating and non-sensitizing	[54,74]
Glucosamine	Product containing 0.01% Glucosamine	tested neat; 2 cm x 2 cm; est. exposure under patch = 1.25 µg/cm^2	25	Maximization assay; induction phase – 0.25% SLS for 24 h; subjects then exposed to the test substance for 48-72 h (5 total induction applications); 10-d rest period; challenge phase – 5% SLS for 1 h; subject then exposed to test material for 48 h; all patches under occlusive conditions; sites evaluated 15 min, 30 min, and 24 h after patch-removal	Non-sensitizing	[56,74]
Glucosamine HCl	Product containing 0.25% Glucosamine HCl	tested neat; 0.05 g; est. exposure under patch = 55.6 µg/cm^2	25	Maximization assay performed according to the same procedures as above; occlusive conditions	Non-sensitizing	[57,74]

DPRA = direct peptide reactivity assay; h-CLAT = human cell line activation test; HRIPT = human repeated insult patch test; IC_{50} = half maximal inhibitory concentration; OECD TG = Organisation for Economic Cooperation and Development test guidelines; PBS = phosphate-buffered saline; SLS = sodium lauryl sulfate; THP-1 = human monocytic cell line

REFERENCES

1. Nikitakis J, Kowcz A. wINCI: *International Cosmetic Ingredient Dictionary and Handbook*. http://webdictionary.personalcarecouncil.org/jsp/Home.jsp. Washington, DC: Personal Care Products Council. Last Updated: 2021. Accessed: November 5, 2021.

2. European Chemicals Agency (ECHA). Glucosamine hydrochloride. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/11641/7/9/1. Last Updated: 2020. Accessed: October 7, 2020.

3. European Chemicals Agency (ECHA). N-acetyl-β-D-glucosamine. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/30216. Last Updated: 2020. Accessed: October 7, 2020.

4. Kelly GS. The role of glucosamine sulfate and chondroitin sulfates in the treatment of degenerative joint disease. *Alternative Medicine Review.* 1998;3(1):27-39.

5. Garner ST, Jr., Israel BJ, Achmed H, Capomacchia AC, Abney T, Azadi P. Transdermal permeability of N-acetyl-D-glucosamine. *Pharm Dev Technol.* 2007;12(2):169-174.

6. Chen J, Shen C, Liu C. *N*-Acetylglucosamine: production and applications. *Mar Drugs.* 2010;8:2493-2516.

7. French Agency for Food, Environmental, and Occupational Health & Safety. 2019. Opinion of the French Agency on Food, Environmental, and Occupational Health & Safety on the risks associated with the consumption of food supplements for joint conditions containing glucosamine and/or chondroitin sulphate. (ANSES Opinion Request No 2015-SA-0069.)

8. European Food Safety Authority (EFSA). Opinion on the safety of glucosamine hydrochloride from *Aspergillus niger* as food ingredient. *The EFSA Journal.* 2009;1099:1-19.

9. Kim YS, Lee SJ, Choi JY, et al. Determination of process-related impurities in N-acetylglucosamine prepared by chemical and enzymatic methods: structural elucidation and quantification. *Arch Pharm Res.* 2016;39(7):937-945.

10. U.S. Pharmacopeia. United States Pharmacopeia - National Formulary Online. https://www.usp.org/. Last Updated: 2021. Accessed: August 10, 2021.

11. Jung AY, Heo MJ, Kim YH. Glucosamine has an antiallergic effect in mice with allergic asthma and rhinitis. *Int Forum Allergy Rhinol.* 2017;7(8):763-769.

12. 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.

13. Personal Care Products Council. 2020. Concentration of use by FDA product category: Glucosamine ingredients. (Unpublished data submitted by the Personal Care Products Council on February 28, 2020.)

14. Bremmer HJ, Prud'homme de Lodder L, van Engelen J. Cosmetics Fact Sheet: To assess the risks for the consumer, Updated version for ConsExpo4. Bilthoven, Netherlands 2006. RIVM 320104001/2006. 2006. http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf. Accessed June 25, 2019. Pages1-77.

15. Rothe H, Fautz R, Gerber, E, et al. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. Netherlands National Institute for Public Health and Environment; Bilthoven, Netherlands. *Toxicol Lett.* 2011;205(2):97-104.

16. CIR Science and Support Committee of the Personal Care Products Council (CIR SCC). 2015. Cosmetic powder exposure. (Unpublished data submitted by the Personal Care Products Council on November 3, 2015.)

17. Aylott R, Byrne G, Middleton J, Roberts M. Normal use levels of respirable cosmetic talc: preliminary study. *Int J Cosmet Sci.* 1979;1(3):177-186.

18. Russell R, Merz R, Sherman W, Siverston J. The determination of respirable particles in talcum powder. *Food Cosmet Toxicol.* 1979;17(2):117-122.

19. European Commission. CosIng database; following Cosmetic Regulation No. 1223/2009. http://ec.europa.eu/growth/tools-databases/cosing/. Last Updated: 2020. Accessed: October 10, 2020.

20. Dahmer S, Schiller RM. Glucosamine. *Am Fam Physician.* 2008;78(4):471-476.

21. Bhathal A, Spryszak M, Louizos C, Frankel G. Glucosamine and chondroitin use in canines for osteoarthritis: a review. *Open Vet J.* 2017;7(1):36-49.

22. Bissett DL, Robinson LR, Raleigh PS, et al. Reduction in the appearance of facial hyperpigmentation by topical N-acetyl glucosamine. *J Cosmet Dermatol.* 2007;6(1):20-26.

23. Tekko I, Bonner MC, Williams AC. An optimized reverse-phase high performance liquid chromatographic method for evaluating percutaneous absorption of glucosamine hydrochloride. *J Pharm Biomed Anal.* 2006;41(2):385-392.

24. Han IH, Choi S, Nam DY, et al. Identification and assessment of permeability enhancing vehicles for transdermal delivery of glucosamine hydrochloride. *Arch Pharm Res.* 2010;33(2):293-299.

25. Kanwischer M, Kim SY, Kim JS, Bian S, Kwon KA, Kim DD. Evaluation of the physicochemical stability and skin permeation of glucosamine sulfate. *Drug Dev Ind Pharm.* 2005;31(1):91-97.

26. Kong M, Hashim KB, Lin P, et al. Penetration of topical glucosamine sulfate into the synovial fluid of patients with knee osteoarthritis: a non-randomized, open-label, single dose, bioavailability study. *Journal of Biosciences and Medicines.* 2019;7(11):76-90.

27. Martinez SE, Lillico R, Lakowski TM, Martinez SA, Davies NM. Pharmacokinetic analysis of an oral multicomponent joint dietary supplement (Phycox(®)) in dogs. *Pharmaceutics.* 2017;9(3):30.

28. Setnikar I, Giachetti C, Zanolo G. Absorption, distribution and excretion of radioactivity after a single intravenous or oral administration of [14C] glucosamine to the rat. *Pharmatherapeutica.* 1984;3(8):538-550.

29. Wu H, Liu M, Wang S, et al. Comparative fasting bioavailability and pharmacokinetic properties of 2 formulations of glucosamine hydrochloride in healthy Chinese adult male volunteers. *Arzneimittelforschung.* 2012;62(8):367-371.

30. Persiani S, Rovati L, Pastorini E, Locatelli M. Pharmacokinetics of glucosamine in man after oral administration of crystalline glucosamine sulfate or glucosamine hydrocholoride alone or in combination with chondroitin sulfate. Osteoarthritis and Cartilage 15 (Suppl C):C223; 2007.

31. Anderson JW, Nicolosi RJ, Borzelleca JF. Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. *Food Chem Toxicol.* 2005;43(2):187-201.

32. Echard BW, Talpur NA, Funk KA, Bagchi D, Preuss HG. Effects of oral glucosamine and chondroitin sulfate alone and in combination on the metabolism of SHR and SD rats. Mol Cell Biochem. 2001 Sep:225(1):85-91.

33. Lee KY, Shibutani M, Takagi H, et al. Subchronic toxicity study of dietary N-acetylglucosamine in F344 rats. *Food Chem Toxicol.* 2004;42(4):687-695.

34. Kubomura D, Ueno T, Yamada M, Tomonaga A, Nagaoka I. Effect of N-acetylglucosamine administration on cartilage metabolism and safety in healthy subjects without symptoms of arthritis: A case report. *Exp Ther Med.* 2017;13(4):1614-1621.

35. Leffler CT, Philippi AF, Leffler SG, Mosure JC, Kim PD. Glucosamine, chondroitin, and manganese ascorbate for degenerative joint disease of the knee or low back: a randomized, double-blind, placebo-controlled pilot study. *Mil Med.* 1999;164(2):85-91.

36. Takahashi M, Inoue K, Yoshida M, Morikawa T, Shibutani M, Nishikawa A. Lack of chronic toxicity or carcinogenicity of dietary N-acetylglucosamine in F344 rats. *Food Chem Toxicol.* 2009;47(2):462-471.

37. Feng C, Yuan T, Wang S, et al. Glucosamine supplementation in premating drinking water improves within-litter birth weight uniformity of rats partly through modulating hormone metabolism and genes involved in implantation. *Biomed Res Int.* 2020;2020:1630890.

38. Schelbach CJ, Robker RL, Bennett BD, Gauld AD, Thompson JG, Kind KL. Altered pregnancy outcomes in mice following treatment with the hyperglycaemia mimetic, glucosamine, during the periconception period. *Reprod Fertil Dev.* 2013;25(2):405-416.

39. Tsai J-H, Schulte M, O'Neill K, Chi MMY, Frolova AI, Moley KH. Glucosamine inhibits decidualization of human endometrial stromal cells and decreases litter sizes in mice. *Biology of reproduction.* 2013;89(1):16-16.

40. Jamialahmadi K, Soltani F, Nabavi Fard M, Behravan J, Mosaffa F. Assessment of protective effects of glucosamine and N-acetyl glucosamine against DNA damage induced by hydrogen peroxide in human lymphocytes. *Drug Chem Toxicol.* 2014;37(4):427-432.

41. Cheki M, Jafari S, Najafi M, Mahmoudzadeh A. Glucosamine protects rat bone marrow cells against cisplatin-induced genotoxicity and cytotoxicity. *Anticancer Agents Med Chem.* 2019;19(14):1695-1702.

42. Wang LS, Chen SJ, Zhang JF, Liu MN, Zheng JH, Yao XD. Anti-proliferative potential of Glucosamine in renal cancer cells via inducing cell cycle arrest at G0/G1 phase. *BMC Urol.* 2017;17(1):38.

43. Zhang L, Liu W, Han B, Peng Y, Wang D. Antitumor activities of D-glucosamine and its derivatives. *J Zheijang Univ Sci B.* 2006;7(8):608-614.

44. Bissett DL, Farmer T, McPhail S, et al. Genomic expression changes induced by topical N-acetyl glucosamine in skin equivalent cultures in vitro. *J Cosmet Dermatol.* 2007;6(4):232-238.

45. Wuttikul K, Boonme P, Thammarat C, Khongkow P. N-acetylglucosamine microemulsions: Assessment of skin penetration, cytotoxicity, and anti-melanogenesis. *J Cosmet Dermatol.* 2021;20(1):304-309.

46. Hwang JS, Lee HY, Lim TY, Kim MY, Yoon TJ. Disruption of tyrosinase glycosylation by N-acetylglucosamine and its depigmenting effects in guinea pig skin and in human skin. *J Dermatol Sci.* 2011;63(3):199-201.

47. Kimball AB, Kaczvinsky JR, Li J, et al. Reduction in the appearance of facial hyperpigmentation after use of moisturizers with a combination of topical niacinamide and N-acetyl glucosamine: results of a randomized, double-blind, vehicle-controlled trial. *Br J Dermatol.* 2010;162(2):435-441.

48. Schlessinger J, Green B, Edison BL, Murphy L, Sabherwal Y. Scientific poster: A firming neck cream containing N-acetyl glucosamine significantly improves signs of aging on the challenging neck and décolletage. March 20-24, 2015. San Francisco, CA.

49. Yoon HS, Byun JW, Shin J, Kim YH, Choi GS. Therapeutic Effect of Glucosamine on an Atopic Dermatitis Animal Model. *Ann Dermatol.* 2019;31(5):538-544.

50. Sakai S, Sugawara T, Kishi T, Yanagimoto K, Hirata T. Effect of glucosamine and related compounds on the degranulation of mast cells and ear swelling induced by dinitrofluorobenzene in mice. *Life Sci.* 2010;86(9-10):337-343.

51. Jin S, Lim W, Sung NH, Cheong KA, Lee A. Combination of glucosamine and low-dose cyclosporine for atopic dermatitis treatment: A randomized placebo-controlled, double-blind, parallel clinical trial. *Dermatol Ther.* 2015;28(1):44-51.

52. Anonymous. 2006. 21-Day cumulative irritation patch test (eye cream with 2% Acetyl Glucosamine).

53. Anonymous. 2018. Repeat insult patch test (mask contains 0.005% Acetyl Glucosamine). (Unpublished data submitted by the Personal Care Products Council on February 19, 2021.)

54. Anonymous. 2012. Clinical safety evaluation repeated insult patch test (leave-on product containing 0.005% Glucosamine HCl). (Unpublished data submitted by Personal Care Products Council on April 29, 2021.)

55. TKL Research. 2011. Human repeat insult patch test (liquid foundation with 2% Acetyl Glucosamine).

56. Anonymous. 2005. An evaluation of the contact sensitization potential of a topical coded product in human skin by means of the maximization assay (product contains 0.01% Glucosamine). (Unpublished data submitted by Personal Care Products Council on February 19, 2021.)

57. Anonymous. 2007. An evaluation of the contact sensitization potential of a topical coded product in human skin by means of the maximization assay (product contains 0.25% Glucosamine HCl). (Unpublished data submitted by Personal Care Products Council on February 19, 2021.)

58. Institute for In Vitro Sciences Inc. 2009. Tissue equivalent assay with Epiocular™ cultures (face serum with 2% Acetyl Glucosamine).

59. Villacis J, Rice TR, Bucci LR, et al. Do shrimp-allergic individuals tolerate shrimp-derived glucosamine? *Clin Exp Allergy.* 2006;36(11):1457-1461.

60. Tallia AF, Cardone DA. Asthma exacerbation associated with glucosamine-chondroitin supplement. *J Am Board Fam Pract.* 2002;15(6):481-484.

61. Gueye S, Saint-Cricq M, Coulibaly M, et al. Chronic tubulointerstitial nephropathy induced by glucosamine: a case report and literature review. *Clin Nephrol.* 2016;86(2):106-110.

62. Matheu V, Gracia Bara MT, Pelta R, Vivas E, Rubio M. Immediate-hypersensitivity reaction to glucosamine sulfate. *Allergy.* 1999;54(6):643.

63. Kantor ED, Newton CC, Giovannucci EL, McCullough ML, Campbell PT, Jacobs EJ. Glucosamine use and risk of colorectal cancer: results from the cancer prevention study II nutrition cohort. *Cancer Causes Control.* 2018;29(3):389-397.

64. Brasky TM, Lampe JW, Slatore CG, White E. Use of glucosamine and chondroitin and lung cancer risk in the vitamins and lifestyle (VITAL) cohort. *Cancer Causes Control.* 2011;22(9):1333-1342.

65. Norwegian Food Safety Authority. Risk Profile *Glucosamine (GlcN)*. Oslo, Norway 2012. https://www.mattilsynet.no/kosmetikk/stoffer_i_kosmetikk/risk_profile_glucosamine_2012.9871/binary/Risk%20Profile%20Glucosamine%20(2012). Pages1-21.

66. Human Metabolome Database (HMDB). Metabocard for glucosamine (HMDB0001514). https://hmdb.ca/metabolites/HMDB0001514. Last Updated: 2020. Accessed: November 20, 2020.

67. 2004. Estimation Programs Interface Suite™ Ver 3.12. Washington, DC, USA: United States Environmental Protection Agency.

68. Bichsel Y, Von Gunten U. 2000. Environ Sci Technol Vol 34. 2784-2791.

69. Technical Services Branch of the USDA National Organic Program. Technical Evaluation Report: Glucosamine Hydrochloride Handling/Processing. https://www.ams.usda.gov/sites/default/files/media/Glucosamine%20Hydrochloride%20TR.pdf. Last Updated: 2010. Accessed: January 29, 2020.

70. National Center for Biotechnology National Library of Medicine. D-(+)-Glucosamine hydrochloride. https://pubchem.ncbi.nlm.nih.gov/compound/D-_-Glucosamine-hydrochloride. PubChem. Last Updated: 2020. Accessed: November 20, 2020.

71. ThermoFisher Scientific. Safety Data Sheet - D-Glucosamine sulfate. https://www.fishersci.com/store/msds?partNumber=AAJ6627106&productDescription=D-GLUCOSAMINE+SULFATE+98%25+5G&vendorId=VN00024248&countryCode=US&language=en. Last Updated: 2020. Accessed: November 20, 2020.

72. Guidechem Chemical Trading Guide. D-Glucosamine sulfate. https://www.guidechem.com/encyclopedia/d-glucosamine-sulfate-dic21885.html#Properties. Last Updated: 2020. Accessed: November 20, 2020.

73. PerkinElmer Inc. ChemDraw Pro. 14.0. Waltham, MA: 2021.

74. Bjerke DL, Kern P, DeConinck W. 2022. Skin sensitization next generation risk assessment framework and case study. Washington, D.C.: The Procter & Gamble Company. (Unpublished information presented to the Expert Panel for Cosmetic Ingredient Safety on March 7, 2022.)