Safety Assessment of Glucosamine Ingredients as Used in Cosmetics

Status:	Draft Report for Panel Review
Release Date:	November 10, 2021
Panel Meeting Date:	December 6 – 7, 2021

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Priya Cherian, Scientific Analyst/Writer.

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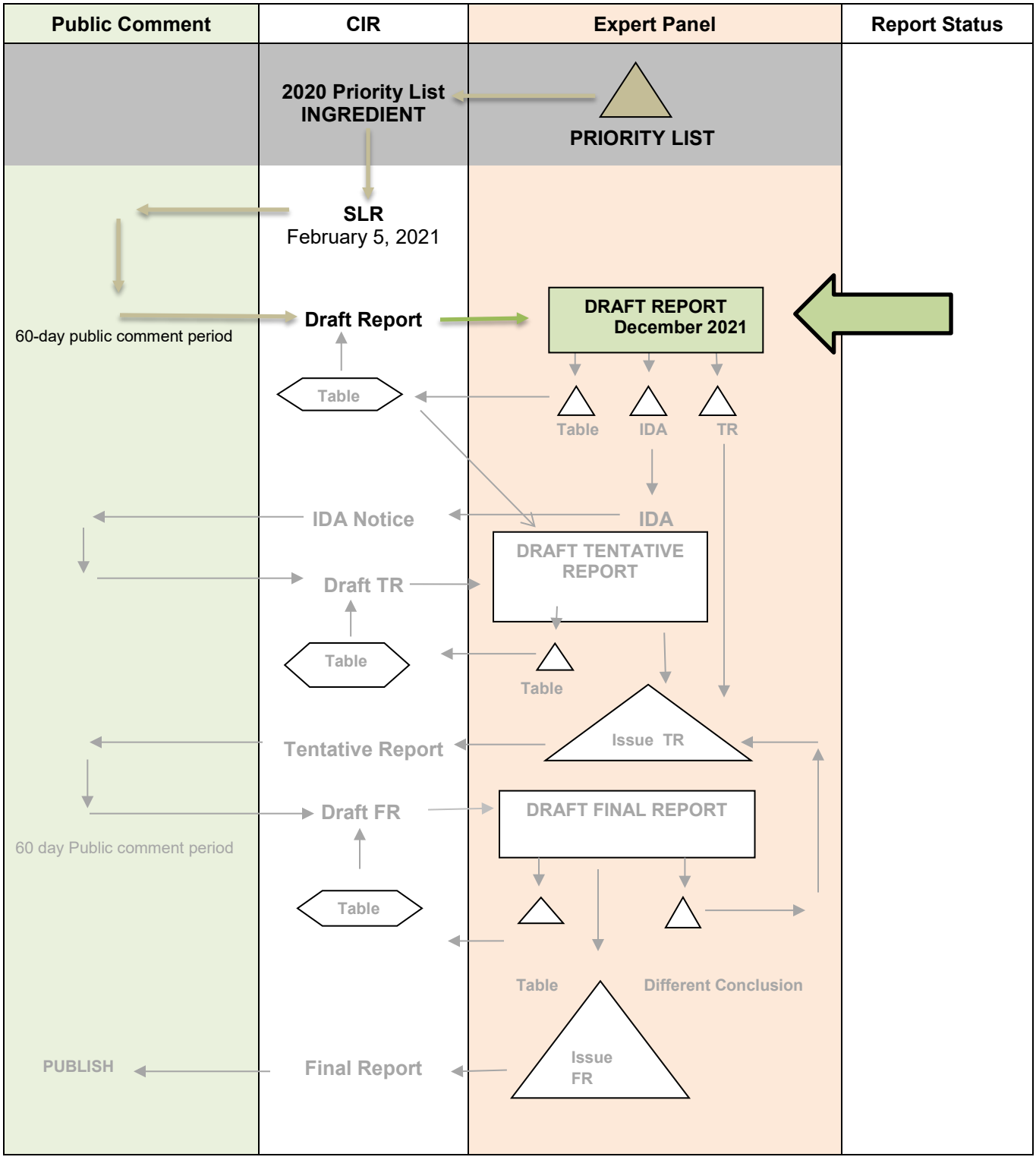
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Glucosamine Ingredients

MEETING December 2021



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, Scientific Analyst/Writer, CIR
Date: November 10, 2021
Subject: Safety Assessment of Glucosamine Ingredients as Used in Cosmetics

Enclosed is the Draft Report of the Safety Assessment of Glucosamine Ingredients as Used in Cosmetics (*report_Glucosamine_122021*). The 4 ingredients reviewed in this report include Acetyl Glucosamine, Glucosamine, Glucosamine HCl, and Glucosamine Sulfate. This is the first time the Expert Panel is reviewing this ingredient group. The Scientific Literature Review (SLR) was announced on February 5, 2021. Since the issuing of the SLR, the following unpublished data were received

- Repeated insult patch test performed in 108 subjects using a mask containing 0.005% Acetyl Glucosamine; non-sensitizing; Anonymous 2018; submitted February 19, 2021 (*data1_Glucosamine_122021*)
- Maximization assay performed in 25 subjects using a leave-on product containing 0.25% Glucosamine HCl; non-sensitizing; Anonymous 2007; submitted February 19, 2021 (*data1_Glucosamine_122021*)
- Maximization assay performed in 25 subjects using a product containing 0.01% Glucosamine; non-sensitizing; Anonymous 2005; submitted February 19, 2021 (*data1_Glucosamine_122021*)
- Repeated insult patch test performed in 51 subjects using a leave-on product containing 0.005% Glucosamine HCl; Anonymous 2012; submitted February 29, 2021 (*data2_Glucosamine_122021*)

Included in this packet are concentration of use data (*data3_Glucosamine_122021*), 2021 VCRP frequency of use data (*VCRP_Glucosamine_122021*), report history (*history_Glucosamine_122021*), data profile (*dataprofile_Glucosamine_122021)*, search strategy (*search_Glucosamine_122021*), and flow chart (*flow_Glucosamine_122021*). In addition, attached are comments on the SLR that were provided from Council (*PCPCcomments_Glucosamine_122021*), as well as responses to these comments (*response-PCPCcomments_Glucosamine_122021*.

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Report with a safe as used, safe with qualifications, or unsafe conclusion, and Discussion items should be identified. If the available data are insufficient, the Panel should issue an Insufficient Data Announcement (IDA), specifying the data needs therein.

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: February 10, 2021

SUBJECT: Scientific Literature Review: Safety Assessment of Glucosamine Ingredients as Used in Cosmetics (release date February 5, 2021)

The Personal Care Products Council respectfully submits the following comments on the scientific literature review, Safety Assessment of Glucosamine Ingredients as Used in Cosmetics.

Key Issues

Method of Manufacture – It is not clear why the method of manufacture of Acetyl Glucosamine is not mentioned in this section as a paper titled "N-Acetylglucosamine production and application" that describes various methods of manufacture is included in the reference section as reference 9.

Dermal Sensitization – The only study currently in the Sensitization section is a DPRA on Acetyl Glucosamine. Because a battery of *in vitro* tests is generally completed to assess dermal sensitization, the ECHA dossier on Acetyl Glucosamine (reference 3 and 47) was checked to see if any assays were missed. The DPRA presented in the CIR report was key study 1 in the sensitization section of the dossier. The ECHA dossier includes two additional *in vitro* assays not yet presented in the CIR report; key study 2, the Keratinosense assay, and key study 3, the hCLAT assay. Both assays were negative and did not predict a sensitization potential for Acetyl Glucosamine. Please add these additional assays to the Sensitization section of CIR report.

Additional Considerations

Impurities – The USP/NF specifications for Glucosamine HCl should be cited to USP/NF not EFSA (reference 7). Reference 7 does state that purity of Glucosamine HCl sourced from *Aspergillus niger* is 83.1% free base glucosamine. This should be presented in the Impurities section.

Cosmetic Use – The reported use from the PCPC survey of 5% Glucosamine HCl in face masks and mud packs should also be stated in the Cosmetic Use section.

Non-Cosmetic Use – Please include the typical dose of Glucosamine used in dietary supplements in the United States.

Dermal Penetration – Please state the analytical method used in reference 20.

As "permeation" may mean something different for different authors, please clarify what is meant. Does this refer to the glucosamine compound recovered only in the receptor fluid, or does it also include the amount in the skin?

ADME, Animal, Oral, Glucosamine HCl and Glucosamine Sulfate; Summary – Did the investigators (reference 24) confirm that the radioactivity in "all tissues, including cartilage" was still in the form of Glucosamine, or were they just measuring ^{14}C from labeled Glucosamine?

Acute Toxicity; Summary – When LD_{50} values are all greater than the doses tested, stating the "lowest" value does not make sense. It would be clearer to state: "The reported oral median lethal doses (LD_{50}) for Glucosamine were higher than the doses tested (>15,000 mg/kg in mice and >8000 mg/kg/day in rats and rabbits)."

Genotoxicity, In Vivo, Glucosamine HCl; Summary – Please also state that there was no significant increase in micronucleated PCEs observed, and that the treatment was not toxic to bone marrow.

Effects on Pigmentation, In Vitro, Animal and Human, Acetyl Glucosamine – As the study (reference 40) was done in humans and guinea pigs, "species" not specified is not correct.

IgE-Mediated Hypersensitivity – As the study looked at the reduction of effects, please change the subheading to "Reduction of IgE-Mediated Hypersensitivity". The first sentence of this section is not necessary.

What type, e.g., sc, of injection was used in the study described in reference 8?

Hypersensitivity to Shrimp-Derived Glucosamine – As no effect was observed in this study, it would be helpful to revise the subheading to "Lack of Hypersensitivity to Shrimp-Derived Glucosamine". The first sentence of this section is not needed. Please consider moving this study to the Clinical Studies section.

Risk Assessment – Please state the source of the NOAEL value used in the Norwegian risk assessment (NOAEL of 2149 mg/kg from a dog study with a 20% absorption factor). It would also be helpful to state the maximum use concentrations recommended in this risk assessment (18% face cream, 10% leg cream and 3.5% body lotion).

Summary – Please also indicate that the 67-year-old man with renal insufficiency also had type 2 diabetes.

It should be made clear that the lower risk of colon cancer was only apparent for shorter duration of use of Glucosamine.

Table 4 – It should be made clear that the study of Glucosamine HCl cited to reference 2 (ECHA dossier) was not considered very reliable.

References- Reference 3 and reference 47 appear to be the same reference (the ECHA dossier for N-Acetyl-β-D-Glucosamine).

Glucosamine – December 2021 – Priya Cherian	
Comment Submitter: Council **Date of Submission: 02/10/2021**	
Comment	**Response/Action**
Method of Manufacture – It is not clear why the method of manufacture of Acetyl Glucosamine is not mentioned in this section as a paper titled "N-Acetylglucosamine production and application" that describes various methods of manufacture is included in the reference section as reference 9.	Addressed
Dermal Sensitization – The only study currently in the Sensitization section is a DPRA on Acetyl Glucosamine. Because a battery of in vitro tests is generally completed to assess dermal sensitization, the ECHA dossier on Acetyl Glucosamine (reference 3 and 47) was checked to see if any assays were missed. The DPRA presented in the CIR report was key study 1 in the sensitization section of the dossier. The ECHA dossier includes two additional in vitro assays not yet presented in the CIR report; key study 2, the Keratinosense assay, and key study 3, the hCLAT assay. Both assays were negative and did not predict a sensitization potential for Acetyl Glucosamine. Please add these additional assays to the Sensitization section of CIR report.	Addressed
Impurities – The USP/NF specifications for Glucosamine HCl should be cited to USP/NF not EFSA (reference 7). Reference 7 does state that purity of Glucosamine HCl sourced from Aspergillus niger is 83.1% free base glucosamine. This should be presented in the Impurities section.	Addressed
Cosmetic Use – The reported use from the PCPC survey of 5% Glucosamine HCl in face masks and mud packs should also be stated in the Cosmetic Use section.	This was not reported in text because it is rinse-off formulation.
Non-Cosmetic Use – Please include the typical dose of Glucosamine used in dietary supplements in the United States.	Addressed
Dermal Penetration – Please state the analytical method used in reference 20.	Addressed
As "permeation" may mean something different for different authors, please clarify what is meant. Does this refer to the glucosamine compound recovered only in the receptor fluid, or does it also include the amount in the skin?	Addressed
ADME, Animal, Oral, Glucosamine HCl and Glucosamine Sulfate; Summary – Did the investigators (reference 24) confirm that the radioactivity in "all tissues, including cartilage" was still in the form of Glucosamine, or were they just measuring 14C from labeled Glucosamine?	The source states "Analyses of radioactivity in tissues and organs showed that [14C]-glucosamine quickly entered into all tissues including cartilage"
Acute Toxicity; Summary – When LD50 values are all greater than the doses tested, stating the "lowest" value does not make sense. It would be clearer to state: "The reported oral median lethal doses (LD50) for Glucosamine were higher than the doses tested (>15,000 mg/kg in mice and >8000 mg/kg/day in rats and rabbits)."	Addressed
Genotoxicity, In Vivo, Glucosamine HCl; Summary – Please also state that there was no significant increase in micronucleated PCEs observed, and that the treatment was not toxic to bone marrow.	Addressed
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IgE-Mediated Hypersensitivity – As the study looked at the reduction of effects, please change the subheading to "Reduction of IgE-Mediated Hypersensitivity". The first sentence of this section is not necessary.	Addressed
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Summary – Please also indicate that the 67-year-old man with renal insufficiency also had type 2 diabetes.	This is already stated in the report.
It should be made clear that the lower risk of colon cancer was only apparent for shorter duration of use of Glucosamine.	Addressed
Table 4 – It should be made clear that the study of Glucosamine HCl cited to reference 2 (ECHA dossier) was not considered very reliable.	Reliability factors are not typically included in reports.
References- Reference 3 and reference 47 appear to be the same reference (the ECHA dossier for N-Acetyl-β-D-Glucosamine)	Addressed

History – Glucosamine Ingredients

February 2021

- SLR posted
- Comments on SLR received
- Concentration of use data received
- Data received:
    - Repeat insult patch test; mask containing 0.005% Acetyl Glucosamine
    - Human maximization assay; product containing 0.25% Glucosamine HCl
    - Human maximization assay; product containing 0.01% Glucosamine

April 2021

- Data received
    - Repeat insult patch test; leave-on product containing 0.005% Glucosamine HCl

October 2021

- Panel reviews Draft Report

Glucosamine Ingredients Profile – December 2021 – Writer, Priya Cherian																														
				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Acetyl Glucosamine	x	x		x	x						x					x		x	x			x		x		x			
Glucosamine	x	x		x				x						x										x		x		x	x
Glucosamine HCL	x	x	x	x	x	x		x			x					x								x					
Glucosamine Sulfate		x			x	x																							x

* "X" indicates that data were available in a category for the ingredient

Glucosamine Ingredients

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Acetyl Glucosamine	10036-64-3; 72-87-7; 7512-17-6	yes	yes	no	no	no	no	no	yes	no	no	no	no	no	no	no	yes
Glucosamine	3416-24-8	yes	no	no	no	no	yes	no	yes	no	no	no	no	no	no	no	yes
Glucosamine HCL	66-84-2	yes	no	no	no	no	yes	no	yes	yes	no	no	no	no	no	no	yes
Glucosamine Sulfate	29031-19-4	yes	no	no	no	no	no	no	yes	yes	no	no	no	no	no	no	yes

Search Strategy

Search terms below were searched for in the websites listed above. If useful information was found, a "yes" is noted.

Search Terms

- INCI names
  - Acetyl Glucosamine
  - Glucosamine
  - Glucosamine HCl
  - Glucosamine Sulfate
- CAS numbers
  - 10036-64-3
  - 72-87-7
  - 7512-17-6
  - 3416-24-8
  - 66-84-2
  - 29031-19-4
- chemical/technical names
- metabolism
- dermal
- inhalation
- skin
- toxicity
- drugs
- medicine
- irritation
- ocular
- eye
- sensitization
- allergy
- manufacture
- cancer

- carcinogenicity
- mutagenicity
- Ames
- Reproductive
- Teratogenicity
- Synthesis

LINKS

Search Engines

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)

Pertinent Websites

- wINCI - http://webdictionary.personalcarecouncil.org
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/

- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

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ABBREVIATIONS

AUC_{ss}	area under the curve; extent of exposure
BAL	bronchoalveolar lavage
BUN	blood urea nitrogen
CAS	Chemical Abstracts Service
CI	confidence interval
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
C_{max}	peak serum concentration
C_{ss}	peak concentration
DART	Developmental and Reproductive Toxicity
Dictionary	*International Cosmetic Ingredient Dictionary and Handbook*
DNFB	dinitrofluorobenzene
DPRA	Direct Peptide Reactivity Assay
ECHA	European Chemicals Agency
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
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
UV	ultraviolet
VCRP	Voluntary Cosmetic Registration Program

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.

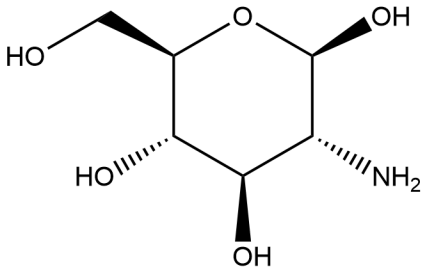
This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Expert Panel for Cosmetic Ingredient Safety (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; molecular weight (MW) = 179.17 g/mol; log K_{ow} = -4.2; 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; formula weight (FW) = 215.63 g/mol; log K_{ow} = -1.91) and Glucosamine Sulfate (CAS No. 29031-19-4; FW = 277.25 g/mol), are metabolized to Acetyl Glucosamine (CAS Nos. 10036-64-3, 72-87-7, 7512-17-6; MW = 222.21 g/mol; log K_{ow} = -2.2) via the hexosamine pathway.[5]



Chemical Properties

Glucosamine HCl is a charged, highly polar, and water-soluble salt.[5] The acetylated glucosamine metabolite, Acetyl Glucosamine, 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

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.[9] 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.[10] 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. Use concentration 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 by product category

According to 2021 VCRP survey data, Acetyl Glucosamine is reported to be used in 117 formulations (105 leave-on formulations and 12 rinse-off formulations; Table 3), and Glucosamine HCl is reported to be used in 69 formulations (57 leave-on formulations and 12 rinse-off formulations).[11] Glucosamine is reported to be used in 4 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).[12] No VCRP or concentration of use data were reported for Glucosamine Sulfate.

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. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 μm, with propellant sprays yielding a greater fraction of droplets/particles < 10 μm compared with pump sprays.[13,14] Therefore, 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] Acetyl Glucosamine is also reportedly used in face powders at concentrations up to 0.07%. 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]

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 Acetyl 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. Test formulations (n = 8) contained either 2% Acetyl Glucosamine alone with the vehicle (vehicle not stated), or a combination of 4% niacinamide and 2% Acetyl Glucosamine with the vehicle. Approximately 5 µl of the test formulation was applied to the cells using a positive displacement pipette. 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 only Acetyl Glucosamine 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.

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).[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 [^{14}C]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 4.

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).[28] 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/species/group).[31] 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.[32] 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.[33] 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.[34] 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).[35] 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 Glucosamine treatment were evaluated in 8-wk old and 16-wk old adult female C57B1/6 mice (24 mice/group).[36] Each age group received either 0 or 20 mg/kg Glucosamine in the diet for 3 wk. After the 3-wk feeding

period, treated animals were given an intraperitoneal injection of a solution containing PBS and Glucosamine (20 mg/kg). Mice that received no Glucosamine treatment during the feeding period received injections of PBS only. Mice were injected for 3 consecutive days. On the third day, each female was mated with a male. All mice were again treated accordingly with an injection of the Glucosamine and PBS solution, or PBS only, on the fourth day. Females that did not successfully mate were re-introduced to males and daily injections were repeated until mating was achieved, followed by a final injection on the day following successful mating, or until mating had been attempted for a maximum of 4 nights. Pregnancy outcomes were assessed at day 18 of gestation. 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 periconception 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 ($p < 0.05$).

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.

Intrauterine

Glucosamine

The effects of intrauterine Glucosamine were evaluated in female ICR mice (3 mice/group).[38] 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.[39] 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 control (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.[40] 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).[35] 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.[41] 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.[42] 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.

In Vivo

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).[42] 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.[43] 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.

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.[44] 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 pigmentation 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.[45] 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 was significantly ($p < 0.05$) caused a more pronounced decrease in detectable areas of facial spots and the appearance of pigmentation, compared to those that used the control formulation.

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.[46] 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).[47] 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.[10] 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).[48] 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. [49] 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 5.

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] HRIPTs performed using a mask containing 0.005% Acetyl Glucosamine (108 subjects) and a leave-on product containing 0.005% Glucosamine HCl (51 subjects) yielded negative results.[50,51] 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.[52,53]

OCULAR IRRITATION STUDIES

In Vitro

Acetyl Glucosamine

An ocular irritation 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.[54] 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.[55] 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).[56] 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.[57] 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.[58] 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.[59] 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).[60] 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 2021 VCRP survey data, Acetyl Glucosamine, Glucosamine HCl, and Glucosamine are reported to be used in 117, 69, and 4 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 penetration ability 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 ability of 5% Glucosamine HCl in different vehicles was evaluated in rat skin. Transdermal flux of Glucosamine HCl zwas 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 [^{14}C]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 Glucosamine (20 mg) treatment via oral ingestion and peritoneal injection was evaluated in 8-wk old and 16-wk old adult female C57B1/6 mice. Mice were fed the test substance via diet for 3 wk, and injected with Glucosamine for 3 consecutive days. On the third day of injection, mice were mated. Pregnancy outcomes were assessed at day 18 of gestation. 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 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 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 in-vivo anti-carcinogenicity assay, Kunming male mice were inoculated with sarcoma 180 tumor cells. Mice were orally treated with up to 500 mg/kg Glucosamine HCl dissolved in saline for 10 d. 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.

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. HRIPTs performed using a mask containing 0.005% Acetyl Glucosamine (108 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.

An in vitro ocular irritation assay was performed in bovine corneas using a saline solution containing 20% Acetyl Glucosamine. The mean in vitro irritancy scores for the test substance, negative control (saline), and positive control (20% imidazole in saline) were 0.42, 0.70, and 105.42, respectively.

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

To be developed.

CONCLUSION

To be determined.

TABLES

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

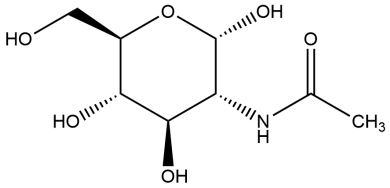
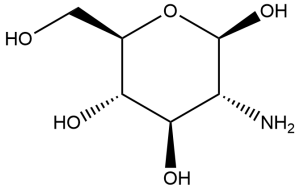
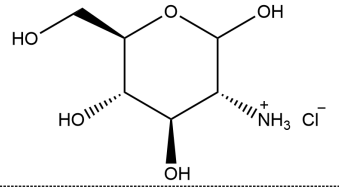
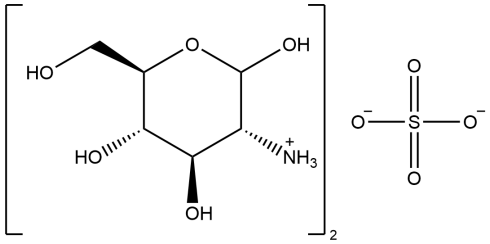
Ingredient	Definition	Function
Acetyl Glucosamine (10036-64-3; 72-87-7; 7512-17-6)	Acetyl Glucosamine is the organic compound that conforms to the structure: 	Skin-Conditioning Agents – Miscellaneous
Glucosamine (3416-24-8)	Glucosamine is the organic compound that conforms to the structure: 	Not Reported
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	61
Molecular Weight (g/mol)	179.17	61
Vapor pressure (mmHg @ 25ºC)	0.0000000902	62
Melting Point (ºC)	88	61
Water Solubility (g/L)	551	61
log K_{ow}	-4.2	62
Disassociation constants (pKa)	7.58	63

Table 2. Chemical properties

Property	Value	Reference
Glucosamine HCl		
Physical Form	Crystalline	64
Formula Weight (g/mol)	215.63	65
Color	Off-White	64
Odor	Odorless	2
Specific Gravity (@ 38 ºC)	1.42	64
Melting Point (ºC)	190 - 194	64
Water Solubility	Soluble	2
log K_{ow}	-1.91	23
Disassociation constant (pKa) (@ 37 ºC)	7.75	23
Glucosamine Sulfate		
Physical Form	Solid	66
Color	Off-White	66
Formula Weight (g/mol)	277.25	66
Density(g/ml)	1.56	67
Boiling Point (ºC)	449.9	67
Water Solubility (g/l)	Freely soluble	67
Disassociation constants (pKa)	12.51 (estimated)	68

Table 3. Frequency (2021)[11] and concentration (2020)[12] 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*	**117**	**0.001 – 5**	**4**	**0.04**	**69**	**0.0001 – 5**
Duration of Use						
Leave-On	*105*	*0.002 – 5*	*4*	*0.04*	57	*0.0001 – 0.9*
Rinse-Off	*12*	*0.001 – 5*	*NR*	*NR*	12	*0.07 – 5*
5Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	NR	*NR*
Exposure Type						
Eye Area	10	0.2 – 2	1	NR	4	0.0001 – 0.2
Incidental Ingestion	2	0.002 – 2	NR	NR	NR	NR
Incidental Inhalation-Spray	39[a]; 35[b]	0.1; 0.005 – 0.07[b]	3[a]	NR	21[a]; 23[b]	NR
Incidental Inhalation-Powder	39[a]	0.07; 0.12 – 5[c]	3[a]	0.04[c]	1; 21[a]	0.0006 – 0.38[c]
Dermal Contact	114	0.01 – 5	4	0.04	59	0.0001 – 5
Deodorant (underarm)	NR	0.01	NR	NR	NR	NR
Hair - Non-Coloring	1	0.001 – 0.55	NR	NR	10	0.55
Hair-Coloring	NR	0.01	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	3	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. 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	28
Glucosamine	CD-1 Mice	NR	8000 mg/kg; gavage	LD_{50} > 8000 mg/kg	28
Glucosamine	Mice (strain unspecified)	NR	15,000 mg/kg; gavage	LD_{50} > 15,000 mg/kg	28
Glucosamine	Sprague-Dawley Rat	NR	8000 mg/kg; gavage	LD_{50} > 8000 mg/kg; no adverse effects reported	28
Glucosamine	Rabbit (strain unspecified)	NR	8000 mg/kg; gavage	LD_{50} > 8000 mg/kg	28
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 5. 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)	100%; 16 mg	3	reconstructed human epidermis; OECD TG 439; positive control: 5% sodium dodecyl sulfate; negative control: PBS; 42 min incubation	Non-irritating	[3]
SENSITIZATION						
				In Vitro		
Acetyl Glucosamine	Acetyl Glucosamine (99.42% purity)	100%	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	100%; 2cm x 2 cm	108	HRIPT; occlusive conditions	Non-sensitizing	[50]
Glucosamine	Product containing 0.01% Glucosamine	100%; 2 cm x 2 cm	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	[52]
Glucosamine	Leave-on product containing 0.005% Glucosamine HCl	100%; 25-38 mg/cm^2	51	HRIPT; occlusive conditions	Non-irritating and Non-sensitizing	[51]
Glucosamine HCl	Product containing 0.25% Glucosamine HCl	100%: 0.05 g	25	Maximization assay performed according to the same procedures as above; occlusive conditions	Non-sensitizing	[53]

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

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: February 19, 2021

SUBJECT: Products Containing Glucosamine Ingredients

Anonymous. 2018. Repeated insult patch test (mask contains 0.005% Acetyl Glucosamine).

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).

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).

[redacted]

REPEATED INSULT PATCH STUDY

mask contains 0.005% Acetyl Glucosamine

[redacted]

CONDUCTED FOR:

[redacted]

DATE OF ISSUE:

August 24, 2018

[redacted] [redacted]

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APPENDICES

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[redacted]

SIGNATURES

This study was conducted in compliance with the requirements of the protocol and [redacted]s Standard Operating Procedures, and in the spirit of GCP ICH Topic E6.[1] The report accurately reflects the raw data for this study.

[redacted]

STATEMENT OF QUALITY CONTROL

The Quality Control Unit of the Dermatological Safety Department conducted a 100% review of all study-related documents. The protocol was reviewed prior to the start of the study, and the medical screening forms and informed consent documents were reviewed in-process of the study. The regulatory binder and study data were reviewed post-study to ensure accuracy. The study report was reviewed and accurately reflects the data for this study.

[1] ICH Topic E6 "Note for guidance on Good Clinical Practices (CPMP/ICH/135/95)" – ICH Harmonised Tripartite Guideline for Good Clinical Practices having reached Step 5 of the ICH Process at the ICH Steering Committee meeting on 1 May 1996.

TITLE OF STUDY

Repeated Insult Patch Study

SPONSOR

[redacted]

STUDY MATERIAL

GE Face Mask, F# [redacted]

DATE STUDY INITIATED

June 18, 2018

DATE STUDY COMPLETED

July 27, 2018

DATE OF ISSUE

August 24, 2018

INVESTIGATIVE PERSONNEL

[redacted]

CLINICAL SITE

[redacted]

SUMMARY

One (1) product, F# [redacted], was evaluated as supplied to determine its ability to sensitize the skin of volunteer subjects with normal skin using an occlusive repeated insult patch study. One hundred eight (108) subjects completed the study.

Under the conditions employed in this study, there was no evidence of sensitization to product, F# [redacted]

1.0 OBJECTIVE

The objective of this study was to determine the ability of the study material to cause sensitization by repeated topical applications to the skin of humans under controlled patch study conditions.

2.0 RATIONALE

Substances that come into contact with human skin need to be evaluated for their propensity to irritate and/or sensitize. Once an appropriate pre-clinical safety evaluation has been performed, a reproducible, standardized, quantitative patch evaluation procedure must be used to demonstrate that a particular material can be applied safely to human skin without significant risk of adverse reactions. The method herein employed is generally accepted for such a purpose.

Repeated insult patch evaluation is a modified predictive patch study that can detect weak sensitizers that require multiple applications to induce a cell-mediated (Type IV) immune response sufficient to cause an allergic reaction. Irritant reactions may also be detected using this evaluation method, although this is not the primary purpose of this procedure. Results are interpreted according to interpretive criteria based upon published works, as well as the clinical experience of , . These interpretive criteria are periodically reviewed and amended as new information becomes available.

3.0 STUDY DESIGN

3.1 STUDY POPULATION

A sufficient number of subjects were enrolled to provide 100 completed subjects. In the absence of any sensitization reactions in this sample size (100 evaluable subjects), a 95% upper confidence bound on the population rate of sensitization would be 3.5%.

3.1.1 Inclusion Criteria

Individuals eligible for inclusion in the study were those who:

1. Were males or females, 18 years of age or older, in general good health;
2. Were free of any systemic or dermatologic disorder which, in the opinion of the investigative personnel, would have interfered with the study results or increased the risk of adverse events (AEs);
3. Were of any skin type or race, providing the skin pigmentation would allow discernment of erythema;
4. Had completed a medical screening procedure; and
5. Had read, understood, and signed an informed consent (IC) agreement.

3.1.2 Exclusion Criteria

Individuals excluded from participation in the study were those who:

1. Had any visible skin disease at the study site which, in the opinion of the investigative personnel, would have interfered with the evaluation;

2. Were receiving systemic or topical drugs or medication which, in the opinion of the investigative personnel, would have interfered with the study results;
3. Had psoriasis and/or active atopic dermatitis/eczema;
4. Were females who were pregnant, planning to become pregnant during the study, or breast-feeding; and/or
5. Had a known sensitivity to cosmetics, skin care products, or topical drugs as related to the material being evaluated.

3.1.3 Informed Consent

A properly executed IC document was obtained from each subject prior to entering the study. The signed IC document is maintained in the study file. In addition, the subject was provided with a copy of the IC document (see Appendix III).

3.2 DESCRIPTION OF STUDY

3.2.1 Outline of Study Procedures

Subjects participated in the study over a 6-week period involving 3 phases: (1) Induction, (2) Rest, and (3) Challenge. Prior to study entry, the subjects were screened to assure that they met the inclusion/exclusion criteria. Informed consent was obtained. Each subject was provided with a schedule of the study activities. All subjects were told to avoid wetting the patches and were asked not to engage in activities that caused excessive perspiration. They were instructed to notify the staff if they experienced any discomfort beyond mild itching or observed any adverse changes at the patch sites, while on the study or within 2 weeks of completing the study.

The Induction Phase consisted of 9 applications of the study material and subsequent evaluations of the patch sites. Prior to application of the patches, the sites were outlined with a skin marker, eg, gentian violet. Patches were applied on Mondays, Wednesdays, and Fridays for 3 consecutive weeks. The subjects were required to remove the patches approximately 24 hours after application. They returned to the facility at 48-hour intervals to have the sites evaluated and identical patches applied to the same sites. Patches applied on Friday were removed by subjects after 24 hours. The sites were evaluated on the following Monday, ie, 72 hours after patch application.[2] Following the 9th evaluation, the subjects were dismissed for a Rest Period of approximately 10-15 days.

Subjects who were absent once during the Induction Phase received a make-up (MU) patch at the last Induction Visit. The MU applications were graded 48 hours later at the MU visit, or were recorded as N9G (no ninth grading). Subjects who missed the 9th evaluation (N9G) but have had 9 patch applications were considered to have completed the Induction Phase.

The Challenge Phase was initiated during the sixth week of the study. Identical patches were applied to sites previously unexposed to the study material. The patches were removed by subjects after 24 hours and the sites graded after additional 24-hour and 48-hour periods (ie, 48 and 72 hours after application). Following a negative Induction, a 48/72-hour sequence of "-/+," "?/+," or "+/+" resulted in an additional reading being performed at the 96-hour interval. Rechallenge was performed whenever there was evidence of possible sensitization.

[2] A Monday or Friday holiday could result in evaluation at 96 hours after patch application.

To be considered a completed case, a subject must have had 9 applications and no fewer than 8 subsequent readings during Induction, and a single application and 2 readings at Challenge. Only completed cases were used to assess sensitization.

Due to the holiday occurring on Wednesday, July 4, 2018, subjects were instructed to return on Thursday, July 5, 2018.

3.2.2 Study Flow Chart

WEEK 1

DAY	ACTIVITIES
1[3]	Staff obtained informed consent, reviewed completed medical screening form, applied patches
2	Subject removed patches
3	Staff graded sites, applied patches
4	Subject removed patches
5	Staff graded sites, applied patches
6	Subject removed patches

WEEK 2

1	Staff graded sites, applied patches
2-6	Same as Week 1

WEEK 3

1-6	Same as Week 2

WEEK 4

1	Staff graded sites; applied make-up (MU) induction patches, if required
2	Subject removed MU induction patches
3	Staff graded MU induction sites at MU visit
2-7	Rest Period

WEEK 5

1-7	Rest Period

WEEK 6

1	Staff applied patches
2	Subject removed patches
3	Staff graded sites
4	Staff graded sites

[3] Study flow starting with Week 1, Day 1, will be altered when enrollment occurs other than on Monday. Study flow could be altered when a holiday occurs during the study.

3.2.3 Definitions Used for Grading Responses

The symbols found in the scoring scales below were used to express the response observed at the time of examination:

- = No reaction
? = Minimal or doubtful response, slightly different from surrounding normal skin
+ = Definite erythema, no edema
++ = Definite erythema, definite edema
+++ = Definite erythema, definite edema and vesiculation

SPECIAL NOTATIONS

E = Marked/severe erythema
S = Spreading of reaction beyond patch site (ie, reaction where material did not contact skin)
p = Papular response > 50%
pv = Papulovesicular response > 50%
D = Damage to epidermis: oozing, crusting and/or superficial erosions
I = Itching
X = Subject absent
PD = Patch dislodged
NA = Not applied
NP = Not patched (due to reaction achieved)
N9G = No ninth grading

3.2.4 Evaluation of Responses

All responses were graded by a trained dermatologic evaluator meeting [redacted] strict certification requirements to standardize the assignment of response grades.

4.0 NATURE OF STUDY MATERIAL

4.1 STUDY MATERIAL SPECIFICATIONS

Identification : GE Face Mask, F# [redacted]
Amount Applied : 2cm x 2cm Piece
Special Instructions : The study material was cut to fit the patch.

4.2 STORAGE, HANDLING, AND DOCUMENTATION OF STUDY MATERIAL

Receipt of the material used in this study was documented in a general logbook, which serves as a permanent record of the receipt, storage, and disposition of all study material received by [redacted] On the basis of information provided by the Sponsor, the study material was considered reasonably safe for evaluation on human subjects. A sample of the study material was reserved and will be stored for a period of 6 months. All study material is kept in a locked product storage room accessible to

clinical staff members only. At the conclusion of the clinical study, the remaining study material was discarded or returned to the Sponsor and the disposition documented in the logbook.

4.3 APPLICATION OF STUDY MATERIAL

All study material was supplied by the Sponsor. Material was applied in an amount proportionate to the patch type or as requested by the Sponsor, generally 0.2 mL or g or an amount sufficient to cover the 2 cm x 2 cm patch. The patches were applied to the infrascapular area of the back, either to the right or left of the midline, or to the upper arm. Unless otherwise directed by the Sponsor, the study material was discarded upon completion of the study.

4.4 DESCRIPTION OF PATCH CONDITIONS

Material evaluated under occlusive patch conditions is applied to a 2 cm x 2 cm Webril™ pad attached to a non-porous, plastic film adhesive bandage (3M medical tape). The patch is secured with hypoallergenic tape (Micropore), as needed.

Material evaluated under semi-occlusive patch conditions is applied to a 2 cm x 2 cm Webril™ pad. The pad is affixed to the skin with hypoallergenic tape (Micropore).

5.0 INTERPRETATION

Sensitization is characterized by an acute allergic contact dermatitis. Typical sensitization reactions begin with an immunologic response in the dermis resulting in erythema, edema formation, and secondary epidermal damage (vesiculation), sometimes extending beyond the patch site and often accompanied by itching. Sensitization reactions tend to be delayed. The reaction typically becomes evident between 24 and 48 hours, peaks at 48-72 hours and subsequently subsides. The reaction is often greater at 72 hours than at 48 hours. The severity of the reaction is generally greater during the Challenge Phase of a Repeated Insult Patch Test (RIPT) than that seen during Induction.

Irritant reactions are characterized as a non-immunologic, localized, superficial, exudative, inflammatory response of the skin due to an externally applied material. The typical initial reaction does not develop much edema or vesiculation but results in scaling, drying, cracking, oozing, crusting, and erosions. The reaction is usually sharply delineated, not spreading beyond the patch site. Irritant reactions are typically evident by 24 hours and diminish over the next 48-72 hours. Removal of the offending agent results in gradual improvement of the epidermal damage. The reaction seen at 72 hours is, therefore, less severe than that seen at 48 hours. Finally, the severity of the reaction experienced in the Challenge Phase is generally similar to that seen during Induction.

If the results of the study indicate the likelihood of sensitization, the recommended practice is to rechallenge the subjects who have demonstrated sensitization-like reactions to confirm that these reactions are, indeed, associated with the product. [redacted]s preferred Rechallenge procedure involves the application of the product to naive sites, under both occlusive and semi-occlusive patch conditions. Use of the semi-occlusive patch condition helps to differentiate irritant and sensitization reactions. Generally speaking, if a product is a sensitizer it will produce a similar reaction under both occlusion and semi-occlusion. Whereas, if the product has caused an irritant reaction, the reactions will be less pronounced under the semi-occlusive condition.

6.0 DOCUMENTATION AND RETENTION OF DATA

The case report forms (CRFs) were designed to identify each subject by subject number and initials, and to record demographics, examination results, AEs, and end of study status. Originals or copies of all CRFs, correspondence, study reports, and all source data will be kept on hard-copy file for a minimum of 5 years from completion of the study. Storage was maintained either at a [redacted] facility in a secured room accessible only to [redacted] employees, or at an offsite location which provided a secure environment with burglar/fire alarm systems, camera detection and controlled temperature and humidity. Documentation will be available for the Sponsor's review on the premises of [redacted]

7.0 RESULTS AND DISCUSSION

One hundred thirty (130) subjects between the ages of 19 and 69 were enrolled and 108 completed the study (see Tables 1 and 2 in Appendix I and Data Listings 1 and 2 in Appendix II). The following table summarizes subject enrollment and disposition:

Number enrolled:		130
Number discontinued:		22
Lost to follow-up:	19	
Voluntary withdrawal:	3	
Number completed:		108

Source: Table 1, Appendix I

There were no adverse events (AEs) reported during the study.

A summary of response data is provided in Table 3, Appendix I. Individual dermatological response grades are provided in Data Listing 3, Appendix II.

8.0 CONCLUSION

Under the conditions employed in this study, there was no evidence of sensitization to product, F# [redacted].

9.0 REFERENCES

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APPENDIX I

SUMMARY TABLES

[redacted]

Table 1: Summary of Subject Enrollment and Disposition

	N (%)
Subjects enrolled	130
Subjects completed induction phase	110 (84.6)
Subjects completed all phases	108 (83.1)
Total subjects discontinued	22 (16.9)
Lost to follow-up	19 (14.6)
Voluntary withdrawal	3 (2.3)

Note: All percentages are relative to total subjects enrolled.

See data listing 1 for further detail.

[redacted]

[redacted]

Table 2: Summary of Subject Demographics
All Enrolled Subjects

Age	
N (%) 18 to 44	51 (39.2)
N (%) 45 to 65	68 (52.3)
N (%) 66 and up	11 (8.5)
Mean (SD)	47.5 (13.9)
Median	50.1
Range	19.0 to 69.8
Sex	
N (%) Male	27 (20.8)
N (%) Female	103 (79.2)
Race	
Amer Ind	2 (1.5)
Black	56 (43.1)
Caucasian	71 (54.6)
Other	1 (0.8)
Ethnicity	
Hispanic/Latino	19 (14.6)
Not Hispanic/Not Latino	111 (85.4)

See data listing 2 for further detail.

[redacted]

[redacted]

Table 3: Summary of Dermatologic Response Grades
Number of Subjects by Product

Product = F#[redacted]

	Induction Reading										Challenge Phase	
Response	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Make Up**	**48hr**	**72hr**
-	122	111	117	111	110	106	108	109	106	21	108	108
Total evaluable	122	111	117	111	110	106	108	109	106	21	108	108
Number absent	3	9	1	3	4	5	2	1	4		0	0
Number discontinued	5	10	12	16	16	19	20	20	20		22	22

Maximum Elicited Response During Induction
All Subjects Completing Induction (N=110)

Response	**n(%) Subjects**
-	110 (100.0%)

(*) when required

See Table 3.1 for Key to Symbols and Scores

[redacted]

[redacted]

Table 3.1: Key To Symbols and Scores

Score or Symbol	Response or Description of Reaction
	Erythema Results
-	No reaction
?	Minimal or doubtful response, slightly different from surrounding normal skin
+	Definite erythema, no edema
++	Definite erythema, definite edema
+++	Definite erythema, definite edema and vesiculation
	Additional Comments
X	Reading not performed due to missed visit or subject discontinuation
D	Damage to epidermis: oozing, crusting and/or superficial erosions
E	Marked/severe erythema
I	Itching
p	Papular response >50%
pv	Papulovesicular response >50%
S	Spreading of reaction beyond patch site
NP	Not patched due to reaction achieved
PD	Patch dislodged
N9G	No ninth grading
NA	Not applied

APPENDIX II

DATA LISTINGS

[redacted]

Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
001	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
002	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
003	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
004	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
005	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
006	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
007	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
008	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
009	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
010	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
011	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
012	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
013	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
014	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
015	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
016	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
017	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
018	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
019	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
020	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
021	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
022	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
023	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
024	06/18/18	06/18/18	--	07/02/18	I5	S	15
025	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
026	06/18/18	06/18/18	--	06/25/18	I1	L	8
027	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
028	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
029	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
030	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
031	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

[redacted]

[redacted]

Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
032	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
033	06/18/18	06/18/18	--	06/27/18	I3	L	10
034	06/18/18	06/18/18	--	06/29/18	I3	L	12
035	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
036	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
037	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
038	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
039	06/18/18	06/18/18	--	07/06/18	I6	L	19
040	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
041	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
042	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
043	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
044	06/18/18	06/18/18	--	06/25/18	I1	L	8
045	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
046	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
047	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
048	06/18/18	06/18/18	--	06/22/18	I0	L	5
049	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
050	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
051	06/18/18	06/18/18	--	06/27/18	I3	L	10
052	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
053	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
054	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
055	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
056	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
057	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
058	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
059	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
060	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
061	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
062	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

[redacted]

[redacted]

Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
063	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
064	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
065	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
066	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
067	06/18/18	06/18/18	--	06/27/18	I2	S	10
068	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
069	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
070	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
071	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
072	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
073	06/18/18	06/18/18	--	06/25/18	I1	L	8
074	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
075	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
076	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
077	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
078	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
079	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
080	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
081	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
082	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
083	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
084	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
085	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
086	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
087	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
088	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
089	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
090	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
091	06/18/18	06/18/18	--	06/22/18	I0	L	5
092	06/18/18	06/18/18	--	06/22/18	I0	L	5
093	06/18/18	06/18/18	--	07/05/18	I5	L	18

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

[redacted]

[redacted]

Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
094	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
095	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
096	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
097	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
098	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
099	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
100	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
101	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
102	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
103	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
104	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
105	06/18/18	06/18/18	--	07/02/18	I5	L	15
106	06/18/18	06/18/18	--	06/25/18	I1	L	8
107	06/18/18	06/18/18	--	06/27/18	I3	L	10
108	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
109	06/18/18	06/18/18	--	06/22/18	I0	L	5
110	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
111	06/18/18	06/18/18	--	06/22/18	I0	L	5
112	06/18/18	06/18/18	07/24/18	07/27/18	C	C	40
113	06/18/18	06/18/18	--	06/27/18	I2	L	10
114	06/20/18	06/20/18	--	07/24/18	I9	S	35
115	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
116	06/20/18	06/20/18	--	07/24/18	I9	L	35
117	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
118	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
119	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
120	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
121	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
122	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
123	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
124	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
125	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
126	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
127	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
128	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
129	06/20/18	06/20/18	07/24/18	07/27/18	C	C	38
130	06/20/18	06/20/18	--	06/27/18	I1	L	8

[redacted]

Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Ethnicity	Race
001	60.5	Female	Not Hispanic/Not Latino	Caucasian
002	41.2	Female	Not Hispanic/Not Latino	Caucasian
003	47.9	Male	Not Hispanic/Not Latino	Black
004	65.3	Female	Not Hispanic/Not Latino	Caucasian
005	54.6	Female	Not Hispanic/Not Latino	Caucasian
006	42.4	Female	Not Hispanic/Not Latino	Black
007	68.8	Female	Not Hispanic/Not Latino	Black
008	60.4	Female	Hispanic/Latino	Caucasian
009	60.3	Female	Not Hispanic/Not Latino	Black
010	56.9	Female	Hispanic/Latino	Caucasian
011	54.2	Female	Not Hispanic/Not Latino	Black
012	65.0	Female	Not Hispanic/Not Latino	Black
013	30.1	Female	Hispanic/Latino	Caucasian
014	34.2	Female	Not Hispanic/Not Latino	Black
015	67.4	Female	Not Hispanic/Not Latino	Black
016	54.2	Female	Not Hispanic/Not Latino	Black
017	59.9	Female	Not Hispanic/Not Latino	Caucasian
018	50.4	Female	Not Hispanic/Not Latino	Caucasian
019	33.4	Male	Hispanic/Latino	Caucasian
020	29.6	Female	Not Hispanic/Not Latino	Caucasian
021	69.4	Female	Not Hispanic/Not Latino	Caucasian
022	61.8	Female	Not Hispanic/Not Latino	Caucasian
023	62.5	Female	Not Hispanic/Not Latino	Black
024	51.5	Male	Not Hispanic/Not Latino	Black
025	50.1	Male	Not Hispanic/Not Latino	Black
026	64.3	Female	Not Hispanic/Not Latino	Caucasian
027	34.6	Male	Not Hispanic/Not Latino	Caucasian
028	45.2	Female	Not Hispanic/Not Latino	Black
029	28.6	Male	Not Hispanic/Not Latino	Black
030	59.7	Female	Not Hispanic/Not Latino	Black
031	59.3	Female	Not Hispanic/Not Latino	Caucasian
032	54.2	Male	Not Hispanic/Not Latino	Caucasian
033	59.0	Female	Not Hispanic/Not Latino	Black
034	19.3	Male	Not Hispanic/Not Latino	Caucasian
035	61.8	Female	Not Hispanic/Not Latino	Caucasian
036	69.5	Female	Not Hispanic/Not Latino	Caucasian
037	47.0	Female	Not Hispanic/Not Latino	Caucasian

[redacted]

[redacted]

Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Ethnicity	Race
038	50.7	Female	Not Hispanic/Not Latino	Black
039	25.6	Female	Not Hispanic/Not Latino	Caucasian
040	30.5	Male	Hispanic/Latino	Caucasian
041	36.2	Female	Not Hispanic/Not Latino	Caucasian
042	34.5	Female	Not Hispanic/Not Latino	Caucasian
043	61.3	Female	Not Hispanic/Not Latino	Caucasian
044	20.7	Male	Not Hispanic/Not Latino	Black
045	45.5	Female	Hispanic/Latino	Caucasian
046	57.9	Female	Hispanic/Latino	Caucasian
047	67.3	Female	Not Hispanic/Not Latino	Caucasian
048	30.3	Female	Hispanic/Latino	Black
049	44.3	Female	Not Hispanic/Not Latino	Caucasian
050	56.3	Female	Not Hispanic/Not Latino	Caucasian
051	53.6	Female	Not Hispanic/Not Latino	Black
052	66.8	Male	Not Hispanic/Not Latino	Caucasian
053	50.6	Female	Not Hispanic/Not Latino	Caucasian
054	58.2	Female	Not Hispanic/Not Latino	Black
055	21.0	Male	Not Hispanic/Not Latino	Black
056	54.6	Female	Not Hispanic/Not Latino	Caucasian
057	19.2	Female	Not Hispanic/Not Latino	Black
058	27.7	Female	Hispanic/Latino	Caucasian
059	53.5	Female	Not Hispanic/Not Latino	Caucasian
060	50.0	Female	Not Hispanic/Not Latino	Black
061	67.4	Female	Not Hispanic/Not Latino	Black
062	39.0	Female	Not Hispanic/Not Latino	Black
063	51.4	Male	Not Hispanic/Not Latino	Black
064	35.0	Female	Not Hispanic/Not Latino	Black
065	50.5	Female	Not Hispanic/Not Latino	Black
066	39.7	Female	Not Hispanic/Not Latino	Black
067	19.0	Female	Not Hispanic/Not Latino	Black
068	21.4	Male	Hispanic/Latino	Caucasian
069	49.3	Female	Not Hispanic/Not Latino	Black
070	62.4	Female	Not Hispanic/Not Latino	Caucasian
071	65.0	Female	Not Hispanic/Not Latino	Caucasian
072	37.0	Female	Hispanic/Latino	Caucasian
073	30.1	Female	Hispanic/Latino	Caucasian
074	65.7	Female	Not Hispanic/Not Latino	Caucasian

[redacted]

[redacted]

Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Ethnicity	Race
075	48.3	Male	Hispanic/Latino	Caucasian
076	27.2	Male	Not Hispanic/Not Latino	Caucasian
077	50.4	Female	Not Hispanic/Not Latino	Black
078	40.0	Female	Not Hispanic/Not Latino	Black
079	55.5	Male	Not Hispanic/Not Latino	Caucasian
080	38.8	Female	Not Hispanic/Not Latino	Caucasian
081	69.8	Male	Not Hispanic/Not Latino	Caucasian
082	49.7	Female	Not Hispanic/Not Latino	Caucasian
083	57.1	Female	Not Hispanic/Not Latino	Caucasian
084	60.5	Female	Not Hispanic/Not Latino	Caucasian
085	66.7	Female	Not Hispanic/Not Latino	Black
086	38.0	Female	Not Hispanic/Not Latino	Caucasian
087	30.5	Male	Not Hispanic/Not Latino	Black
088	51.9	Female	Not Hispanic/Not Latino	Black
089	49.8	Female	Not Hispanic/Not Latino	Caucasian
090	43.3	Male	Not Hispanic/Not Latino	Black
091	51.4	Male	Not Hispanic/Not Latino	Black
092	46.7	Female	Not Hispanic/Not Latino	Black
093	53.3	Female	Not Hispanic/Not Latino	Black
094	42.6	Female	Hispanic/Latino	Caucasian
095	33.0	Female	Hispanic/Latino	Amer Ind
096	32.4	Female	Not Hispanic/Not Latino	Caucasian
097	50.9	Female	Hispanic/Latino	Amer Ind
098	58.4	Male	Not Hispanic/Not Latino	Caucasian
099	57.4	Female	Not Hispanic/Not Latino	Caucasian
100	61.0	Female	Not Hispanic/Not Latino	Caucasian
101	42.0	Female	Not Hispanic/Not Latino	Black
102	35.1	Female	Not Hispanic/Not Latino	Caucasian
103	49.4	Female	Not Hispanic/Not Latino	Black
104	42.6	Female	Not Hispanic/Not Latino	Caucasian
105	23.9	Female	Not Hispanic/Not Latino	Black
106	24.0	Female	Hispanic/Latino	Black
107	39.0	Female	Not Hispanic/Not Latino	Caucasian
108	69.4	Male	Not Hispanic/Not Latino	Caucasian
109	21.6	Female	Hispanic/Latino	Caucasian
110	43.9	Female	Not Hispanic/Not Latino	Black
111	41.1	Female	Not Hispanic/Not Latino	Black

[redacted]

[redacted]

Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Ethnicity	Race
112	51.7	Female	Not Hispanic/Not Latino	Caucasian
113	21.4	Female	Hispanic/Latino	Caucasian
114	62.1	Female	Not Hispanic/Not Latino	Caucasian
115	30.2	Female	Not Hispanic/Not Latino	Caucasian
116	29.2	Male	Not Hispanic/Not Latino	Black/Hawaiian/Pacific Islander
117	29.1	Female	Not Hispanic/Not Latino	Black
118	59.2	Female	Not Hispanic/Not Latino	Black
119	56.5	Female	Not Hispanic/Not Latino	Black
120	63.3	Female	Not Hispanic/Not Latino	Caucasian
121	39.1	Female	Not Hispanic/Not Latino	Caucasian
122	47.6	Female	Not Hispanic/Not Latino	Black
123	32.9	Female	Not Hispanic/Not Latino	Caucasian
124	40.2	Female	Not Hispanic/Not Latino	Black
125	54.9	Female	Not Hispanic/Not Latino	Black
126	47.6	Female	Not Hispanic/Not Latino	Black
127	63.3	Male	Not Hispanic/Not Latino	Caucasian
128	66.9	Male	Not Hispanic/Not Latino	Caucasian
129	49.3	Male	Not Hispanic/Not Latino	Black
130	51.6	Female	Not Hispanic/Not Latino	Black

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = F# [redacted]

Subject No.	Induction Reading										Challenge Phase	
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr
001	-	-	-	-	-	-	-	-	-		-	-
002	-	-	-	-	-	-	-	-	-		-	-
003	-	-	-	-	-	-	-	-	-		-	-
004	-	-	-	-	-	-	-	-	-		-	-
005	-	-	-	-	-	-	-	-	-		-	-
006	-	-	-	-	-	-	-	-	-		-	-
007	-	-	-	-	-	-	-	-	-		-	-
008	-	-	-	-	-	-	-	-	-		-	-
009	-	-	-	-	-	-	-	-	-		-	-
010	-	-	-	-	-	-	-	-	-		-	-
011	-	-	-	-	-	-	-	-	N9G		-	-
012	-	-	-	-	-	-	-	-	-		-	-
013	-	-	-	X	-	-	-	-	-	N9G	-	-
014	-	-	-	-	-	-	-	-	-		-	-
015	-	-	-	-	-	-	X	-	-	-	-	-
016	-	-	-	-	-	-	-	-	-		-	-
017	-	-	-	-	-	-	-	-	-		-	-
018	-	-	-	-	-	-	-	-	-		-	-
019	-	-	-	-	-	-	-	-	-		-	-
020	-	-	-	-	-	-	-	-	-		-	-
021	-	-	-	-	-	-	-	-	-		-	-
022	-	-	-	-	-	-	-	-	-		-	-
023	-	-	-	-	-	-	-	-	-		-	-

See Table 3.1 for Key to Symbols and Scores

MU = Make-up reading for missed induction visit

(*) When required

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = F# [redacted]

Subject No.	Induction Reading										Challenge Phase	
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr
024	-	X	-	-	-	X	X	X	X		X	X
025	-	-	-	-	-	-	-	-	-		-	-
026	-	X	X	X	X	X	X	X	X		X	X
027	-	-	-	-	-	-	-	-	-		-	-
028	-	-	-	-	-	-	-	-	-		-	-
029	-	-	-	-	-	-	-	-	-		-	-
030	-	-	-	-	-	-	-	-	-		-	-
031	-	-	-	-	-	-	-	-	-		-	-
032	-	-	-	-	-	-	-	-	-		-	-
033	X	-	-	X	X	X	X	X	X		X	X
034	-	-	-	X	X	X	X	X	X		X	X
035	-	-	-	-	-	-	-	-	-		-	-
036	-	-	-	-	-	-	-	-	-		-	-
037	-	-	-	-	-	-	-	-	-		-	-
038	-	-	-	-	-	-	-	-	-		-	-
039	-	-	-	-	-	-	X	X	X		X	X
040	-	-	-	-	-	-	-	-	-		-	-
041	-	X	-	-	-	-	-	-	-	-	-	-
042	-	X	-	-	-	-	-	-	-	-	-	-
043	-	-	-	-	-	-	-	-	-		-	-
044	-	X	X	X	X	X	X	X	X		X	X
045	-	-	-	-	-	-	-	-	-		-	-
046	-	-	-	-	-	-	-	-	-		-	-

(*) When required

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = F# [redacted]

Subject No.	Induction Reading										Challenge Phase	
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr
047	-	-	-	-	-	X	-	-	-	-	-	-
048	X	X	X	X	X	X	X	X	X		X	X
049	-	-	-	-	-	-	-	-	-		-	-
050	-	-	-	-	-	-	-	-	-		-	-
051	X	-	-	X	X	X	X	X	X		X	X
052	-	-	-	-	-	-	-	-	N9G		-	-
053	-	-	-	-	-	-	-	-	-		-	-
054	-	-	-	-	-	-	-	-	-		-	-
055	-	-	-	-	-	-	-	-	-		-	-
056	-	-	-	-	-	-	-	-	-		-	-
057	-	-	-	-	-	-	-	-	-		-	-
058	-	-	-	-	-	-	-	X	-	-	-	-
059	-	-	-	-	-	-	-	-	-		-	-
060	-	-	-	-	-	-	-	-	-		-	-
061	-	-	-	-	-	-	-	-	-		-	-
062	-	-	-	-	-	-	-	-	-		-	-
063	-	-	-	-	-	-	-	-	-		-	-
064	-	-	-	-	-	-	-	-	-		-	-
065	-	-	-	-	-	-	-	-	-		-	-
066	-	-	-	-	-	-	-	-	-		-	-
067	-	-	X	X	X	X	X	X	X		X	X
068	-	-	-	-	-	-	-	-	N9G		-	-
069	-	-	-	-	-	-	-	-	-		-	-

(*) When required

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = F# [redacted]

Subject No.	Induction Reading										Challenge Phase	
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr
070	-	-	-	-	-	-	-	-	-		-	-
071	-	X	-	-	-	-	-	-	-	-	-	-
072	-	-	-	-	-	-	-	-	-		-	-
073	-	X	X	X	X	X	X	X	X		X	X
074	-	-	-	-	-	-	-	-	N9G		-	-
075	-	-	-	-	X	-	-	-	-	-	-	-
076	-	-	-	-	-	-	-	-	-		-	-
077	-	-	-	-	-	-	-	-	-		-	-
078	-	-	-	-	-	-	-	-	-		-	-
079	-	-	-	-	-	X	-	-	-	-	-	-
080	-	-	-	-	-	-	-	-	-		-	-
081	-	-	-	-	-	-	-	-	-		-	-
082	-	-	-	-	-	-	-	-	-		-	-
083	-	-	-	-	-	X	-	-	-	-	-	-
084	-	-	-	-	-	-	-	-	-		-	-
085	-	-	-	-	-	-	-	-	-		-	-
086	-	-	-	-	-	-	-	-	-		-	-
087	-	-	-	-	-	-	-	-	-		-	-
088	-	-	-	-	-	X	-	-	-	-	-	-
089	-	-	-	-	-	-	-	-	-		-	-
090	-	-	-	-	-	-	-	-	-		-	-
091	X	X	X	X	X	X	X	X	X		X	X
092	X	X	X	X	X	X	X	X	X		X	X

(*) When required

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = F# [redacted]

Subject No.	Induction Reading										Challenge Phase	
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr
093	-	-	-	-	-	X	X	X	X		X	X
094	-	-	-	-	-	-	-	-	-		-	-
095	-	-	-	-	-	-	-	-	-		-	-
096	-	-	-	-	-	-	-	-	-		-	-
097	-	-	-	-	-	-	-	-	-		-	-
098	-	-	-	-	-	-	X	-	-	-	-	-
099	-	-	-	-	-	-	-	-	-		-	-
100	-	-	-	-	-	-	-	-	-		-	-
101	-	-	-	-	-	-	-	-	-		-	-
102	-	-	-	-	-	-	-	-	-		-	-
103	-	-	-	-	-	-	-	-	-		-	-
104	-	-	-	-	-	-	-	-	-		-	-
105	-	X	-	-	-	X	X	X	X		X	X
106	-	X	X	X	X	X	X	X	X		X	X
107	-	X	-	X	X	X	X	X	X		X	X
108	-	-	-	-	-	-	-	-	-		-	-
109	X	X	X	X	X	X	X	X	X		X	X
110	-	-	-	-	-	X	-	-	-	-	-	-
111	X	X	X	X	X	X	X	X	X		X	X
112	X	-	-	-	-	-	-	-	-	-	-	-
113	-	-	X	X	X	X	X	X	X		X	X
114	-	-	-	-	-	-	-	-	-		X	X
115	-	-	-	-	-	-	-	-	-		-	-

(*) When required

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = F# [redacted]

Subject No.	Induction Reading 1	2	3	4	5	6	7	8	9	MU	Challenge Phase 48hr	72hr
116	-	X	-	-	-	-	-	-	-	N9G	X	X
117	-	-	-	-	-	-	-	-	-		-	-
118	-	X	-	-	-	-	-	-	-	-	-	-
119	-	-	-	-	X	-	-	-	-	-	-	-
120	-	-	-	-	-	-	-	-	-		-	-
121	-	-	-	X	-	-	-	-	-	-	-	-
122	-	-	-	X	-	-	-	-	-	-	-	-
123	-	X	-	-	-	-	-	-	-	-	-	-
124	-	-	-	-	-	-	-	-	-		-	-
125	-	-	-	-	X	-	-	-	-	-	-	-
126	-	-	X	-	-	-	-	-	-	-	-	-
127	-	-	-	-	-	-	-	-	-		-	-
128	-	-	-	-	-	-	-	-	-		-	-
129	-	-	-	-	X	-	-	-	-	-	-	-
130	-	X	X	X	X	X	X	X	X		X	X

(*) When required

[redacted]

APPENDIX III

INFORMED CONSENT DOCUMENT

[redacted] [redacted] [redacted]

INFORMED CONSENT
REPEATED INSULT PATCH TEST

STUDY NO.: [redacted]

PURPOSE

You are invited to participate in this Repeated Insult Patch Test (RIPT), which is a research study to determine if these products can be applied to human skin without causing an allergic reaction. The study will involve a minimum of 100 participants.

STUDY PRODUCT

The study product include or may be components of cosmetics, moisturizers, lipsticks, skin care products, shampoos, shower gel/body wash, antiperspirants/deodorants, disinfectants, antibacterial, fragrances, soaps, sunscreens, fibers, adhesives, antimicrobials (an ingredient used as a preservative), and/or any other products which are intended for and/or may come into contact with human skin. Included is sodium lauryl sulfate (SLS) which is a caustic soap solution used as a control for comparison.

STUDY DURATION

This study consists of 13 visits (14 visits, if required) over 6 weeks, most visits lasting approximately 10 minutes. You will receive a schedule of visit dates and instructions.

PROCEDURE

Before you can start the study, the study staff will explain the study and answer any questions you may have. You will be asked to read and sign this form stating that you understand the study procedures. The study staff will begin screening you to see if you meet all study entrance requirements. This study consists of three phases, which include Induction, Rest and Challenge which are explained below.

Each patch received during this study will contain one cosmetic study product. However, more than one patch will be applied with several different cosmetic study products. The dose of the study product will be about 0.2mL, covering a 2cm by 2cm area. You will wear the study product and patch(s) on your back.

Induction: The first three weeks of the study are called the induction phase. During the induction phase you will report to [redacted] on Mondays, Wednesdays and Fridays. At each visit study staff will apply a set of patches to your back. Each patch will be removed 24 hours after application and new patch(s) will be applied at each visit. Your skin will be examined before any study product is applied. The patch(s) applied on Monday and Wednesday and Friday will remain on your back for 24 hours. At each of these induction visits, a clinical evaluator will examine your back to see if you are reacting to any of the products. If you have a strong reaction at the study site (where the study product is applied), the study product will not be applied to that site, but may be applied to another site. The induction period consists of 10 visits.

Rest: During week four of the study, you will begin a rest period during which study product will not be applied to your back and you will not have to report to [redacted]. This rest period will last through weeks four and five.

Challenge: After the rest period is over and week six begins (the final week of the study), you will receive the same products applied on a new area of the back. The study products (with patches) will be put on the part of your back that has not received study product before. During this phase of the study, you will have to return to [redacted] for three more visits. The first visit during the challenge phase you will have your back evaluated and identical patches re-applied. You will return to [redacted] 48 hours after initial challenge patch application for skin evaluation. Finally you will return to [redacted] for your final visit, 72 hour after initial challenge patch application, for your final evaluation. If the study doctor/staff determines that it is necessary to make additional evaluations, due to reactions, you will be asked to come back for an additional visit.

[redacted] [redacted] [redacted]

INFORMED CONSENT
REPEATED INSULT PATCH TEST

STUDY NO.: [redacted]

If you are a female of childbearing potential (i.e., not surgically sterile or have not experienced menopause), you must agree to prevent pregnancy throughout this study by using at least one form of accepted birth control [e.g., oral/ injectable/transdermal contraceptive pill, IUD, condom/diaphragm with spermicide, abstinence (no sexual intercourse)].

If you are breastfeeding a child, you will not be permitted to participate in this study. Pregnancy and breastfeeding are prohibited to prevent any unforeseen risk to an unborn child or breast-feeding child.

SUBJECT REQUIREMENTS

You must agree to make all your scheduled visits to [redacted]. You must not apply products such as creams, lotions and moisturizers on or near the test sites. You must avoid sun exposure or the use of tanning beds on your back (including the rest period). You must agree to refrain from swimming during the course of the study. You must agree to minimize water exposure on the patch area while showering or bathing by taking a low tub bath or frontal shower. You will receive written instructions for this study.

POTENTIAL RISKS

Some of the study products may be irritating under certain conditions but the degree of irritation is not expected to be greater than that described below. Individuals participating in this study may experience side effects such as redness, swelling, itching, cracking, peeling, or in rare cases, small blisters or sores. Reactions usually occur only where the study products or patch products (such as the patch tape adhesive) touch the skin. On rare occasions, the reactions may spread beyond the patch. A reaction may result in localized lightening or darkening of the skin, which may persist in an occasional individual. Reactions may be due to either skin irritation or allergy to either study products or patch products (e.g., patch tape adhesive). This study may include taking photographs of part(s) of your back that received study product.

It may be necessary to do additional application (rechallenge) to determine if an allergic reaction has occurred. If you should prove to be allergic, you can expect to react to this product if you encounter it at a later date. Whenever possible, you will be informed as to the identity of the product in order that you may avoid contact with it in the future.

For any significant reactions that may occur as a direct result of your participation in this study, appropriate and reasonable medical treatment will be provided by [redacted]. at no cost to you to resolve the immediate problem. Provision of such medical care is not an admission of legal liability or responsibility for the condition being treated. If such reactions occur, [redacted] personnel should be contacted immediately at [redacted] during business hours and at [redacted] at, night or weekends. Extended medical care will not be provided.

POTENTIAL BENEFITS

You may receive no direct benefit from being in this study. However, taking part in this study may benefit society by gaining new knowledge

SIGNIFICANT NEW FINDINGS

You will be informed of any significant new findings that may affect your willingness to continue your participation.

ALTERNATIVE TREATMENT

Since this study is for research only, the only alternative is for you not to participate.

WITHDRAWAL FROM STUDY

Participation in the study is voluntary and you may refuse to participate or may withdraw at any time. Voluntary withdrawal from the study for reasons unrelated to the study or failure to follow test procedures

[redacted] [redacted] [redacted]

INFORMED CONSENT
REPEATED INSULT PATCH TEST

STUDY NO.: [redacted]

will result in some loss of payment based on the number of visits completed. Subjects will be paid $5.00 per visit for early withdrawal. Your participation may also be discontinued at any time without your consent by the study doctor, or the study sponsor(s) (the company(s) that makes the product(s) being evaluated). If you fail to comply with study procedures, your participation may be terminated.

COST

Your participation in the study will not incur any cost to you.

FINANCIAL INCENTIVE

Your participation is voluntary. You may discontinue participation at any time without prejudice. You will be compensated for you participation. A payment of $160.00 will be made only upon completion of all phases of the study. If in the judgment of the investigating personnel, it is best to discontinue your participation in this study due to an adverse experience or severe reaction you will be paid in full for your participation. Voluntary withdrawal from the study for reasons unrelated to the study or failure to follow test procedures will result in some loss of payment based on the number of visits completed. Subjects will be paid $5.00 per visit. Other than the compensation described above, you will not directly benefit from this study. This study is for scientific information. Not participating in the study would be your alternative.

CONFIDENTIALITY AND AUTHORIZATION

[redacted] will protect information about you and your taking part in this research study to the best of our ability. If information about this study is published, your identity will remain confidential. Reports prepared by [redacted] will utilize statistical information only and at no time will your name be used. However, the U.S. Food and Drug Administration (FDA), the sponsor and [redacted] may sometimes inspect the research record and study information of those who take part in this study. By signing this consent form, you are authorizing such access. A court of law could also order research records shown to other people, but that is unlikely. Therefore, absolute confidentiality cannot be guaranteed.

WHO TO CALL

Additional information regarding this research is available either before or during the course of this study. If you have any questions or research related side effect or injury, you may contact the study coordinator, [redacted] during business hours. After business hours the emergency phone number is [redacted]

A copy of this consent form will be given to you.

****

I have read and understand the information given in this consent form. I have had an opportunity to ask questions and my questions have been answered. I voluntarily consent to participate. By signing this form I have not given up any of my legal rights which I would otherwise have as a research subject.

_____	_____	_____	_____
Entry Number	Print Name	Signature	Date

_____ _____

Signature of Person Explaining the Consent Form Date

APPENDIX IV

DERMATOLOGIST SIGNED LETTER

[redacted]

February 20, 2017

[redacted]

Dear [redacted],

All Dermatologic Safety studies at [redacted] are conducted under the supervision and coordination of a board-certified dermatologist. [redacted] is a board-certified dermatologist and site Medical Director who serves as the Principal Investigator for all Dermatologic Safety studies. As Principal Investigator, [redacted] follows NIH and Good Clinical Practices (GCP) in his responsibility for delegating authority to trained and qualified personnel, whose credentials are documented on their curriculum vitae (CV) on file with site standard operating procedures. All subject's grading is performed under the supervision of the dermatologist. The dermatologist is responsible for all Clinical Grading Assessments, and for reviewing and signing all laboratory reports.

[redacted]
Assoc. Director Dermatologic Safety

[redacted]

FINAL REPORT dated December 14, 2007

[redacted]

Sample: Cream Makeup coded [redacted]

[redacted]

Title: 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

Sponsor: [redacted]

Principal Investigator: [redacted] (Board Certified Dermatologist)

Testing Facility: [redacted]

Protocol: [redacted] Authorization Letter dated October 29, 2007

Final Report Date: **December 14, 2007**

[redacted]

December 14, 2007
Date

Principal Investigator

"The names of [redacted] any officer, employee, or collaborating scientist are not to be used for any advertising, promotional or sale purposes without the written consent of [redacted]."

FINAL REPORT

[redacted] PROTOCOL:

[redacted]

SPONSOR:

[redacted]

[redacted]

SPONSOR STUDY:

Authorization Letter Dated: October 29, 2007

STUDY TITLE:

Evaluation of the contact-sensitizing potential of a coded topically-applied test agent.

STUDY OBJECTIVE:

The objective of this study is to assess the skin sensitizing potential of any preparation designed for topical use by means of the Maximization Test (see references #1 and #2).

TEST MATERIAL:

The test sample, supplied by the sponsor, was a product labeled Cream Makeup coded [redacted]. The test product was tested as supplied viz., neat. A fresh jar of the test material was used for each patching day both during the induction phase and the challenge phase of the study.

TEST PRODUCT ACCOUNTABILITY:

All test samples and materials were received in good condition by our Quality Assurance Department. The test materials and quantities were checked for (1) amount (2) product number or code (3) material container etc. The materials were individually listed on a special sheet (drug/test product log form) signed by the receiver, the laboratory supervisor and the investigator (physician). All test materials were stored under ambient conditions in an inaccessible location under the supervision of the investigator.

PRINCIPAL INVESTIGATOR:

[redacted] (Board Certified Dermatologist)

Medical Director, [redacted]

[redacted]

[redacted]

[redacted]

KGL ADMINISTRATIVE STRUCTURE:

[redacted] (Screening, Patch Applications/Removals, Recognize AE's)

[redacted] (Expert Grader)

[redacted] (Panel Recruitment/Receptionist)

TESTING FACILITY:

[redacted]

[redacted]

[redacted]

[redacted]

CONDUCTION DATES:

This study was conducted from November 5, 2007 through December 7, 2007

PANEL COMPOSITION:

Healthy, adult volunteers over the age of 18 years were recruited for this study. None of the subjects had a medical or dermatological illness and none were sensitive to sunlight or to topical preparations and/or cosmetics. The criteria for exclusion were:

1 - History of sun hypersensitivity and photosensitive dermatoses

2 - History of drug hypersensitivity or recurrent dermatological diseases

3 - Pregnancy or mothers who are breastfeeding

4 – History of recurrent urticaria or hives

5 - Scars, moles or other blemishes over the test site which can interfere with the study

6 - Subjects receiving systemic or topical drugs or medications, including potential sensitizers within the previous 4 weeks

7 - Other medical conditions considered by the investigator as sound reasons for disqualification from enrollment into the study.

INFORMED CONSENT:

After the protocol, reasons for the study, possible associated risks and potential benefits or risks of the treatment had been completely explained, signed, informed subject consent was obtained from each volunteer prior to the start of the study. Copies of all consent forms are on file at [redacted]

METHOD:

Patches were applied to the upper outer arm, volar forearm or the back of each subject. The entire test was composed of two distinct phases: (1) an Induction phase and (2) a Challenge phase.

(1) **Induction Phase**:

Approximately 0.05ml of aqueous SLS (0.25%) was applied to a designated site under a 15mm disc of Webril cotton cloth and the patch was fastened to the skin with occlusive tape for a period of 24 hours. After 24 hours, the SLS patch was removed and 0.05gm of the test material was applied to the same site before the site was again covered with occlusive tape (induction patch). The induction patch was left in place for 48 hours (or for 72 hours when placed over a weekend) following which it was removed and the site again examined for irritation. If no irritation was present, a 0.25% aqueous SLS patch was again reapplied to the same site for 24 hours, followed by reapplication of a fresh induction patch with the test material to the same site. This sequence viz. 24 hour SLS pre-treatment followed by 48 hours of test material application was continued for a total of 5 induction exposures.

If irritation developed at any time-point during the induction phase as previously outlined, the 24-hour SLS pre-treatment patch was eliminated and only the test material was reapplied to the same site after a 24-hour rest period during which no patch was applied.

The aim during this phase of the study was to maintain at least a minimal degree of irritation in order to enhance penetration through the corneum barrier.

(2) **Challenge Phase**:

After a ten day rest period which follows the last induction patch application, the subjects were challenged with a single application of the test material to a new skin site on the opposite arm, forearm or side of back in order to determine if sensitization had developed.

Pre-treatment with SLS was performed prior to challenge. Approximately 0.05ml of a 5.0% aqueous solution was applied to a fresh skin site under a 15mm disc of Webril cotton and covered with occlusive tape. The SLS patch was left in place for one hour. It was then removed and the test material was applied to the same site, as outlined above. The challenge patch was then covered by occlusive tape and left in place for 48 hours. After that period, the patch was removed and the site graded 15-30 minutes later and again 24 hours later for any reaction.

SCORING SCALE:

0 = not sensitized

1 = mild sensitization (viz. erythema and a little edema)

2 = moderate sensitization (erythema with infiltration, raised, spreading beyond the borders of the patch, with or without vesiculation)

3 = strong sensitization (large vesiculo-bullous reaction).

Based on these findings the number of subjects with positive responses were tabulated for the test material. The test system shown below was used to classify the allergenic

potential of the test substance.

SENSITIZATION RATES:	**GRADES:**	**CLASSIFICATION**:
0 - 2/25	1	Weak
3 - 7/25	2	Mild
8 - 13/25	3	Moderate
14 - 20/25	4	Strong
21 - 25/25	5	Extreme

RESULTS:

A total of twenty-five (25) healthy, adult volunteers of both sexes who satisfied the inclusion criteria were enrolled into this study. There were 20 females and 5 males. Their ages ranged from 19 to 62 years. The demographic data are shown in Table 1.

All 25 subjects completed this investigation as outlined in the standard protocol. No adverse or unexpected reactions were seen in any of the panelists during the induction phase.

The results of the challenge are shown in the enclosed table (Table 2). No instances of contact allergy were recorded at either 48 or 72 hours after the application of the challenge patches.

CONCLUSION:

Under the conditions of this test, the test sample labeled Cream Makeup and coded [redacted] does not possess a detectable contact-sensitizing potential and hence is not likely to cause contact sensitivity reactions under normal use conditions.

References:

(1) Kligman, A.M.: The Maximization Test. J.I.D., Vol. 47, No. 5, pp. 393-409, 1966.

(2) Kligman, A.M. and Epstein W.: Updating the Maximization Test for Identifying Contact Allergens. Contact Dermatitis. Vol. 1, 231-239, 1975.

TABLE 1

DEMOGRAPHIC DATA

Subject Number:	Subject Initials:	Age:	Sex:	Race:
01	WHK	47	M	C
02	G-E	49	F	C
03	J-B	22	M	C
04	N-D	57	F	C
05	R-S	25	F	C
06	K-K	36	F	C
07	C-C	47	F	C
08	F-M	44	M	C
09	S-A	32	F	C
10	CLL	62	F	B
11	G-H	36	F	C
12	C-E	45	F	C
13	G-U	23	F	C
14	C-K	24	M	C
15	S-W	19	F	C
16	M-D	37	F	C
17	D-W	48	F	C
18	L-A	22	F	C
19	J-N	44	F	C
20	A-N	19	F	C
21	C-D	47	F	C
22	D-A	26	F	C
23	M-G	53	M	C
24	D-K	54	F	C
25	S-D	47	F	C

C = Caucasian
B = Black

TABLE 2

MAXIMIZATION TESTING RESULTS

Sample: Cream Makeup coded [redacted]

Subject Number:	48-Hour Grading	72-Hour Grading
01	0	0
02	0	0
03	0	0
04	0	0
05	0	0
06	0	0
07	0	0
08	0	0
09	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	0	0
17	0	0
18	0	0
19	0	0
20	0	0
21	0	0
22	0	0
23	0	0
24	0	0
25	0	0

Challenge Readings:

48-Hour Reading – December 6, 2007
72-Hour Reading – December 7, 2007

[redacted]

FINAL REPORT dated October 25, 2005
[redacted] Protocol: [redacted]
Sample: Patch coded [redacted]

[redacted]

Title: 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

Sponsor: [redacted]

Principal Investigator: [redacted] (Board Certified Dermatologist)

Testing Facility: [redacted]

Protocol: [redacted]

Final Report Date: October 25, 2005

[redacted]

Principal Investigator

October 25, 2005
Date

"The names of [redacted], any officer, employee, or collaborating scientist are not to be used for any advertising, promotional or sale purposes without the written consent of [redacted]"

F I N A L R E P O R T

[redacted]PROTOCOL:

[redacted]

SPONSOR:

[redacted]

[redacted]

SPONSOR STUDY:

Authorization Letter Dated: September 7, 2005

STUDY TITLE:

Evaluation of the contact-sensitizing potential of a coded topically-applied test agent.

STUDY OBJECTIVE:

The objective of this study is to assess the skin sensitizing potential of any preparation designed for topical use by means of the Maximization Test (see references #1 and #2).

TEST MATERIAL:

The test sample, supplied by the sponsor, was a product labeled ”Patch” and coded [redacted]which was tested in accordance with the sponsor’s instructions viz., a new packette was opened daily and the remaining unused sheet was discarded.

TEST PRODUCT ACCOUNTABILITY:

All test samples and materials were received in good condition by our Quality Assurance Department. The test materials and quantities were checked for (1) amount (2) product number or code (3) material container etc. The materials were individually listed on a special sheet (drug/test product log form) signed by the receiver, the laboratory supervisor and the investigator (physician). All test materials were stored under ambient conditions in an inaccessible location under the supervision of the investigator.

PRINCIPAL INVESTIGATOR:

[redacted] (Board Certified Dermatologist)

Medical Director, [redacted]

Telephone: [redacted]

FAX: [redacted]

[redacted] **ADMINISTRATIVE STRUCTURE:**

[redacted] Screening, Patch Applications/Removals, Recognize AE's)

[redacted] (Expert Grader)

[redacted] (Panel Recruitment/Receptionist)

TESTING FACILITY:

[redacted]

[redacted]

[redacted]

CONDUCTION DATES:

This study was conducted from September 12, 2005 through October 14, 2005

PANEL COMPOSITION:

Healthy, adult volunteers over the age of 18 years were recruited for this study. None of the subjects had a medical or dermatological illness and none were sensitive to sunlight or to topical preparations and/or cosmetics. The criteria for exclusion were:

1 - History of sun hypersensitivity and photosensitive dermatoses

2 - History of drug hypersensitivity or recurrent dermatological diseases

3 - Pregnancy or mothers who are breastfeeding

4 - Scars, moles or other blemishes over the test site which can interfere with the study

5 - Recent sunburn

6 - Subjects receiving systemic or topical drugs or medications, including potential sensitizers within the previous 4 weeks

7 - Other medical conditions considered by the investigator as sound reasons for disqualification from enrollment into the study.

INFORMED CONSENT:

After the protocol, reasons for the study, possible associated risks and potential benefits or risks of the treatment had been completely explained, signed, informed subject consent was obtained from each volunteer prior to the start of the study. Copies of all consent forms are on file at [redacted]

METHOD:

Approximately 0.05ml of aqueous SLS (0.25%) was applied to a designated site under a 2x2cm^2 disc of Webril cotton cloth and the patch was fastened to the skin with occlusive tape for a period of 24 hours. After 24 hours, the SLS patch was removed and a 2x2 cm^2 of the test patch coded [redacted] was then applied to the test site and covered with a 2x2 cm^2 of Webril (non-woven cotton cloth), and the entire area sealed with occlusive tape (Blenderm, 3M) and further secured to the skin with Scanpor Tape (induction patch). The induction patch was left in place for 48 hours (or for 72 hours when placed over a weekend) following which it was removed and the site again examined for irritation. If no irritation was present, a 0.25% aqueous SLS patch was again reapplied to the same site for 24 hours, followed by a re-treatment with the test product, as described above, to the same site. This sequence viz. 24 hour SLS pre-treatment followed by 48 hours of test material application was continued for a total of 5 induction exposures.

Patches were applied to the upper outer arm, volar forearm or the back of each subject. The entire test was composed of two distinct phases: (1) an Induction phase and (2) a Challenge phase.

If irritation developed at any time-point during the induction phase as previously outlined, the 24-hour SLS pre-treatment patch was eliminated and only the test material was reapplied to the same site after a 24-hour rest period during which no patch was applied.

The aim during this phase of the study was to maintain at least a minimal degree of irritation in order to enhance penetration through the corneum barrier.

(2) **Challenge Phase**:

After a ten day rest period which follows the last induction patch application, the subjects were challenged with a single application of the test material to a new skin site on the opposite arm, forearm or side of back in order to determine if sensitization had developed.

Pre-treatment with SLS was performed prior to challenge. Approximately 0.05ml of a 5.0% aqueous solution was applied to a fresh skin site under a 2x2cm^2 disc of Webril cotton and covered with occlusive tape. The SLS patch was left in place for one hour. It was then removed and the test material was applied to the same site, as outlined above. The challenge patch was then covered by occlusive tape and left in place for 48 hours. After that period, the patch was removed and the site graded 15-30 minutes later and again 24 hours later for any reaction.

SCORING SCALE:

0 = not sensitized

1 = mild sensitization (viz. erythema and a little edema)

2 = moderate sensitization (erythema with infiltration, raised, spreading beyond the borders of the patch, with or without vesiculation)

3 = strong sensitization (large vesiculo-bullous reaction).

Based on these findings the number of subjects with positive responses were tabulated for the test material. The test system shown below was used to classify the allergenic potential of the test substance.

SENSITIZATION RATES:	**GRADES:**	**CLASSIFICATION**:
0 - 2/25	1	Weak
3 - 7/25	2	Mild
8 - 13/25	3	Moderate
14 - 20/25	4	Strong
21 - 25/25	5	Extreme

RESULTS:

A total of twenty-seven (27) healthy, adult volunteers of both sexes who satisfied the inclusion criteria were enrolled into this study. There were 20 females and 7 males. Their ages ranged from 18 to 61 years. Two subjects #04 and #06 voluntarily withdrew for personal reasons unrelated to the study. The remaining 25 subjects completed this investigation as outlined in the standard protocol.

The demographic data are shown in Table 1. No adverse or unexpected reactions were seen in any of the panelists during the induction phase.

The results of the challenge are shown in the enclosed table (Table 2). No instances of contact allergy were recorded at either 48 or 72 hours after the application of the challenge patches.

CONCLUSION:

Under the conditions of this test, the test sample labeled Patch and coded [redacted] does not possess a detectable contact-sensitizing potential and hence is not likely to cause contact sensitivity reactions under normal use conditions.

References:

(1) Kligman, A.M.: The Maximization Test. J.I.D., Vol. 47, No. 5, pp. 393-409, 1966.

(2) Kligman, A.M. and Epstein W.: Updating the Maximization Test for Identifying Contact Allergens. Contact Dermatitis. Vol. 1, 231-239, 1975.

TABLE 1

DEMOGRAPHIC DATA

Subject Number:	Subject Initials:	Age:	Sex:	Race:
01	M-H	23	F	C
02	J-R	24	F	C
03	M-G	60	F	C
04	T-B Sr	44	M	B
05	T-B	19	F	B
06	T-B Jr	21	M	B
07	S-T	50	M	C
08	J-H	37	F	C
09	L-C	55	F	C
10	J-M	61	F	C
11	E-I	55	F	C
12	J-J	44	M	C
13	L-W	35	F	B
14	JEM	47	F	C
15	B-V	22	M	C
16	C-G	36	F	C
17	T-H	41	F	C
18	R-N	53	M	C
19	T-A	45	F	B
20	B-S	44	F	C
21	R-S	23	F	C
22	G-M	44	F	C
23	J-M	26	F	C
24	J-K	24	F	C
25	L-R	55	F	C
26	H-H	50	F	C
27	C-M	18	M	C

C = Caucasian
B = Black

[redacted] **Patch coded** [redacted]

TABLE 2

MAXIMIZATION TESTING RESULTS

Sample: Patch coded [redacted]

Subject Number:	48-Hour Grading	72-Hour Grading
01	0	0
02	0	0
03	0	0
04	Voluntarily withdrew from the study	
05	0	0
06	Voluntarily withdrew from the study	
07	0	0
08	0	0
09	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	0	0
17	0	0
18	0	0
19	0	0
20	0	0
21	0	0
22	0	0
23	0	0
24	0	0
25	0	0
26	0	0
27	0	0

Challenge Readings:

48-Hour Reading – October 13, 2005
72-Hour Reading – October 14, 2005

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: April 29. 2021

SUBJECT: Glucosamine HCl

Anonymous. 2012. Clinical safety evaluation repeated insult patch test (leave-on product containing 0.005% Glucosamine HCL)

[redacted] Comment Only -- Do Not Cite or Quote

FINAL REPORT

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

Leave-on product containing 0.005% Glucosamine HCl

Sponsor

[redacted]

Sponsor Representatives

[redacted]

Clinical Testing Facility

[redacted]

Sponsor Code: [redacted]
Panel No.:
Entry No.: [redacted]

Date of Final Report

6-1-12

[redacted]

[redacted] Panel No.: [redacted]
Entry No.:

SIGNATURE PAGE

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

[redacted]

3 mayor
Date

[redacted]

12/12
Date

[redacted]

12/30/12
Date

[redacted]

QUALITY ASSURANCE STATEMENT

This study ([redacted] Panel No.: [redacted] Entry No.: [redacted]) was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in 21 CFR Part 50 (Protection of Human Subjects – Informed Consent) and the Standard Operating Procedures of [redacted]

For purposes of this clinical study:

_X__ Informed Consent was obtained.

____ Informed Consent was not obtained.

_X__ An IRB review was not required.

____ An IRB review was conducted and approval to conduct the proposed clinical research was granted.

To assure compliance with the study protocol, the Quality Assurance Unit completed an audit of the applicable study records and report. This report is considered a true and accurate reflection of the testing methods and source data.

[redacted]

1 June 2012
Date

[redacted]

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[redacted]

[redacted] Panel No.: [redacted]
Entry No.:

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

1.0 OBJECTIVE

The objective of this study was to determine the irritation and/or sensitization potential of the test article after repeated application under occlusive patch test conditions to the skin of human subjects (exclusive panel).

2.0 SPONSOR

[redacted]

2.1 Sponsor Representatives

[redacted]

3.0 CLINICAL TESTING FACILITY

The study was conducted by:

[redacted]

4.0 CLINICAL INVESTIGATORS

[redacted]

5.0 STUDY DATES

Study initiation: April 11, 2012

Final evaluation: May 18, 2012

[redacted]

6.0 ETHICS

6.1 Ethical Conduct of the Study

This study was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in Title 21 of the U.S. Code of Federal Regulations (CFR), the Declaration of Helsinki and/or [redacted] Standard Operating Procedures.

6.2 Subject Information and Consent

This study was conducted in compliance with CFR Title 21, Part 50 (Informed Consent of Human Subjects). Informed Consent was obtained from each subject in the study and documented in writing before participation in the study. A copy of the Informed Consent was provided to each subject.

7.0 TEST MATERIAL

The test article used in this study was provided by:

[redacted]

It was received on March 19, 2012 and identified as follows:

[redacted] Entry No.	Test Article I.D.	Description
[redacted]	[redacted]	[redacted]

*The test article was volatilized at least 30 minutes, but less than 90 minutes, on the patch prior to application to the skin.

8.0 TEST SUBJECTS

Approximately 50 male or female subjects ranging in age from 18 to 79 years were to be empanelled for this test. Subject demographics are listed in Table 1.

The subjects chosen were to be dependable and able to read and understand instructions. The subjects were not to exhibit any physical or dermatological condition that would have precluded application of the test article or determination of potential effects of the test article.

9.0 TEST PROCEDURE

The 9 Repeated Insult (occlusive) Patch Test (9-RIPT)[1] was conducted as follows:

9.1 Induction Phase

A sufficient amount of the test article (approximately 0.1 g – 0.15 g) was placed onto a Parke-Davis Readi-Bandage® occlusive patch (approximately 25 - 38 mg/cm^2 of test material) and applied to the back of each subject between the scapulae and waist, adjacent to the spinal mid-line. This procedure was performed by a trained technician/examiner and repeated every Monday, Wednesday and Friday until 9 applications of the test article had been made.

The subjects were instructed to remove the patch 24 hours after application. Twenty-four hour rest periods followed the Tuesday and Thursday removals and 48-hour rest periods followed each Saturday removal. Subjects returned to the Testing Facility and the site was scored by a trained examiner just prior to the next patch application.

If a subject developed a positive reaction of a level 2 erythema or greater during the Induction phase or if, at the discretion of the Study Director, the skin response warranted a change in site, the patch was applied to a previously unpatched, adjacent site for the next application. If a level 2 reaction or greater occurred at the new site, no further applications were made. However, any reactive subjects were subsequently Challenge patch tested.

9.2 Challenge Phase

After a rest period of approximately 2 weeks (no applications of the test article), the Challenge patch was applied to a previously unpatched (virgin) test site. The site was scored 24 and 72 hours after application. All subjects were instructed to report any delayed skin reactivity that occurred after the final Challenge patch reading. When warranted, selected test subjects were called back to the Clinic for additional examinations and scoring to determine possible increases or decreases in Challenge patch reactivity.

Dermal responses for both the Induction and Challenge phases of the study were scored according to the following 6-point scale:

0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

All other observed dermal sequelae (eg, edema, dryness, hypo- or hyperpigmentation) were appropriately recorded on the data sheet and described as mild, moderate or severe.

[1] Marzulli FN, Maibach HI. (1976) Contact allergy: predictive testing in man. *Contact Dermatitis.* 2, 1-17.

[redacted]

9.0 TEST PROCEDURE (CONT'D)

9.3 Data Interpretation

Edema, vesicles, papules and/or erythema that persist or increase in intensity either during the Induction and/or Challenge phase may be indicative of allergic contact dermatitis. Allergic responses normally do not resolve or improve markedly at 72-96 hours.

Exceptions to typical skin reactions may occur. These may include, but not be limited to, symptoms of allergic contact sensitivity early in the Induction period to one or more test products. When this occurs in one subject, such a reaction usually suggests either an idiosyncratic response or that the subject had a pre-exposure/sensitization to the test material or component(s) of the test material or a cross-reactivity with a similar product/component. Data for such reactions will be included in the study report but will not be included in the final study analysis/conclusion of sensitization.

10.0 RESULTS AND DISCUSSION

(See Table 2 for Individual Scores)

A total of 55 subjects (18 males and 37 females ranging in age from 20 to 76 years) were empanelled for the testing procedure. Fifty-one (51/55) subjects satisfactorily completed the test procedure on Test Article: [redacted] Four (4/55) subjects discontinued for personal reasons unrelated to the conduct of the study. Discontinued subject data are shown up to the point of discontinuation, but are not used in the Conclusions section of this final report.

Induction Phase Summary

Test Article	Induction Scores (Number of Responses)						Evidence of Irritation
	0.5	1	2	3	4	Other	
[redacted]	0	0	0	0	0	0	No

Challenge Phase Summary

Test Article	Challenge Scores (Number of Responses)						Evidence of Sensitization
	0.5	1	2	3	4	Other	
[redacted]	0	0	0	0	0	0	No

There was no skin reactivity observed at any time during the course of the study.

11.0 CONCLUSIONS

Under the conditions of a repeated insult (occlusive) patch test procedure conducted in 51 subjects, Test Article: [redacted] was "Dermatologist-Tested" and was not associated with skin irritation or allergic contact dermatitis in human subjects.

[redacted]

[redacted] Panel No.:
Entry No.: [redacted]

TABLE 1

SUBJECT DEMOGRAPHICS

Test Article [redacted]

Subject No.	Initials	Age	Sex	Race	Subject No.	Initials	Age	Sex	Race
1	LAF	51	F	CA	29	KDV	58	M	CA
2	R-B	44	F	CA	30	J-B	63	F	CA
3	K-A	54	F	CA	31	MLA	56	F	CA
4	LJR	58	F	AS	32	EBB	20	M	CA
5	J-B	69	F	CA	33	M-V	38	F	CA
6	ALP	48	M	HS	34	R-P	58	F	CA
7	CLK	56	F	CA	35	FVC	60	F	CA
8	B-B	56	F	HS	36	M-H	20	M	CA
9	M-H	59	F	CA	37	EWH	45	M	CA
10	JAB	73	F	CA	38	A-D	22	M	HS
11	OIC	44	F	HS	39	RBM	21	M	HS
12	PAR	66	F	CA	40	RSC	60	M	CA
13	S-E	49	F	CA	41	BFA	46	F	BA
14	KAT	34	F	CA	42	S-F	42	F	BA
15	MCM	73	M	CA	43	S-H	53	F	BH
16	M-C	75	F	CA	44	SEP	25	M	BA
17	JGH	70	F	CA	45	T-P	24	M	BA
18	M-H	71	F	CA	46	S-W	55	F	CA
19	DWS	52	M	CA	47	GSB	55	F	CA
20	DRM	22	M	HS	48	D-A	55	F	CA
21	C-F	70	F	CA	49	J-M	25	F	CA
22	V-M	39	F	HS	50	L-I	54	F	CA
23	JML	47	M	CA	51	FPI	60	M	CA
24	RTR	57	M	AS	52	CLH	59	F	CA
25	BJB	51	M	CA	53	VRM	58	M	HS
26	J-K	61	F	CA	54	DMM	56	F	CA
27	JAD	56	F	CA	55	CMC	74	F	CA
28	RMB	76	F	CA					

AS = Asian or Pacific Islander
BA = Black/African American
BH = Black Hispanic
CA = Caucasian
HS = Hispanic

Shaded area = Discontinued subject

[redacted]

TABLE 2

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST -- OCCLUSIVE

Test Article: [redacted]

Subj. No.	Induction Evaluation Number									Challenge Virgin Site	
	1	2	3	4	5	6	7	8	9	24hr	72hr
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0
10	0	Discontinued									
11	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

[redacted]

TABLE 2 (CONT'D)

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - OCCLUSIVE

Test Article: [redacted]

Subj. No.	Induction Evaluation Number 1	2	3	4	5	6	7	8	9	Challenge Virgin Site 24hr	72hr
31	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	Discontinued					
37	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0
39	0	Discontinued									
40	Discontinued										
41	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = Barely perceptible (Minimal, faint, uniform or spotty erythema)
1 = Mild (Pink, uniform erythema covering most of the contact site)
2 = Moderate (Pink-red erythema uniform in the entire contact site)
3 = Marked (Bright red erythema with/without petechiae or papules)
4 = Severe (Deep red erythema with/without vesiculation or weeping)

[redacted]

Concentration of Use by FDA Product Category – Glucosamine Ingredients*

Acetyl Glucosamine
Glucosamine
Glucosamine HCl
Glucosamine Sulfate

Ingredient	Product Category	Maximum Concentration of Use
Acetyl Glucosamine	Eye lotions	2%
Acetyl Glucosamine	Other eye makeup preparations	0.2%
Acetyl Glucosamine	Hair conditioners	0.001-0.55%
Acetyl Glucosamine	Hair sprays Pump sprays	0.1%
Acetyl Glucosamine	Tonics, dressings and other hair grooming aids	0.005-0.07%
Acetyl Glucosamine	Other hair preparations (noncoloring)	0.005%
Acetyl Glucosamine	Hair rinses (coloring)	0.01%
Acetyl Glucosamine	Face powders	0.07%
Acetyl Glucosamine	Foundations	0.2-2%
Acetyl Glucosamine	Lipstick	0.002-2%
Acetyl Glucosamine	Makeup bases	2%
Acetyl Glucosamine	Other makeup preparations	2%
Acetyl Glucosamine	Other makeup preparations	2%
Acetyl Glucosamine	Deodorants Not spray	0.01%
Acetyl Glucosamine	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.07-0.1%
Acetyl Glucosamine	Face and neck products Not spray	2-5%
Acetyl Glucosamine	Body and hand products Not spray	0.12-2%
Acetyl Glucosamine	Moisturizing products Not spray	0.1-0.15%
Acetyl Glucosamine	Night products Not spray	0.1-2%
Acetyl Glucosamine	Paste masks and mud packs	5%
Acetyl Glucosamine	Suntan products Not spray	0.1%
Glucosamine	Face and neck products Not spray	0.04%
Glucosamine HCl	Eye lotions	0.0001-0.2%
Glucosamine HCl	Hair conditioners	0.55%
Glucosamine HCl	Foundations	0.0001-0.075%
Glucosamine HCl	Makeup bases	0.0001-0.02%
Glucosamine HCl	Aftershave lotions	0.03%
Glucosamine HCl	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.07%
Glucosamine HCl	Face and neck products Not spray	0.0006-0.38%

Glucosamine HCl	Moisturizing products Not spray	0.15-0.38%
Glucosamine HCl	Night products Not spray	0.15%
Glucosamine HCl	Paste masks and mud packs	5%
Glucosamine HCl	Other skin care preparations	0.12-0.9%
Glucosamine HCl	Suntan products Not spray	0.021%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.

Information collected in 2019-2020
Table prepared: February 27, 2020

2021 FDA VCRP data – Glucosamine

Acetyl Glucosamine – 117 total uses

Eye Lotion	8
Other Eye Makeup Preparations	2
Tonics, Dressings, and Other Hair Grooming Aids	1
Foundations	1
Lipstick	2
Makeup Bases	1
Other Makeup Preparations	1
Other Personal Cleanliness Products	1
Cleansing	8
Face and Neck (exc shave)	23
Body and Hand (exc shave)	16
Moisturizing	26
Night	4
Paste Masks (mud packs)	3
Skin Fresheners	4
Other Skin Care Preps	16

Glucosamine – 4 total uses

Eye Lotion	1
Face and Neck (exc shave)	2
Body and Hand (exc shave)	1

Glucosamine HCl – 69 total uses

Eye Lotion	3
Other Eye Makeup Preparations	1
Hair Conditioner	2
Shampoos (non-coloring)	2
Tonics, Dressings, and Other Hair Grooming Aids	3
Other Hair Preparations	3
Face Powders	1
Foundations	1
Makeup Bases	1
Other Makeup Preparations	1
Aftershave Lotion	1
Shaving Cream	1
Other Shaving Preparation Products	1
Cleansing	3
Face and Neck (exc shave)	21
Moisturizing	16
Night	4
Paste Masks (mud packs)	3
Other Skin Care Preps	1