Safety Assessment of *Rosa damascena*-Derived Ingredients as Used in Cosmetics

Status:	Draft Report for Panel Review
Release Date:	August 20, 2021
Panel Meeting Date:	September 13 - 14, 2021

The members of the Expert Panel for Cosmetic Ingredient Safety 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 Preethi S. Raj, M.Sc., Senior Scientific Analyst/ Writer, CIR.

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Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons

From: Preethi S. Raj, M.Sc.
Senior Scientific Analyst/Writer, CIR

Date: August 20, 2021

Subject: Safety Assessment of *Rosa damascena*-Derived Ingredients as Used in Cosmetics

Enclosed is the Draft Report of the Safety Assessment of *Rosa damascena*-Derived Ingredients as Used in Cosmetics (identified as *rosdam092021rep* in the pdf). This is the first time the Panel is seeing a safety assessment of these 10 cosmetic ingredients. A Scientific Literature Review (SLR) was announced on November 19, 2020.

Concentration of use data were received from the Council in 2019 (*rosdam092021data1*). The following data were received in response to the SLR, and have been incorporated in the report:

1. Information for a trade name mixture that contains 0.1 - 1.0% Rosa Damascena Flower Water and 0.1 - 1% Rosa Damascena Flower Oil in pentylene glycol (*rosdam092021data2*)
    a. Composition Breakdown (2020)
    b. Specification Criteria (2020)
    c. Allergens certificate (2019)
    d. Characteristic molecules certificate (2019)
    e. Toxicological File (2020)
2. Rosa Damascena Flower Water in a trade name mixture with butylene glycol (method of manufacture and impurities) (2020; *rosdam092021data3*)
3. HRIPT of a fragrance product containing 0.1068% Rosa Damascena Flower Water and of a fragrance product containing 0.7794% Rosa Damascena Flower Extract (2012; *rosdam092021data4*)
4. HRIPT of a mask formulation containing 0.1260% Rosa Damascena Flower Oil (2019; *rosdam092021data5*)

Comments on the SLR (*rosdam092021pcpc*) that were received from the Council have been addressed. Also included in this package, for your review, are a flow chart (*rosdam092021flow*), literature search strategy (*rosdam092021strat*), ingredient data profile (*rosdam092021prof*), ingredient history (*rosdam092021hist*), and 2021 FDA VCRP data (*rosdam092021FDA*).

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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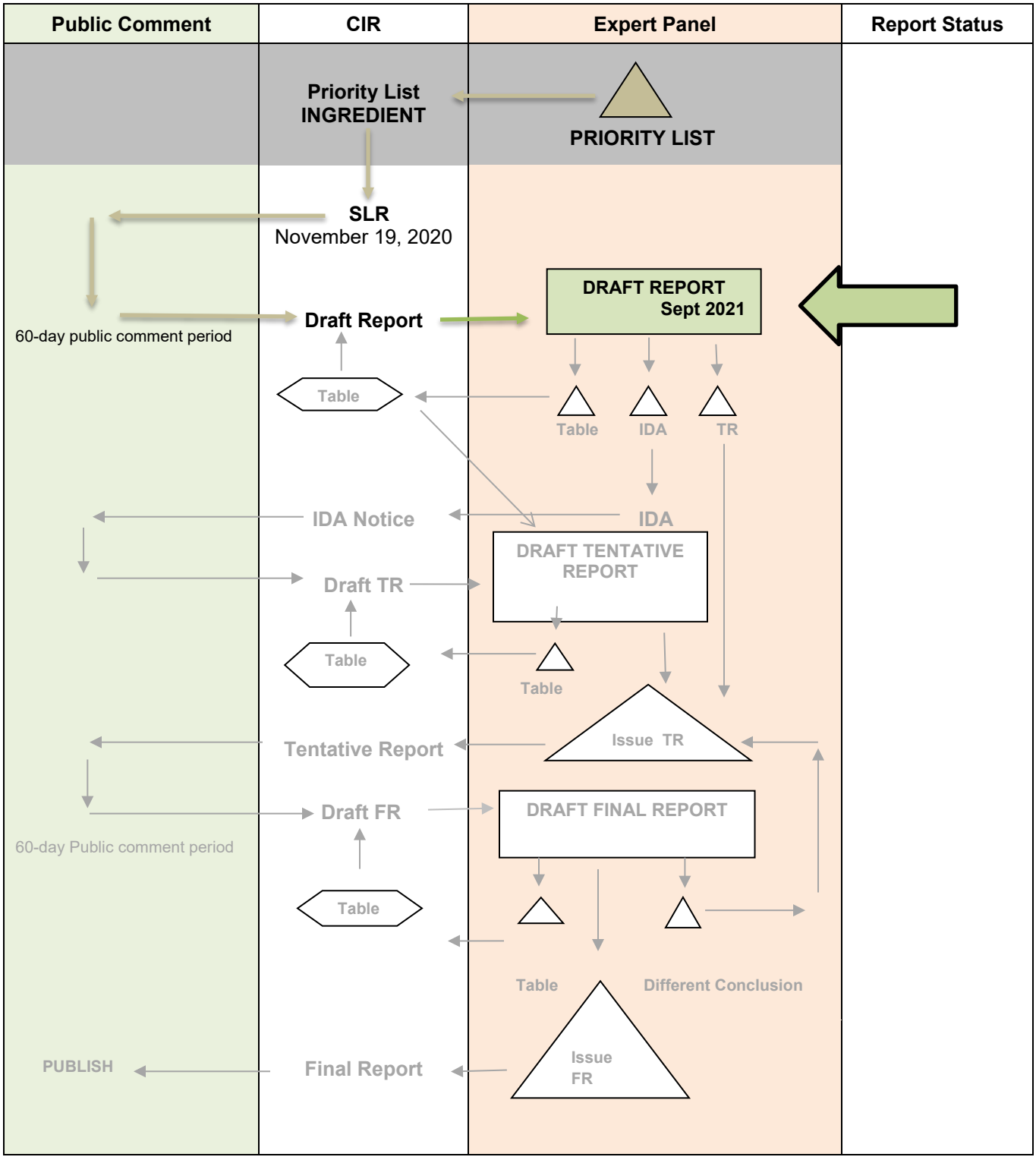
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Rosa damascena-derived ingredients

MEETING September 2021



CIR History of:

Rosa damascena-derived Ingredients

July 2019

-Concentration of use data submitted by Council

November 2020

- SLR posted on the CIR website

December 2020

Data received:

- December 2, 2020: Information for a trade name mixture that contains 0.1-1.0% Rosa Damascena Flower Water and 0.1-1% Rosa Damascena Flower Oil in Pentylene Glycol
- December 10, 2020: Rosa Damascena Flower Water in a trade name mixture with Butylene Glycol (method of manufacture and impurities).

February 2021

Data received:

- February 18, 2021: Two HRIPTs of fragrance products, containing 0.1068% Rosa Damascena Flower Water and 0.7794% Rosa Damascena Flower Extract
- February 18, 2021: HRIPT of a mask formulation, containing 0.1260% Rosa Damascena Flower Oil

January 2021

New VCRP data were received

September 2021

A Draft Report is being presented to the Panel.

Rosa damascena-derived Ingredients Data Profile* – September 13-14, 2021 – Writer, Preethi Raj																															
				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/log K_{ow}	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
Hydrolyzed Rosa Damascena Flower Extract																													
Rosa Damascena Bud Extract		X																											
Rosa Damascena Extract	X																												
Rosa Damascena Flower	X		X																										
Rosa Damascena Flower Extract	X	X						X			X													X						
Rosa Damascena Flower Oil	X	X	X				X	X							X				X	X	X	X		X	X	X		X	X
Rosa Damascena Flower Powder	X	X																											
Rosa Damascena Flower Water	X	X	X					X							X				X		X	X		X	X	X			
Rosa Damascena Flower Water Extract	X	X						X			X																		
Rosa Damascena Flower Wax	X	X																											

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

[*Rosa damascena*- derived ingredients – (10 ingredients - September 13-14, 2021 Panel Meeting]

Ingredient/CAS #	InfoB	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Hydrolyzed Rosa Damascena Flower Extract	✓	NR	NR	NR	✓*	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	
Rosa Damascena Bud Extract 90106-38-0	✓	NR	NR	✓	✓*	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	
Rosa Damascena Extract 90106-38-0	✓	✓*	✓	NR	✓*	✓	NR	NR	NR	NR	✓*	✓	NR	NR	NR	NR	NR	✓
Rosa Damascena Flower 90106-38-0	✓	✓*	✓	✓	✓*	NR	NR	NR	NR	NR	✓*	✓	NR	NR	NR	NR	NR	✓
Rosa Damascena Flower Extract 90106-38-0	✓	✓*	✓*	NR	✓*	NR	NR	NR	NR	NR	✓*	✓	NR	NR	NR	NR	NR	
Rosa Damascena Flower Oil 8007-01-0 90106-38-0	✓	✓*	✓*	✓	✓*	✓*	NR	NR	NR	NR	✓*	✓	NR	NR	✓	NR	✓	✓
Rosa Damascena Flower Powder 90106-38-0	✓	NR	NR	NR	✓*	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	
Rosa Damascena Flower Water 90106-38-0	✓	✓*	✓*	NR	✓*	NR	NR	NR	NR	NR	✓*	✓	NR	NR	NR	NR	NR	
Rosa Damascena Flower Water Extract 90106-38-0	✓	✓*	✓*	NR	✓*	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	✓
Rosa Damascena Flower Wax 90106-38-0	✓	NR	NR	NR	✓*	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	

✓ - data pertaining to safety was found
✓* - reported, but no data relevant to safety was found
NR- not reported

Botanical and/or Fragrance Websites (if applicable)

Ingredient	CAS #	Dr. Duke's	Taxonomy	GRIN #	Sigma-Aldrich	IFRA	RIFM
Hydrolyzed Rosa Damascena Flower Extract							
Rosa Damascena Bud Extract	90106-38-0						
Rosa Damascena Extract	90106-38-0	✓	✓	5328	✓*	✓	
Rosa Damascena Flower	90106-38-0						
Rosa Damascena Flower Extract	90106-38-0						
Rosa Damascena Flower Oil	8007-01-0 90106-38-0					✓	✓
Rosa Damascena Flower Powder	90106-38-0						
Rosa Damascena Flower Water	90106-38-0						
Rosa Damascena Flower Water Extract	90106-38-0						
Rosa Damascena Flower Wax	90106-38-0						

Search Strategy in PubMed [*# useful hits / total # of hits*]
Hydrolyzed Rosa damascena flower extract – 0/0
Rosa damascena bud extract – 0/1
Rosa damascena extract - 18/90
Rosa damascena flower – 11/49
Rosa damascena flower extract – 8/26
Rosa damascena flower oil – 15/19
Rosa damascena flower powder – 0/1
Rosa damascena flower water – 7/14
Rosa damascena flower water extract – 2/5
Rosa damascena flower wax – 0/0
Rosa damascene/a HRIPT – 0/0
Rosa damascena toxicity – 2/17
Rosa damascene – 3/31
Rosa damascena oil allergy – 0/0
Rose oil dermatitis - 3/8
Rose oil contact allergen- 1/1
Rose oil sensitization – 0/5
Rose oil photosensitization – 0/5
Rose oil depigmentation – 0/0
((((((((((hydrolyzed rosa damascena flower extract) OR rosa damascena bud extract) OR rosa damascena extract) OR rosa damascena flower) OR rosa damascena flower extract) OR rosa damascena flower oil) OR rosa damascena flower powder) OR rosa damascena flower water) OR rosa damascena flower water extract) OR rosa damascena flower wax) OR 90106-38-0) OR 8007-01-0) AND:
Toxicity -0/1
Cosmetic toxicity – 1/2
Reproductive effects- 0/4
Ocular irritation- 0/0
Skin irritation – 0/0
Inhalation toxicity – 0/0
Ocular toxicity – 0/0
Teratogenicity – 0/0
Immune – 2/3
(rosa damascene) OR (90106-38-0) AND (toxicity) – 2/20
((rosa damascena extract) OR (90106-38-0)) AND (toxicity) – 0/15
tox [subset] AND (rosa damascena extract) OR (90106-38-0) – 7/34

General Web Search Strategy [*# useful hits / total # of hits*]
Hydrolyzed rosa damascene flower extract – 0/159,000
Rosa damascene/a HRIPT -0/0; 2/310
Rosa damascena skin or dermal sensitization/irritation -0/0
Rosa damascena oil allergy – 0/263,000

LINKS

Search Engines

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)
- Toxnet (https://toxnet.nlm.nih.gov/); (includes Toxline; HSDB; ChemIDPlus; DART; IRIS; CCRIS; CPDB; GENE-TOX)

Connected Papers - https://www.connectedpapers.com/

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;,
- EAFUS: http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true
- 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
- http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf

- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- OTC ingredient list: https://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm135688.pdf
- (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/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
- 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/
- IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
- 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
- NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.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

Botanical Websites, if applicable

- Dr. Duke's - https://phytochem.nal.usda.gov/phytochem/search
- Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
- GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
- Sigma Aldrich plant profiler- http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html
- American Herbal Products Association Botanical Safety Handbook (database) - http://www.ahpa.org/Resources/BotanicalSafetyHandbook.aspx
- European Medicines Agency Herbal Medicines - http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/herbal_search.jsp
- National Agricultural Library NAL Catalog (AGRICOLA) https://agricola.nal.usda.gov/
- The Seasoning and Spice Association List of Culinary Herbs and Spices
- http://www.seasoningandspice.org.uk/ssa/background_culinary-herbs-spices.aspx

Fragrance Websites, if applicable

- IFRA (International Fragrance Association) – http://www.ifraorg.org/
- Research Institute for Fragrance Materials (RIFM)

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ABBREVIATIONS

ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
Cyt B	cytochalasin B
Dictionary	*International Cosmetic Ingredient Dictionary and Handbook*
DMEM	Dulbecco's modified Eagle's medium
DMSO	dimethyl sulfoxide
EC	maximal effective concentration
ECHA	European Chemicals Agency
FBS	fetal bovine serum
FDA	Food and Drug Administration
GAE	gallic acid equivalents
GC-MS	gas chromatography – mass spectroscopy
GRAS	generally recognized as safe
HCA	hydrocitric acid
h-CLAT	human cell line activation test assay
HeLa	human cervical cancer cell line
HDL	high-density lipoprotein
HRIPT	human repeat insult patch test
IFRA	International Fragrance Association
LD	lethal dose
LDL	low-density lipoprotein
MIT	minimum induction threshold
MMC	mitomycin C
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
N/A	not applicable
NR	not reported/none reported
NS	not specified
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate-buffered saline
PHA	phytohemagglutinin
RIFM	Research Institute for Fragrance Materials
RPMI	Roswell Park Memorial Institute
SCCS	Scientific Committee on Consumer Safety
SLS	sodium lauryl sulfate
TG	test guideline
TG	triglyceride
THP-1	human monocytic leukemia cell lines
TNBS	trinitrobenzenesulfonic acid
US	United States
VCRP	Voluntary Cosmetic Registration Program

INTRODUCTION

This assessment reviews the safety for the following 10 *Rosa damascena*-derived ingredients, as used in cosmetic formulations:

Hydrolyzed Rosa Damascena Flower Extract
Rosa Damascena Bud Extract
Rosa Damascena Extract
Rosa Damascena Flower
Rosa Damascena Flower Extract
Rosa Damascena Flower Oil
Rosa Damascena Flower Powder
Rosa Damascena Flower Water
Rosa Damascena Flower Water Extract
Rosa Damascena Flower Wax

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), some *Rosa damascena*-derived ingredients are reported to function as skin conditioning agents and fragrance ingredients in cosmetic products (Table 1).[1] Additionally, these ingredients are sometimes reported to function as antioxidants and cosmetic astringents. Common names for *Rosa damascena* include damask rose, pink rose, Turkish rose, and Bulgarian rose.[2]

The Expert Panel for Cosmetic Ingredient Safety (Panel) does not review ingredients that function only as fragrance ingredients because, as fragrances, the safety of these ingredients is evaluated by the Research Institute for Fragrance Materials (RIFM). Rosa Damascena Extract, Rosa Damascena Flower Extract, Rosa Damascena Flower Powder, and Rosa Damascena Flower Wax are reported to function only as fragrance ingredients, according to the wINCI *Dictionary*. However, according to personal communications with RIFM in May-June 2020, these ingredients have not been reviewed, and are not currently scheduled for review by RIFM; thus, the Panel is reviewing the safety of these ingredients.

Rosa damascena fruit, *Rosa damascena* seeds, *Rosa damascena* concrete oil, and *Rosa damascena* absolute oil are not cosmetic ingredients, and, therefore, the safety of these materials is not being reviewed. However, information regarding these materials has been included as it may be helpful in determining the safety of the other named ingredients. The relevancy of this data has yet to be determined by the Panel.

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

Botanicals, such as *Rosa damascena*-derived ingredients, may contain hundreds of constituents. However, in this assessment, the Panel is evaluating the potential toxicity of each botanical ingredient as a whole, complex substance; potential toxicity from exposures to mixtures of different chemical compounds may not replicate the biological activity of the individual components.

Also, with botanicals, it is often not known how the substance being tested in a study compares to the cosmetic ingredient. In the report text, if it is known that the material being tested is a cosmetic ingredient, the INCI naming convention is used (i.e., the names of cosmetic ingredients are capitalized, without italics (e.g., Rosa Damascena Extract)). If it is not known that the test substance is the same as the cosmetic ingredient, the taxonomic naming conventions (i.e. with genus and species name italicized (e.g., a *Rosa damascena* extract)) is used.

CHEMISTRY

Definition and Plant Identification

Definitions of the 10 *Rosa damascena*-derived ingredients reviewed in this safety assessment are presented in Table 1.[1] Generically, the bud is defined as a not yet developed shoot in the axil of a leaf, often covered with scales, or a young flower that has not bloomed. The flower is defined as the reproductive shoot in flowering plants, usually with sepals, petals, stamens, and pistil(s).

Rosa damascena is an ornamental, old garden rose hybrid, belonging to the Rosaceae family, with more than 200 species and 18,000 cultivars around the world.[3] Thought to originate in the Mediterranean or Asia, *Rosa damascena* is mainly grown in Turkey, Bulgaria, Morocco, Iran, Egypt, France, China, and India, with Turkey and Bulgaria reported to be the largest producers.[2] Commonly known as the damask rose, *Rosa damascena* is one of the few rose species which possesses the characteristic rose fragrance, owing to its highly valuable aromatic oil.[2] The total world production of *Rosa damascena* oil and *Rosa damascena* concrete (flower wax) is estimated to be 15 - 20 tons.

Rosa damascena is a thorny shrub, up to 2.5 m in height, that blooms in the spring.[4] The stem has numerous stout and hooked prickles, occasionally mixed with glandular bristles, while the leaves are pinnate and compound with 5 - 7 leaflets that are 2.5 - 6.3 cm long, ovate-oblong, and have serrated edges.[5] Flowers have an average of 33 petals, which are arranged in a corymb, and can range in color from white to light red; most *Rosa damascena* flowers are light pink or magenta in hue.[4,5]

Chemical Properties

A summary of chemical properties described for *Rosa damascena*-derived ingredients are provided in Table 2.

Solid residues, containing mainly straight-chain saturated hydrocarbons and the esters of carboxylic acids, were identified in a gas chromatography-mass spectrometry (GC-MS) analysis of *Rosa damascena* flower extracts (absolute), produced from a *Rosa damascena* flower wax (concrete).[6]

Rosa Damascena Flower Oil and Rosa Damascena Flower Water

A supplier described a trade mixture, comprising 0.1 - 1 % Rosa Damascena Flower Oil and 0.1 - 1% Rosa Damascena Flower Water formulated in pentylene glycol, as a transparent, colorless liquid with a characteristic odor.[7] At 20 °C, the refractive index of this trade mixture is 1.434 - 1.444.

Method of Manufacture

Most of the methods below are general to the processing of *Rosa damascena*-derived materials, and it is unknown if these apply to cosmetic ingredient manufacturing. In some cases, the definition of the ingredients, as given in the *Dictionary*, provides insight as to the method of manufacture.[1]

Rosa Damascena Bud Extract

In a method of preparing a *Rosa damascena* bud extract, 1700 g of air-dried, whole buds of *Rosa damascena* were coarsely powdered and extracted with distilled water at 100 °C for 2 h.[8] Upon removal of the water under vacuum, 720 g of a *Rosa damascena* bud extract were obtained, suspended in distilled water, and sequentially partitioned with n-hexane, chloroform, ethyl acetate, and n-butanol to create multiple fractions.

Rosa Damascena Flower Extract

In a pharmacological analysis of *Rosa damascena* petals, 100 g of dried *Rosa damascena* flower powder was passed through a sieve and macerated, separately, with water, ethanol, chloroform, ethyl acetate, and petroleum ether for 7 d, with occasional agitation.[9] The extracts were filtered through muslin cloth, and the filtrates were evaporated under reduced pressure, vacuum dried, and stored.

A *Rosa damascena* extract was produced from rose blossoms spent in a hydrodistillation process.[6] Sufficient amounts of citronellol, nerol, geraniol, and β-phenethyl alcohol were found, suggesting the utility of rose waste in obtaining valuable extracts.

Rosa Damascena Flower Oil

A large quantity of *Rosa damascena* flowers yields a relatively small amount of a *Rosa damascena* flower oil (e.g., 4000 kg of flowers yields 1 kg of oil).[10] Optimal yield and higher quality *Rosa damascena* flower oil is produced from roses freshly picked in either the early morning hours or colder temperatures, compared to roses subject to heat or fermentation, due to minimal evaporation.[2,11]

A *Rosa damascena* flower oil has been manufactured traditionally for centuries, using copper stills, loosely connected to a condensing apparatus.[12] In the present-day, a *Rosa damascena* flower oil is often produced industrially in well-sealed, steel stills, producing oils with a richer constituent profile, which are of higher quality.[13]

Rosa Damascena Flower Oil and Rosa Damascena Flower Water

A *Rosa damascena* flower water is often a by-product of the hydrodistillation process to produce a *Rosa damascena* flower oil.[2] Both fresh and dried *Rosa damascena* flowers can be utilized in the manufacture of a *Rosa damascena* flower oil and water.[14,15] In a study using fresh *Rosa damascena* flowers, 400 g of fresh flower petals were hydrodistilled with 2 l of water for 4 h in a Clevenger apparatus, to yield 800 ml of a *Rosa damascena* flower water.[14] In a study using shade-dried *Rosa damascena* petals, 60 g of rose petals (with 79.3% moisture removed) were hydrodistilled with 1.5 l of water for 4 h to prepare 800 ml of a *Rosa damascena* flower water.[15]

Rosa damascena flower oil, and consequently *Rosa damascena* flower water, are often produced by the hydrodistillation of *Rosa damascena* flowers in a Clevenger apparatus, or via an analogous steam distillation procedure.[15] In a method of preparing a *Rosa damascena* flower oil, a cauldron was filled with 200 kg of fresh *Rosa damascena* flowers and water, and boiled for approximately an hour.[10] After boiling, steam transported through an attached condensing pipe to a refrigerator yielded a distillation product of a *Rosa damascena* flower water. This *Rosa damascena* flower water moved from the first Floridian container, where a very small quantity of oil (~15%) was segregated, and the water was boiled for about 2.5 h, twice, before condensing in a separate refrigerated pipe, where it passed through a second Floridian container, and separated from the remaining oil. After repeated distillations, rose oil from both Floridian containers was combined and passed through a clean filter; a final yield of 50 - 60 g of a *Rosa damascena* flower oil was obtained.

Rosa Damascena Flower Powder

Rosa damascena flower petals were separated from the sepals and shade-dried.[9] The dried petals were then ground into a fine powder, resulting in a *Rosa damascena* flower powder.

Rosa Damascena Flower Water

A supplier has reported that Rosa Damascena Flower Water is also produced from dried raw material.[16] The water phase of dried *Rosa damascena* flowers processed via steam distillation is further concentrated and added to a 80%, 1,3-butylene glycolic solution.

Rosa Damascena Flower Water Extract

Fresh *Rosa damascena* flowers (2.0 kg) were soaked in 12.5 l water overnight to yield 1.0 l of a *Rosa damascena* flower water.[17] Well-stirred *Rosa damascena* flower water (500 ml) was extracted with dichloromethane (250 ml x 5) and dried over fused calcium chloride/anhydrous sodium sulfate. After the solvent was removed by distillation, a *Rosa damascena* flower water extract (dichloromethane) was stored at -5 °C.

Rosa Damascena Flower Wax

Volatile, hydrocarbon solvents, such as ethyl alcohol, hexane, petroleum ether, and benzene, are often used to extract *Rosa damascena* absolute and concrete, a semisolid, waxy substance from *Rosa damascena* flowers.[12,18] During the industrial production of this *Rosa damascena* flower wax, 600 - 750 kg of *Rosa damascena* flowers were added to a 3000 l extraction vessel, filled half-way with n-hexane, and extracted in two cycles for 20 min at 60 - 65 °C.[18] The resulting extracts were combined in an evaporator, and traces of the solvent were removed in a vacuum evaporator, to yield > 1 kg of a *Rosa damascena* flower wax.

Composition and Impurities

Of the 26 allergens defined by the European Union Cosmetic Directive, benzyl alcohol, eugenol, geraniol, citronellol, limonene, linalool, and farnesol are present in *Rosa damascena*-derived ingredients.[2,14,15,17-22] The components identified in *Rosa damascena*-derived ingredients can vary greatly, depending upon extraction solvent and method,[15] part of the plant,[8] or growth and harvest conditions.[23,24] A percent-composition profile of constituents found in a *Rosa damascena* flower oil, a flower water, a flower water extract, and a flower wax, produced from dried and fresh flowers, is presented in Table 3.

Rosa Damascena Bud Extract

A *Rosa damascena* bud extract (720 g), obtained via hydrodistillation, was used to create concentrated fractions with n-hexane (0.5 g), chloroform (2.8 g), ethyl acetate (124.7 g), n-butanol (274.4 g), and water (317.6 g).[8] Repeated silica gel, octadecyl silane, and Sephadex LH-20 column chromatography of the ethyl acetate fraction yielded five main flavonoids, including: isoquercitrin, afzelin, cyanidin-3-*O*-β-glucoside, quercetin gentiobioside, and kaempferol-3-*O*-β-D-glucopyranosyl(1→4)-β-D-xylopyranoside.

Rosa Damascena Extract

Flavonoids, such as kaempferol, quercetin, and pectolinargenin, were identified as the major components in hydroalcoholic, ethyl acetate: ethanol, and ether extracts of dried and powdered *Rosa damascena* flowers.[25]

In an compositional analysis of *Rosa damascena* fruit extracts, a 5 g sample of *Rosa damascena* fruit yielded 332 mg/100 g ascorbic acid, while a 2 g sample of *Rosa damascena* fruit yielded 7.10 µg/g α- tocopherol, and 3.70 µg/g β- carotene.[26] The fatty acid content was determined to be 93.18% in *Rosa damascena* fruit seed oil.

Rosa Damascena Flower

In a reversed phase high performance liquid chromatography test of fresh *Rosa damascena* flowers, the following components were identified, in µg/ml: gallic acid (125.41), rutin (84.98), quercitrin (360.87), myricetin (170.43), quercetin (81.35), and kaempferol (2.36).[27] Trace amounts of catechin were also identified.

Rosa Damascena Flower Extract

The total phenolic content of fresh and spent *Rosa damascena* flowers, used in the hydrodistillation process, was measured in gallic acid equivalents (GAE/g).[28] The GAE of these *Rosa damascena* flowers, extracted with methanol, were measured at 276.02 ± 2.93 mg GAE for fresh flowers, and 248.97 ± 2.96 mg GAE, for spent flowers.

Rosa Damascena Flower Oil

Rosa damascena flower oil is characterized by high percentages of monoterpene alcohols, including citronellol (35.1%), geraniol (17.9%), nerol (8.4%), phenethyl alcohol (2.5%), and linalool (1%).[2] Additionally, various hydrocarbons, oxides, ethers, esters, aldehydes, and phenols are found in *Rosa damascena* flower oil. Citronellol is the major component which determines rose oil quality. Methyl eugenol levels may be over 2.5%, especially in the oils distilled from rose flowers subject to excess or long-term fermentation.[2]

According to International Fragrance Research Association (IFRA) standards, *Rosa damascena* absolute can comprise 0.5% methyl eugenol, 5% geraniol, and 6% citronellol.[29-32] Additionally, *Rosa damascena* oil is reported to contain 1% farnesol, 2% methyl eugenol, 20% geraniol, and 34% citronellol, according to IFRA standards.

Rosa Damascena Flower Oil and Rosa Damascena Flower Water

Specifications provided by a supplier indicate that a trade mixture containing 0.1 - 1% Rosa Damascena Flower Oil and 0.1 - 1% Rosa Damascena Flower Water, formulated in pentylene glycol, should contain 0.15 – 0.35 % phenethyl alcohol and < 10 ppm methyl eugenol.33 In this trade mixture, most of the 26 allergens defined by the European Union Cosmetic Directive are below the level of detection (< 1ppm), with the following exceptions: benzyl alcohol (41 ppm), citral (16 ppm), citronellol (1080 ppm), farnesol (6 ppm), geraniol (365 ppm), and linalool (33 ppm).[34]

This supplier measured the mean concentration of several constituents in the *Rosa damascena* fraction mixture, using 3 batches of the same trade mixture.[35] It was determined that the *Rosa damascena* fraction mixture could contain 1 ppm of benzaldehyde, 2 ppm of pinene, 36 ppm of isobutenyl methyltetrahydropyran, 40 ppm of terpineols, 48 ppm of β-caryophyllene, 50 ppm of citronellal, 150 ppm of 1-nonadecene, 350 ppm of nonadecane, and 400 ppm of nerol.

Rosa Damascena Flower Water

Due to an increased solubility in water, phenethyl alcohol is the major component collected in a *Rosa damascena* flower water, during the hydrodistillation of roses to produce *Rosa damascena* flower oil.[2] In a GC-MS analysis of *Rosa damascena* flower water samples, phenethyl alcohol was present at up to 39.53%, geraniol at up to 24.01%, and β-citronellol at up to 10.26%.[20]

Methanol and ethanol, produced via plant fiber fermentation, were measured in 90 commercial herbal distillates, including 9 *Rosa damascena* water samples, by GC-MS.[36] The methanol content in *Rosa damascena* flower water samples was 9.04 mg/dl. Two *Rosa damascena* flower water samples were found to have the highest average ethanol content (56.77 mg/dl and 38.97 mg/dl).

A supplier reported that Rosa Damascena Flower Water, formulated in a trade mixture with butylene glycol, contained no more than 20 ppm heavy metals and 2 ppm arsenic.[16] No further details were provided.

Rosa Damascena Flower Water Extract

Dichloromethane extracts of a *Rosa damascena* flower water produced from fresh flowers contained up to 50% more *Rosa damascena* flower oil than flower water produced from dried flowers.[17] These fresh flower dichloromethane extracts also contained mostly phenethyl alcohol (69.7 - 81.6%), linalool (1.5 - 3.3%), citronellol (1.8 - 7.2%), nerol (0.2 - 4.2%), and geraniol (0.9 - 7.0%).

Rosa Damascena Flower Wax

Phenethyl alcohol is present at over 50% in a *Rosa damascena* flower wax.[2] Although citronellol, geraniol, and nerol contents are relatively lower, the phenethyl alcohol content is higher in a *Rosa damascena* flower wax than in a *Rosa damascena* flower oil. As per IFRA standards, *Rosa damascena* concrete can naturally comprise 0.5% methyl eugenol, 2.7% geraniol, and 4.7% citronellol.[29-31]

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, Rosa Damascena Flower Water is reported to be used in 308 formulations, and Rosa Damascena Flower Oil is reported to be used in 223 formulations, of which 245 and 180 uses are in leave-on products, respectively (Table 4).[37] Results from the concentration of use survey, conducted in 2019 by the Council, indicate that Rosa Damascena Flower Water and Rosa Damascena Flower Oil have the highest concentrations of use, at up to 32.7% in face and neck products and at up to 10.8% in other skincare preparations, respectively.[38] Hydrolyzed Rosa Damascena Flower Extract and Rosa Damascena Bud Extract are not in reported to be in use, according to the VCRP and industry survey.

These ingredients have been reported to be used in products that may lead to incidental ingestion and exposure to mucous membranes, such as in lipstick, and bath soaps and detergents. For example, Rosa Damascena Flower Oil and Rosa Damascena Flower Wax are reported to be used at up to 0.01% and 1.1% in lipsticks, respectively. Rosa Damascena Flower

Water is reported to be used at up to 0.09% in bath soaps and detergents. Additionally, some of these ingredients are reported to be used in products applied near the eye (e.g., up to 0.13% Rosa Damascena Flower Wax in eyeliners).

Additionally, some of these ingredients are used in cosmetic sprays and could possibly be inhaled; for example, Rosa Damascena Flower Oil is reported to be used at up to 0.0003% in aerosol hair spray. 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.[39,40] 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.[41,42] Rosa Damascena Extract is reported to be used at up to 0.00007% in aerosol spray deodorant formulations. There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[41] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. Rosa Damascena Flower Extract is reported to be used in face powder formulations (concentration of use not reported), and, could therefore possibly be inhaled. 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.[43-45]

The *Rosa damascena*-derived ingredients named in this report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[46] However, according to Regulation EC No 1223/2009, methyl eugenol, a minor component of *Rosa damascena*, is restricted to a maximum concentration of 0.01% in fine fragrances, 0.004% in eau de toilette products, 0.002% in fragrance creams, 0.001% in rinse-off products, and at 0.0002% in other leave-on and oral products that are ready for use.[47]

Non-Cosmetic

According to the US FDA, the essential oils, oleoresins (solvent-fee), and natural extractives/distillates of *Rosa damascena* rose absolute, rose otto, rose buds, rose flowers, and rose fruit are generally recognized as safe (GRAS) for their intended use in foods [21CFR182.20].

Traditionally, *Rosa damascena* flowers and derived products have a wide range of uses in religious ceremonies, pharmaceuticals, and food, especially in the Middle East and Southeast Asia.[4,15,20] Dried *Rosa damascena* flower petals and flower water are added to flavor and embellish food, and are consumed in Iran as a digestive aide.[4,15] In traditional medicine, *Rosa damascena* flower oil and flower water are considered to possess antibacterial, analgesic, antioxidant, and anti-inflammatory properties.[48] Consequently, these ingredients have been used in aromatherapy,[49,50] and for the treatment of many conditions, including skin, eye, and oral ailments,[48] arthritis,[51] dysmennorhea,[52] pediatric seizures,[53] depression, and cognitive decline.[54]

TOXICOKINETIC STUDIES

No relevant toxicokinetic studies on *Rosa damascena*-derived ingredients were found in the published literature, and unpublished data were not submitted. In general, toxicokinetic data are not expected to be found on botanical ingredients because each botanical ingredient is a complex mixture of constituents.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The acute in vitro, dermal, and oral toxicity studies summarized below are described in Table 5.

The acute dermal LD_{50} of *Rosa damascena* flower oil was determined to be ≥ 2500 mg/kg in rabbits.[55-57] No toxic effects were observed and the acute oral LD_{50} of a *Rosa damascena* flower extract, prepared in 0.7% carboxymethylcellulose, was > 2000 mg/kg in male and female Swiss albino mice.[58] In one study, the acute oral LD_{50} of *Rosa damascena* flower oil was determined to be > 5000 mg/kg in rats.[55-57] In subsequent oral toxicity studies, the acute oral LD_{50} of *Rosa damascena* flower oil was determined to be 5525 mg/kg in male rats, and 2975 mg/kg and 3972 mg/kg, in mature and immature female rats, respectively.[56] Swiss albino mice dosed orally with up to 6000 mg/kg of a *Rosa damascena* flower water extract did not die during the 24-h post-treatment observation period, and the acute LD_{50} was determined to be > 6000 mg/kg.[59]

Short-Term and Subchronic Toxicity Studies

Details of the short-term and subchronic oral toxicity studies summarized below are provided in Table 6.

Groups of 10 Wistar rats were administered 0, 2.5, 5, 25, or 50 mg/kg/d aqueous *Rosa damascena* flower extract, via gavage, for 30 d.[60] Body weight gain was greater in all test groups compared to controls, but the percent weight gains were not statistically significant. Groups of 5 dogs were administered distilled water or lactulose (controls), or, 90, 180, 360, 720, or 1440 mg/kg/d aqueous *Rosa damascena* flower extract for 10 d.[61] No significant differences were observed between groups for respiration, temperature, or cardiac response. A dose-dependent increase of diarrhea was observed, starting with the lowest dose of 90 mg/kg/d. Animals in the 720 and 1440 mg/kg/d groups exhibited slight weight loss after day 7, which was

attributed to possible diarrhea-induced malabsorption, or dehydration; no further changes or adverse effects were observed. Fifteen Swiss albino mice (compared to 10 controls) were administered 300 mg/kg/d *Rosa damascena* flower water extract for 28 d.[59] No significant differences from controls in body or organ weights, organ tissue, mortality, or hematological biomarkers were observed upon sacrifice. Groups of 25 Swiss albino mice were dosed with 0 or 300 mg/kg/d *Rosa damascena* flower water extract for 90 d.[59] Two control and 2 treated mice died in the first month, one control mouse and 2 treated mice died in the second month, and no mortality occurred in the third month of observation. Mice killed after the first, second, and third month (number not specified), progressively exhibited mild hydroponic degeneration in the liver, congestion in coronary blood vessels, and peribronchiolar aggregation of round cells in the lungs. No significant differences were observed in body and weights, and various hematological markers, compared to the control group.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Developmental and reproductive toxicity studies were not found in the published literature, and unpublished data were not submitted.

GENOTOXICITY

In Vitro

Rosa Damascena Flower Oil

In a micronucleus assay, doses of 1, 10, 50, 100, 150, or 200 µg/ml *Rosa damascena* flower oil were added to whole blood samples treated with Roswell Park Memorial Institute (RPMI) culture medium supplemented with fetal bovine serum (FBS) containing L-glutamine, antibiotics, and phytohemagglutinin (PHA).[62] Cytochalasin B (Cyt B) was added at a concentration of 6 µg/ml 44 h after PHA stimulation. The frequency of micronuclei in binucleated lymphocytes was significantly greater
($p < 0.05$) in samples treated with > 50 µg/ml *Rosa damascena* flower oil, compared to negative and 1% dimethyl sulfoxide (DMSO)-treated controls.

No inhibition of mitotic activity was observed when a *Rosa damascena* flower oil (absolute) and a *Rosa damascena* flower oil (extracted from fresh flowers) were tested on cultures of normal human blood lymphocytes at doses of 10 µg/ml.[63] *Rosa damascena* flower absolute oil showed significant antimutagenic activity ($p < 0.001$) when added at a dose of 10 µg/ml to a blood lymphocyte culture treated with 300 ng/ml mitomycin C (MMC).

Rosa Damascena Flower Oil and Rosa Damascena Flower Water

A trade mixture of 0.1 - 1% Rosa Damascena Flower Oil and 0.1 - 1% Rosa Damascena Flower Water, in pentylene glycol, was tested in an Ames test using *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535, TA 1537, at up to 5000 µg/plate, with and without metabolic activation.[64] No signs of precipitate or dose responses were found at any concentration. The test material was not deemed genotoxic.

CARCINOGENICITY STUDIES

In Vitro Cell Transformation

Rosa Damascena Flower Oil

Human colon carcinoma SW742 cell lines and human fibroblast cell lines were prepared for an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay with RPMI-1640 medium, combined with FBS (10% v/v), streptomycin (100 µg/ml), and penicillin (100 µg/ml).[65] Doses of 0, 1, 2, 3, 4, 5, or 10 µl of a *Rosa damascena* flower oil were induced, in triplicate, to cells for 48 h. Outer and inner controls were used, in which cells not exposed to the flower oil were cultured in separate, or the same, dishes as cell lines treated with flower oil. Both morphology and cell survival rates of cancer and fibroblast cells were affected by *Rosa damascena* flower oil exposure. The evaporated (non-soluble) phase of the oil was shown to have an inhibitory effect on cell growth, especially in the inner controls, while the water-soluble phase of the oil significantly increased cell growth by nine-fold, compared to the inner controls. Both SW742 cells and fibroblasts showed cell growth induction when exposed to 10 µl of *Rosa damascena* flower oil, while at lower concentrations a potent induction effect was only seen in fibroblasts.

OTHER RELEVANT STUDIES

Cytotoxicity

Rosa Damascena Flower Extract

A methanolic extract of dried *Rosa damascena* flowers was used in an MTT colorimetric assay to evaluate in vitro activity against human cervical cancer (HeLa) and African green monkey kidney epithelial (Vero) cell lines.[66] Studies examining the effect of increasing doses of the extract upon cytotoxicity exhibited IC_{50} values at 265 µg/ml and > 1000 µg/ml

Rosa damascena flower extract on the HeLa and Vero cells, respectively. Additionally, a selectivity index (SI), of > 3.8 for the *Rosa damascena* flower extract indicated minimal concerns for concurrent cytotoxic effects in normal cells.

Rosa Damascena Flower Oil and Rosa Damascena Flower Water

The cytotoxicity of a trade mixture of 0.1 - 1.0% Rosa Damascena Flower Water and 0.1 - 1.0% Rosa Damascena Flower Oil formulated in pentylene glycol was estimated by measuring the intake of neutral red dye by murine fibroblast cells treated with either 1.4 - 50 mg/ml of the test article or 10 - 100 µg/ml sodium lauryl sulfate (SLS), for 48 h, in an vitro cytotoxicity assay, in accordance with the Organisation for Economic Cooperation and Development (OECD) test guideline (TG) 129.[64] A mean IC_{50} value of 6.68 mg/ml was determined.

Hematological and Clinical Effects

Rosa Damascena Flower Extract

Groups of 10 Wistar rats were administered 0, 2.5, 5, 25, or 50 mg/kg/d aqueous *Rosa damascena* flower extract, via gavage, for 30 d.[60] Blood samples were collected on days 0 and 30 to assess hematological parameters and biochemical changes. Significant decreases in total white blood cell count was noted in the 2.5 and 50 mg/kg/d groups, while platelet counts were significantly increased in all test groups. Fasting glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels were significantly decreased and alkaline phosphatase (ALP) levels were significantly increased in all test groups. Increased triglyceride (TG) levels were statistically significant in only the 50 mg/kg/d group, while the cholesterol/high-density lipoprotein (HDL) ratio and low-density lipoprotein (LDL)/HDL ratios were significantly decreased in the 2.5, 5, and 25 mg/kg/d groups.

A methanolic, *Rosa damascena* flower extract was administered at 1.5 g, via diet, to rabbits (number not specified) for 45 d.[67] Animals were anesthetized at the end of the experiment, and under intubation, had pressure transducer cannulae inserted into the left carotid artery and left ventricle, to record heart rate, arterial blood pressure, and left ventricular pressure, respectively. A fasting blood sample was taken on days 1 and 46 to measure the total cholesterol, TG, LDL, and HDL levels. TG levels were significantly higher than controls at the end of the experiment. No other significant differences in lipid profiles, pulse, or cardiac indices were observed.

Groups of 5 dogs were administered distilled water or lactulose (controls), or, 90, 180, 360, 720, or 1440 mg/kg/d aqueous *Rosa damascena* flower extract for 10 d.[61] Serum levels of urea, creatinine, ALP, ALT, bilirubin, albumin, and protein were measured in all experimental groups at day 0, 1, 3, 7, and 10. Except for a significant increase in bilirubin levels on day 3 and ALT on day 10 in animals in the 1440 mg/kg bw/d group, there were no statistically significant differences with controls.

Rosa Damascena Flower Water

Groups of 10 male albino rabbits were dosed with either 250 or 500 mg/kg bw/d *Rosa damascena* flower water for 60 d.[68] Blood samples were collected for hematological testing on day 31 and day 61; compared to controls, no significant differences were observed between hemoglobin, white blood cells, red blood cells, and platelets after 30 and 60 days of dosing. The 250 mg/kg/d group had a significant increase in red blood cell count, and a higher platelet count was observed for both doses, at day 60 compared to day 30.

DERMAL IRRITATION AND SENSITIZATION

The dermal irritation and sensitization studies summarized below are described in Table 7.

In an in vitro study, 30 µl of a trade mixture containing 0.1 - 1% Rosa Damascena Flower Oil and 0.1 - 1% Rosa Damascena Flower Water, in pentylene glycol, was predicted to be non-sensitizing when applied neat to an EpiSkin™ model.[64] Human monocytic leukemia cell lines (THP-1) exposed to up to 5000 µg/ml of the same trade mixture, undiluted, in a human cell line activation test (h-CLAT) in vitro assay were considered to be sensitized (minimum induction threshold of 923 µg/ml).[64] The trade mixture of 0.1 - 1% Rosa Damascena Flower Oil and 0.1 - 1% Rosa Damascena Flower Water was not considered sensitizing when evaluated in a luciferase assay (KeratinoSens™ model), undiluted, at up to 400 µg/ml.[64]

Undiluted *Rosa damascena* flower oil was not irritating to the skin of mice and pigs, but was moderately irritating when applied to the intact or abraded skin of rabbits for 24 h.[55,57] No further details were provided.

A trade mixture of 0.1-1% Rosa Damascena Flower Oil and 0.1-1% Rosa Flower Water, in pentylene glycol, was not irritating when applied as a single, semi-occlusive application of 160 µl, at a concentration of 20% in distilled water, to 11 subjects.[64] A single, occlusive, 48-h application of *Rosa damascena* flower oil, dissolved in 2% petrolatum, was not irritating in 25 subjects.[55,57] Two fragrance products, one containing 0.7794% Rosa Damascena Flower Extract and one containing 0.1068% Rosa Damascena Flower Water, and one mask formulation containing 0.1260% Rosa Damascena Flower Oil, were not sensitizing in HRIPTs using either 100 or 107 subjects.[69-71]

Photosensitization/Phototoxicity

In Vitro

Rosa Damascena Flower Oil and Rosa Damascena Flower Water

In an ultraviolet-visible spectrophotometric analysis of a trade mixture of 0.1 - 1% Rosa Damascena Flower Oil and 0.1 - 1% Rosa Damascena Flower Water, formulated in pentylene glycol, diluted to 10% in water, a very low UV absorption was observed between 290 - 400 nm.[64] The test article was not considered to have phototoxic potential.

Animal

Rosa Damascena Flower Oil

No phototoxic effects were reported when undiluted *Rosa damascena* flower oil was applied on hairless mice and swine.[55,57] No further details were provided.

OCULAR IRRITATION STUDIES

In Vitro

Rosa Damascena Flower Oil and Rosa Damascena Flower Water

The potential of a trade mixture comprising 0.1 - 1.0% Rosa Damascena Flower Oil and 0.1 - 1.0% Rosa Damascena Flower Water formulated in pentylene glycol to cause ocular irritation was investigated in a neutral red release assay.[64] Rabbit cornea fibroblast cells (SIRC cell line) were preloaded with neutral red dye (amount not specified) for 3 h at 37 °C. The dyed cells were then treated with either 500 µl of the test article diluted at 0, 25, or 50% (in water), sodium dodecyl sulfate diluted at 0.2%, 0.05%, or 0.01% in saline solution (positive control), or saline solution (negative control), for 60 s. Upon removal of the test article and controls, the amount of dye, released solely by surviving cells, was measured at an optical density of 540 nm. The resulting cell death percentages were plotted against the corresponding test article concentrations to determine IC_{50} values. Under these experimental conditions, the trade mixture exhibited negligible cytotoxicity (≤ 20% cell death at 50% dilution), with the positive controls producing expected results. The test article was not considered an ocular irritant.

CLINICAL STUDIES

Retrospective and Multicenter Studies

Rosa Damascena Flower Oil

Patients in Japan (n= 1483), suspected of having contact dermatitis, were enrolled in a 9-yr study (1990-1998), in which they were annually patch tested with a series of essential oils.[72] A *Rosa damascena* flower oil (2% pet.) was one of the 10 fragrance oils applied on the upper back of patients, in a 2-d close patch test, using Finn Chambers and Scanpor tape. Readings were taken at 1 h and 1 d after removal, according to International Contact Dermatitis Group recommendations. The average patch test positivity rate for this *Rosa damascena* flower oil, over 8 yr, was 0.4%.

Case Reports

A 48-yr old woman experienced an intense scalp itch and immunoresponse to a hair dye application 3 mo prior to presenting with symptoms of contact dermatitis.[73] These contact dermatitis symptoms were observed at sites of repeated and novel application of a cologne containing *Rosa damascena* flower oil. Of 326 patients patch tested with the Chemotechnique fragrance series for contact dermatitis from perfumes, this was the first patient to show a positive reaction to a *Rosa damascena* flower oil in 2% petroleum. Upon chromatographic analysis of the allergenic material, citronellol and geraniol were the 2 major components (33.4% and 18.5%, respectively). *Rosa damascena* flower oil contains approximately 20% geraniol, which led the researchers to attribute the woman's possible reaction to this component in the cologne.

A 71-yr old Asian woman reported painful, pruritic, palmar eruptions, lasting 3 mo, shortly after novel exposure to a household cleaner.[74] The patient presented with hyperkeratotic plaques and bilateral fissures on her palms, which did not improve upon over-the-counter treatment with tar soaks, betamethasone dipropionate ointment, hydroxyzine, and use of a fragrance-free soap. Patch test results, graded according to the North American Contact Dermatitis Group, revealed positive reactions at 48 and 72 h to cinnamic aldehyde (2+), balsam of Peru (1+), fragrance mix (2+), cinnamic alcohol (2+), geraniol (1+), ylang-ylang oil (1+), *Rosa damascena* flower oil (1+), lavender absolute (1+), geranium oil bourbon (1+), methyl methacrylate (1+), and ethylenediamine at 72 h (1+). The observed dermatitis partly diminished after discontinuing use of the fragrance-free soap and tar soaks, minimizing handling of spices, and minimizing the consumption of Chinese herbal preparations and balsam. The subject was considered sensitized to multiple fragrance plant allergens, including *Rosa damascena* flower oil.

SUMMARY

The safety of 10 *Rosa damascena*-derived ingredients as used in cosmetics is reviewed in this safety assessment. According to the *Dictionary*, some of these ingredients are reported to function as skin-conditioning and fragrance ingredients, while a few are reported to function as antioxidants and cosmetic astringents, in cosmetic products. Constituents such as monoterpene alcohols, various hydrocarbons, oxides, ethers, esters, aldehydes, and phenols are found in the *Rosa damascena* flower, with amounts varying based on time of harvest, as well as the timing and method of extraction.

According to 2021 VCRP survey data, Rosa Damascena Flower Water is reported to be used in 308 formulations, at a maximum concentration of 32.7% in face and neck products, while Rosa Damascena Flower Oil is reported to be used in 223 formulations, at a maximum concentration of 10.8% in other skincare preparations. Incidental ingestion and mucous membrane exposure are possible; for example, Rosa Damascena Flower Wax is reported to be used at a maximum of up to 1.1% in lipsticks. Additionally, these ingredients are used in cosmetic sprays and powders, and could possibly be inhaled. For example, Rosa Damascena Flower Extract is reported to be used at up to 0.00007% in aerosol spray deodorant formulations, and Rosa Damascena Flower Oil is reported to be used at up to 0.0003% in hair spray. Rosa Damascena Flower Extract is reported to be used in face powder formulations (concentration of use not reported)

The acute dermal LD_{50} of *Rosa damascena* flower oil was determined to be ≥ 2500 mg/kg in rabbits. A single oral dose of 2000 mg/kg ethyl acetate *Rosa damascena* flower extract did not cause toxic effects in groups of 3 male and 3 female Swiss albino mice. The acute oral LD_{50} of *Rosa damascena* flower oil was determined to be > 5000 mg/kg in rats, and in another study, was determined to be up to 5525 mg/kg in male rats and 2975 mg/kg in mature, and 3972 mg/kg in immature, female rats, respectively. No deaths were observed in groups of 6 Swiss albino mice orally dosed with up to 6000 mg/kg *Rosa damascena* flower water extract, and the acute LD_{50} in mice was determined to be > 6000 mg/kg.

Groups of 10 Wistar rats administered 0, 2.5, 5, 25, or 50 mg/kg/d aqueous *Rosa damascena* flower extract, via gavage, for 30 d, exhibited greater body weight gain in all test groups compared to controls, but the percent weight gain was not statistically significant. Groups of 5 dogs administered 0, 90, 180, 720, or 1440 mg/kg/d aqueous *Rosa damascena* flower extract for 10 d exhibited a dose dependent increase of diarrhea, and animals in the 720 and 1440 mg/kg/d groups exhibited slight, but not significant, weight loss after day 7. No significant differences in body or organ weights, organ tissue, mortality, or hematological biomarkers were observed in 15 Swiss albino mice administered 300 mg/kg/d *Rosa damascena* flower water extract for 28 d (compared to 10 controls). Groups of 25 Swiss albino mice were dosed with 0 or 300 mg/kg/d *Rosa damascena* flower water extract for 90 d. Two control mice and 2 treated mice died in the first month, 1 control mouse and 2 treated mice died in the second month, and no mortality occurred during the third month of observation. Mice killed after the first, second, and third month (number not specified), progressively exhibited mild hydroponic degeneration in the liver, congestion in coronary blood vessels, and peribronchiolar aggregation of round cells in the lungs. No significant differences were observed in body and weights, and various hematological markers, compared to the control group.

Whole blood samples exposed to up to 200 µg/ml of a *Rosa damascena* flower oil, followed by PHA stimulation and the addition of Cyt B, exhibited a significantly greater frequency of micronuclei at doses > 50 µg/ml, compared to controls. Concentrations of 10 µg/ml of a *Rosa damascena* flower oil (absolute) and a *Rosa damascena* flower oil (extracted from whole flowers) did not inhibit mitotic activity in normal human blood lymphocytes. *Rosa damascena* flower oil (absolute) exhibited significant ($p < 0.001$) antimutagenic activity when added to a blood lymphocyte culture treated with 300 ng/ml MMC. A trade mixture of 0.1 - 1% Rosa Damascena Flower Oil and 0.1 - 1% Rosa Damascena Flower Water, in pentylene glycol was not considered genotoxic when tested in an Ames test using *S. typhimurium* strains TA 98, TA100, TA 102, TA 1535, TA 1537, at up to 5000 µg/plate.

Human colon carcinoma cell lines dosed with up to 10 µl of a *Rosa damascena* flower oil in an MTT assay exhibited a significant cell growth induction at the highest dose, while an induction effect was observed at lower concentrations in human fibroblast cells. In two separate MTT assays, a methanolic *Rosa damascena* flower extract exhibited IC_{50} values of 265 µg/ml and > 1000 µg/ml on HeLa and Vero cell lines, respectively. Murine fibroblast cell lines treated with up to 50 mg/ml of a trade mixture comprising 0.1 – 1% Rosa Damascena Flower Oil and 0.1 – 1% Rosa Damascena Flower Water formulated in pentylene glycol, yielded a mean IC_{50} value of 6.68 mg/ml.

Wistar rats administered up to 50 mg/kg/d aqueous *Rosa damascena* flower extract for 30 d exhibited significant decreases in the cholesterol/HDL and LDL/HDL ratios in the 2.5 and 50 mg/kg/d groups, and a significant TG increase in the 50 mg/kg/d group. A methanolic, *Rosa damascena* flower extract was administered at 1.5 g, via diet, to rabbits (number not specified) for 45 d. Comparison of fasting blood samples on day 1 and 1 d after the experiment revealed significantly higher TG levels in treated animals compared to the controls. No other significant differences in lipid profiles, pulse, or cardiac indices were observed. Groups of 5 dogs were administered up to 1440 mg/kg bw/d aqueous *Rosa damascena* flower extract for 10 d, via gavage. Except for a significant increase in bilirubin levels on day 3 and ALT on day 10 in animals in the 1440 mg/kg bw/d group, there were no statistically significant differences from controls. Blood samples collected from groups of 10 male albino rabbits dosed with either 250 or 500 mg/kg bw/d *Rosa damascena* flower water for 60 d only showed a statistically significant increase in red blood cell counts for the 250 mg/kg group, and an increase in platelet counts for both groups, at day 60, compared to day 30 of dosing.

In an in vitro study, 30 µl of a trade mixture of 0.1-1% Rosa Damascena Flower Oil and 0.1-1% Rosa Damascena Flower Water, formulated in pentylene glycol did not cause irritation when applied to an EpiSkin™ model. Human monocytic leukemia cell lines (THP-1) exposed to up to 5000 µg/ml of the same trade mixture, undiluted, in an h-CLAT in vitro assay were sensitized, with a minimum induction threshold of 923 µg/ml. The aforementioned trade mixture was not considered sensitizing when evaluated in a luciferase assay (KeratinoSens™ model), undiluted, at up to 400 µg/ml. No dermal irritation or phototoxic effects were observed when *Rosa damascena* flower oil was applied to the backs of hairless mice and swine. Rabbits with intact or abraded skin exposed to undiluted *Rosa damascena* flower oil for 24 h under occlusion showed signs of moderate irritation. The same trade mixture of Rosa Damascena Flower Oil and Rosa Damascena Flower Water, in pentylene glycol was not irritating when applied, at a concentration of 20% in distilled water, at160 µl in a single, semi-occlusive patch test of 11 subjects. *Rosa damascena* flower oil, dissolved in 2% petrolatum, did not produce irritation or sensitization reactions in a 48-h closed patch maximization test using 25 human subjects. Sensitization was not observed in 3 separate HRIPTs testing the sensitizing potential of two fragrance products containing 0.7794% Rosa Damascena Flower Extract, 0.1068% Rosa Damascena Flower Water, and a mask formulation containing 0.1260% Rosa Damascena Flower Oil, in either 100 or 107 subjects.

A trade mixture of 0.1 - 1% Rosa Damascena Flower Oil and 0.1 - 1% Rosa Damascena Flower Water, in pentylene glycol, diluted to 10% in water, had very low UV absorption between 290 - 400 nm, and was not considered phototoxic. Undiluted *Rosa damascena* flower oil was not considered phototoxic to mice and swine skin. The same trade mixture, applied at a dose of 500 µl, neat or 25 or 50%, in water, to neutral red dye treated-rabbit cornea fibroblast cells for 60 s did not exhibit cytotoxicity and was not considered an ocular irritant.

In an 8-yr, annual essential oil patch study of 1483 Japanese patients susceptible to contact dermatitis, among the 10 fragrance oils used, the average patch test positivity rate for *Rosa damascena* flower oil (2% pet.) was 0.4%. A 48-yr old woman experiencing contact dermatitis, at the same and novel application sites of a cologne containing *Rosa damascena* flower oil, was posited to test positive to the geraniol in *Rosa damascena* flower oil, in 2% petrolatum. The painful, pruritic palmar eruptions of a 71-yr old Asian woman, who patch-tested positive to multiple botanical fragrance allergens, including *Rosa damascena* flower oil, diminished after her discontinued use of a fragrance-free soap containing *Rosa damascena* flower oil, among other fragrance allergens.

DISCUSSION

To be developed.

CONCLUSION

To be determined.

TABLES

Table 1. Definitions and reported functions of *Rosa damascena* ingredients [1]

Ingredient/CAS No.	Definition	Function
Hydrolyzed Rosa Damascena Flower Extract	Hydrolyzed Rosa Damascena Flower Extract is the hydrolysate of Rosa Damascena Flower Extract derived by acid, enzyme, or other method of hydrolysis. The accepted scientific name for *Rosa damascena* is *Rosa* x *damascena.*	Antioxidants
Rosa Damascena Bud Extract 90106-38-0	Rosa Damascena Bud Extract is the extract of the buds of *Rosa damascena*. The accepted scientific name for *Rosa damascena* is *Rosa* x *damascena.*	Skin-conditioning agents - miscellaneous
Rosa Damascena Extract 90106-38-0	Rosa Damascena Extract is the extract of the rose, *Rosa damascena*. The accepted scientific name for *Rosa damascena* is *Rosa* x *damascena.*	Fragrance ingredients
Rosa Damascena Flower 90106-38-0	Rosa Damascena Flower are the flowers of *Rosa damascena.* The accepted scientific name for *Rosa damascena* is *Rosa* x *damascena.*	Skin-conditioning agents - miscellaneous
Rosa Damascena Flower Extract 906106-38-0	Rosa Damascena Flower Extract is the extract of the flowers of *Rosa damascena*. The accepted scientific name for *Rosa damascena* is *Rosa* x *damascena.*	Fragrance ingredients
Rosa Damascena Flower Oil 8007-01-0 90106-38-0	Rosa Damascena Flower Oil is the volatile oil obtained from the flowers of *Rosa damascena.* The accepted scientific name for *Rosa damascena* is *Rosa* x *damascena.*	Fragrance ingredients; Skin-conditioning agents – miscellaneous
Rosa Damascena Flower Powder 90106-38-0	Rosa Damascena Flower Powder is the powder obtained from the dried, ground flowers of *Rosa damascena.* The accepted scientific name for *Rosa damascena* is *Rosa* x *damascena.*	Fragrance ingredients
Rosa Damascena Flower Water 90106-38-0	Rosa Damascena Flower Water is an aqueous solution of the steam distillate obtained from the flowers of *Rosa damascena.* The accepted scientific name for *Rosa damascena* is *Rosa* x *damascena.*	Fragrance ingredients; Skin-conditioning agents - miscellaneous
Rosa Damascena Flower Water Extract 90106-38-0	Rosa Damascena Flower Water Extract is the extract of Rosa Damascena Flower Water. The accepted scientific name for *Rosa damascena* is *Rosa* x *damascena.*	Antioxidants; Cosmetic astringents
Rosa Damascena Flower Wax 90106-38-0	Rosa Damascena Flower Wax is a wax obtained from the flower of *Rosa damascena.* The accepted scientific name for *Rosa damascena* is *Rosa* x *damascena.*	Fragrance ingredients

Table 2. Chemical properties of *Rosa damascena*-derived ingredients

Property	Value	Reference
	Rosa Damascena Extract	
Physical Form (@ 20°C and 1013 hPa)	Viscous liquid; can contain crystallized product	[75]
Color	Orange-red	[75]
Density (g/ml)	0.9804	[75]
Vapor pressure (mmHg @ 20 ºC; 25°C)	3.053; 3.960	[75]
Boiling Point	Decomposed before boiling	[75]
	Rosa Damascena Flower	
Physical Form (fresh petal width and length, in cm)	Heart/pear shape, soft and smooth petals; 0.9-3.8; 1.8-4.2	[9]
Color	Magenta on base, light yellow near apex pink	[2,9]
Odor	Aromatic distinct	[9]
Density (g/ml)	0.202	[9]
pH	6.56	[9]
Ash (% w/w upon burning); Total Acid insoluble; Water soluble	6.34 1.51; 2.48	[9] [9]
	Rosa Damascena Flower Extract	
UV Wavelengths and Absorbance (nm; AU)* Hydroalcoholic extract Ether extract Ethyl acetate:ethanol 	 228; 2.57 (λ_{max}) 226; 2.42 355; 0.9 269; 1.59 (λ_{max}) 238; 1.35 350; 0.85 270; 1.16 (λ_{max}) 354; 0.64	[25]
	Rosa Damascena Flower Oil	
Physical Form	Liquid or crystallized	[76]
Color	Colorless, light yellow to yellow-green	[2,76,77]
Odor	Floral, rose	[76]
Density (g/ml @ 20 ºC)	0.848-0.880	[76]

Table 2. Chemical properties of *Rosa damascena*-derived ingredients

Rosa Damascena Flower Water		
Density (g/ml @ 20 ºC)	0.9916; 0.9927	[4,10]
Viscosity (cm^2/s) @ 25 ºC)	104	[10]
Melting Point (ºC)	93	[10]
pH	7.2; 6.55	[4,10]
Rosa Damascena Flower Wax		
Melting Point (ºC)		[18]
Turkish rose wax	42	
Bulgarian rose wax	41.0-46.5	

Table 3. Percent composition of constituents found in *Rosa damascena*-derived ingredients[2,14,15,17,18,20-22]

	Shade-dried petals of *Rosa damascena*		**Fresh flowers of *Rosa damascena***			
Constituent	**Hydrodistilled Essential Oil**	**Hexane Extract of Rose Water**	**Hydrodistilled Essential Oil**	**Rose Water**	**Rose Water Extract***	**Rose Wax****
α-bulnesene	0.4%	0.1%	NR	NR	NR	NR
α-guaiene	NF	0.1%	2.0%	NR	NR	NR
α-humulene	0.3%	0.2%	0.6%	NR	NR	NR
α-pinene	0.1%	0.3%	2.8%	0.71%[20]	NR	NR
α-selinene	trace	trace	NR	NR	NR	NR
α-terpinene	trace	trace	NR	NR	NR	NR
α-terpineol	0.1%	0.2%	1.6%	0.12%	NR	NR
β-caryophyllene	trace	-	trace	NR	NR	NR
β-citronellol	NR	NR	NR	28.70%	NR	NR
β-copaene	0.1%	0.2%	2.0%	NR	NR	NR
β-damascenone	NR	NR	0.5%	NR	NR	NR
β-elemene	0.1%	-	NR	NR	NR	NR
β-myrcene	0.2%	0.2%	2.4%	NR	NR	NR
β-pinene	NR	0.1%	0.3%	NR	NR	NR
β-selinene	0.1	-	NR	NR	NR	NR
δ-cadinene	-	0.1%	NR	NR	NR	NR
δ-elemene	0.1%	trace	NR	NR	NR	NR
(2*e*,6*e*)-farnesol	0.4%	0.3%	0.6%	NR	NR	NR
e-β-ocimene	0.3	-	0.8%	1.06%	NR	NR
(*e*)-rose oxide	NR	NR	0.7%	NR	trace	NR
(z)-rose oxide	NR	NR	0.2%	NR	trace	NR
z-β-farnesene	0.3%	0.1%	NR	NR	NR	NR
z-β-ocimene	trace	-	0.3%	0.18%	NR	NR
(z)-9-nonadecene	NR	NR	0.6%	NR	NR	NR
1-eicosene	0.1%	0.1%	NR	NR	NR	NR
1-nonadecene	1.6%	0.8%	10.2%[2]	NR	NR	NR
10-*epi*-γ-eudesmol	0.1%	0.1%	NR	NR	NR	NR
benzaldehyde	trace	0.1%	NR	NR	NR	NR
benzyl alcohol	NR	NR	NR	0.85%	NR	NR
caryophyllene oxide	0.1%	0.1%	NR	NR	NR	NR
cis-geraniol	NR	NR	NR	10.81%	NR	NR
citronellal	0.1%	0.2%	NR	NR	NR	NR
citronellol	7.1%	2.2%	35.3%	29.44%[22]	1.8-7.2%	17%
citronellyl acetate	0.1%	0.3%	0.5%	NR	NR	NR
citronellyl butyrate	0.1 %	-	NR	NR	NR	NR
citronellyl formate	0.2%	0.3%	NR	NR	NR	NR
docasane	1.1%	1.4%	0.6%	0.4%[20]	NR	NR
eicosane	2.5%	2.4%	0.5%	0.45%	0.2%	NR
ethanol	NR	NR	2.1%[2]	NR	NR	NR
eugenol	NR	NR	1.6%[2]	2.26-17.75%[20]	0.4%	1%
farnesol	NR	NR	NR	0.89%	NR	NR
geranial	0.1%	trace	0.7%	NR	NR	NR
geraniol	4.1%	2.5%	18.7%	30.74%[22]	0.9-7.0%	5%
geranyl acetate	0.8%	0.1%	1.7%	7.33%	NR	NR
geranyl formate	0.5%	1.5%	1.0%	NR	NR	NR
geranyl propionate	trace	-	NR	NR	NR	NR
germacrene-d	trace	-	NR	NR	NR	NR
heneicosane	19.7%	15.7%	2.6%	0.56%	1.4%	NR
heptadecane	0.6%	0.5%	0.3%	1.08%[20]	NR	NR

Table 3. Percent composition of constituents found in *Rosa damascena*-derived ingredients[2,14,15,17,18,20-22]

	Shade-dried petals of *Rosa damascena*		Fresh flowers of *Rosa damascena*			
Constituent	**Hydrodistilled Essential Oil**	**Hexane Extract of Rose Water**	**Hydrodistilled Essential Oil**	**Rose Water**	**Rose Water Extract***	**Rose Wax****
hexadecane	0.1%	0.4%	NR	2.14%	NR	NR
isomenthone	0.2%	0.3%	NR	NR	NR	NR
limonene	NR	NR	0.8%	NR	NR	NR
linalool	0.5%	0.7%	2.6%	0.65-8.99%[20]	1.5-3.3%*	NR
linalyl acetate	trace	trace	NR	NR	NR	NR
methyl eugenol	trace	0.1%	1.3%	1.83%	0.4%	2%
methyl geranate	trace	-	NR	NR	NR	NR
n-decanal	NR	NR	trace	NR	NR	NR
neral	trace	0.1%	0.3%	NR	NR	NR
nerol	0.1%	-	7.2%	16.12%[22]	0.2-4.2%	4%
nerol oxide	0.2%	0.1%	NR	NR	NR	NR
neryl acetate	0.4%	-	NR	NR	NR	NR
nonane	NR	NR	NR	0.31%[20]	NR	NR
n-nonanal	0.4%	0.4%	0.2%	NR	NR	NR
nonadecane	13.0%	8.4%	4.5%	2.05%	0.9%	0.1%
nonadecene	NR	NR	NR	NR	0.7%	-
octadecane	0.2%	0.9%	NR	NR	NR	NR
p-cymene	0.6%	0.6%	NR	NR	NR	NR
pentacosane	5.3%	5.1%	0.5%	NR	NR	NR
pentadecane	-	0.2%	NR	0.73%	NR	NR
phenethyl alcohol	0.4%	7.1%	2.9%	4.95%[21]; 23.70%[22]	69.7-81.6%	43%
terpinen-4-ol	0.1%	0.2%	0.5%	NR	NR	NR
terpinolene	0.1%	0.1%	NR	NR	NR	NR
tetracosane	0.9%	1.1%	trace	NR	NR	NR
tetradecanol	0.1%	-	NR	NR	NR	NR
trans-geraniol	NR	NR	NR	16.44%	NR	NR
tricosane	11.3%	9.3%	0.6%	NR	NR	NR

* *Rosa damascena* flower extracts prepared with listed solvents (20 mg%)

Abbreviations: - (not found); NR- not reported; *dichloromethane extract; ** solid phase microextraction (SPME) analysis

Table 4. Frequency (2021)[37] and concentration (2019)[38] of use of *Rosa damascena*-derived ingredients

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Rosa Damascena Extract		**Rosa Damascena Flower**		**Rosa Damascena Flower Extract**	
Totals*	**59**	**0.000005-0.05**	**6**	**NR**	**194**	**0.0012-0.0018**
Duration of Use						
Leave-On	*39*	*0.00001-0.014*	*5*	*NR*	*164*	*NR*
Rinse-Off	*18*	*0.000005-0.05*	*1*	*NR*	*28*	*0.0012-0.0018*
Diluted for (Bath) Use	*2*	*NR*	*NR*	*NR*	*2*	*NR*
Exposure Type						
Eye Area	4	NR	NR	NR	59	NR
Incidental Ingestion	0	NR	NR	NR	22	NR
Incidental Inhalation-Spray	5; 14[a]; 14[b]	0.00001-0.0027; 0.00013-0.0065[a]	2; 1[a]; 1[b]	NR	18[a]; 20[b]	NR
Incidental Inhalation-Powder	14[b]	0.00077-0.014[c]	1[b]	NR	7; 20[b]	NR
Dermal Contact	56	0.000005-0.05	6	NR	154	0.0018
Deodorant (underarm)	1[a]	aerosol: 0.00007; 0.00007 (not spray)	NR	NR	2[a]	NR
Hair - Non-Coloring	3	0.00001-0.0065	NR	NR	15	0.0012
Hair-Coloring	NR	0.0023	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	7	0.000005-0.05	NR	NR	33	NR
Baby Products	NR	NR	NR	NR	NR	NR
	Rosa Damascena Flower Oil		**Rosa Damascena Flower Powder**		**Rosa Damascena Flower Water**	
Totals*	**223**	**0.000059-10.8**	**1**	**NR**	**308**	**0.009-32.7**
Duration of Use						
Leave-On	*180*	*0.00013-10.8*	*1*	*NR*	*245*	*0.09-32.7*
Rinse Off	*35*	*0.000059-0.31*	*NR*	*NR*	*63*	*0.009-0.99*
Diluted for (Bath) Use	*8*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	8	0.0095	NR	NR	20	NR
Incidental Ingestion	1	0.0002-0.01	NR	NR	9	NR
Incidental Inhalation-Spray	47; 54[a]; 51[b]	0.00013-0.0003; 0.0012[a]	NR	NR	1; 84[a]; 99[b]	NR
Incidental Inhalation-Powder	51[b]; 2[c]	NR; 0.005-0.16[c]	NR	NR	99[b]	32.7[c]
Dermal Contact	213	0.00014-10.8	1	NR	289	0.09-32.7
Deodorant (underarm)	2[a]	NR	NR	NR	1[a]	NR
Hair - Non-Coloring	7	0.00013-0.0017	NR	NR	10	0.009
Hair-Coloring	NR	0.000059	NR	NR	NR	0.03-0.09
Nail	NR	0.005	NR	NR	NR	NR
Mucous Membrane	19	0.00014-0.01	NR	NR	16	0.09
Baby Products	2	NR	NR	NR	NR	NR

	Rosa Damascena Flower Water Extract		**Rosa Damascena Flower Wax**	
Totals*	**1**	**NR**	**7**	**0.015-1.1**
Duration of Use				
Leave-On	*NR*	*NR*	*6*	*0.015-1.1*
Rinse-Off	*1*	*NR*	*1*	*0.05*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	NR	NR	1	0.13
Incidental Ingestion	NR	NR	2	1.1
Incidental Inhalation-Spray	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	1[b]	0.05[c]
Dermal Contact	1	NR	4	0.015-0.13
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	NR	NR	3	1.1
Baby Products	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] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

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

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

NR – not reported

Table 5. Acute toxicity studies

Ingredient	Animals	No./Group	Vehicle	Concentration/Dose/Protocol	LD_{50}/ Results	Reference
DERMAL						
Rosa damascena flower oil	Rabbits	NR	NR	NR	LD_{50} > 2500 mg/kg	[55-57]
ORAL						
Rosa damascena flower extract	Male and female Swiss albino mice	3/sex	0.7% carboxy-methylcellulose	2000 mg/kg ethyl acetate extract	LD_{50} > 2000 mg/kg	[58]
Rosa damascena flower oil	rats	NR	NR	NR	LD_{50} > 5000 mg/kg	[55-57]
Rosa damascena flower oil	Male and female rats	NR	NR	NR	LD_{50} was determined to be 5525 mg/kg in male rats, and 2975 mg/kg and 3972 mg/kg in mature and immature female rats, respectively.	[55-57]
Rosa damascena flower water extract	Swiss albino mice	6/group	NR	500, 1000, 2000, 3000, 4000, 5000, or 6000 mg/kg	LD_{50} > 6000 mg/kg	[59]

DMEM – Dulbecco's modified Eagle's medium; NR – not reported; SLS – sodium lauryl sulfate

Table 6. Short-term and subchronic oral toxicity studies

Ingredient	Animals/Group	Study Duration	Vehicle/Control	Dose/Concentration	Protocol/Results	Reference
Rosa damascena flower extract Aqueous extract	Wistar rats; 10/group	30 d	0.9 % saline	0, 2.5, 5, 25, or 50 mg/kg/d, via gavage	Body weight increased more in test groups than in controls, but the percent weight gains were not statistically significant.	[60]
Rosa damascena flower extract Aqueous extract	Dogs (strain not specified); 5/group (test substance) 4/group (controls)	10 d	Distilled water; Negative control: distilled water Positive control: lactulose	0, 90, 180, 360, 720, or 1440 mg/kg/d	Animals were monitored for changes in gastrointestinal performance, weight, electrocardiogram, temperature, respiration, and heart rates. No significant differences were observed between groups for respiration, temperature, or cardiac response. A dose-dependent increase of soft feces and diarrhea was observed, starting from the 90 mg/kg/d group. The animals in the 1440 mg/kg/d group showed sedation, and animals in the 720 and 1440 mg/kg/d groups exhibited slight weight loss, especially after day 7, which was statistically significant. However, animals treated with lactulose also experienced slight weight loss, and this effect was, therefore, attributed to possible diarrhea-induced malabsorption, or dehydration. No further changes or adverse effects were observed.	[61]
Rosa damascena flower water extract	Swiss albino mice; control: 10; treated: 15	28 d	NS	0, 300 mg/kg/d)	Animal body weights were recorded prior to, and during, treatment. Animals were sacrificed 24 h after the end of treatment and vital organs were weighed and examined for histopathological changes. No significant differences in body or organ weights, organ tissue, or mortality were observed in treated mice. ALT, ALP, AST, urea, and creatinine levels were not significantly different from controls.	[59]

Table 6. Short-term and subchronic oral toxicity studies

Ingredient	Animals/Group	Study Duration	Vehicle/Control	Dose/Concentration	Protocol/Results	Reference
Rosa damascena flower water extract	Swiss albino mice; 25/group	90 d	NS	0, 300 mg/kg/d	Mortality rates were recorded, and every month a group of mice (# not specified) was sacrificed. Total body and organ weights, and histopathological changes in the kidney and liver were assessed. Two control and 2 treated mice died in the first month (8%, both groups), one control and 2 treated mice in the second month (12.5% treated mice vs. 6.25% control mice), and no mortality occurred in the third month of observation. In the mice killed after the first month, the liver of treated mice showed mild hydropic degeneration and the heart showed slight congestion in coronary blood vessels with mild perivascular edema. In mice killed after the second month, the liver showed mild hydropic degeneration, and slight congestion of hepatic blood vessels, the kidneys had mild vacuolations and hydropic degeneration in the tubular epithelia, the heart showed granular eosinophilic sarcoplasm and slightly congested coronary blood vessels, and the lung had peribronchiolar aggregations of round cells with thickening of the adjacent interalveolar septa. In addition to the aforementioned effects, mice killed after the third month had focal hyaline degeneration in cardiac muscle fibers, the intestine showed an increase in the numbers of goblet cells and slight activation of Paneth cells, and the spleen exhibited sub capsular edema. No significant differences were observed in body and organ weights, or ALT, ALP, AST, urea, and creatinine levels in treated mice compared to the control group. (statistical significance not provided).	[59]

ALT- alanine aminotransferase, ALP- alkaline phosphatase, AST- aspartate aminotransferase; HDL- high-density lipoprotein; LDL – low-density lipoprotein; NS- not specified; TG – triglyceride; TC – plasma total cholesterol

Table 7. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			IN VITRO STUDIES		
0.1-1% Rosa Damascena Flower Oil and 0.1-1% Rosa Damascena Flower Water, in pentylene glycol	Undiluted, 10%, or 50% v/v in DMSO; in PBS at 10% v/v 30 µl of each dilution	EpiSkin™, Sens-IS	Test articles were applied to the model for 15 min, rinsed with PBS, and then incubated at 37 °C for 6 h. After incubation, the epidermis was collected, and RNA was extracted to analyze the expression of irritation biomarker genes. Overexpression of at least 15 of 23 genes associated with irritation would classify the substance as an irritant.	Not sensitizing. Three negative controls (PBS, olive oil, and DMSO - treated skins), a positive irritation control (5% SLS), and two positive sensitization controls (50% HCA and 1% TNBS) were used for each experiment.	[64]
0.1-1% Rosa Damascena Flower Oil and 0.1-1% Rosa Damascena Flower Water, in pentylene glycol	Undiluted; up to 5000 µg/ml	Human monocytic leukemia cell line (THP-1)	OECD TG 442E. In an h-CLAT in vitro assay, THP-1 cell lines were exposed to 8 concentrations of the test article ranging from 19.5 to 5000 µg/ml for 24 h. Post-exposure, the expression of two cell surface antigens, CD86 and CD54, was measured by flow cytometry. Vehicle control (RPMI), negative control (lactic acid), and positive controls (2,4-dinitrochlorobenzene or nickel sulfate) were also run in parallel.	Sensitizing; Based on linear regression, the median concentrations to induce a 150/200% expression of CD86/CD54 relative fluorescence intensity, were an EC_{200} of 923 µg/ml and an EC_{150} of 2125 µg/ml. The MIT was calculated as 923 µg/ml, from these EC_{200} and EC_{150} values.	[64]

Table 7. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
0.1-1% Rosa Damascena Flower Oil and 0.1-1% Rosa Damascena Flower Water, in pentylene glycol	Undiluted, 0.2 µg/ml – 400 µg/ml (12 concentrations)	KeratinoSens™, transformed keratinocytes	OECD TG 442D. Luciferase induction was measured in keratinocytes transformed with the AKR1C2 gene (a gene which identifies skin sensitizers). Cinnamaldehyde and 1% DMSO were run in parallel as positive and negative controls, respectively. The experiment was repeated twice to calculate average values for luciferase induction, i.e., test article concentrations at which the luciferase activity was 1.5-fold higher than basal luciferase activity ($EC_{1.5}$), and cell viability (IC_{70}).	Not sensitizing. Luciferase induction was lower than 1.5-fold of base values, and $EC_{1.5}$ values were not determined.	[64]
			ANIMAL		
Rosa damascena flower oil	NR	Mice and pigs (# and strain not stated)	NR	Not irritating	[55,57]
Rosa damascena flower oil	NR	Rabbits (# and strain not stated)	Intact or abraded rabbit skin was exposed to undiluted test article for 24 h, under occlusion. No further details provided.	Moderately irritating	[55,57]
			HUMAN		
0.1-1% Rosa Damascena Flower Oil and 0.1-1% Rosa Damascena Flower Water, in pentylene glycol	20%, diluted in distilled water/ 160 µl	11 subjects	A single, semi-occlusive application of the test article was made for 48 h. Readings were taken 30-40 min after removal of the patches.	Not irritating	[64]
Rosa damascena flower oil	NR; 2%, in petrolatum	25 subjects	A one-time, occlusive application of the test article, was made for 48 h. No further details provided.	Not irritating	[55,57]
Fragrance; 0.7794% Rosa Damascena Flower Extract	0.2 ml	100 subjects	In an HRIPT, 9 occlusive induction applications were applied for 24 h using 2 cm^2 patches, over 3 wk. Prior to each patch application, the test article was evaporated for 30 min. Test sites were scored and retested every 48 - 72 h. After a rest period of 10-15 d, a previously unexposed site was challenged with the test substance for 24 h. Challenge sites were scored 48 and 72 h after application.	Not sensitizing; 1 adverse event, which was not test article related, was reported.	[69]
Mask; 0.1260% Rosa Damascena Flower Oil	0.2 ml	107 subjects	In an HRIPT, 9 occlusive induction applications were applied for 24 h using 2 cm^2 patches, over 3 wk. Test sites were scored and retested every 48 h. After a rest period of 10-15 d, a previously unexposed site was challenged with the test substance for 24 h. Challenge sites were scored 48 and 72 h after application.	Not sensitizing	[71]
Fragrance; 0.1068% Rosa Damascena Flower Water	0.2 ml	100 subjects	In an HRIPT, 9 occlusive induction applications were applied for 24 h using 2 cm^2 patches , over 3 wk. Prior to each patch application, the test article was evaporated for 30 min. Test sites were scored and retested every 48 h. After a rest period of 10-15 d, a previously unexposed site was challenged with the test substance for 24 h. Challenge sites were scored 48 and 72 h after application.	Not sensitizing	[70]

DMSO – dimethyl sulfoxide; EC - maximal effective concentration ; HCA- hydrocitric acid; HRIPT – human repeated insult patch test; MIT – minimum induction threshold; NR – not reported; PBS- phosphate-buffered saline; RPMI – Roswell Park Memorial Institute; SLS – sodium lauryl sulfate; TNBS – trinitrobenzenesulfonic acid

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2021 VCRP Frequency of Use Data for *Rosa damascena*-derived Ingredients

INGREDIENT_NAME	CATEGORY CODE- DESCRIPTION	CPIS_COUNT
Rosa Damascena (Damask Rose)Extract Total Uses: 59		
Rosa Damascena (Damask Rose) Extract	02A- Bath Oils, Tablets, And Salts	2
Rosa Damascena (Damask Rose) Extract	03D- Eye Lotion	1
Rosa Damascena (Damask Rose) Extract	03E - Eye Makeup Remover	1
Rosa Damascena (Damask Rose) Extract	03G - Other Eye Makeup Preparations	2
Rosa Damascena (Damask Rose) Extract	04A - Cologne And Toilet Waters	2
Rosa Damascena (Damask Rose) Extract	04B - Perfumes	3
Rosa Damascena (Damask Rose) Extract	05A - Hair Conditioner	1
Rosa Damascena (Damask Rose) Extract	05F - Shampoos (Non-Coloring)	1
Rosa Damascena (Damask Rose) Extract	05G - Tonics, Dressings, And Other Hair Grooming Aids	1
Rosa Damascena (Damask Rose) Extract	10A - Bath Soaps And Detergents	4
Rosa Damascena (Damask Rose) Extract	10B - Deodorants (Underarm)	1
Rosa Damascena (Damask Rose) Extract	10D - Feminine Deodorants	1
Rosa Damascena (Damask Rose) Extract	12A - Cleansing	8
Rosa Damascena (Damask Rose) Extract	12C - Face And Neck (Exc Shave)	9
Rosa Damascena (Damask Rose) Extract	12D - Body And Hand (Exc Shave)	4
Rosa Damascena (Damask Rose) Extract	12F - Moisturizing	7
Rosa Damascena (Damask Rose) Extract	12G - Night	5
Rosa Damascena (Damask Rose) Extract	12H - Paste Masks (Mud Packs)	3
Rosa Damascena (Damask Rose) Extract	12I - Skin Fresheners	1
Rosa Damascena (Damask Rose) Extract	12J - Other Skin Care Preps	2
Rosa Damascena (Damask Rose) Flower Total Uses: 6		
Rosa Damascena (Damask Rose) Flower	04B - Perfumes	2
Rosa Damascena (Damask Rose) Flower	12C - Face And Neck (Exc Shave)	1
Rosa Damascena (Damask Rose) Flower	12F - Moisturizing	1
Rosa Damascena (Damask Rose) Flower	12H - Paste Masks (Mud Packs)	1
Rosa Damascena (Damask Rose) Flower	12J - Other Skin Care Preps	1
Rosa Damascena (Damask Rose) Flower Extract Total Uses: 194		
Rosa Damascena (Damask Rose) Flower Extract	02B - Bubble Baths	2
Rosa Damascena (Damask Rose) Flower Extract	03A - Eyebrow Pencil	1
Rosa Damascena (Damask Rose) Flower Extract	03B - Eyeliner	6
Rosa Damascena (Damask Rose) Flower Extract	03C - Eye Shadow	46
Rosa Damascena (Damask Rose) Flower Extract	03D - Eye Lotion	1
Rosa Damascena (Damask Rose) Flower Extract	03F - Mascara	2
Rosa Damascena (Damask Rose) Flower Extract	03G - Other Eye Makeup Preparations	3
Rosa Damascena (Damask Rose) Flower Extract	05A - Hair Conditioner	4
Rosa Damascena (Damask Rose) Flower Extract	05E - Rinses (Non-Coloring)	1
Rosa Damascena (Damask Rose) Flower Extract	05F - Shampoos (Non-Coloring)	6
Rosa Damascena (Damask Rose) Flower Extract	05G - Tonics, Dressings, And Other Hair Grooming Aids	2
Rosa Damascena (Damask Rose) Flower Extract	05I - Other Hair Preparations	2
Rosa Damascena (Damask Rose) Flower Extract	07A - Blushers (All Types)	15
Rosa Damascena (Damask Rose) Flower Extract	07B - Face Powders	7

Rosa Damascena (Damask Rose) Flower Extract	07C - Foundations	1
Rosa Damascena (Damask Rose) Flower Extract	07E - Lipstick	22
Rosa Damascena (Damask Rose) Flower Extract	07G - Rouges	8
Rosa Damascena (Damask Rose) Flower Extract	07I - Other Makeup Preparations	2
Rosa Damascena (Damask Rose) Flower Extract	10A - Bath Soaps And Detergents	5
Rosa Damascena (Damask Rose) Flower Extract	10B - Deodorants (Underarm)	2
Rosa Damascena (Damask Rose) Flower Extract	10C - Douches	1
Rosa Damascena (Damask Rose) Flower Extract	10D - Feminine Deodorants	1
Rosa Damascena (Damask Rose) Flower Extract	10E - Other Personal Cleanliness Products	2
Rosa Damascena (Damask Rose) Flower Extract	12A - Cleansing	8
Rosa Damascena (Damask Rose) Flower Extract	12C - Face And Neck (Exc Shave)	11
Rosa Damascena (Damask Rose) Flower Extract	12D - Body And Hand (Exc Shave)	8
Rosa Damascena (Damask Rose) Flower Extract	12F - Moisturizing	15
Rosa Damascena (Damask Rose) Flower Extract	12H - Paste Masks (Mud Packs)	1
Rosa Damascena (Damask Rose) Flower Extract	12I - Skin Fresheners	1
Rosa Damascena (Damask Rose) Flower Extract	12J - Other Skin Care Preps	8

Rosa Damascena (Damask Rose) Flower Oil
Total Uses: 223

Rosa Damascena (Damask Rose) Flower Oil	01B - Baby Lotions, Oils, Powders, And Creams	2
Rosa Damascena (Damask Rose) Flower Oil	02A - Bath Oils, Tablets, And Salts	4
Rosa Damascena (Damask Rose) Flower Oil	02B - Bubble Baths	2
Rosa Damascena (Damask Rose) Flower Oil	02D - Other Bath Preparations	2
Rosa Damascena (Damask Rose) Flower Oil	03D - Eye Lotion	5
Rosa Damascena (Damask Rose) Flower Oil	03E - Eye Makeup Remover	1
Rosa Damascena (Damask Rose) Flower Oil	03G - Other Eye Makeup Preparations	2
Rosa Damascena (Damask Rose) Flower Oil	04A - Cologne And Toilet Waters	1
Rosa Damascena (Damask Rose) Flower Oil	04B - Perfumes	38
Rosa Damascena (Damask Rose) Flower Oil	04E - Other Fragrance Preparation	8
Rosa Damascena (Damask Rose) Flower Oil	05A - Hair Conditioner	2
Rosa Damascena (Damask Rose) Flower Oil	05F - Shampoos (Non-Coloring)	4
Rosa Damascena (Damask Rose) Flower Oil	05I - Other Hair Preparations	1
Rosa Damascena (Damask Rose) Flower Oil	07E - Lipstick	1
Rosa Damascena (Damask Rose) Flower Oil	10A - Bath Soaps And Detergents	6
Rosa Damascena (Damask Rose) Flower Oil	10B - Deodorants (Underarm)	2
Rosa Damascena (Damask Rose) Flower Oil	10C - Douches	2
Rosa Damascena (Damask Rose) Flower Oil	10D - Feminine Deodorants	1
Rosa Damascena (Damask Rose) Flower Oil	10E - Other Personal Cleanliness Products	1
Rosa Damascena (Damask Rose) Flower Oil	12A - Cleansing	14
Rosa Damascena (Damask Rose) Flower Oil	12C - Face And Neck (Exc Shave)	35
Rosa Damascena (Damask Rose) Flower Oil	12D - Body And Hand (Exc Shave)	15
Rosa Damascena (Damask Rose) Flower Oil	12F - Moisturizing	37
Rosa Damascena (Damask Rose) Flower Oil	12G - Night	10
Rosa Damascena (Damask Rose) Flower Oil	12H - Paste Masks (Mud Packs)	5
Rosa Damascena (Damask Rose) Flower Oil	12I - Skin Fresheners	4
Rosa Damascena (Damask Rose) Flower Oil	12J - Other Skin Care Preps	15
Rosa Damascena (Damask Rose) Flower Oil	13B - Indoor Tanning Preparations	2

Rosa Damascena (Damask Rose) Flower Oil	13C - Other Suntan Preparations	1
Rosa Damascena (Damask Rose) Flower Powder **Total Uses: 1**		
Rosa Damascena (Damask Rose) Flower Powder	12J - Other Skin Care Preps	1
Rosa Damascena (Damask Rose) Flower Water **Total Uses: 308**		
Rosa Damascena (Damask Rose) Flower Water	03D - Eye Lotion	13
Rosa Damascena (Damask Rose) Flower Water	03E - Eye Makeup Remover	2
Rosa Damascena (Damask Rose) Flower Water	03G - Other Eye Makeup Preparations	5
Rosa Damascena (Damask Rose) Flower Water	04B - Perfumes	1
Rosa Damascena (Damask Rose) Flower Water	05A - Hair Conditioner	2
Rosa Damascena (Damask Rose) Flower Water	05F - Shampoos (Non-Coloring)	2
Rosa Damascena (Damask Rose) Flower Water	05G - Tonics, Dressings, And Other Hair Grooming Aids	5
Rosa Damascena (Damask Rose) Flower Water	05I - Other Hair Preparations	1
Rosa Damascena (Damask Rose) Flower Water	07C - Foundations	7
Rosa Damascena (Damask Rose) Flower Water	07E - Lipstick	7
Rosa Damascena (Damask Rose) Flower Water	07F - Makeup Bases	5
Rosa Damascena (Damask Rose) Flower Water	07H - Makeup Fixatives	3
Rosa Damascena (Damask Rose) Flower Water	07I - Other Makeup Preparations	1
Rosa Damascena (Damask Rose) Flower Water	09A - Dentifrices	1
Rosa Damascena (Damask Rose) Flower Water	09B - Mouthwashes And Breath Fresheners	1
Rosa Damascena (Damask Rose) Flower Water	10A - Bath Soaps And Detergents	3
Rosa Damascena (Damask Rose) Flower Water	10B - Deodorants (Underarm)	1
Rosa Damascena (Damask Rose) Flower Water	10E - Other Personal Cleanliness Products	4
Rosa Damascena (Damask Rose) Flower Water	11A - Aftershave Lotion	1
Rosa Damascena (Damask Rose) Flower Water	12A - Cleansing	31
Rosa Damascena (Damask Rose) Flower Water	12C - Face And Neck (Exc Shave)	83
Rosa Damascena (Damask Rose) Flower Water	12D - Body And Hand (Exc Shave)	16
Rosa Damascena (Damask Rose) Flower Water	12F - Moisturizing	58
Rosa Damascena (Damask Rose) Flower Water	12G - Night	3
Rosa Damascena (Damask Rose) Flower Water	12H - Paste Masks (Mud Packs)	17
Rosa Damascena (Damask Rose) Flower Water	12I - Skin Fresheners	17
Rosa Damascena (Damask Rose) Flower Water	12J - Other Skin Care Preps	18
Rosa Damascena (Damask Rose) Flower Water Extract **Total Uses: 1**		
Rosa Damascena (Damask Rose) Flower Water Extract	12H - Paste Masks (Mud Packs)	1
Rosa Damascena (Damask Rose) Flower Wax **Total Uses: 7**		
Rosa Damascena (Damask Rose) Flower Wax	03F - Mascara	1
Rosa Damascena (Damask Rose) Flower Wax	07E - Lipstick	2
Rosa Damascena (Damask Rose) Flower Wax	10A - Bath Soaps And Detergents	1
Rosa Damascena (Damask Rose) Flower Wax	12D - Body And Hand (Exc Shave)	1
Rosa Damascena (Damask Rose) Flower Wax	12J - Other Skin Care Preps	2

Concentration of Use by FDA Product Category – *Rosa damascena*-Derived Ingredients*

Rosa Damascena Flower Oil
Hydrolyzed Rosa Damascena Flower Extract
Rosa Damascena Bud Extract
Rosa Damascena Extract
Rosa Damascena Flower
Rosa Damascena Flower Extract
Rosa Damascena Flower Powder
Rosa Damascena Flower Water
Rosa Damascena Flower Water Extract
Rosa Damascena Flower Wax

Ingredient	Product Category	Maximum Concentration of Use
Rosa Damascena Flower Oil	Eye makeup removers	0.0095%
Rosa Damascena Flower Oil	Hair conditioners	0.0017%
Rosa Damascena Flower Oil	Hair sprays Aerosol	 0.00013-0.0003%
Rosa Damascena Flower Oil	Shampoos (noncoloring)	0.0011%
Rosa Damascena Flower Oil	Tonics, dressings and other hair grooming aids	0.0012%
Rosa Damascena Flower Oil	Hair dyes and colors	0.000059%
Rosa Damascena Flower Oil	Foundations	0.027%
Rosa Damascena Flower Oil	Lipstick	0.0002-0.01%
Rosa Damascena Flower Oil	Cuticle softeners	0.005%
Rosa Damascena Flower Oil	Bath soaps and detergents	0.00014%
Rosa Damascena Flower Oil	Skin cleansing (cold creams, cleansing lotions liquids and pads)	0.001-0.31%
Rosa Damascena Flower Oil	Face and neck products Not spray	 0.16%
Rosa Damascena Flower Oil	Body and hand products Not spray	 0.005-0.01%
Rosa Damascena Flower Oil	Other skin care preparations	10.8%
Rosa Damascena Extract	Hair conditioners	0.003%
Rosa Damascena Extract	Hair sprays Aerosol	 0.00001-0.00027%
Rosa Damascena Extract	Shampoos (noncoloring)	0.0003-0.003%
Rosa Damascena Extract	Tonics, dressings and other hair grooming aids	0.0065%
Rosa Damascena Extract	Hair dyes and colors	0.0023%
Rosa Damascena Extract	Foundations	0.00026%
Rosa Damascena Extract	Bath soaps and detergents	0.000005-0.05%
Rosa Damascena Extract	Deodorants Not spray Aerosol	 0.00007% 0.00007%
Rosa Damascena Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0025%
Rosa Damascena Extract	Face and neck products Not spray	 0.00077-0.014%
Rosa Damascena Extract	Moisturizing products	

	Not spray	0.00005%
Rosa Damascena Extract	Skin fresheners	0.00013%
Rosa Damascena Flower Extract	Hair conditioners	0.0012%
Rosa Damascena Flower Extract	Shampoos (noncoloring)	0.0012%
Rosa Damascena Flower Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0018%
Rosa Damascena Flower Water	Hair conditioners	0.009%
Rosa Damascena Flower Water	Shampoos (noncoloring)	0.009%
Rosa Damascena Flower Water	Hair dyes and colors	0.03-0.09%
Rosa Damascena Flower Water	Other hair coloring preparations	0.09%
Rosa Damascena Flower Water	Foundations	0.09-1.9%
Rosa Damascena Flower Water	Makeup bases	1.9%
Rosa Damascena Flower Water	Bath soaps and detergents	0.09%
Rosa Damascena Flower Water	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.99%
Rosa Damascena Flower Water	Face and neck products Not spray	32.7%
Rosa Damascena Flower Water	Moisturizing products Not spray	0.09%
Rosa Damascena Flower Water	Other skin care preparations	0.9%
Rosa Damascena Flower Wax	Eyeliners	0.13%
Rosa Damascena Flower Wax	Foundations	0.015%
Rosa Damascena Flower Wax	Lipstick	1.1%
Rosa Damascena Flower Wax	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.05%
Rosa Damascena Flower Wax	Face and neck products Not spray	0.05%

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

Information collected in 2019
Table prepared: July 23, 2019

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: December 2, 2020

SUBJECT: Rosa Damascena Flower Water and Rosa Damascena Flower Oil

The attached information concerns a trade name mixture, Rosality™, that contains 0.1-1% Rosa Damascena Flower Water and 0.1-1% Rosa Damascena Flower Oil in Pentylene Glycol.

Lucas Meyer Cosmetics by IFF. 2020. Composition Breakdown - Rosality™.

Lucas Meyer Cosmetics by IFF. 2020. Rosality™ - Specification Criteria.

IFF Lucas Meyer Cosmetics. 2019. Allergans certificate - Rosality™.

IFF Lucas Meyer Cosmetics. 2019. Characteristic molecules certificate - Rosality™.

Lucas Meyer Cosmetics by IFF. 2020. Toxicological File - Rosality™.

LUCASMEYER COSMETICS by IFF

COMPOSITION BREAKDOWN – Rosality™

Table 1: Rosality™ composition breakdown

INGREDIENTS	CAS n°	EINECS n°	% (W/W)
Pentylene Glycol	5343-92-0	226-285-3	QSP 100
Rosa Damascena Flower Water	90106-38-0	290-260-3	0.1-1.0
Rosa Damascena Flower Oil	90106-38-0 / 8007-01-0	290-260-3	0.1-1.0

Anne-Valérie CORNET (ex. SERGENT), Ing.

Regulatory Affairs Manager

IFF Lucas Meyer Cosmetics France & Canada

October 19th, 2020

Date

Lucas Meyer Cosmetics S.A.S.
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ROSALITY™ - SPECIFICATION CRITERIA
SP-ROS00010-4

DEFINITION	Lipophilic and hydrophilic olfactory compounds from Damask roses, stabilized in vegetable-derived pentylene glycol
INCI NAME (US)	Pentylene Glycol (and) Rosa Damascena Flower Water (and) Rosa Damascena Flower Oil
INCI NAME (EU)	Pentylene Glycol (and) Rosa Damascena Flower Water (and) Rosa Damascena Flower Oil
REGULATORY STATUS	No specific regulation
ANTI-OXIDANT(S)	None
PRESERVATIVE(S)	None
OTHER(S)	None
APPLICATIONS	Cosmetic products
COUNTRY OF ORIGIN	France

ORGANOLEPTIC CHARACTERISTICS	METHOD	SPECIFICATIONS
Aspect	*Visual*	*Transparent liquid*
Color	*Visual*	*Colorless*
Odour	*Olfactive*	*Characteristic*
PHYSICO-CHEMICAL CHARACTERISTICS	**METHOD**	**SPECIFICATIONS**
Refractive index @ 20°C	*-*	*1.434 - 1.444*
Phenylethyl alcohol (%)	*GC-FID*	*0.15 - 0.35*
Methyl eugenol (ppm)	*GC-MS*	*< 10*

STORAGE / PACKAGING :

SHELF LIFE	24 months in its original unopened packaging
STORAGE TEMPERATURE	25°C
STORAGE CONDITIONS	Protect from direct light and air. Reclose immediately after use.
USE CONDITIONS	-
PACKAGING	1 Kg and 5 Kg aluminium cans

April 23rd, 2020

Lucas Meyer Cosmetics S.A.S.
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www.lucasmeyercosmetics.com

IFF | LUCASMEYER COSMETICS

CERTIFICATE

Allergens

Rosality™

We, the undersigned, Lucas Meyer Cosmetics SAS, hereby declare that our product **Rosality™** contains the following substances considered as allergens according to Annex III of Regulation (EC) n° 1223/2009:

INCI DESCRIPTION	CAS n°	Total Content (ppm)
Alpha-Isomethyl Ionone	127-51-5	< 1*
Amyl Cinnamal	122-40-7	< 1*
Amylcinnamyl Alcohol	101-85-9	< 1*
Anise Alcohol	105-13-5	< 1*
Benzyl Alcohol	100-51-6	41
Benzyl Benzoate	120-51-4	< 1*
Benzyl Cinnamate	103-41-3	< 1*
Benzyl Salicylate	118-58-1	< 1*
Butylphenyl Methylpropional	80-54-6	< 1*
Cinnamal	104-55-2	< 1*
Cinnamyl Alcohol	104-54-1	< 1*
Citral	5392-40-5	16
Citronellol	106-22-9	1080
Coumarin	91-64-5	< 1*
Eugenol	97-53-0	< 1*
Evernia Furfuracea (Treemoss) Extract	90028-67-4	Negative (qualitative result)
Evernia prunastri (Oakmoss) Extract	90028-68-5	Negative (qualitative result)
Farnesol	4602-84-0	6
Geraniol	106-24-1	365
Hexyl Cinnamal	101-86-0	< 1*
Hydroxycitronellal	107-75-5	< 1*
Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde	31906-04-4	< 1*
Isoeugenol	97-54-1	< 1*
Limonene	5989-27-5	< 1*
Linalool	78-70-6	33
Methyl 2-Octynoate	111-12-6	< 1*

*Below indicated quantification level

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This declaration is based on the results obtained from the analytical test carried out by GC-MS on one industrial batch of Rosality™.

Anne-Valérie CORNET (ex. SERGENT), Ing.	**February 06th, 2019**
Regulatory Affairs Manager	Date
Lucas Meyer Cosmetics SAS	

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CERTIFICATE

Characteristic molecules

Rosality™

We, the undersigned Lucas Meyer Cosmetics SAS, hereby declare that **Rosality™** could contain the following molecules:

- ✓ Beta-caryophyllene (CAS n°: 87-44-5) : 48 ppm
- ✓ Pinene (CAS n°: 80-56-8) : 2 ppm
- ✓ Isobutenyl Methyltetrahydropyran (CAS n° : 16409-43-1) : 36 ppm
- ✓ Terpineols (CAS n° : 586-81-2, 7299-41-4, 7299-40-3, 7299-42-5; 8000-41-7, 10482-56-1, 98-55-5, 138-87-4, 562-74-3) : 40 ppm
- ✓ Benzaldehyde (CAS n°: 100-52-7): 1 ppm
- ✓ Nerol (CAS n°: 106-25-2): 400 ppm
- ✓ Citronellal (CAS n°: 106-23-0): 50 ppm
- ✓ 1-Nonadecene (CAS n°: 18435-45-5): 150 ppm
- ✓ Nonadecane (CAS n°: 629-92-5): 350 ppm

These values were calculated based on:

- the content of Rose fractions in **Rosality™**
- the mean concentrations of these molecules measured in three production batches of the mixture of Rose fractions.

Furthermore, **Rosality™** contains also:

- ✓ EU allergenic substances: please refer to Allergens certificate
- ✓ Methyl eugenol (CAS n°: 93-15-2): part of our specifications
- ✓ Phenethyl alcohol (CAS n°: 60-12-8): part of our specifications

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March 1st, 2019

Anne-Valérie CORNET (ex. SERGENT), Ing.

Regulatory Affairs Manager

Lucas Meyer Cosmetics SAS

Date

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LUCASMEYER COSMETICS
by IFF

TOXICOLOGICAL FILE

ROSALITY™

FIGHTING AGAINST THE STRESSOSPHERE™ TO RECOVER SKIN VITALITY

Synergistic combination of the full spectrum of olfactory compounds from Damask rose

-

Regulates cell metabolism disrupted by various stressors

-

Reduces the visible signs of skin fatigue for a glow, more rested and refreshed look

Tolerance/Toxicological File

ROSALITY™

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OVERVIEW OF ROSALITY™

ASSESSMENT OF THE ACUTE ORAL TOXICITY BY USING A 3T3 NRU CYTOTOXICITY ASSAY

Study n° CYTO-CHEM 18.493 performed by BIO-HC, Pessac, France.
This study is performed according to the OECD test guidelines 129 and to EURL ECVAM DB-ALM Protocol n°139.

In the experimental conditions described, the results of the NRU assay indicate an IC50 = 6.68 mg/mL allowing to estimate the acute oral Lethal Dose 50 (LD_{50}) of Rosality™ as **LD_{50} = 2798 mg/Kg** (**> 2000 mg/Kg**).

IN VITRO ALTERNATIVE TO OCULAR IRRITATION TEST – NEUTRAL RED RELEASE METHOD

Study n° 6.01_S-40500-ID-17/07763 performed by IDEA, Martillac, France.
This study is conducted according to the protocol proposed in the Journal Officiel de la République Française (N°302) as of December 30th 1999.

Under the retained experimental conditions, Rosality™ **tested at 20%** presents a **negligible cytotoxicity**.

SKIN TOLERANCE – 48-HOURS SINGLE PATCH TEST

Study n° 1.01_48H-ID-17/07763 performed by IDEA, Talence, France.
The study follows the "Guidelines for the Assessment of Skin Tolerance of Potentially Irritant Cosmetic Ingredients", COLIPA, 1997.

The irritation potential of Rosality™ tested at **20%** was clinically evaluated following a single contact application under a semi-occlusive patch that was maintained on the skin for 48 hours. The average irritation index obtained is equal to 0.

Results obtained under these experimental conditions indicate that Rosality™ **diluted at 20%** can be considered as **non-irritant** regarding its primary skin tolerance.

PHOTOTOXICITY

Test made by IEB-Lucas Meyer Cosmetics, Toulouse, France.
The study follows the Guidelines of OECD 101 "UV-VIS Absorption Spectra" (Spectrophotometric Method)", adopted by the council on 12th May 1981.

An UV spectrum was made on Rosality™ (10% in water) and it shows a low UV absorption between 290-400 nm. Therefore, according to ANSM recommendations, **no phototoxic potential could be suspected**.

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MUTAGENIC POTENTIAL *IN VITRO* TEST - AMES TEST

Study n° 6.46_5S-46372-ID-18/07230 performed by IDEA Lab, Plouzane, France.
The test is conducted in accordance with OECD Guideline 471 for the Testing of Chemicals (Bacterial Reverse Mutation Test. adopted 21th July 1997) and the test Method B13/B14 of Commission Regulation (EC) N°440/2008, dated May 30, 2008.
The test is performed according to the European Directive 2004/10/EC and the Good Laboratory Practice (GLP) principles of France.

No cytotoxicity was observed at any dose. No mutagenic response was observed for any of the bacterial strains, at the concentrations tested, with or without the addition of S9. Under the experimental conditions used, Rosality™ does not show mutagenic nor pro-mutagenic activity in the bacterial reverse mutation test.

IN VITRO SKIN SENSITIZATION STUDIES

-Sens-Is
Study n°L03F003a performed by Immunosearch, Grasse, France.

When tested pure or diluted in DMSO at 50 % v/v and 10% v/v and in PBS at 10% v/v, Rosality™ induced less than 7 genes in either the SENS-IS or the ARE groups of genes.

Under the experimental conditions of this study, Rosality™ can be therefore classified as a **non-irritant** and a **non-sensitizer**.

- **h-CLAT**
Study n°DC20494/CL02 performed by EUROSAFE, Saint Grégoire, France.
This study is performed according to the OECD test guideline 442E adopted July, 29th 2016 and to the h-CLAT DB-ALM protocol n°158.

No cytotoxicity was induced on THP-1 cells by Rosality™.
Under the assay conditions, a reproducible increase of the CD54/CD86 expression compared with the vehicle control for at least two and one dose-levels respectively of Rosality™ was noticed.
In all three experiments, a dose-response relationship was noticed for CD54/CD86 markers with an increase of 2.50 to 72.30 and 1.51 to 4.44-fold of expression compared to the vehicle control, respectively.
Based on these results, Rosality™ **demonstrated an in-vitro sensitizing potential with a Minimum Induction Threshold (MIT) of 923 µg/mL** under the conditions used during this study.

- **KeratinoSens™**
Study n°6.52-47205-ID-18/07230 performed by IDEA Lab, Martillac, France.
The study is conducted according to the OECD test guideline 442D dated February, 4th 2015 and the ECVAM DB-ALM protocol 155: KeratinoSens™.

The maximal average fold induction of luciferase activity value (I_{max}) was **lower than 1.50 for two independent repetitions**. Therefore, the **$EC_{1.5}$ value**, representing the concentration for which induction of luciferase activity is above the 1,5-fold threshold, **cannot be determined.**

So, under the experimental conditions of this test, Rosality™ may be classified as **not skin sensitizer**.

Conclusion on *in-vitro* studies: The KeratinoSens™ and Sens-IS assays are concordant so we are able to conclude based on these three sensitization tests.

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CONCLUSION ON SKIN SENSITIZATION

Based on the *in-vitro* results obtained, we can conclude that Rosality™ can be classified as **not skin sensitizer**.

BIODEGRADABILITY STUDY (CLOSED BOTTLE)

Study made by Alcycor, Limoges, France.
The test was performed in accordance with OECD Guideline 301 D permitting the screening of chemicals for ready biodegradability in an aerobic aqueous medium. This method was adopted by the council on 17th July 1992.

After 28 days, the biodegradability of **Rosality™** reached **27.68**%. **Rosality™ doesn't fulfill ready biodegradability criteria** at a concentration value of 5 mg/L in the reaction medium.

DAPHNIA SP. ACUTE IMMOBILIZATION TEST

Study made by Alcycor, Limoges, France.
The test was performed in accordance with OECD Guideline 202 (adopted on 4th April 1984 – last version dated 13th April 2004) and the European Directive (EC) 440/2008 adopted on 30thMay 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH).
OECD Guideline 202 describes an acute toxicity test to assess effects of chemicals towards daphnids.

According to the results obtained under the experimental conditions adopted, **Rosality™** can be considered as **non-toxic**, **(EC_{50}, 48h) > 100 mg/L**.

FRESHWATER ALGA AND CYANOBACTERIA, GROWTH INHIBITION TEST

Study made by Alcycor, Limoges, France.
The test was performed in accordance with OECD Guideline 201 (original adoption: 12th May 1981 – most recently updated: 23th March 2006).
The purpose of this assay is to determine the effects of a substance on the growth of freshwater microalgae and/or cyanobacteria.

Under the experimental conditions used, **Rosality™** has **no toxicity to the algal growth rate** (*Pseudokirchnerilla subcapitata)* at **concentrations up to 100 mg/L**; **E_rC_{50} .72h >100 mg/L**.

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TOLERANCE STUDIES

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ASSESSMENT OF THE ACUTE ORAL TOXICITY BY USING A 3T3 NRU CYTOTOXICITY ASSAY

Study n° CYTO-CHEM 18.493 performed by BIO-HC, Pessac, France.
This study is performed according to the OECD test guidelines 129 and to EURL ECVAM DB-ALM Protocol n°139.

INTRODUCTION

The purpose of the *in vitro* 3T3 NRU Cytotoxicity assay is to estimate the acute oral toxicity of Rosality™ with a basal cytotoxicity assay in BALB/3T3 murine fibroblasts.
The cytotoxicity is expressed as a dose dependent reduction of the uptake of the vital dye Neutral Red[1] (NR) when measured 48 hours after exposure to increased concentrations of the test item. The test is based on the determination of IC_{50} value, i.e. the concentration of the test item that decreases cell viability by 50%.
The 3T3 NRU Cytotoxicity test is proposed as an *in vitro* method used to estimate an IC_{50} value which in turn is used to predict a LD_{50} value that can serve as the starting dose for the acute oral toxicity test *in vivo* (OECD n°129 Guidance document, 2010[2]).

PROTOCOL

Test system: Murine fibroblasts BALB/3T3 clone A31, from the ATCC cultured in [DMEM-GlutaMAX-1] medium supplemented with fetal calf serum (10% FCS) and antibiotics ([complete DMEM] medium), maintained under the following conditions:
- temperature: 37°C (± 1°C)
- humidified athmosphere with 5% (± 1%) CO_2.

Test procedure[3]:

Plating: BALB/3T3 cells are detached by trypsinization and counted according to standard operating procedures of the laboratory. The cell suspension is seeded into 96-well plates at a density of 3-4 x 10^3 cells per well in [complete DMEM] medium. Two 96-well microplates are used: 1 microplate for the test item and 1 microplate for the positive control tested concurrently in the assay.
After seeding, the 96-well plates are then incubated under the following conditions:
- incubation: 24 hours (± 2 hrs).
- temperature: 37°C (± 1°C)
- humidified athmosphere with 5% (± 1%) CO_2.

Experimental group distribution:
[Rosality™] groups: 9 concentrations (initial test) and 8 concentrations (Main test).
[Negative control] group: [DMEM + 5% FCS] ± solvent
[Positive control] group: 8 concentrations between 10 and 100 µg/mL of Sodium Lauryl Sulfate (SLS) (Main test).

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Rosality™ dilutions: they are prepared on a weight/volume basis using culture medium with 5% FCS. Two separate consecutive experiments are performed to determine the IC_{50} value for each test product.

1. Initial test: range finding experiment

The range of the 9 tested concentrations (6 wells/conc.) is determined adequately by logarithmic serial dilutions from the highest soluble concentration of the test substance in one 96-well plate.
9 concentrations are tested between 0.01 and 100 mg/mL.

2. Main test: IC_{50} determination

The choice of the concentrations to be tested in the main test depends on the results of the initial test. The range of the 8 tested concentrations (6 wells/conc.) is determined adequately around the IC_{50} estimated from the range finder test. The main test should be performed twice.
8 concentrations are tested between 1.4 and 50 mg/mL.

Treatment of cells: 24 hours (± 2 hrs) after seeding, cells are treated with the 8 test-solutions of Rosality™ and/or with the positive control (SLS) concentrations range and incubated for 48 hours (± 0.5h).

Cell viability: after contact with Rosality™ or SLS, the incubation media are removed from plates and neutral red (NR) solution (50 µg/mL) is added into each well. After 180 min (± 10 min) incubation, the NR solution is removed and NR desorb solution (acidified ethanol solution) is added. After 10 minutes (± 1 min) incubation under shaking at room temperature, the optical density (OD) of the NR extract is measured at 540 nm.

Cytotoxicity evaluation:
The optical densities (OD) of each well are corrected by subtracting the mean OD value of respective blanks (n=6) measured in parallel. From the corrected optical densities (OD), the percentage of cell death is calculated for each concentration tested, according to the formula:

$$cell\ death\ \% = \frac{OD_{corr} mean[treated\ wells]}{OD_{corr} mean[control\ wells]} \times 100$$

The concentration of Rosality™ reducing cell viability to 50% (IC_{50}) is calculated from the dose-response curve using the linear probit-log regression model.

Interpretation of results:
Based on the validation study[4], the LD_{50} value of Rosality™ is estimated from the mean value of IC_{50} values obtained in 2 separate assays, according to the following regression formula:

$$\log DL_{50}\ (mmol/kg) = 0{,}439 \log CI_{50}\ (mM) + 0{,}621$$

or

$$\log DL_{50}\ (mg/kg) = 0{,}372 \log CI_{50}(\mu g/mL) + 2{,}024$$

Test validation
The 3T3 NRU cytotoxicity test is validated if:

- The positive control (PC) IC_{50} value is within ±2,5 standard derivations (SD) of the historical mean established by the laboratory.
- The PC fitted dose-response curve has an R^2 (coefficient of determination) ≥ 0,85.
- The corrected OD_{corr} of each control well ($|VCi|$) is ≤ 15% from the mean corrected OD of the negative control.
- For the test item, at least one calculated cytotoxicity value > 0% and ≤ 50% viability and at least one calculated cytotoxicity value > 50% and < 100% viability should be present.
- These results allow to validate the test.

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RESULTS

Cytotoxicity of Rosality™:
Initial test:
A dose dependent cytotoxic effect was observed in the range of tested concentrations.
Highest tested concentration in final test: 50 mg/mL.
Main test:
A dose dependent cytotoxicity was observed in the range of tested concentrations. The percentages of cytotoxicity induced by test concentrations in each assay are collected in the following table:

Conc. (mg/mL)	1.4	2.33	3.89	6.48	10.8	18	30	50
Cytotoxicity assay n°1	3%	11%	23%	42%	68%	93%	100%	99%
Cytotoxicity assay n°2	2%	12%	24%	40%	66%	89%	99%	99%

The IC_{50} value was calculated in each assay using the probit-log [concentration] regression model.
Assay n°1: IC_{50} = 6.70 mg/mL
Assay n°2: IC_{50} = 6.67 mg/mL
IC_{50} (mean of the 2 assays) = 6.68 mg/mL

Cytotoxicity of the positive control:
Main test: Results showed a dose dependent cytotoxicity in the range of tested concentrations.
The IC_{50} value was calculated in each assay using the probit-log [concentration] regression model:
Assay n°1: IC_{50} = 50.76 µg/mL
Assay n°2: IC_{50} = 47.39 µg/mL

LD 50 estimation:
The mean value of IC_{50} obtained (6.68 mg/mL) allows to estimate the LD_{50} value of **Rosality™:**
LD_{50} = 2798 mg/Kg

CONCLUSION

In the experimental conditions described, the results of the NRU assay indicate an **IC_{50} = 6.68 mg/mL** allowing to estimate the acute oral Lethal Dose 50 (LD_{50}) of Rosality™ as **LD_{50} = 2798 mg/Kg (> 2000 mg/Kg).**

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IN VITRO ALTERNATIVE TO OCULAR IRRITATION TEST NEUTRAL RED RELEASE METHOD

Study n° 6.01_S-40500-ID-17/07763 performed by IDEA, Martillac, France.
This study is conducted according to the protocol proposed in the Journal Officiel de la République Française (N°302) as of December 30th 1999.

OBJECTIVE

The Neutral Red Release assay is a quantitative colorimetric cytotoxicity test used to evaluate the ocular irritant potential of cosmetic products through a determination of the concentration of product leading to 50% of cell mortality (IC_{50}).
Rosality™ is applied during a fixed length on rabbit cornea fibroblasts (SIRC cells), preloaded with a vital dye, the neutral red.
Once Rosality™ is removed, only living cells appear colored. The quantity of neutral red remaining in the surviving cells is released by cell lysis and evaluated by optical density measurement. The percentage of cell mortality or cytotoxicity is established in comparison with a negative control.

STUDY RELEVANCE

The Neutral Red Release assay is an alternative to animal experimentation (such as the Draize rabbit eye test), included in a group of tests used to assess the ocular irritancy of finished cosmetics as well as chemical substances.

PROTOCOL

Material and Methods: rabbit cornea fibroblasts lineage SIRC (ATCC – CCL60). Cells were preloaded with neutral red dye for 3 hours at 37°C.

Rosality™ **(20%)** was used as a stock solution, from which 3 different concentrations were tested on the SIRC cells: 50% - 25% and 0%. The cells were exposed to 500 µL of test substance; the contact time was of 60 seconds.

Negative control:
Reference item diluent: saline solution (NaCl 9 g/L)
Test item diluent: water
Positive control: 0.2%, 0.05% and 0.01% Sodium Dodecyl Sulfate (CAS: 151-21-3) in saline solution.

Reading procedure:
The amount of released neutral red dye was assessed by measurement of the Optical Density (OD), at 540 nm.

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The cell death rate was calculated for each dilution according to the formula:

$$\% \text{ death rate} = \frac{Abs._{\text{negative control}} - Abs._{\text{test item}}}{Abs._{\text{negative control}}} \times 100$$

This percentage was then plotted against product concentration in a graph. The IC_{50} corresponds to the concentration of tested product inducing 50% of cell death.
If no dose-response is observed (in case the product is not cytotoxic at all the dilutions), the IC_{50} is not calculated and is just reported to be superior to 50% (IC_{50}> 50).

Interpretation of results:
The amount of neutral red released is related to the cytotoxicity of the test substance. The correlation with the ocular irritant potential is given by the prediction model below:

IC_{50}	% cell death rate obtained at 50% dilution of the test item	Classification
IC_{50} > 50%	≤ 20	Negligible cytotoxicity
IC_{50} > 50%	> 20 and < 50	Slight cytotoxicity
25 < IC_{50} ≤ 50%	-	Moderate cytotoxicity
IC_{50} ≤ 25%	-	Severe cytotoxicity

RESULTS

Under the retained experimental conditions:

Negative control: Negligible cytotoxicity

Positive Controls (Sodium Dodecyl Sulfate at 0.01%, 0.05% and 0.2%): Severe cytotoxicity (IC_{50} = 0.116%)

Product	IC_{50}	% cell death rate at 50% dilution of the tested product	Classification
Rosality™ (20%)	IC_{50} > 50%	20	Negligible cytotoxicity

CONCLUSION

Under the retained experimental conditions, Rosality™ tested by the Neutral Red Release method at **20%**, and according to the JORF classification, presented a **negligible cytotoxicity**. Regarding its ocular irritant potential, Rosality™ is **well tolerated at recommended usage (1% and below)**.

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PRIMARY SKIN IRRITATION
48-HOURS SINGLE PATCH TEST

Study n° 1.01_48H-ID-17/07763 performed by IDEA, Talence, France.
The study follows the "Guidelines for the Assessment of Skin Tolerance of Potentially Irritant Cosmetic Ingredients", COLIPA, 1997.

OBJECTIVE

The objective of the study is to check the skin compatibility of Rosality™ after single application on the external face of the arm under exaggerated experimental conditions (under semi-occlusive patch, for 48 hours).

STUDY RELEVANCE

Cutaneous irritation is a general phenomenon of inflammatory origin which can be defined as a loss of skin integrity. It leads to inflammatory reactions within the dermis and the epidermis that translate into redness (erythema) and oedema.

The human Single Patch Test (semi-occlusive application of Rosality™ to the skin for 48 hours), allows to check for the absence of cutaneous primary irritation after a single sustained application. Visual scoring is performed according to a pre-established numeric scale.

PROTOCOL

Inclusion Criteria:

- Number of included subjects: 11
- Number of exclusions: 0
- Number of valid cases: 11
- Sex: 9 women and 2 men.
- Age: 19 to 63 years old (Mean = 35)
- Skin type: normal skin – There is no dermatological lesion on the experimental area.

Test molecule mode of application:

- *Area*: external face of the arm of the test subject, taking into account the skin appearance and avoiding the areas of friction with clothes
- *Quantity*: 160 µL of Rosality™ **diluted at 20% in distilled water**
- *Frequency and duration:* single application during 48 hours.
- *Conditions of application*: use of a semi-occlusive patch (True Med®)

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Modalities of evaluation:

The skin compatibility is based on visual skin examination.

- *Clinical observations*: 30-40 minutes after removal of the patches, readings were performed by the dermatologist and results obtained in the treated area compared to those obtained for a "negative" control (empty patch).
- *Quantification of cutaneous irritation:* The clinical marking is given according to a numerical scale established depending on the intensity of the irritation phenomena observed (erythema, oedema, dryness, blister, etc.). According to their intensity, the quotation is spread out from 0 to 3.

Analysis of results:

- *Determination of the Average Irritation Index:* the total sum of scores, divided by the number of volunteers, defines the **Average Irritation Index**.
- The ranking of the irritant potential is determined according to the Average Irritation Index obtained as described below:

Average Irritation Index (A.I.I.)	Classification
A.I.I. ≤ 0.20	Non-irritant
0.20 < A.I.I. ≤ 0.50	Slightly irritant
0.50 < A.I.I. ≤ 2.0	Moderately irritant
2.0 < A.I.I. ≤ 3.0	Very irritant

RESULTS

Results from 11 volunteers have been included in the analysis giving an Average Irritation Index of **0.0** for Rosality™ **tested at 20%.**
None of the volunteers selected took a treatment contraindicated with the study. No withdrawal of the study happened.

CONCLUSION

Considering the results obtained under these experimental conditions, Rosality™ **(20%)** shows very good skin compatibility and can be considered as **non-irritant regarding its primary skin tolerance at recommended usage level of 1% and below**.

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PHOTOTOXICITY

Test made by IEB-Lucas Meyer Cosmetics, Toulouse, France.
The study follows the Guidelines of OECD 101 "UV-VIS Absorption Spectra" (Spectrophotometric Method)", adopted by the council on 12th May 1981.

OBJECTIVE

The primary environmental purpose in determining the ultraviolet-visible (UV-VIS) absorption spectrum of a chemical compound is to have some indication of the wavelengths at which the compounds may be susceptible to photochemical degradation.

Since photochemical degradation is likely to occur on both the atmosphere and the aquatic environment, spectra appropriate to these media will be informative concerning the need for further persistence testing.

Degradation will depend upon the total energy absorbed in specific wavelength regions. Such energy absorption is characterized by both molar absorption coefficient (molar extinction coefficient) and band width. However, the absence of measurable absorption does not preclude the possibility of photodegradation.

PROTOCOL

Material:

Acquisitions are done on a spectrophotometer UV Perkin Elmer lamda12 and drove by the UV Winlab software.

Product tested:

Rosality™ diluted at 10% in water.

Methodology: MSC.spectre

The acquisition methodology with the SPECTRE.MSC is a methodology which covers the spectral wavelength from 200 nm to 800 nm with a recording every 2 nm and a speed screening of 480 nm/min.
For the solution tested, a spectrogram is edited from 200 nm to 800 nm.
The absorbance scale is chosen in order to obtain the maximum amplitude.

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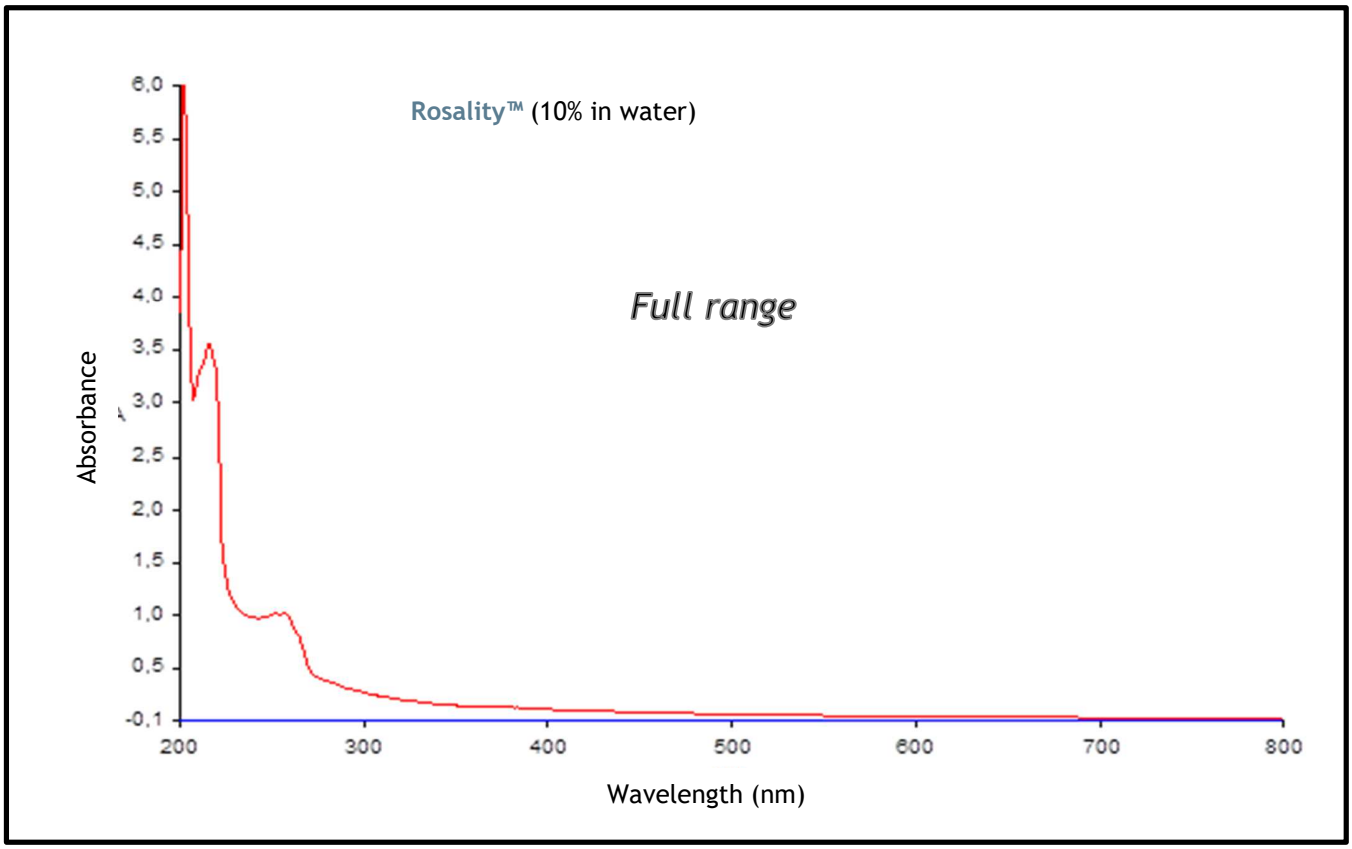
RESULTS



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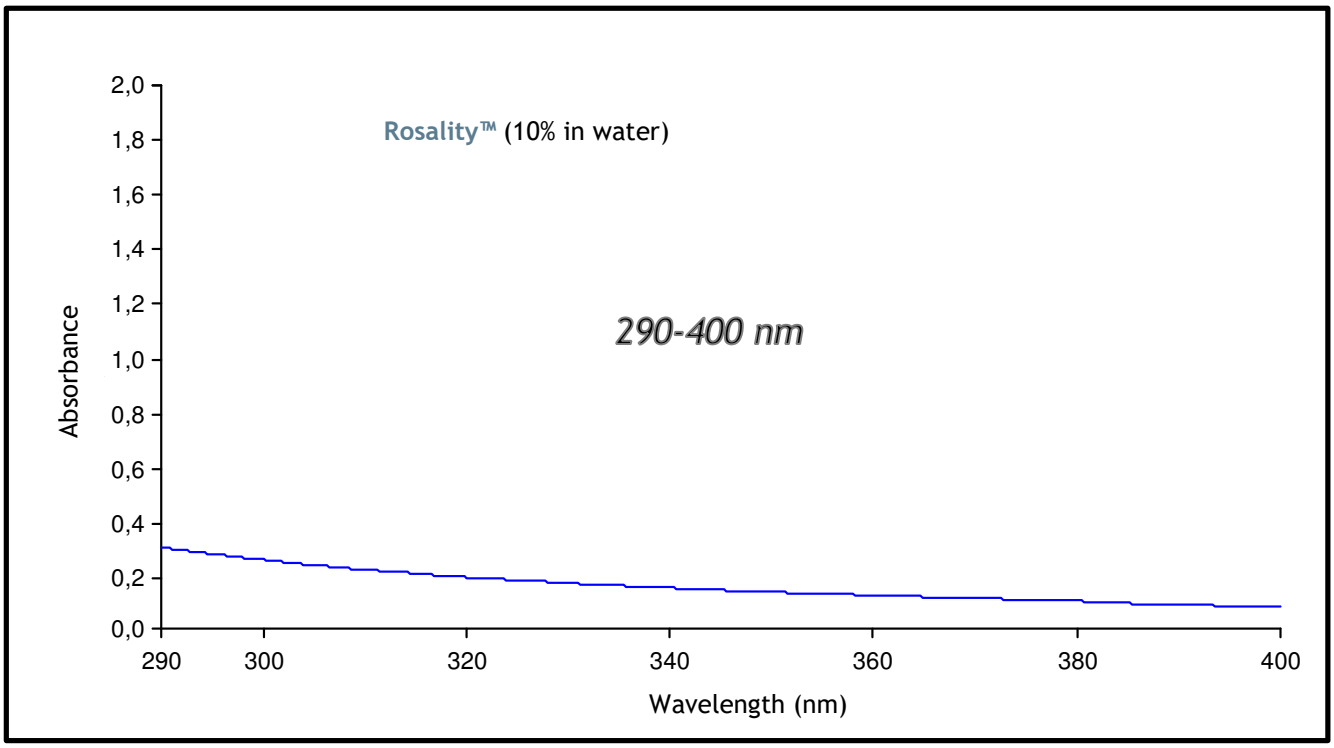
Zoom on 290-400 nm:



CONCLUSION

Rosality™ at 10% in water shows a very low UV absorption between 290-400 nm. Therefore, according to ANSM recommendations, **no phototoxic potential** of **Rosality™ could be suspected.**

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MUTAGENIC POTENTIAL IN VITRO TEST - AMES TEST

Study n° 6.46_5S-46372-ID-18/07230 performed by IDEA Lab, Plouzane, France.
The test is conducted in accordance with OECD Guideline 471 for the Testing of Chemicals (Bacterial Reverse Mutation Test. adopted 21th July 1997) and the test Method B13/B14 of Commission Regulation (EC) N°440/2008, dated May 30, 2008.
The test is performed according to the European Directive 2004/10/EC and the Good Laboratory Practice (GLP) principles of France.

OBJECTIVE

The purpose of this assay was to test whether Rosality™ is mutagenic or pro-mutagenic.

STUDY RELEVANCE

Mc Cann et al. proved the great specificity and sensitivity of this test by establishing the connection between the carcinogenic and mutagenic potential of 300 products. This test is commonly used as a first evaluation test for the mutagenic potential of a test article, in the pharmaceutical, cosmetic, veterinary, as well as chemical industries.

The assay is based on the detection of point mutations (substitution, addition or deletion of one or a few DNA base pairs) or frameshift-mutations in five bacterial strains (*S.typhimurium* TA 98, *S.typhimurium* TA 100, *S.typhimurium* TA 102, *S.typhimurium* TA 1535 and *S.typhimurium* TA 1537) by incubation with one concentration of the product (Rosality™). These strains have several features that make them most sensitive for the detection of mutations. The mutagenic effect was analyzed in the presence and in the absence of a metabolic system, namely rat liver microsome fraction (S9).

PROTOCOL

Materials: Five bacterial strains (*S.typhimurium* TA 98, *S.typhimurium* TA 100, *S.typhimurium* TA 102, *S.typhimurium* TA 1535 and *S.typhimurium* TA 1537) were used for this assay.

Metabolic Activation system: The S9 mix is prepared from rat liver microsomial fractions and contains metabolic enzymes. The S9 mix is buffered and supplemented with essential co-factors.

Test item: Rosality™

Method:

Solubility test: Rosality™ was soluble in deionized water at the highest concentration of 5000 µg/plate.

Preliminary cytotoxicity assay: it was performed on *S.typhimurium* TA 100 strain at the concentrations of 5000, 1600, 500, 160 and 50 µg/plate, with and without S9. No cytotoxicity of the test item was observed.
Therefore, this concentrations range was used for the main study.

Two experiments were carried out using each tester strain with plating in triplicates at each concentration.

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Reference items were included in experiments:

-Negative controls: the spontaneous revertant count with the solvent (deionized water), with and without metabolic activation, was included in each experiment.
-Negative control without treatment: the spontaneous revertant count without the solvent (deionized water), with and without metabolic activation was included in each experiment for the control of absence of mutagen activity of vehicle.
-Positive controls: known mutagens were used for each strain.

Evaluation of genetic mutations: Following incubation, the number of colonies per plate was counted and recorded.

Data are presented as the number of revertant colonies present per plate (mean ± standard deviation). The R ratio is calculated as follows:

$$R = \frac{\text{Number of revertant colonies in the presence of } \textbf{Rosality™}}{\text{Number of revertant colonies with solvent in the absence of } \textbf{Rosality™}}$$

Interpretation of data: All of the following were considered as positive results:

1) A dose-response R increase in the range tested in at least one strain, with or without the metabolic activation system. The mutagenicity is taken into account for a given concentration only when the number of revertants is equal at least to the double of the spontaneous rate of reversion for TA 98, TA 100 and TA 102 ($R \geq 2$) and the triple of the spontaneous rate of reversion for TA 1535 and TA 1537 ($R \geq 3$).

2) A reproducible R increase at one or more concentration in at least one strain, with or without the metabolic activation system.

Any positive result from the bacterial reverse mutation test indicates that the test item may induce point mutations or reading frame shifts in the bacterial genome.

A negative result indicates that, under the test conditions, the test item is not mutagenic for the bacterial strains tested.

RESULTS

Test controls were in concordance with the expected results:

-No cytotoxicity activity was observed by Rosality™ in the bacterial system at a concentration of 5000 µg/plate.

-All positive controls performed showed a valid ratio (R) above 2.5.

-Positive and negative controls showed absolute numbers of revertant colonies comparable to historical data.

-At the concentrations tested, Rosality™ showed no significant increase in the number of revertant colonies either with or without S9 metabolic activation.

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-No dose response was observed in any of the tested bacterial strains.

-In addition, no sign of precipitate was observed.

CONCLUSION

Based on the results obtained in this study, Rosality™ was found to be **NON-MUTAGENIC** and **NON PRO-MUTAGENIC** under the experimental conditions assayed.

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IN-VITRO SKIN SENSITIZATION STUDIES

-SENS-IS

Study n°L03F003a performed by Immunosearch, Grasse, France.

OBJECTIVE

The study assessed the potential of the test item **Rosality™** to specifically induce the expression of irritation and/or sensitization biomarkers in an *in-vitro* 3D skin model.

STUDY PRINCIPLE

Application of irritant and/or sensitizer onto the skin induces the reversible destruction of tissue as well as the activation of the innate and adaptative immune system. These biological changes are induced by the specific expression (or repression) of distinct set of genes with a kinetic dependent of the type of reaction observed.

The SENS-IS test is based on the analysis by RT-PCR (reverse transcription polymerase chain reaction) of two set of genes, one specifically reflecting the irritant potential of a chemical after application to the skin ("IRRITATION" set of genes) and the other set of genes correlating with the sensitization potential (splited in 2 subsets of genes namely "SENS_IS" and "REDOX"). By measuring the level of expression of these two separate sets of genes at a given time point after application and by comparison with internal negative, irritant and sensitizer positive controls, the SENS-IS test can determine the irritant and sensitizer potential of a chemical.

STUDY RELEVANCE

Although studies performed in this laboratory have shown a very good correlation between SENS-IS test results and sensitization potency for a number of tested chemicals, it has to be noted that the test has not been thoroughly evaluated by competent authorities yet.

PROTOCOL

Test item: **Rosality™** was tested pure and dissolved in DMSO at 50% v/v and 10% v/v and in PBS at 10% v/v.

Test system:
Episkin large is a three-dimensional human skin model comprising a reconstructed epidermis with a functional *stratum corneum* provided by SkinEthic Laboratories.
The EpiSkin models are obtained by culturing adult human keratinocytes on a collagen substrate in conditions which permit their terminal differentiation and the reconstruction of an epidermis with a functional horny layer. After 3 days of immersed culture conditions, the epidermis is airlifted during 10 days allowing differentiation and formation of a horny layer.

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The human keratinocytes come from mammary samples obtained from healthy consenting donors during plastic surgery. HIV 1 & 2, B and C hepatitis tests are carried out on the donor bloods as well as verification of the bacteriological & fungal sterility of the cells and absence of mycoplasma.
The reconstructed human epidermis expresses the major differentiation markers (filaggrin and involucrin in granular cell layers, transglutaminase I and keratin 10 in supra basal cell layers and loricrin in upper granular cell layers), as well as expressing the basement membrane markers (type IV collagen; integrin alpha 6, integrin beta 4, antigen BP, laminin I and laminin V).
Free fatty acids and ceramides are detected in the lipid profile. The ultra-structural features show secretion and normal arrangement of bi-layered lipid content into the intercellular spaces of the cornified cell layers (formation of normal permeability barrier).

Experimental procedure:
30 µl (26.3 µl/cm^2) of each dilution was applied on the top of each reconstituted epidermis (EpiSkin model), using a positive displacement pipette. The tested product was gently spread on the epidermis surface to ensure it covers all the surface.
After 15 mn exposure, the Episkin were rinsed with PBS. Epidermis were then incubated at 37°C for 6 hours.
After incubation, the complete epidermis was collected, placed in a RNAzol solution and the total RNA was isolated by homogenization of the skin. After reverse transcription, quantitative gene expression was measured by RT-PCR using a sybr green buffer using the LC480 Roche's apparatus and specific biomarkers primers defined for the Sens-IS test.
For each analysis three negative controls (PBS, olive oil and DMSO treated skins), a positive irritation control (5% SLS) and two positive sensitization controls (HCA at 50% and TNBS at 1%) were performed.
The test product and the controls are tested in at least 2 experiments (using different batches of Episkin models). Further experiments can be conducted if invalid results are obtained in the previous experiments.

EVALUATION OF THE RESULTS

Acceptance criteria:
The first acceptance criteria is the IC_{50} value of SLS on Episkin that must be ≥ 1.2 mg/mL (ImmunoSearch defined specification).
Tissue destruction or over irritation induced by the chemical at a given concentration are then analyzed as follow:
- Tissue destruction is measured by the expression of the gene HSPAA1. The cycle threshold (CT) value of HSPAA1 gene for the sample must not be above 10% of that of the olive oil or PBS controls.
- Over irritation due to cell stress is measured by the number of irritation biomarker genes over expressed. If more than 20 genes are overexpressed for a given concentration of the product, the sample is not accepted and the substance is analysed at a lower concentration.

Evaluation criteria:
The results for a test substance are considered to be valid if the controls are correctly classified based on the number of gene overexpressed.
A substance is considered irritant if it induces the overexpression of at least 15 genes among a group of 23 genes named "IRRITATION".
A test substance is considered a sensitizer if it induces the over expression (fold value >1.25) (compared to the mean of the PBS and olive oil controls) of at least 7 genes among two groups of respectively 21 and 17 genes named "SENS_IS" and "REDOX". Both groups of genes are involved in skin sensitization but are related to different pathways: the "REDOX" group gathers genes involved in the oxidative stress responses, whereas the "SENS-IS" group gathers genes biomarkers of skin sensitization but not involved in the oxidative stress response.

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The sensitizing response is analyzed under different concentration to measure the concentration at which no positive response is induced.

RESULTS

The results of the positive and negative controls reached the acceptance criteria in the 3 experiments; they were therefore considered to be valid.

Results obtained with **Rosality™** are the following ones:

Number of overexpressed genes	**Rosality™** tested Pure	**Rosality™** dissolved in DMSO at 50% v/v	**Rosality™** dissolved in DMSO at 10% v/v	**Rosality™** dissolved in PBS at 10% v/v
IRRITATION	12	4	3	3
SENS_IS	4	0	2	5
ARE	1	4	2	1
IRRITATION	**NEGATIVE**	**NEGATIVE**	**NEGATIVE**	**NEGATIVE**
SENSITIZATION	**NEGATIVE**	**NEGATIVE**	**NEGATIVE**	**NEGATIVE**

CONCLUSION

Under the experimental conditions of this study, **Rosality™** can be therefore classified as a **non-irritant** and a **non-sensitizer**.

-H-CLAT

Study n°DC20494/CL02 performed by EUROSAFE, Saint Grégoire, France.
This study is performed according to the OECD test guideline 442E adopted July, 29th 2016 and to the h-CLAT DB-ALM protocol n°158.

OBJECTIVE

The h-CLAT method is proposed to address the third key event (Dendritic Cells activation) of the skin sensitization Adverse Outcome Pathway by quantifying changes in the expression of cell surface markers associated with the process of activation of DC (i.e. CD86 and CD54), in the human monocytic leukaemia cell line THP-1, following exposure to sensitizers (Ashikaga et al. (2006). The measured expression levels of CD86 and CD54 cell surface markers are then used for supporting the discrimination between skin sensitizers and non-sensitizers.
This test is part of a tiered strategy for skin sensitization assessment.

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STUDY PRINCIPLE

The h-CLAT method is an *in vitro* assay that quantifies changes of cell surface marker expression (i.e. CD86 and CD54) on a human monocytic leukemia cell line, THP-1 cells, following 24 hours exposure to the test item.
These surface molecules are typical markers of monocytic THP-1 activation and may mimic DC activation, which plays a critical role in T-cell priming. The changes of surface marker expression are measured by flow cytometry following cell staining with fluorochrome-tagged antibodies.
Cytotoxicity measurement is also conducted concurrently to assess whether upregulation of surface marker expression occurs at sub-cytotoxic concentrations. The relative fluorescence intensity of surface markers compared to solvent/vehicle control are calculated and used in the prediction model to support the discrimination between sensitizers and non-sensitizers.

STUDY RELEVANCE

The study design and data interpretation are based on the OECD test guideline 442E: *In Vitro* Skin Sensitization: human Cell Line Activation Test (h-CLAT) adopted on July 2016.
This test is considered scientifically valid to be used as part of an IATA strategy (IATA: Integrated Approaches to Testing and Assessment), to support the discrimination between skin sensitizers and non-sensitizers for the purpose of hazard classification and labelling.
Results generated in the validation study (ECVAM, 2012) overall indicate that, compared with the murine Local lymph node assay (LLNA) results, the accuracy in distinguishing skin sensitizers from non-sensitizers is 85% with a sensitivity of 93% and a specificity of 66%.

PROTOCOL

Test item: **Rosality™** was tested **pure**

Vehicle control used for all the assays in this study: RPMI-1640 medium.

Reference controls:
-Positive controls: DNCB (CAS n°: 97-00-7) and NiSO4 (CAS n°: 10101-97-0).
-Negative control: Lactic Acid (CAS n°: 50-21-5).

Test system:
THP-1 (monocytic leukeamia cell line, TIB-202™) cells were provided from American Type Culture Collection.
Cells were stored in liquid nitrogen and the assays were performed thanks to a master bank.
Cryopreserved cells were thawed. Cells were cultured, at 37°C under 5% CO_2 and humidified atmosphere, in RPMI-1640 medium supplemented with 10% Foetal Calf Serum, 100 units/mL penicillin and 100 µg/mL streptomycin. THP-1 were routinely seeded every 3-4 days at the density of 0.15 to 0.2 x 10^6 cells/mL.
For testing, THP-1 cells were seeded at a density of 0.2 x 10^6 and pre-cultured in culture flasks for 72 hours.
The quality of each batch of THP-1 cells should be checked. Cell viability for negative controls must be above 90%.
DNCB and $NiSO_4$ produced a positive response for both CD86 and CD54.
Lactic Acid produced a negative response for both markers.

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Preliminary study: Cytotoxicity assay
The cytotoxicity of the test item was evaluated in order to select at least 4-5 concentrations able to induce cytotoxicity, around 50%, for the highest one. Assessment of cell toxicity was performed by determining cell viability on THP-1 cells, using the 7-Aminoactinomycin D (7-AAD) inclusion method.
Eight concentrations of test item were prepared by a two-fold serial dilution and a final maximum concentration of 1 000 µg/mL, obtaining a final range of concentrations in the plate of 7.81 to 1 000 µg/mL.
In the day of testing, cells harvested from culture flask were re-suspended with fresh culture medium at 2 x 10^6 cells/mL. Then, THP-1 cells were distributed into a 24 well flat-bottom plate with 500 µL (1 x 10^6 cells/well) with various concentrations of test item (1:1 ratio) for 24±0.5 hours at 37°C under 5% CO_2. After treatment cells were transferred into sample tubes and collected by centrifugation. The cells were stained with 7-AAD (5 µg/mL final concentration). Then cells were analyzed with flow cytometry and a software to measure cell viability. The living cells (7-AAD-) gate was set in the 7-AAD negative area. 10^4 7-AAD- cells were counted as the living population.
According to the results the dose levels for the main study were selected.

Main study: Activation test
Based on the cytotoxicity assay the eight final test item concentrations were selected.
In case of non-toxic concentration for the top dose used in preliminary experiment, the maximum concentration selected for activation test reached 5 000 µg/mL when the test item could be dissolved or stably dispersed in saline or medium. The doses range for activation test was therefore the following (two fold dilution factor): from 39.1 to 5000 µg/mL in the experiment 1, then from 19.5 to 2500 µg/mL in the experiments 2 and 3.
The range of concentrations was adjusted for activation test 2 and 3 depending of results obtained in the first experiment to calculate the minimum induction threshold.
Each experiment of activation test was performed on eight concentrations.
THP-1 cells were plated at 1*10^6 cells/mL/well in 24 well plates and treated for 24±0.5 hours at 37 ± 1°C under 5±1% $CO_2$with selected test item concentrations. After treatment cells were washed once with Fb. Then cells were stained for 30 min at about 4°C with the following fluorescein isothiocyanate (FITC) conjugated monoclonal antibodies (mAbs): anti-human CD54, anti-human CD86; FITC labelled-mouse IgG1. Using the manufacturer's recommended dilutions, cells were incubated with above mAbs at 6 µL/3*10^5 cells /50µL for the anti-human CD86 mAb, and 3 µL/3*10^5 cells /50µL for the anti-human CD54 mAb. FITC labelled-mouse IgG1 was used as an isotype control at a dilution of 3 µL/3*10^5 cells /50µL. Then, the cells were stained also with 7-AAD for at least 30 min at about 4°C. After washing and resuspension with Fb, the fluorescence intensities of the THP-1 cell surface markers were then analyzed by flow cytometry and a software, on 10000 living cells.

RESULTS CALCULATION

Calculation of RFI

The Relative Fluorescence Intensity (RFI) is used as an indicator of CD86 and CD54 expression.
RFI is calculated with the following formula:

$$RFI = \frac{\text{MFI of test item-treated cells} - \text{MFI of test item-treated isotype control cells}}{\text{MFI of vehicle control cells} - \text{MFI of vehicle isotype control cells}} \times 100$$

MFI: Mean Fluorescence Intensity

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When the cell viability was less than 50%, RFI was not calculated because of the diffuse labelling of cytoplasmic structures that are generated following cell membrane destruction.

Calculation of EC (Effective Concentration)

For the test items predicted as positive with the h-CLAT, the EC150 for CD86 and EC200 for CD54, i.e. the concentration at which the test item induced a RFI of 150 or 200, may be determined. They are calculated by the following equations:

$EC150\ (CD86) = B_{concentration} + [(150 - B_{RFI})/(A_{RFI} - B_{RFI}) \times (A_{concentration} - B_{concentration})]$
$EC200\ (CD54) = B_{concentration} + [(200 - B_{RFI})/(A_{RFI} - B_{RFI}) \times (A_{concentration} - B_{concentration})]$

$A_{concentration}$: the lowest concentration in µg/mL, with RFI > 150 (CD86) or 200 (CD54)
$B_{concentration}$: the highest concentration in µg/mL, with RFI < 150 (CD86) or 200 (CD54)
A_{RFI} :RFI at the lowest concentration with RFI> 150 (CD86) or 200 (CD54)
B_{RFI} : RFI at the highest concentration with RFI< 150 (CD86) or 200 (CD54)

The ECs are calculated for each experiment. If three experiments are performed, the final EC150/EC200 values are then determined as the median value of the ECs calculated from the three independent runs. When only two experiments are performed or two of three independent runs meet the criteria for positivity, the highest EC150 or EC200 of the two calculated values are adopted.

Determination of MIT (Minimum Induction Threshold)

The MIT is determined as the smallest of either EC150 or EC200.

RESULTS INTERPRETATION

For CD86/CD54 expression measurement, each test item is tested in at least two independent runs to derive a single prediction (POSITIVE or NEGATIVE). An h-CLAT prediction is considered POSITIVE if at least one of the following conditions is met in 2 of 2 or in at least 2 of 3 independent runs, otherwise the h-CLAT prediction is considered NEGATIVE:

- The RFI of CD86 is equal to or greater than 150% at any tested concentration (with cell viability ≥ 50%);
- The RFI of CD54 is equal to or greater than 200% at any tested concentration (with cell viability ≥ 50%).

Based on the above, if the first two runs are both positive for CD86 and/or are both positive for CD54, the h-CLAT prediction is considered POSITIVE and a third run does not need to be conducted. Similarly, if the first two runs are negative for both markers, the h-CLAT prediction is considered NEGATIVE without the need for a third run. If however, the first two runs are not concordant for at least one of the markers (CD54 or CD86), a third run is needed and the final prediction will be based on the majority result of the three individual runs (i.e. 2 out of 3).

In this respect, it should be noted that if two independent runs are conducted and one is only positive for CD86 and the other is only positive for CD54, a third run is required. If this third run is negative for both markers, the h-CLAT prediction is considered NEGATIVE. On the other hand, if the third run is positive for either marker or for both markers, the h-CLAT prediction is considered POSITIVE.

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VALIDATION OF THE STUDY

Acceptability criteria for evaluating results induced by the positive and negative controls, the vehicle controls and the test item, were based on the guideline for h-CLAT (OECD 442E, 2017).
This study is considered valid if the following criteria are fully met:

- In the positive controls:
RFI values of both CD86 and CD54 must be over the positive criteria (CD54 ≥ 200% and CD86 ≥ 150%).
The cell viability must be more than 50%.

-In the negative control:
RFI values of both CD86 and CD54 must be under the positive criteria (CD54 < 200% and CD86 < 150%).
The cell viability must be more than 50%.

- In the vehicle control (medium, 0.9% NaCl, DMSO, Ethanol etc.):
Cell viability must be more than 90%.
In the solvent/vehicle control, RFI values of both CD86 and CD54 must not exceed the positive criteria (CD86 ≥ 150% and CD54 ≥ 200%) compared to the medium control.
The MFI ratio of both CD86 and CD54 to isotype control must be>105%.

- In the test item:
Cell viability should be more than 50% in at least four tested concentrations in each run.

RESULTS

Rosality™ was tested **pure.**
Solubility of Rosality™ was assessed before performing the assay. Rosality™ was soluble in RPMI, the highest concentration that has been reached was 5000 µg/mL. The highest dose used in this study was **5000 µg/mL** after dilution in medium.

Preliminary study: Cytotoxicity assay
Cytotoxicity profile of the test item was assessed with the 7-AAD dye.
According to the results obtained, **no CV75 value could be determined**. Based on the cell toxicity assays, the maximum dose level selected for the main study (Activation test) was **5000 µg/mL**.

Main study: Activation test:
Under the assay conditions, a reproducible increase of the CD54/CD86 expression compared with the vehicle control for at least two and one dose-levels respectively of Rosality™ was noticed.
In all three experiments, a dose-response relationship was noticed for CD54/CD86 markers with an increase of 2.50 to 72.30 and 1.51 to 4.44-fold of expression compared to the vehicle control, respectively.
Based on linear regression and according to the guideline, the concentrations inducing 150/200% of CD86/CD54 RFI (EC150/200, EC: Effective Concentration) were calculated for each experiment.
The median value of the EC200 is 923 µg/mL.
The median value of the EC150 is 2125 µg/mL.
The MIT is 923 µg/mL.

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CONCLUSION

Based on these results, the test item Rosality™ **demonstrated an *in-vitro* sensitizing potential with a Minimum Induction Threshold (MIT) of 923 µg/mL** under the conditions used during this study.

-KERATINOSENS™

Study n°6.52-47205-ID-18/07230 performed by IDEA Lab, Martillac, France.
The study is conducted according to the OECD test guideline 442D dated February, 4th 2015 and the ECVAM DB-ALM protocol 155: KeratinoSens™.

OBJECTIVE

The objective of this study was to evaluate the activation of the Keap1-Nrf2-ARE pathway in transformed keratinocytes by monitoring the induction of the luciferase.
This test is part of a tiered strategy for skin sensitization assessment.

STUDY PRINCIPLE

A skin sensitizer refers to a substance that will lead to an allergic response following skin contact. One of the biological keys takes place in the keratinocytes and includes inflammatory responses as well as gene expression associated with specific cell signaling pathways such as the antioxidant/electrophile response element (ARE) dependent pathways.
The genes under the ARE control, including AKR1C2 gene identified as a target gene for detecting skin sensitizers in dendritic cells, are induced by the protein Nrf2 via Keap1.
The test consists in evaluating the activation of AKR1C2 in transformed keratinocytes (KeratinoSens™), by monitoring the induction of the luciferase gene fused to AKR1C2. The luciferase produced by the cells complexes with luciferin which, in the presence of ATP, produces light measured in Relative Light Units (RLU).
After contact between a sensitizing potential substance with a KeratinoSens™ monolayer, the induction of the luciferase is quantified. In parallel, the cytotoxicity is measured, in order to exclude a false positive generated by a skin irritation.

STUDY RELEVANCE

The study design and data interpretation are based on the OECD test guideline 442D: *In Vitro* Skin Sensitization: ARE-Nrf2 Luciferase Test Method, adopted on February 2015.
This test is considered scientifically valid to be used as part of an IATA strategy (IATA: Integrated Approaches to Testing and Assessment), to support the discrimination between skin sensitizers and non-sensitizers for the purpose of hazard classification and labelling.

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PROTOCOL

Test system:
Cells: KeratinoSens™ (Givaudan). They were exempt of mycoplasma.
Media and reagents:
-Treatment medium: DMEM 1g/L glucose, 1% non-heat inactivated foetal calf serum – stored at 5°C ± 3°C.
-Seeding medium: DMEM 1g/L glucose, 9.1% non-heat inactivated foetal calf serum – stored at 5°C ± 3°C.
-Staining solution: 5 mg/mL MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5 diphenyl-tetrazolium bromide] solution in PBS.
-Test item: **Rosality™**, batch XO12617.41-2

Reference items:
-Positive control: cinnamaldehyde (CAS n°: 104-55-2)
-Negative control: 1% DMSO (CAS n°: 67-68-5) in treatment medium.

Series definition:
The test item was tested at 12 concentrations according to a geometric progression of ratio 2 from 0.2 µg/mL to 400 µg/mL.
Negative control: 6 wells of solvent control (1% DMSO in treatment medium) with cells and one well of solvent control without cell by culture plate.
Positive control: five concentrations of cinnamaldehyde on each culture plate. The concentration varied from 4 to 64 µM according to a geometric progression of ratio 2.

The study was composed of three independent repetitions. For each repetition the test item and the reference items were replicated on three independent plates for the measurement of induction and two plates for the measurement of cytotoxicity. Each repetition was performed on a different day with fresh stock solution of the test item.

Test protocol:

First day: cells seeding
The cells were trypsinized and cells suspension were adjusted to a density of 8.10^4 cells/mL in seeding medium.
125 µL of the cell suspension at 8.10^4 cells/mL (i.e. 10^4 cells/well) were distributed in three white plates for the induction measurement and two transparent plates to assess the cytotoxicity. The seeded plates were incubated 24 hours ± 1 hour at 37°C, 5% CO_2.

Second day: preparation of test item dilutions
The test item was diluted in sterile water. The stock solution was prepared at 40 mg/mL.
Preparation of the positive control stock solution: The positive control was prepared at 200 mM in DMSO then diluted to the final concentration of 6.4 mM.

Preparation of the 100 X plate: a 100-fold concentrated dilutions series was prepared in 96-well plate.
Test item: it was placed in one of the rows B to F. 100 µL of sterile water were distributed from column 1 to 11. 200 µL of the 40 mg/mL stock solution were placed in column 12. Then the series dilutions were prepared by transferring 100 µL of the column 12 in the column 11 and so on until the column 1.
Positive control: 100 µL of DMSO were distributed in row G from columns 7 to 10. 200 µL of the 6.4 mM stock solution were placed in column 11. Then the series dilutions were prepared by transferring 100 µL of the column 11 in the column 10 and so on until the column 7.

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Negative control: 100 µL of DMSO were distributed in row G columns 1 to 6 and 12 and in the well H12.

Preparation of the 4 X dilution plate:
The 100 X plate was diluted 25 times in a new plate (4 X) with treatment medium.

Second day: contact between the cells and the test and reference items
In the five seeded plates, the medium was aspirated and replaced with 150 µL of treatment medium. Then the 4 X plate was replicated five times: 50 µL from the 4 X plate were placed in each of the three white plates and in the two transparent plates. The plates (1 X) were covered with an adhesive plastic foil to prevent evaporation and incubated for 48 hours ± 1 hour (37°C, 5% CO_2).

Day 4: Luciferase activity
After 48 hours, the medium was aspirated and each well was gently washed once with 200 µL of PBS. Then 100 µL of luciferase substrate (luciferine + ATP + lysine agent) were then added in each well. The plates were incubated at least 15 minutes at room temperature to ensure cell lysis.
The plates were placed in the luminometer and the luciferase activity was measured.

Day 4: Cell viability assessment with MTT method
After 48 hours, the medium was aspirated and each well was gently washed once with 200 µL of PBS. Then, 225 µL of staining solution diluted at 0.6 mg/mL in treatment medium were distributed in each well. The plates were covered with an adhesive plastic foil and incubated for 4 hours ± 30 minutes (37°C, 5% CO_2).
After this contact time, the staining solution was eliminated and the cells were treated with 200 µL of 10% SDS one night in the dark (37°C, 5% CO_2). After a 10 minutes homogenization, the absorbances were measured at 540 nm.

RESULTS CALCULATION

Two parameters are measured: the luciferase induction and the cytotoxicity.

Luciferase induction:

I_{max}, maximal fold induction of luciferase activity value observed at any concentration of the test item and positive control. The induction value I is calculated according to the following formula:

$$I = \frac{\text{Luminescence}_{\text{Test item}} - \text{Luminescence}_{\text{Blank}}}{\text{Luminescence}_{\text{Negative control}} - \text{Luminescence}_{\text{Blank}}}$$

The I_{max} of an item is the average of the I_{max} calculated for each of the repetitions.

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EC1.5, value representing the concentration for which induction of luciferase activity is above 1.5 threshold, is obtained according to the following equation:

$$EC1.5 = (C_b - C_a) \times \left[\frac{(1.5 - I_a)}{(I_b - I_a)} \right] + C_a$$

Where:
C_a = the lowest concentration (µM or µg/mL) with more than 1.5-fold the induction
C_b = the highest concentration (µM or µg/mL) with less than 1.5-fold the induction
I_a = induction factor for the lowest concentration with more than 1.5-fold the induction
I_b = induction factor for the highest concentration with less than 1.5-fold the induction

The retained EC1.5 value is the geometric average of the EC1.5 calculated for each of the repetitions.

Cytotoxicity

IC_{70}, concentration in µg/mL for which we obtained 70% cell viability:

$$IC_X = (C_b - C_a) \times \left[\frac{(X) - V_a)}{(V_b - V_a)} \right] + C_a$$

Where:
X = is the % viability at the concentration to be calculated (70)
C_a = the lowest concentration for which the % viability is lower than X%
C_b = the highest concentration for which the % viability is higher than X%
V_a = the % viability at the lowest concentration for which the % viability is lower than X%
V_b = the % viability at the highest concentration for which the % viability is higher than X%

The data processing is carried out by a locked Excel sheet provided by Givaudan. The raw data generated from the reading of the plates are directly introduced into the dedicated fields, and a data processing is performed automatically.
A graph showing the gene induction and the cytotoxicity of each element, the I_{max} and EC1.5 values are automatically generated.

RESULTS INTERPRETATION

The test item is identified as potential skin sensitizer if the four following conditions are met in two of two or in two of three repetitions. Otherwise, the KeratinoSens™ prediction is considered as negative:

- the I_{max} is strictly 1.5-fold higher of the basal luciferase activity statistically significantly to the value obtained for the negative control,
  If the I_{max} is exactly equal to 1.5, the test item is rated as negative and no EC1.5 value is calculated.

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- the EC1.5 value is strictly below 200 µg/mL,

- at the lowest concentration with a gene induction above 1.5, the cell viability must be strictly above 70% (i.e. EC1.5 < IC_{70}),

- There is an apparent overall dose-response for luciferase induction, which is similar between the repetitions.

RESULTS

Results obtained with the positive and the negative controls allowed us to validate the test.

Study plan deviation: In repetition 2, the original electronic file of the RLU reading of plate 2 was accidentally erased. Even if data could be exploited, this calls into question raw data integrity. Results of repetition 2 were therefore not taken into account for the study conclusion. However, they correlated with the two other repetitions.

Test item:
In repetition 1 and 3, **I_{max} is lower than 1.5** and the **EC1.5 values are not determined**. The repetitions are **negative**.

CONCLUSION

The two repetitions are considered **negative**. So, under the experimental conditions of this test, Rosality™ may be classified as **not skin sensitizer**.

-CONCLUSION ON *IN-VITRO* STUDIES

The KeratinoSens™ and Sens-IS assays are concordant so we are able to conclude based on these three sensitization tests.

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CONCLUSION ON SKIN SENSITIZATION

Based on the *in-vitro* results obtained, we can conclude that Rosality™ can be classified as **not skin sensitizer**.

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BIODEGRADABILITY STUDY (CLOSED BOTTLE)

Study made by Alcycor, Limoges, France.
The test was performed in accordance with OECD Guideline 301 D permitting the screening of chemicals for ready biodegradability in an aerobic aqueous medium. This method was adopted by the council on 17th July 1992.

OBJECTIVE

The purpose of this assay was to assess the ready biodegradability of **Rosality™** in an aerobic aqueous medium.

PRINCIPLE OF THE TEST

For this method, the formula of the product and its purity, or relative proportions of major components, should be known so that the Theoretical Oxygen Demand (ThOD) may be calculated. If the ThOD cannot be calculated, the Chemical Oxygen Demand (COD) should be determined.

The solution of the test product in mineral medium (usually 2-5 mg/L) is inoculated with a relatively small number of micro-organisms from a mixed population and kept in completely full, closed bottles in the dark at constant temperature.
Degradation is followed by analysis of dissolved oxygen over a 28-day period and measurements are taken at sufficiently frequent intervals to allow the identification of the beginning and end of biodegradation.
The amount of oxygen taken up by the microbial population during biodegradation of the test product, corrected for uptake by the blank inoculum run in parallel, is expressed as a percentage of ThOD or, less satisfactorily COD.

The OECD 301D method evaluates the ability of the inoculum to really degrade the product.

PROTOCOL

The inoculum is derived from the secondary effluent of a treatment plant. The concentration of inoculum introduced in the reaction medium is 1 mL/L.
The concentration of **Rosality™** in reaction medium is 5 mg/L.
The Chemical Oxygen Demand is evaluated and equal to 2.20 mg O_2/mg.
Positive control: Sodium acetate at 10 mg/L.
Analytical method used: Dissolved oxygen measured by electrode method (electrode FDO® 925 WTW).

RESULTS

The biodegradability of **Rosality™** reached **27.68**% after 28 days.

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CONCLUSION

The pass level for ready biodegradability is 60% of ThOD. This pass value has to be reached in a 10-day window within the 28-day period of the test. The 10-day window begins when the degree of biodegradation has reached 10% ThOD and must end before day 28 of the test.

Based on the results obtained in this study, Rosality™ **doesn't fulfill ready biodegradability criteria** at a concentration value of 5 mg/L in the reaction medium.

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DAPHNIA SP. ACUTE IMMOBILIZATION TEST

Study made by Alcycor, Limoges, France.
The test was performed in accordance with OECD Guideline 202 (adopted on 4th April 1984 – last version dated 13th April 2004) and the European Directive (EC) 440/2008 adopted on 30thMay 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH).
OECD Guideline 202 describes an acute toxicity test to assess effects of chemicals towards daphnids.

OBJECTIVE

The objective of this study was the assessment of the acute toxicity effects of the test item Rosality™ to invertebrates, measured as immobilization of *Daphnia magna*.

STUDY RELEVANCE

Young daphnids, aged less than 24 hours at the start of the test, are exposed to the test substance at a range of concentrations for a period of 48 hours. Immobilization is recorded at 24 hours and 48 hours and compared with control values. The results are analyzed in order to calculate the EC_{50} at 48h.
EC_{50} is the concentration estimated to immobilize 50 per cent of the daphnids within a stated exposure period. Immobilization: Animals that are not able to swim within 15 seconds, after gentle agitation of the test vessel are considered to be immobilized (even if they can still move their antennae).

PROTOCOL

Material: *Daphnia magma* is used for this assay.
Rosality™ was tested at 5 concentrations: 1 mg/L; 3.5 mg/L; 10 mg/L; 35 mg/L and 100 mg/L.

Method:

Daphnids (*Daphnia magna*), not older than 24 hours were exposed to 5 concentrations of Rosality™ under semi-static conditions for a period of 48 hours. The numbered test vessels were completely filled with the test media, the test organisms were added and the vessels were closed with a gas-tight stopper directly afterwards by avoiding air bubbles. No feeding and no aeration occurred throughout the test.

The test media was renewed after 24 hours by transferring the test organisms to new vessels with freshly prepared test media under sterile conditions.

Immobility and abnormal behavior were recorded after 24 and 48 hours. Immobile animals were eliminated from the vessels as soon as they were discovered.

The temperature should be within the range of 18°C - 22°C, and for each single test it should be constant within ±1°C.The beakers were subjected to a light cycle of 16 hours followed by a dark cycle of 8 hours and this light/dark cycles lasted 48 hours.

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Data are analyzed by an appropriate statistical method (e.g. probit analysis, etc.) to calculate the slopes of the curve and the EC_{50} with 95% confidence limit (p = 0.95).

RESULTS

Mortality or immobility of the control: 0%

Rosality™ conc. (mg/L)	Efficacy %
1	0
3.5	0
10	0
35	0
100	0

(EC_{50}, 48h) of Rosality™ is **upper than 100 mg/L**.

CONCLUSION

According to the results obtained under the experimental conditions adopted, Rosality™ can be considered as **non-toxic.**

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FRESHWATER ALGA AND CYANOBACTERIA, GROWTH INHIBITION TEST

Study made by Alcycor, Limoges, France.
The test was performed in accordance with OECD Guideline 201 (original adoption: 12th May 1981 – most recently updated: 23th March 2006).
The purpose of this assay is to determine the effects of a substance on the growth of freshwater microalgae and/or cyanobacteria.

OBJECTIVE

The purpose of this assay was to assess the effects of **Rosality™** on the growth of freshwater microalgae and/or cyanobacteria.

PRINCIPLE OF THE TEST

Exponentially growing test algae are exposed to the test substance in batch cultures over a period of normally 72 hours.
The system response is the reduction of growth in a series of algal cultures (test units) exposed to various concentrations of a test substance. The response is evaluated as a function of the exposure concentration in comparison with the average growth of control cultures. For full expression of the system response to toxic effects (optimal sensitivity), the cultures are allowed unrestricted exponential growth under nutrient sufficient conditions and continuous light for a sufficient period of time to measure reduction of the specific growth rate. Growth and growth inhibition are quantified from measurements of the algal biomass density as a function of time.
The test endpoint is inhibition of growth, expressed as logarithmic algal biomass increase (average growth rate) during the exposure period. From the average specific growth rates recorded in a series of test solutions, the concentration bringing about a specified 50 % inhibition of growth rate is determined and expressed as the E_rC_{50}.
In addition, the no observed effect concentration (NOEC) may be statistically determined.

PROTOCOL

Type of specie used: Unicellular green algae, *Pseudokirchneriella subcapitata*.
Culture conditions used:
Culture medium: LC-Oligo medium
Temperature: 21-24°C
Light intensity: light cycle of 16 hours followed by a dark cycle of 8 hours; around 4500 lux
Ventilation: bubbling

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Pre-culture conditions: The algae are incubated for about 3 days under test conditions and used to inoculate the test solutions. This is made to adapt the test alga to the test conditions and ensure that the algae are in the exponential growth phase when used to inoculate the test solutions.

Test conditions:
Duration of test: 72h
Renewal of test solutions: none (static mode)
Growth medium: OECD medium (original medium of OECD TG 201)
Temperature: 21-25°C
Light intensity: constant, 400-700 nm, about 7500 lux
Ventilation: none
Stirring: constant, about 250 rpm
Initial biomass concentration: around 10^4 cells/mL.

This study is made in two steps:

- Screening step: this allows us to determine the concentrations of tested product which inhibit between 5% and 75% of algal growth rate.
  The tests were carried out by using a stock solution of Rosality™ at 100 mg/L diluted in the test medium.
  Concentrations tested in % of the stock solution: 100%; 35%; 10%; 3.5% and 1%.
- Limit test: when the preliminary test indicates that the test substance has no toxic effects at concentrations up to 100 mg/L, a limit test involving a comparison of responses in a control group and one treatment group (100 mg/L) is undertaken. It could allow us to determine E_rC_{50} .72h; E_rC_{20} .72h; E_rC_{10} .72h and the NOEC.
  [ErCx: Concentration in mg/L of the tested product which causes a reduction of x% of the algal growth rate compared to the control.
  NOEC: Highest tested concentration that did not cause significant inhibition of the algal growth rate compared to the control].

RESULTS

Data processing:
After 72h±2h, measurement of biomass is done for each concentration tested by manual cells counting by microscope. Any abnormal observations are reported.
Specific growth rate and specific growth inhibition rate are calculated for each tested concentration.
ErCx determination is made by using a logistic model based on Hill's equation.
The NOEC is evaluated by statistical model using the Bonferroni t test.

Reference substance used: Potassium dichromate, $K_2Cr_2O_7$ (reference substance for green algae).
Its E_rC_{50} .72h = 1.16 mg/L (value compliant with results previously obtained by the laboratory and between 0.92 mg/L and 1.46 mg/L - acceptable range of sensitivity of algae *P. subcapitata* as defined in standard NF EN ISO 8692: 2012).

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Results of the screening assay:
The percentage of growth inhibition rate is equal to 0 at all the concentration tested.
Rosality™ has **no toxic effects** at concentrations **up to 100 mg/L**.
Therefore, a limit test is performed at 100 mg/L.

Results of the limit test:
E_rC_{50} .72h > 100 mg/L
E_rC_{20} .72h > 100 mg/L
E_rC_{10} .72h > 100 mg/L
NOEC.72h ≥ 100 mg/L

CONCLUSION

Under the experimental conditions used, Rosality™ has **no toxicity to the algal growth rate** (*Pseudokirchneriella subcapitata)* at concentrations **up to 100 mg/L; E_rC_{50} .72h >100 mg/L.**

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FINAL CONCLUSION

Tolerance and safety studies have shown that **Rosality™ at recommended usage level (1% and below)** presents no risk for cutaneous and/or ocular irritation. Furthermore, **Rosality™** is not mutagenic and has no skin sensitizing properties.

Rosality™ is also considered as non-toxic for the aquatic environment.

In conclusion to these toxicological studies, **Rosality™** is considered as well tolerated and safe for cosmetic purpose at the recommended usage level (1% and below).

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[2] OECD 129 Guidance document on using cytotoxicity tests to estimate starting doses for acute oral systemic toxicity tests, 2010

[3] BALB/c 3T3 Neutral Red Uptake Cytotoxicity assay (3T3 NRU) EURL ECVAM DB-ALM Protocol n°139

[4] NIH, 2006. Publication n°07-4518, November 2006

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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: December 10, 2020

SUBJECT: Rosa Damascena Flower Water

Anonymous. 2020. Rosa Damascena Flower Water in a trade name mixture with Butylene Glycol (method of manufacture and impurities).

December 2020

Rosa Damascena Flower Water in a trade name mixture with Butylene Glycol

1. Method of manufacture and impurities data

Trade name	Damask Rose Water BG80
Method of manufacture	Dried raw material ⇒steam distillation ⇒obtain distillate (water phase) ⇒concentration ⇒obtain concentrate ⇒add 80vol% 1,3-butylene glycolic solution ⇒packaging
Impurities data	Heavy metals: not more than 20ppm Arsenic: not more than 2ppm

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 18, 2021

SUBJECT: *Rosa damascena*-Derived Ingredients

The first two reports are on two different products that were tested on the same panel of volunteers.

Anonymous. 2012. Repeated insult patch test study (fragrance containing 0.1068% Rosa Damascena Flower Water).

Anonymous. 2012. Repeated insult patch test study (fragrance containing 0.7794% Rosa Damascena Flower Extract).

Anonymous. 2019. Repeated insult patch test study (mask containing 0.1260% Rosa Damascena Flower Oil).

[redacted]

REPEATED INSULT PATCH STUDY

fragrance containing 0.1068% Rosa Damascena Flower Water

[redacted]

CONDUCTED FOR:

[redacted]

DATE OF ISSUE:

November 6, 2012

Version 1.0

[redacted]

[redacted] [redacted]

TABLE OF CONTENTS

APPENDICES

[redacted]

SIGNATURES

This study was conducted in compliance with the requirements of the protocol and TKL'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.

[redacted]

TITLE OF STUDY

Repeated Insult Patch Study

SPONSOR

[redacted]

STUDY MATERIAL

[redacted], Fragrance

DATE STUDY INITIATED

August 29, 2012

DATE STUDY COMPLETED

October 5, 2012

DATE OF ISSUE

November 6, 2012

INVESTIGATIVE PERSONNEL

[redacted]

CLINICAL SITE

[redacted]

[redacted]

SUMMARY

One product, [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 (100) subjects completed the study.

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

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

3.2.3 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:

[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.

- = 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]'s strict certification requirements to standardize the assignment of response grades.

4.0 NATURE OF STUDY MATERIAL

4.1 STUDY MATERIAL SPECIFICATIONS

Identification : [redacted]
Amount Applied : 0.2 mL
Special Instructions : Evaporated for 30 minutes prior to patch application.

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 sixteen (116) subjects between the ages of 18 and 70 were enrolled and 100 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:		116
Number discontinued:		16
Lost to follow-up:	12	
Voluntary withdrawal:	3	
Adverse events:	1	
Number completed:		100

Source: Table 1, Appendix I

There was one non-product-related serious adverse event (SAE) reported during the study. See Data Listing 4, Appendix II for details.

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, [redacted].

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APPENDIX I

SUMMARY TABLES

[redacted]

Table 1: Summary of Subject Enrollment and Disposition

	N (%)
Subjects enrolled	116
Subjects completed induction phase	102 (87.9)
Subjects completed all phases	100 (86.2)
Total subjects discontinued	16 (13.8)
Lost to follow-up	12 (10.3)
Voluntary withdrawal	3 (2.6)
Adverse events	1 (0.9)

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	43 (37.1)
N (%) 45 to 64	65 (56.0)
N (%) 65 and up	8 (6.9)
Mean (SD)	47.7 (13.2)
Median	49.9
Range	18.5 to 70.4
Gender	
N (%) Male	30 (25.9)
N (%) Female	86 (74.1)
Race	
Asian	1 (0.9)
Black	8 (6.9)
Caucasian	75 (64.7)
Hispanic	32 (27.6)

See data listing 2 for further detail.

[redacted]

[redacted]

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

Product = [redacted]

	Induction Reading										Challenge Phase		
Response	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Make Up**	**48hr**	**72hr**	**96hr(*)**
-	109	103	105	105	103	102	101	101	96	22	100	100	
Total evaluable	109	103	105	105	103	102	101	101	96	22	100	100	
Number absent	5	9	5	1	2	3	3	2	6		0	0	
Number discontinued	2	4	6	10	11	11	12	13	14		16	16	

Maximum Elicited Response During Induction
All Subjects Completing Induction (N=102)

Response	**n(%) Subjects**
-	102 (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	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
002	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
003	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
004	08/29/12	08/29/12	--	09/04/12	I0	L	7
005	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
006	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
007	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
008	08/29/12	08/29/12	--	09/12/12	I6	S	15
009	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
010	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
011	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
012	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
013	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
014	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
015	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
016	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
017	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
018	08/29/12	08/29/12	--	09/19/12	I7	L	22
019	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
020	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
021	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
022	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
023	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
024	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
025	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
026	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
027	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
028	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
029	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
030	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
031	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38

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	08/29/12	08/29/12	--	09/04/12	I1	AE	7
033	08/29/12	08/29/12	--	09/05/12	I1	L	8
034	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
035	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
036	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
037	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
038	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
039	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
040	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
041	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
042	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
043	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
044	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
045	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
046	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
047	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
048	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
049	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
050	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
051	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
052	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
053	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
054	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
055	08/29/12	08/29/12	--	10/02/12	I9	L	35
056	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
057	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
058	08/29/12	08/29/12	--	09/10/12	I3	L	13
059	08/29/12	08/29/12	--	09/07/12	I3	L	10
060	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
061	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
062	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38

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	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
064	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
065	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
066	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
067	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
068	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
069	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
070	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
071	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
072	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
073	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
074	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
075	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
076	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
077	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
078	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
079	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
080	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
081	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
082	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
083	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
084	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
085	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
086	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
087	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
088	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
089	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
090	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
091	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
092	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
093	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38

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	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
095	08/29/12	08/29/12	--	09/07/12	I3	L	10
096	08/29/12	08/29/12	--	09/07/12	I3	L	10
097	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
098	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
099	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
100	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
101	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
102	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
103	08/29/12	08/29/12	--	09/10/12	I4	L	13
104	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
105	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
106	08/29/12	08/29/12	--	10/02/12	I9	L	35
107	08/29/12	08/29/12	--	09/05/12	I2	S	8
108	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
109	08/29/12	08/29/12	--	09/04/12	I0	L	7
110	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
111	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
112	08/29/12	08/29/12	--	09/19/12	I8	L	22
113	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
114	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
115	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
116	08/29/12	08/29/12	--	09/05/12	I2	S	8

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 2: Subject Demographics

Subject No.	Age	Gender	Race
001	54.0	Female	Hispanic
002	22.0	Male	Asian
003	58.7	Female	Caucasian
004	38.1	Female	Caucasian
005	53.9	Female	Hispanic
006	65.8	Female	Caucasian
007	63.4	Female	Caucasian
008	58.9	Male	Caucasian
009	50.7	Male	Caucasian
010	70.1	Female	Hispanic
011	39.6	Female	Hispanic
012	44.0	Female	Hispanic
013	64.0	Male	Caucasian
014	43.4	Female	Caucasian
015	55.9	Female	Hispanic
016	38.1	Female	Hispanic
017	38.8	Male	Hispanic
018	49.8	Female	Hispanic
019	45.1	Female	Caucasian
020	39.9	Female	Hispanic
021	65.4	Male	Caucasian
022	65.1	Male	Caucasian
023	59.9	Female	Black
024	49.1	Female	Caucasian
025	50.4	Female	Caucasian
026	60.2	Female	Caucasian
027	50.0	Female	Caucasian
028	41.3	Female	Caucasian
029	52.3	Female	Caucasian
030	44.1	Male	Caucasian
031	50.7	Female	Caucasian
032	58.7	Female	Caucasian
033	53.0	Female	Caucasian
034	58.2	Female	Caucasian
035	62.3	Female	Caucasian
036	64.5	Female	Hispanic
037	60.1	Female	Caucasian

[redacted]

[redacted]

Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
038	44.7	Female	Caucasian
039	41.8	Female	Black
040	32.2	Female	Caucasian
041	29.2	Female	Caucasian
042	48.9	Female	Caucasian
043	50.1	Male	Hispanic
044	44.9	Female	Caucasian
045	41.7	Female	Hispanic
046	49.0	Female	Caucasian
047	52.6	Female	Hispanic
048	64.6	Female	Caucasian
049	66.3	Female	Hispanic
050	59.6	Female	Caucasian
051	23.6	Male	Caucasian
052	41.2	Female	Caucasian
053	59.3	Female	Caucasian
054	54.1	Female	Caucasian
055	39.5	Male	Hispanic
056	29.3	Female	Hispanic
057	55.7	Female	Caucasian
058	45.1	Female	Caucasian
059	45.0	Male	Caucasian
060	56.7	Female	Caucasian
061	50.6	Female	Caucasian
062	18.5	Male	Hispanic
063	54.5	Female	Caucasian
064	55.9	Female	Caucasian
065	59.4	Female	Caucasian
066	43.4	Female	Caucasian
067	23.6	Male	Hispanic
068	43.4	Female	Caucasian
069	67.0	Female	Caucasian
070	36.1	Male	Black
071	45.4	Female	Black
072	20.5	Male	Black
073	37.0	Male	Black
074	47.7	Female	Black

[redacted]

[redacted]

Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
075	43.6	Female	Caucasian
076	51.7	Male	Caucasian
077	59.9	Female	Caucasian
078	62.7	Male	Caucasian
079	55.0	Female	Hispanic
080	27.9	Female	Caucasian
081	23.1	Male	Caucasian
082	58.9	Female	Caucasian
083	47.0	Female	Caucasian
084	64.6	Female	Caucasian
085	55.2	Male	Hispanic
086	45.5	Female	Hispanic
087	23.1	Female	Hispanic
088	19.2	Female	Caucasian
089	48.9	Female	Caucasian
090	46.6	Female	Caucasian
091	30.9	Female	Caucasian
092	37.7	Male	Caucasian
093	70.4	Female	Caucasian
094	34.7	Female	Caucasian
095	20.7	Female	Caucasian
096	48.7	Female	Caucasian
097	54.0	Male	Caucasian
098	56.4	Male	Caucasian
099	33.3	Female	Black
100	66.6	Male	Caucasian
101	36.8	Female	Hispanic
102	31.8	Female	Hispanic
103	53.7	Male	Hispanic
104	62.3	Female	Hispanic
105	53.9	Male	Caucasian
106	33.0	Male	Caucasian
107	56.7	Male	Hispanic
108	54.2	Female	Hispanic
109	20.9	Female	Caucasian
110	58.8	Male	Hispanic
111	56.9	Female	Caucasian
112	61.3	Female	Caucasian
113	21.4	Female	Hispanic
114	45.2	Female	Caucasian
115	59.9	Female	Hispanic
116	24.6	Female	Caucasian

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted]

Subject No.	Induction Reading 1	2	3	4	5	6	7	8	9	MU	Challenge Phase 48hr	72hr	96hr(*)
001	-	-	-	-	-	-	-	-	-		-	-	
002	-	-	-	-	-	-	-	-	-		-	-	
003	-	-	-	-	-	-	-	-	-		-	-	
004	X	X	X	X	X	X	X	X	X		X	X	
005	-	-	-	-	-	-	-	-	-		-	-	
006	-	-	-	-	-	-	-	-	-		-	-	
007	-	-	-	-	-	-	-	-	-		-	-	
008	-	-	-	-	-	-	X	X	X		X	X	
009	-	-	-	-	-	-	-	-	-		-	-	
010	-	-	-	-	-	-	-	-	-		-	-	
011	-	-	-	-	-	-	-	-	-		-	-	
012	-	-	-	-	-	-	-	-	-		-	-	
013	-	-	-	-	-	-	-	-	-		-	-	
014	-	-	-	-	-	-	-	X	-	-	-	-	
015	-	-	-	-	-	-	-	-	-		-	-	
016	-	-	-	-	-	-	-	-	-		-	-	
017	-	-	-	-	-	-	-	-	-		-	-	
018	-	-	-	-	-	-	-	X	X		X	X	
019	-	-	-	-	-	-	-	-	-		-	-	
020	-	-	-	-	-	-	-	-	-		-	-	
021	-	-	-	-	-	-	-	-	-		-	-	
022	-	-	-	-	-	-	-	-	-		-	-	
023	-	X	-	-	-	-	-	-	-	-	-	-	

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 = [redacted]

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
024	-	-	-	-	X	-	-	-	-	-	-	-	
025	-	-	-	-	-	-	-	-	-		-	-	
026	-	-	-	-	-	-	-	-	-		-	-	
027	X	-	-	-	-	-	-	-	-	-	-	-	
028	-	-	-	-	-	-	-	-	-		-	-	
029	-	-	-	-	-	-	-	-	-		-	-	
030	-	-	-	-	-	-	-	-	-		-	-	
031	-	-	-	-	-	-	-	-	-		-	-	
032	-	X	X	X	X	X	X	X	X		X	X	
033	-	X	X	X	X	X	X	X	X		X	X	
034	-	-	-	-	-	X	-	-	-	-	-	-	
035	-	-	-	-	-	-	-	-	-		-	-	
036	-	-	-	-	-	-	-	-	-		-	-	
037	-	-	-	-	-	-	-	-	-		-	-	
038	-	-	-	-	-	-	-	-	-		-	-	
039	-	-	-	-	-	-	-	-	-		-	-	
040	-	-	-	-	-	X	-	-	-	-	-	-	
041	-	-	-	-	-	X	-	-	-	-	-	-	
042	-	-	-	-	-	-	-	-	-		-	-	
043	-	-	-	-	-	-	-	-	-		-	-	
044	-	-	-	-	-	-	-	-	-		-	-	
045	-	X	-	-	-	-	-	-	-	-	-	-	
046	-	-	-	-	-	-	-	-	-		-	-	

(*) When required
Generated on 10/11/12:12:01 by DETAIL.SAS/USES: RESPONSE, PRODLIST

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted]

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
047	-	X	-	-	-	-	-	-	-	-	-	-	
048	-	-	-	-	-	-	-	-	N9G		-	-	
049	-	-	-	-	-	-	-	-	-		-	-	
050	-	-	-	-	-	-	-	-	-		-	-	
051	-	X	-	-	-	-	-	-	-	-	-	-	
052	-	-	X	-	-	-	-	-	-	N9G	-	-	
053	-	-	-	-	-	-	X	-	-	-	-	-	
054	-	X	-	-	-	-	-	-	-	-	-	-	
055	-	-	X	-	-	-	-	-	-	-	X	X	
056	-	-	-	-	-	-	-	-	-		-	-	
057	-	-	-	-	-	-	X	-	-	-	-	-	
058	-	-	-	X	X	X	X	X	X		X	X	
059	-	X	-	X	X	X	X	X	X		X	X	
060	-	-	-	-	-	-	-	-	N9G		-	-	
061	-	-	-	-	-	-	-	-	-		-	-	
062	-	-	-	-	-	-	-	-	-		-	-	
063	-	-	X	-	-	-	-	-	-	-	-	-	
064	-	-	-	-	-	-	-	-	-		-	-	
065	-	-	-	-	-	-	-	-	-		-	-	
066	-	-	-	-	-	-	-	-	-		-	-	
067	-	-	-	-	-	-	-	X	-	-	-	-	
068	-	-	-	-	-	-	-	-	-		-	-	
069	-	-	-	-	-	-	-	-	-		-	-	

(*) When required
Generated on 10/11/12:12:01 by DETAIL.SAS/USES: RESPONSE, PRODLIST

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted]

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
070	-	X	-	-	-	-	-	-	-	-	-	-	
071	-	-	-	-	-	-	-	-	N9G		-	-	
072	-	-	-	-	-	-	-	-	N9G		-	-	
073	-	-	-	-	-	-	-	-	-		-	-	
074	-	-	-	-	-	-	-	-	-		-	-	
075	-	-	-	-	-	-	-	-	-		-	-	
076	-	-	-	-	-	-	-	-	-		-	-	
077	-	-	-	-	-	-	-	-	-		-	-	
078	-	-	-	-	-	-	-	-	-		-	-	
079	-	-	-	-	-	-	-	-	-		-	-	
080	-	-	-	-	-	-	X	-	-	-	-	-	
081	-	-	-	-	-	-	-	-	-		-	-	
082	-	-	-	-	-	-	-	-	-		-	-	
083	-	-	-	-	-	-	-	-	-		-	-	
084	-	-	-	-	-	-	-	-	-		-	-	
085	-	-	-	-	-	-	-	-	-		-	-	
086	-	-	-	-	-	-	-	-	-		-	-	
087	-	-	-	-	-	-	-	-	-		-	-	
088	-	-	-	-	-	-	-	-	-		-	-	
089	-	-	-	-	-	-	-	-	-		-	-	
090	-	-	-	-	-	-	-	-	-		-	-	
091	-	-	-	-	-	-	-	-	N9G		-	-	
092	-	-	-	-	-	-	-	-	N9G		-	-	

(*) When required

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted]

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
093	-	-	-	-	-	-	-	-	-		-	-	
094	-	-	-	-	-	-	-	-	-		-	-	
095	-	X	-	X	X	X	X	X	X		X	X	
096	-	X	-	X	X	X	X	X	X		X	X	
097	-	-	-	-	-	-	-	-	-		-	-	
098	X	-	-	-	-	-	-	-	-	N9G	-	-	
099	-	-	X	-	-	-	-	-	-	-	-	-	
100	-	-	-	X	-	-	-	-	-	-	-	-	
101	-	-	-	-	-	-	-	-	-		-	-	
102	-	-	-	-	-	-	-	-	-		-	-	
103	X	-	-	-	X	X	X	X	X		X	X	
104	-	-	-	-	-	-	-	-	-		-	-	
105	-	-	-	-	-	-	-	-	-		-	-	
106	-	-	-	-	-	-	-	-	-		X	X	
107	X	-	X	X	X	X	X	X	X		X	X	
108	X	-	-	-	-	-	-	-	-	-	-	-	
109	X	X	X	X	X	X	X	X	X		X	X	
110	-	-	-	-	-	-	-	-	-		-	-	
111	-	-	-	-	-	-	-	-	-		-	-	
112	-	-	X	-	-	-	-	-	X		X	X	
113	-	-	-	-	-	-	-	-	-		-	-	
114	-	-	-	-	-	-	-	-	-		-	-	
115	-	-	-	-	X	-	-	-	-	-	-	-	
116	-	-	X	X	X	X	X	X	X		X	X	

(*) When required

[redacted]

[redacted]

Data Listing 4: Adverse Events

Subject No. 032

Adverse Event: CROHN'S DISEASE	Date of Onset: 09/04/12	Date of Resolution:
Severity: Moderate		Outcome: Continuing
Relation to Study Product: Not Related	Duration: N/A	Action Taken/Study Product: Discontinued
Serious? YES		Action Taken/Treatment?: YES

Comment: SUBJECT CALLED FROM HOSPITAL AND REPORTED SHE WAS ADMITTED WITH STOMACH PAINS. NO MEDICATIONS WERE GIVEN AND IS WAITING FOR TEST RESULTS TO DETERMINE DIAGNOSIS. SUBJECT CALLED TO SAY SHE WAS DIAGNOSED WITH CROHNS DISEASE AND IS GIVEN IV - METRONIDAZOLE FOR INFLAMATION - MEDS - ASCLOA - BUDESONIDE

[redacted]

[redacted]

REPEATED INSULT PATCH STUDY

fragrance containing 0.7794% Rosa Damascena Flower Extract

[redacted]

CONDUCTED FOR:

[redacted]

DATE OF ISSUE:

November 6, 2012

Version 1.0

[redacted]

[redacted] [redacted]

TABLE OF CONTENTS

APPENDICES

[redacted]

SIGNATURES

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

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.

[redacted]

TITLE OF STUDY

Repeated Insult Patch Study

SPONSOR

[redacted]

STUDY MATERIAL

[redacted], Fragrance

DATE STUDY INITIATED

August 29, 2012

DATE STUDY COMPLETED

October 5, 2012

DATE OF ISSUE

November 6, 2012

INVESTIGATIVE PERSONNEL

[redacted]

CLINICAL SITE

[redacted]

[redacted]

SUMMARY

One product, [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 (100) subjects completed the study.

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

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

3.2.3 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:

[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.

- = 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 : [redacted]
Amount Applied : 0.2 mL
Special Instructions : Evaporated for 30 minutes prior to patch application.

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] 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 sixteen (116) subjects between the ages of 18 and 70 were enrolled and 100 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:		116
Number discontinued:		16
Lost to follow-up:	12	
Voluntary withdrawal:	3	
Adverse events:	1	
Number completed:		100

Source: Table 1, Appendix I

There was one non-product-related serious adverse event (SAE) reported during the study. See Data Listing 4, Appendix II for details.

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, [redacted].

9.0 REFERENCES

Schwartz L, Peck SM. The patch test in contact dermatitis. Publ Health Pep 1944; 59:2.

Draize JH, Woodward G, Calvary HO. Methods for the study of irritation and toxicology of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944; 82: 377-390.

Lanman BM, Elvers WB, Howard CS. The role of human patch testing in a product development program. Joint Conf Cosmet Sci Toilet Goods Assoc 1968; 135-145.

Marzulli FN, Maibach HI. Contact allergy: predictive testing in man. Contact Dermatitis 1976; 2:1.

Zhai H, Maibach HI. Dermatotoxicology. 6th ed. New York:Hemisphere, 1996.

Stotts J. Planning, conduct and interpretation of human predictive sensitization patch tests. In:Drill VA, Lazar P, eds. Current Concepts in Cutaneous Toxicity. New York: Academic Press, 1980: 41-53.

Griffith JF. Predictive and diagnostic testing for contact sensitization. Toxicol Appl Pharmacol, Suppl 1969; 3:90.

Gerberick GF, Robinson MK, Stotts J. An approach to allergic contact sensitization risk assessment of new chemicals and product ingredients. American Journal of Contact Dermatitis 1993; 4(4): 205-211.

APPENDIX I

SUMMARY TABLES

[redacted]

Table 1: Summary of Subject Enrollment and Disposition

	N (%)
Subjects enrolled	116
Subjects completed induction phase	102 (87.9)
Subjects completed all phases	100 (86.2)
Total subjects discontinued	16 (13.8)
Lost to follow-up	12 (10.3)
Voluntary withdrawal	3 (2.6)
Adverse events	1 (0.9)

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	43 (37.1)
N (%) 45 to 64	65 (56.0)
N (%) 65 and up	8 (6.9)
Mean (SD)	47.7 (13.2)
Median	49.9
Range	18.5 to 70.4
Gender	
N (%) Male	30 (25.9)
N (%) Female	86 (74.1)
Race	
Asian	1 (0.9)
Black	8 (6.9)
Caucasian	75 (64.7)
Hispanic	32 (27.6)

See data listing 2 for further detail.

[redacted]

[redacted]

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

Product = [redacted]

	Induction Reading										Challenge Phase		
Response	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Make Up**	**48hr**	**72hr**	**96hr(*)**
-	109	103	105	105	103	102	101	101	96	22	100	100	
Total evaluable	109	103	105	105	103	102	101	101	96	22	100	100	
Number absent	5	9	5	1	2	3	3	2	6		0	0	
Number discontinued	2	4	6	10	11	11	12	13	14		16	16	

Maximum Elicited Response During Induction
All Subjects Completing Induction (N=102)

Response	**n(%) Subjects**
-	102 (100.0%)

(*) when required

See Table 3.1 for Key to Symbols and Scores

[redacted]

TKL Study No. [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	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
002	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
003	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
004	08/29/12	08/29/12	--	09/04/12	I0	L	7
005	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
006	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
007	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
008	08/29/12	08/29/12	--	09/12/12	I6	S	15
009	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
010	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
011	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
012	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
013	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
014	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
015	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
016	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
017	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
018	08/29/12	08/29/12	--	09/19/12	I7	L	22
019	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
020	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
021	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
022	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
023	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
024	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
025	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
026	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
027	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
028	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
029	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
030	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
031	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38

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	08/29/12	08/29/12	--	09/04/12	I1	AE	7
033	08/29/12	08/29/12	--	09/05/12	I1	L	8
034	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
035	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
036	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
037	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
038	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
039	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
040	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
041	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
042	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
043	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
044	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
045	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
046	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
047	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
048	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
049	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
050	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
051	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
052	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
053	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
054	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
055	08/29/12	08/29/12	--	10/02/12	I9	L	35
056	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
057	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
058	08/29/12	08/29/12	--	09/10/12	I3	L	13
059	08/29/12	08/29/12	--	09/07/12	I3	L	10
060	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
061	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
062	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38

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	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
064	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
065	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
066	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
067	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
068	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
069	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
070	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
071	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
072	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
073	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
074	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
075	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
076	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
077	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
078	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
079	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
080	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
081	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
082	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
083	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
084	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
085	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
086	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
087	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
088	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
089	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
090	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
091	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
092	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
093	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38

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	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
095	08/29/12	08/29/12	--	09/07/12	I3	L	10
096	08/29/12	08/29/12	--	09/07/12	I3	L	10
097	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
098	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
099	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
100	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
101	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
102	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
103	08/29/12	08/29/12	--	09/10/12	I4	L	13
104	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
105	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
106	08/29/12	08/29/12	--	10/02/12	I9	L	35
107	08/29/12	08/29/12	--	09/05/12	I2	S	8
108	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
109	08/29/12	08/29/12	--	09/04/12	I0	L	7
110	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
111	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
112	08/29/12	08/29/12	--	09/19/12	I8	L	22
113	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
114	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
115	08/29/12	08/29/12	10/02/12	10/05/12	C	C	38
116	08/29/12	08/29/12	--	09/05/12	I2	S	8

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 2: Subject Demographics

Subject No.	Age	Gender	Race
001	54.0	Female	Hispanic
002	22.0	Male	Asian
003	58.7	Female	Caucasian
004	38.1	Female	Caucasian
005	53.9	Female	Hispanic
006	65.8	Female	Caucasian
007	63.4	Female	Caucasian
008	58.9	Male	Caucasian
009	50.7	Male	Caucasian
010	70.1	Female	Hispanic
011	39.6	Female	Hispanic
012	44.0	Female	Hispanic
013	64.0	Male	Caucasian
014	43.4	Female	Caucasian
015	55.9	Female	Hispanic
016	38.1	Female	Hispanic
017	38.8	Male	Hispanic
018	49.8	Female	Hispanic
019	45.1	Female	Caucasian
020	39.9	Female	Hispanic
021	65.4	Male	Caucasian
022	65.1	Male	Caucasian
023	59.9	Female	Black
024	49.1	Female	Caucasian
025	50.4	Female	Caucasian
026	60.2	Female	Caucasian
027	50.0	Female	Caucasian
028	41.3	Female	Caucasian
029	52.3	Female	Caucasian
030	44.1	Male	Caucasian
031	50.7	Female	Caucasian
032	58.7	Female	Caucasian
033	53.0	Female	Caucasian
034	58.2	Female	Caucasian
035	62.3	Female	Caucasian
036	64.5	Female	Hispanic
037	60.1	Female	Caucasian

[redacted]

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Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
038	44.7	Female	Caucasian
039	41.8	Female	Black
040	32.2	Female	Caucasian
041	29.2	Female	Caucasian
042	48.9	Female	Caucasian
043	50.1	Male	Hispanic
044	44.9	Female	Caucasian
045	41.7	Female	Hispanic
046	49.0	Female	Caucasian
047	52.6	Female	Hispanic
048	64.6	Female	Caucasian
049	66.3	Female	Hispanic
050	59.6	Female	Caucasian
051	23.6	Male	Caucasian
052	41.2	Female	Caucasian
053	59.3	Female	Caucasian
054	54.1	Female	Caucasian
055	39.5	Male	Hispanic
056	29.3	Female	Hispanic
057	55.7	Female	Caucasian
058	45.1	Female	Caucasian
059	45.0	Male	Caucasian
060	56.7	Female	Caucasian
061	50.6	Female	Caucasian
062	18.5	Male	Hispanic
063	54.5	Female	Caucasian
064	55.9	Female	Caucasian
065	59.4	Female	Caucasian
066	43.4	Female	Caucasian
067	23.6	Male	Hispanic
068	43.4	Female	Caucasian
069	67.0	Female	Caucasian
070	36.1	Male	Black
071	45.4	Female	Black
072	20.5	Male	Black
073	37.0	Male	Black
074	47.7	Female	Black

[redacted]

[redacted]

Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
075	43.6	Female	Caucasian
076	51.7	Male	Caucasian
077	59.9	Female	Caucasian
078	62.7	Male	Caucasian
079	55.0	Female	Hispanic
080	27.9	Female	Caucasian
081	23.1	Male	Caucasian
082	58.9	Female	Caucasian
083	47.0	Female	Caucasian
084	64.6	Female	Caucasian
085	55.2	Male	Hispanic
086	45.5	Female	Hispanic
087	23.1	Female	Hispanic
088	19.2	Female	Caucasian
089	48.9	Female	Caucasian
090	46.6	Female	Caucasian
091	30.9	Female	Caucasian
092	37.7	Male	Caucasian
093	70.4	Female	Caucasian
094	34.7	Female	Caucasian
095	20.7	Female	Caucasian
096	48.7	Female	Caucasian
097	54.0	Male	Caucasian
098	56.4	Male	Caucasian
099	33.3	Female	Black
100	66.6	Male	Caucasian
101	36.8	Female	Hispanic
102	31.8	Female	Hispanic
103	53.7	Male	Hispanic
104	62.3	Female	Hispanic
105	53.9	Male	Caucasian
106	33.0	Male	Caucasian
107	56.7	Male	Hispanic
108	54.2	Female	Hispanic
109	20.9	Female	Caucasian
110	58.8	Male	Hispanic
111	56.9	Female	Caucasian
112	61.3	Female	Caucasian
113	21.4	Female	Hispanic
114	45.2	Female	Caucasian
115	59.9	Female	Hispanic
116	24.6	Female	Caucasian

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted]

Subject No.	Induction Reading 1	2	3	4	5	6	7	8	9	MU	Challenge Phase 48hr	72hr	96hr(*)
001	-	-	-	-	-	-	-	-	-		-	-	
002	-	-	-	-	-	-	-	-	-		-	-	
003	-	-	-	-	-	-	-	-	-		-	-	
004	X	X	X	X	X	X	X	X	X		X	X	
005	-	-	-	-	-	-	-	-	-		-	-	
006	-	-	-	-	-	-	-	-	-		-	-	
007	-	-	-	-	-	-	-	-	-		-	-	
008	-	-	-	-	-	-	X	X	X		X	X	
009	-	-	-	-	-	-	-	-	-		-	-	
010	-	-	-	-	-	-	-	-	-		-	-	
011	-	-	-	-	-	-	-	-	-		-	-	
012	-	-	-	-	-	-	-	-	-		-	-	
013	-	-	-	-	-	-	-	-	-		-	-	
014	-	-	-	-	-	-	-	X	-	-	-	-	
015	-	-	-	-	-	-	-	-	-		-	-	
016	-	-	-	-	-	-	-	-	-		-	-	
017	-	-	-	-	-	-	-	-	-		-	-	
018	-	-	-	-	-	-	-	X	X		X	X	
019	-	-	-	-	-	-	-	-	-		-	-	
020	-	-	-	-	-	-	-	-	-		-	-	
021	-	-	-	-	-	-	-	-	-		-	-	
022	-	-	-	-	-	-	-	-	-		-	-	
023	-	X	-	-	-	-	-	-	-	-	-	-	

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 = [redacted]

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
024	-	-	-	-	X	-	-	-	-	-	-	-	
025	-	-	-	-	-	-	-	-	-		-	-	
026	-	-	-	-	-	-	-	-	-		-	-	
027	X	-	-	-	-	-	-	-	-	-	-	-	
028	-	-	-	-	-	-	-	-	-		-	-	
029	-	-	-	-	-	-	-	-	-		-	-	
030	-	-	-	-	-	-	-	-	-		-	-	
031	-	-	-	-	-	-	-	-	-		-	-	
032	-	X	X	X	X	X	X	X	X		X	X	
033	-	X	X	X	X	X	X	X	X		X	X	
034	-	-	-	-	-	X	-	-	-	-	-	-	
035	-	-	-	-	-	-	-	-	-		-	-	
036	-	-	-	-	-	-	-	-	-		-	-	
037	-	-	-	-	-	-	-	-	-		-	-	
038	-	-	-	-	-	-	-	-	-		-	-	
039	-	-	-	-	-	-	-	-	-		-	-	
040	-	-	-	-	-	X	-	-	-	-	-	-	
041	-	-	-	-	-	X	-	-	-	-	-	-	
042	-	-	-	-	-	-	-	-	-		-	-	
043	-	-	-	-	-	-	-	-	-		-	-	
044	-	-	-	-	-	-	-	-	-		-	-	
045	-	X	-	-	-	-	-	-	-	-	-	-	
046	-	-	-	-	-	-	-	-	-		-	-	

(*) When required

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted]

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
047	-	X	-	-	-	-	-	-	-	-	-	-	
048	-	-	-	-	-	-	-	-	N9G		-	-	
049	-	-	-	-	-	-	-	-	-		-	-	
050	-	-	-	-	-	-	-	-	-		-	-	
051	-	X	-	-	-	-	-	-	-	-	-	-	
052	-	-	X	-	-	-	-	-	-	N9G	-	-	
053	-	-	-	-	-	-	X	-	-	-	-	-	
054	-	X	-	-	-	-	-	-	-	-	-	-	
055	-	-	X	-	-	-	-	-	-	-	X	X	
056	-	-	-	-	-	-	-	-	-		-	-	
057	-	-	-	-	-	-	X	-	-	-	-	-	
058	-	-	-	X	X	X	X	X	X		X	X	
059	-	X	-	X	X	X	X	X	X		X	X	
060	-	-	-	-	-	-	-	-	N9G		-	-	
061	-	-	-	-	-	-	-	-	-		-	-	
062	-	-	-	-	-	-	-	-	-		-	-	
063	-	-	X	-	-	-	-	-	-	-	-	-	
064	-	-	-	-	-	-	-	-	-		-	-	
065	-	-	-	-	-	-	-	-	-		-	-	
066	-	-	-	-	-	-	-	-	-		-	-	
067	-	-	-	-	-	-	-	X	-	-	-	-	
068	-	-	-	-	-	-	-	-	-		-	-	
069	-	-	-	-	-	-	-	-	-		-	-	

(*) When required

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted]

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
070	-	X	-	-	-	-	-	-	-	-	-	-	
071	-	-	-	-	-	-	-	-	N9G		-	-	
072	-	-	-	-	-	-	-	-	N9G		-	-	
073	-	-	-	-	-	-	-	-	-		-	-	
074	-	-	-	-	-	-	-	-	-		-	-	
075	-	-	-	-	-	-	-	-	-		-	-	
076	-	-	-	-	-	-	-	-	-		-	-	
077	-	-	-	-	-	-	-	-	-		-	-	
078	-	-	-	-	-	-	-	-	-		-	-	
079	-	-	-	-	-	-	-	-	-		-	-	
080	-	-	-	-	-	-	X	-	-	-	-	-	
081	-	-	-	-	-	-	-	-	-		-	-	
082	-	-	-	-	-	-	-	-	-		-	-	
083	-	-	-	-	-	-	-	-	-		-	-	
084	-	-	-	-	-	-	-	-	-		-	-	
085	-	-	-	-	-	-	-	-	-		-	-	
086	-	-	-	-	-	-	-	-	-		-	-	
087	-	-	-	-	-	-	-	-	-		-	-	
088	-	-	-	-	-	-	-	-	-		-	-	
089	-	-	-	-	-	-	-	-	-		-	-	
090	-	-	-	-	-	-	-	-	-		-	-	
091	-	-	-	-	-	-	-	-	N9G		-	-	
092	-	-	-	-	-	-	-	-	N9G		-	-	

(*) When required

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted]

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
093	-	-	-	-	-	-	-	-	-		-	-	
094	-	-	-	-	-	-	-	-	-		-	-	
095	-	X	-	X	X	X	X	X	X		X	X	
096	-	X	-	X	X	X	X	X	X		X	X	
097	-	-	-	-	-	-	-	-	-		-	-	
098	X	-	-	-	-	-	-	-	-	N9G	-	-	
099	-	-	X	-	-	-	-	-	-	-	-	-	
100	-	-	-	X	-	-	-	-	-	-	-	-	
101	-	-	-	-	-	-	-	-	-		-	-	
102	-	-	-	-	-	-	-	-	-		-	-	
103	X	-	-	-	X	X	X	X	X		X	X	
104	-	-	-	-	-	-	-	-	-		-	-	
105	-	-	-	-	-	-	-	-	-		-	-	
106	-	-	-	-	-	-	-	-	-		X	X	
107	X	-	X	X	X	X	X	X	X		X	X	
108	X	-	-	-	-	-	-	-	-	-	-	-	
109	X	X	X	X	X	X	X	X	X		X	X	
110	-	-	-	-	-	-	-	-	-		-	-	
111	-	-	-	-	-	-	-	-	-		-	-	
112	-	-	X	-	-	-	-	-	X		X	X	
113	-	-	-	-	-	-	-	-	-		-	-	
114	-	-	-	-	-	-	-	-	-		-	-	
115	-	-	-	-	X	-	-	-	-	-	-	-	
116	-	-	X	X	X	X	X	X	X		X	X	

(*) When required

[redacted]

[redacted]

Data Listing 4: Adverse Events

Subject No. 032

Adverse Event: CROHN'S DISEASE	Date of Onset: 09/04/12	Date of Resolution:
Severity: Moderate		Outcome: Continuing
Relation to Study Product: Not Related	Duration: N/A	Action Taken/Study Product: Discontinued
Serious? YES		Action Taken/Treatment?: YES

Comment: SUBJECT CALLED FROM HOSPITAL AND REPORTED SHE WAS ADMITTED WITH STOMACH PAINS. NO MEDICATIONS WERE GIVEN AND IS WAITING FOR TEST RESULTS TO DETERMINE DIAGNOSIS. SUBJECT CALLED TO SAY SHE WAS DIAGNOSED WITH CROHNS DISEASE AND IS GIVEN IV - METRONIDAZOLE FOR INFLAMATION - MEDS - ASCLOA - BUDESONIDE

[redacted]

[redacted]

REPEATED INSULT PATCH STUDY

contains 0.1260% Rosa Damascena Flower Oil [redacted]

CONDUCTED FOR:

[redacted]

DATE OF ISSUE:

July 12, 2019

[redacted]

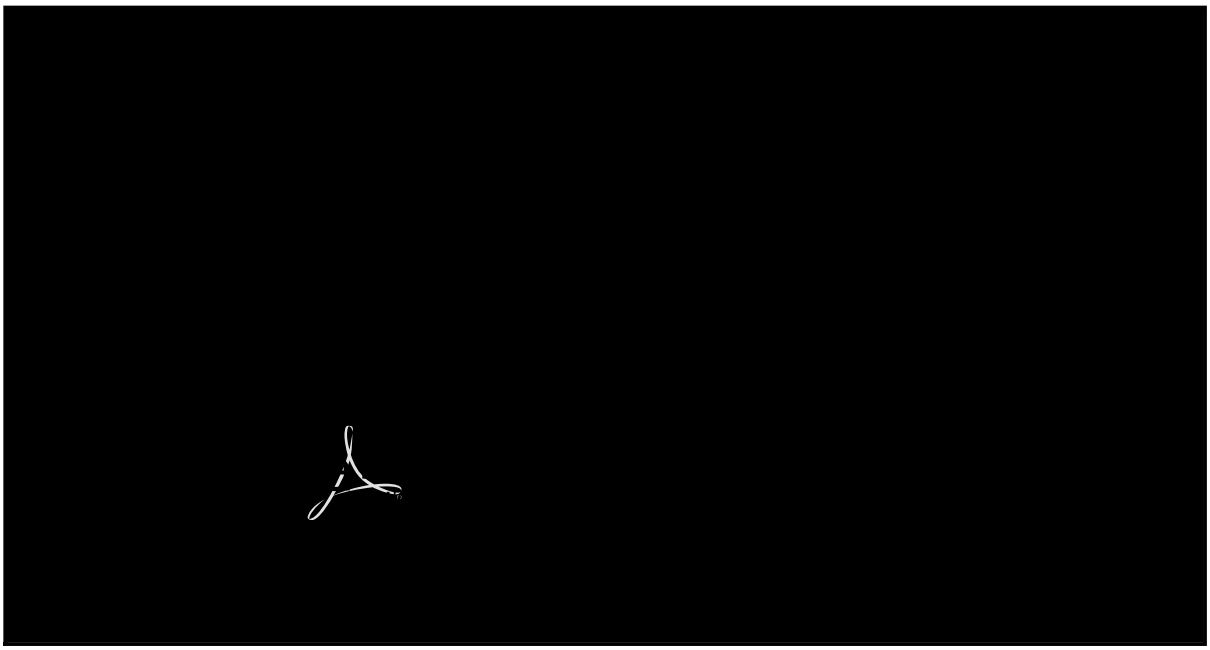
[redacted] [redacted]

TABLE OF CONTENTS

APPENDICES

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.



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.

[redacted] [redacted] [redacted]

TITLE OF STUDY

Repeated Insult Patch Study

SPONSOR

[redacted]

STUDY MATERIAL

BH Mask-Apple, F# [redacted]

DATE STUDY INITIATED

May 15, 2019

DATE STUDY COMPLETED

June 21, 2019

DATE OF ISSUE

July 12, 2019

INVESTIGATIVE PERSONNEL

[redacted]

[redacted]

CLINICAL SITE

[redacted]

SUMMARY

One (1) product, F# , 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 seven (107) subjects completed the study.

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

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.

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

3.2.3 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

[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.

? = 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 : BH Mask-Apple, F# [redacted]
Amount Applied : 0.2mL

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 facility in a secured room accessible only to 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 .

7.0 RESULTS AND DISCUSSION

One hundred twenty-two (122) subjects between the ages of 21 and 74 were enrolled and 107 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:		122
Number discontinued:		15
Lost to follow-up:	14	
Other reason: (002: Tape reaction)	1	
Number completed:		107

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# 1033943-001.

9.0 REFERENCES

Schwartz L, Peck SM. The patch test in contact dermatitis. Publ Health Pep 1944; 59:2.

Draize JH, Woodward G, Calvary HO. Methods for the study of irritation and toxicology of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944; 82: 377-390.

Lanman BM, Elvers WB, Howard CS. The role of human patch testing in a product development program. Joint Conf Cosmet Sci Toilet Goods Assoc 1968; 135-145.

Marzulli FN, Maibach HI. Contact allergy: predictive testing in man. Contact Dermatitis 1976; 2:1.

Zhai H, Maibach HI. Dermatotoxicology. 6th ed. New York:Hemisphere, 1996.

Stotts J. Planning, conduct and interpretation of human predictive sensitization patch tests. In:Drill VA, Lazar P, eds. Current Concepts in Cutaneous Toxicity. New York: Academic Press, 1980: 41-53.

Griffith JF. Predictive and diagnostic testing for contact sensitization. Toxicol Appl Pharmacol, Suppl 1969; 3:90.

Gerberick GF, Robinson MK, Stotts J. An approach to allergic contact sensitization risk assessment of new chemicals and product ingredients. American Journal of Contact Dermatitis 1993; 4(4): 205-211.

APPENDIX I

SUMMARY TABLES

[redacted]

Table 1: Summary of Subject Enrollment and Disposition

	N (%)
Subjects enrolled	122
Subjects completed induction phase	108 (88.5)
Subjects completed all phases	107 (87.7)
Total subjects discontinued	15 (12.3)
Lost to follow-up	14 (11.5)
Other reasons	1 (0.8)

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	24 (19.7)
N (%) 45 to 65	70 (57.4)
N (%) 66 and up	28 (23.0)
Mean (SD)	55.3 (12.1)
Median	56.5
Range	21.9 to 74.7
Sex	
N (%) Male	42 (34.4)
N (%) Female	80 (65.6)
Race	
Amer Ind	2 (1.6)
Black	59 (48.4)
Caucasian	59 (48.4)
Other	2 (1.6)
Ethnicity	
Hispanic/Latino	20 (16.4)
Not Hispanic/Not Latino	102 (83.6)

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**	**96hr(*)**
-	111	106	111	110	107	106	103	107	103	13	108	107	
Total evaluable	111	106	111	110	107	106	103	107	103	13	108	107	
Number absent	5	10	5	4	5	5	7	1	5		0	0	
Number discontinued	6	6	6	8	10	11	12	14	14		14	15	

Maximum Elicited Response During Induction
All Subjects Completing Induction (N=108)

Response	**n(%) Subjects**
-	108 (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	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
002	05/15/19	05/15/19	--	05/31/19	I7	O	17
003	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
004	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
005	05/15/19	05/15/19	06/18/19	06/21/19	C1	L	38
006	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
007	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
008	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
009	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
010	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
011	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
012	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
013	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
014	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
015	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
016	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
017	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
018	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
019	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
020	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
021	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
022	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
023	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
024	05/15/19	05/15/19	--	05/20/19	I0	L	6
025	05/15/19	05/15/19	06/18/19	06/21/19	C	C	38
026	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
027	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
028	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
029	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
030	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
031	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36

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	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
033	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
034	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
035	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
036	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
037	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
038	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
039	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
040	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
041	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
042	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
043	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
044	05/17/19	05/17/19	--	05/30/19	I3	L	14
045	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
046	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
047	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
048	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
049	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
050	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
051	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
052	05/17/19	05/17/19	--	05/30/19	I4	L	14
053	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
054	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
055	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
056	05/17/19	05/17/19	--	05/30/19	I3	L	14
057	05/17/19	05/17/19	--	05/22/19	I0	L	6
058	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
059	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
060	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
061	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
062	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36

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	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
064	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
065	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
066	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
067	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
068	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
069	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
070	05/17/19	05/17/19	--	05/22/19	I0	L	6
071	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
072	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
073	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
074	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
075	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
076	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
077	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
078	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
079	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
080	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
081	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
082	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
083	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
084	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
085	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
086	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
087	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
088	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
089	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
090	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
091	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
092	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
093	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36

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	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
095	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
096	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
097	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
098	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
099	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
100	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
101	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
102	05/17/19	05/17/19	--	05/30/19	I4	L	14
103	05/17/19	05/17/19	--	06/05/19	I6	L	20
104	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
105	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
106	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
107	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
108	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
109	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
110	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
111	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
112	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
113	05/17/19	05/17/19	--	05/22/19	I0	L	6
114	05/17/19	05/17/19	--	05/31/19	I5	L	15
115	05/17/19	05/17/19	--	05/22/19	I0	L	6
116	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
117	05/17/19	05/17/19	--	06/05/19	I7	L	20
118	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
119	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
120	05/17/19	05/17/19	--	05/22/19	I0	L	6
121	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36
122	05/17/19	05/17/19	06/18/19	06/21/19	C	C	36

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 2: Subject Demographics

Subject No.	Age	Gender	Ethnicity	Race
001	74.7	Male	Not Hispanic/Not Latino	Caucasian
002	73.4	Female	Not Hispanic/Not Latino	Caucasian
003	68.8	Female	Not Hispanic/Not Latino	Caucasian
004	39.6	Female	Hispanic/Latino	Caucasian
005	50.2	Female	Not Hispanic/Not Latino	Caucasian
006	46.7	Female	Hispanic/Latino	Caucasian
007	71.1	Male	Not Hispanic/Not Latino	Black
008	52.1	Male	Not Hispanic/Not Latino	Caucasian
009	73.6	Female	Not Hispanic/Not Latino	Caucasian
010	72.8	Female	Not Hispanic/Not Latino	Black
011	72.8	Male	Hispanic/Latino	Caucasian
012	54.3	Female	Not Hispanic/Not Latino	Black
013	69.0	Female	Hispanic/Latino	Black
014	21.9	Female	Not Hispanic/Not Latino	Caucasian
015	52.3	Male	Not Hispanic/Not Latino	Caucasian
016	61.5	Male	Not Hispanic/Not Latino	Black
017	48.1	Female	Not Hispanic/Not Latino	Caucasian
018	54.8	Female	Not Hispanic/Not Latino	Caucasian
019	52.3	Female	Not Hispanic/Not Latino	Black
020	72.5	Female	Not Hispanic/Not Latino	Caucasian
021	50.3	Female	Not Hispanic/Not Latino	Black
022	48.8	Female	Hispanic/Latino	Caucasian
023	56.9	Female	Not Hispanic/Not Latino	Caucasian
024	35.5	Female	Not Hispanic/Not Latino	White/Black
025	61.9	Female	Not Hispanic/Not Latino	Caucasian
026	47.5	Female	Not Hispanic/Not Latino	Black
027	62.0	Male	Not Hispanic/Not Latino	Black
028	54.4	Male	Not Hispanic/Not Latino	Black
029	32.7	Male	Not Hispanic/Not Latino	Black
030	35.1	Male	Not Hispanic/Not Latino	Black
031	62.9	Male	Not Hispanic/Not Latino	Black
032	66.3	Male	Not Hispanic/Not Latino	Black
033	68.8	Female	Not Hispanic/Not Latino	Black
034	66.8	Male	Not Hispanic/Not Latino	Black
035	60.5	Female	Not Hispanic/Not Latino	Black
036	39.4	Male	Hispanic/Latino	Caucasian
037	45.6	Female	Not Hispanic/Not Latino	Caucasian

[redacted]

[redacted]

Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Ethnicity	Race
038	59.2	Female	Not Hispanic/Not Latino	Caucasian
039	47.2	Male	Not Hispanic/Not Latino	Caucasian
040	61.3	Male	Not Hispanic/Not Latino	Black
041	61.6	Female	Hispanic/Latino	Caucasian
042	42.6	Male	Hispanic/Latino	Caucasian
043	56.2	Female	Not Hispanic/Not Latino	Black
044	41.0	Female	Not Hispanic/Not Latino	Caucasian
045	66.1	Female	Not Hispanic/Not Latino	Caucasian
046	67.4	Male	Not Hispanic/Not Latino	Black
047	52.6	Male	Not Hispanic/Not Latino	Black
048	57.9	Female	Not Hispanic/Not Latino	Black
049	42.1	Female	Not Hispanic/Not Latino	Caucasian
050	74.5	Female	Not Hispanic/Not Latino	Caucasian
051	72.9	Male	Not Hispanic/Not Latino	Black
052	72.7	Male	Not Hispanic/Not Latino	Black
053	60.1	Female	Not Hispanic/Not Latino	Caucasian
054	68.1	Female	Not Hispanic/Not Latino	Black
055	57.5	Female	Not Hispanic/Not Latino	Caucasian
056	62.9	Female	Not Hispanic/Not Latino	Caucasian
057	63.6	Female	Not Hispanic/Not Latino	Black
058	68.2	Male	Not Hispanic/Not Latino	Black
059	49.4	Female	Not Hispanic/Not Latino	Black
060	68.8	Female	Not Hispanic/Not Latino	Caucasian
061	36.5	Female	Not Hispanic/Not Latino	Black
062	52.1	Female	Not Hispanic/Not Latino	Black
063	60.3	Male	Not Hispanic/Not Latino	Black
064	56.1	Female	Not Hispanic/Not Latino	Caucasian
065	73.9	Female	Not Hispanic/Not Latino	Caucasian
066	51.7	Female	Not Hispanic/Not Latino	Black
067	51.9	Male	Not Hispanic/Not Latino	Caucasian
068	48.1	Female	Hispanic/Latino	Caucasian
069	64.8	Female	Not Hispanic/Not Latino	Caucasian
070	59.9	Female	Not Hispanic/Not Latino	Black
071	56.5	Female	Hispanic/Latino	Caucasian
072	56.7	Female	Not Hispanic/Not Latino	Black
073	66.0	Female	Not Hispanic/Not Latino	Caucasian
074	41.4	Female	Not Hispanic/Not Latino	Caucasian

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Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Ethnicity	Race
075	53.2	Male	Not Hispanic/Not Latino	Black
076	63.7	Female	Not Hispanic/Not Latino	Caucasian
077	67.7	Female	Not Hispanic/Not Latino	Black
078	55.0	Male	Hispanic/Latino	Black
079	43.2	Female	Not Hispanic/Not Latino	Caucasian
080	52.8	Male	Not Hispanic/Not Latino	Black
081	25.9	Female	Hispanic/Latino	Caucasian
082	49.2	Female	Hispanic/Latino	Caucasian
083	27.6	Male	Hispanic/Latino	Caucasian
084	50.8	Male	Not Hispanic/Not Latino	Black
085	68.8	Female	Not Hispanic/Not Latino	Black
086	49.0	Female	Not Hispanic/Not Latino	Black
087	34.1	Female	Not Hispanic/Not Latino	Black
088	70.1	Female	Not Hispanic/Not Latino	Black
089	56.6	Female	Hispanic/Latino	Caucasian
090	55.8	Female	Not Hispanic/Not Latino	Black
091	47.6	Female	Not Hispanic/Not Latino	Caucasian
092	70.7	Female	Not Hispanic/Not Latino	Caucasian
093	69.8	Male	Not Hispanic/Not Latino	Caucasian
094	61.6	Male	Not Hispanic/Not Latino	Black
095	64.8	Female	Not Hispanic/Not Latino	Caucasian
096	61.4	Female	Not Hispanic/Not Latino	Black
097	54.4	Female	Not Hispanic/Not Latino	Caucasian
098	32.8	Female	Not Hispanic/Not Latino	Black
099	59.9	Female	Not Hispanic/Not Latino	Amer Ind
100	63.7	Male	Not Hispanic/Not Latino	Black
101	44.8	Female	Hispanic/Latino	Caucasian
102	41.5	Male	Hispanic/Latino	Caucasian
103	27.2	Female	Not Hispanic/Not Latino	Black
104	63.3	Male	Not Hispanic/Not Latino	Caucasian
105	55.5	Male	Not Hispanic/Not Latino	Caucasian
106	63.5	Female	Not Hispanic/Not Latino	Black
107	43.1	Male	Not Hispanic/Not Latino	Black
108	50.8	Female	Not Hispanic/Not Latino	Black
109	55.7	Female	Not Hispanic/Not Latino	White/Black/Native American
110	50.7	Male	Not Hispanic/Not Latino	Black
111	66.0	Female	Not Hispanic/Not Latino	Black
112	56.4	Male	Not Hispanic/Not Latino	Black
113	43.2	Male	Hispanic/Latino	Amer Ind
114	44.2	Male	Not Hispanic/Not Latino	Caucasian
115	57.2	Female	Not Hispanic/Not Latino	Black
116	37.0	Female	Not Hispanic/Not Latino	Black
117	58.4	Male	Hispanic/Latino	Caucasian
118	60.3	Female	Not Hispanic/Not Latino	Caucasian
119	46.8	Female	Not Hispanic/Not Latino	Caucasian
120	56.9	Female	Not Hispanic/Not Latino	Black
121	27.4	Male	Hispanic/Latino	Caucasian
122	60.7	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	96hr(*)
001	-	-	-	-	-	-	-	-	-		-	-	
002	-	-	-	-	-	-	-	X	X		X	X	
003	-	X	-	-	-	-	-	-	-	-	-	-	
004	-	X	-	-	-	-	-	-	-	-	-	-	
005	-	-	X	-	-	-	-	-	-	-	-	X	
006	-	-	-	-	-	-	X	-	-	-	-	-	
007	-	-	-	-	-	X	-	-	-	-	-	-	
008	-	-	X	-	-	-	-	-	-	-	-	-	
009	-	-	-	-	-	-	-	-	-		-	-	
010	-	-	-	-	-	X	-	-	-	-	-	-	
011	-	-	-	-	-	-	-	-	-		-	-	
012	-	-	-	-	-	-	X	-	-	-	-	-	
013	-	-	-	-	-	-	-	-	-		-	-	
014	-	-	-	-	-	-	-	-	-		-	-	
015	-	-	-	-	-	X	-	-	-	-	-	-	
016	-	-	-	-	-	-	-	-	-		-	-	
017	-	-	-	-	-	-	-	-	-		-	-	
018	-	-	-	-	-	-	-	-	-		-	-	
019	-	X	-	-	-	-	-	-	-	-	-	-	
020	-	-	-	-	-	X	-	-	-	-	-	-	
021	-	-	-	-	-	-	-	-	-		-	-	
022	-	X	-	-	-	-	-	-	-	-	-	-	
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	96hr(*)
024	X	X	X	X	X	X	X	X	X		X	X	
025	-	-	X	-	-	-	-	-	-	-	-	-	
026	-	-	-	-	-	-	-	-	-		-	-	
027	-	-	-	-	-	-	-	-	-		-	-	
028	-	-	-	-	-	-	-	-	-		-	-	
029	-	X	-	-	-	-	-	-	-	N9G	-	-	
030	-	-	-	-	-	-	-	-	-		-	-	
031	-	-	-	-	-	-	-	-	-		-	-	
032	-	-	-	-	-	-	-	-	-		-	-	
033	-	-	-	-	-	-	-	-	-		-	-	
034	-	-	-	-	-	-	-	-	-		-	-	
035	X	-	-	-	-	-	-	-	-	N9G	-	-	
036	-	-	-	-	-	-	-	-	-		-	-	
037	-	-	-	-	-	-	-	-	-		-	-	
038	-	-	-	-	-	-	-	-	N9G		-	-	
039	-	-	-	-	-	-	-	-	-		-	-	
040	-	-	-	-	X	-	-	-	-	N9G	-	-	
041	-	-	-	-	-	-	-	-	-		-	-	
042	-	-	-	-	-	-	-	-	-		-	-	
043	-	X	-	-	-	-	-	-	-	N9G	-	-	
044	-	-	-	X	X	X	X	X	X		X	X	
045	-	-	-	-	-	-	-	-	-		-	-	
046	-	-	-	-	-	-	X	-	-	N9G	-	-	

(*) 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	96hr(*)
047	-	-	-	-	-	-	-	-	-		-	-	
048	-	-	-	-	-	-	-	-	-		-	-	
049	-	-	-	-	-	-	X	-	-	N9G	-	-	
050	X	-	-	-	-	-	-	-	-	N9G	-	-	
051	-	-	-	-	-	-	X	-	-	N9G	-	-	
052	-	X	-	-	X	X	X	X	X		X	X	
053	-	-	-	-	-	-	-	-	N9G		-	-	
054	-	-	-	-	-	-	-	-	-		-	-	
055	-	-	-	X	-	-	-	-	-	N9G	-	-	
056	-	-	-	X	X	X	X	X	X		X	X	
057	X	X	X	X	X	X	X	X	X		X	X	
058	-	-	-	-	-	-	-	-	N9G		-	-	
059	-	-	-	-	-	-	-	-	-		-	-	
060	-	-	-	-	-	-	-	-	-		-	-	
061	-	-	-	-	-	-	-	-	-		-	-	
062	-	X	-	-	-	-	-	-	-	N9G	-	-	
063	-	-	-	-	-	X	-	-	-	N9G	-	-	
064	-	-	-	-	-	-	-	-	-		-	-	
065	-	-	-	-	-	-	-	-	-		-	-	
066	-	-	-	-	-	-	-	-	-		-	-	
067	-	-	-	-	-	-	-	-	-		-	-	
068	-	-	-	-	-	-	-	-	-		-	-	
069	-	-	-	-	-	-	X	-	-	N9G	-	-	

(*) 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	96hr(*)
070	X	X	X	X	X	X	X	X	X		X	X	
071	-	-	-	-	-	-	-	-	-		-	-	
072	-	-	-	-	-	-	X	-	-	N9G	-	-	
073	-	-	-	-	-	-	-	-	-		-	-	
074	X	-	-	-	-	-	-	-	-	N9G	-	-	
075	-	-	-	-	-	-	-	X	-	N9G	-	-	
076	-	-	-	-	-	-	-	-	-		-	-	
077	-	-	-	-	-	-	-	-	-		-	-	
078	-	-	-	-	X	-	-	-	-	N9G	-	-	
079	-	-	-	-	-	-	-	-	-		-	-	
080	-	-	-	-	-	-	-	-	-		-	-	
081	-	-	-	-	-	-	-	-	-		-	-	
082	-	-	-	-	-	-	-	-	-		-	-	
083	-	-	-	-	-	-	-	-	-		-	-	
084	-	-	-	-	-	-	-	-	-		-	-	
085	-	-	-	-	-	-	-	-	-		-	-	
086	-	-	-	-	X	-	-	-	-	N9G	-	-	
087	-	-	-	-	-	-	-	-	-		-	-	
088	-	-	-	-	-	-	-	-	-		-	-	
089	-	-	-	-	-	-	-	-	-		-	-	
090	-	-	-	-	X	-	-	-	-	N9G	-	-	
091	-	X	-	-	-	-	-	-	-	N9G	-	-	
092	-	X	-	-	-	-	-	-	-	N9G	-	-	

(*) 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	96hr(*)
093	-	-	-	-	-	-	-	-	-		-	-	
094	-	-	-	-	-	-	-	-	-		-	-	
095	-	-	X	-	-	-	-	-	-	N9G	-	-	
096	-	-	-	-	-	-	-	-	-		-	-	
097	-	-	-	-	-	-	-	-	-		-	-	
098	-	-	-	-	-	-	-	-	-		-	-	
099	-	-	-	-	-	-	-	-	N9G		-	-	
100	-	-	-	-	-	-	-	-	N9G		-	-	
101	-	-	-	-	-	-	-	-	-		-	-	
102	-	-	X	-	X	X	X	X	X		X	X	
103	-	-	-	-	-	-	X	X	X		X	X	
104	-	-	-	-	-	-	-	-	-		-	-	
105	-	-	-	-	X	-	-	-	-	N9G	-	-	
106	-	-	-	-	-	-	-	-	-		-	-	
107	-	-	-	-	-	-	-	-	-		-	-	
108	-	-	-	-	-	-	-	-	-		-	-	
109	-	-	-	-	-	-	-	-	-		-	-	
110	-	-	-	X	-	-	-	-	-	N9G	-	-	
111	-	-	-	-	-	-	-	-	-		-	-	
112	-	-	-	-	-	-	-	-	-		-	-	
113	X	X	X	X	X	X	X	X	X		X	X	
114	-	-	-	X	-	X	X	X	X		X	X	
115	X	X	X	X	X	X	X	X	X		X	X	
116	X	-	-	-	-	-	-	-	-	N9G	-	-	
117	-	-	-	X	-	-	-	X	X		X	X	
118	-	-	-	-	-	-	-	-	-		-	-	
119	-	-	-	-	-	-	-	-	-		-	-	
120	X	X	X	X	X	X	X	X	X		X	X	
121	-	-	-	-	-	-	-	-	-		-	-	
122	X	-	-	-	-	-	-	-	-	N9G	-	-	

(*) 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
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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]

September 4, 2018

[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]

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: December 16, 2020

SUBJECT: Scientific Literature Review: Safety Assessment of *Rosa damascena*-derived Ingredients as Used in Cosmetics (release date November 19, 2020)

The Personal Care Products Council has no suppliers listed for Rosa Damascena Flower Powder.

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

Composition and Impurities – For specified fragrance components, 0.01% is not a threshold level in Europe. It is a concentration, if exceeded in rinse-off products, the fragrance ingredient needs to be included on the label. The level for labeling certain fragrance ingredients in leave-on products is 0.001%.

Composition and Impurities, Rosa Damascena Flower Oil – Reference 25 is a review article about methods used to measure pesticides in natural products and the levels of pesticides in natural products. If some pesticides coelute with some naturally occurring constituents using gas chromatography, did they suggest other methods to measure those pesticides? What were the levels of pesticides found in Rosa Damascena Flower Oil?

Composition and Impurities, Rosa Damascena Flower Water, Rosa Damascena Flower Wax - Please be consistent with names of chemicals. Phenyl ethyl alcohol and 2-phenylethanol are the same chemical that has an INCI name Phenylethyl Alcohol. Phenylethyl Alcohol has been reviewed by CIR (conclusion safe as used up to 1%).

Cosmetic Use – The 32.7% use concentration was reported in face and neck products (the report states skincare preparations), while the 10.8% use concentration was reported in other skin care preparations (the report states face and neck products).

Short-Term and Subchronic – The description of the dog study states: “Animals in the highest dose groups showed signs of soft feces and diarrhea in a dose dependent manner.” It would be helpful to be more specific and state the doses at which these observations occurred. Table 5 states: “A dose-dependent increase of soft feces and diarrhea was observed, starting from the 90 mg/kg/d group.” 90 mg/kg/day is the lowest dose tested, so if the table is correct, “in the highest dose groups” as stated in the text is not correct.

Please correct “moth” (should be “month”)

Genotoxicity, In Vitro – Please correct “L-glutamin”

Hematology and Clinical Effects – This does not need to be a separate section. Hematology and clinical chemistry results of the short-term and subchronic toxicity studies should be summarized in the Short-Term and Subchronic Toxicity Studies section and the results should be included in Table 5.

Retrospective and Multicenter Studies; Summary – It is not clear what is meant by an “annual essential oil patch test study”. Were the 1483 patients tested each year, or were there 1483 patients tested over the 8-year study period?

Summary – Since composition information focuses on one plant part, the flower, perhaps “based on plant part” should be changed to “based on time of harvest”, e.g., bud versus fresh and dried flowers.