Safety Assessment of *Punica granatum* -Derived Ingredients as Used in Cosmetics

Status:	Draft Report for Panel Review
Release Date:	March 15, 2019
Panel Meeting Date:	April 8-9, 2019

The 2019 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A. Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer.

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Christina L. Burnett, Senior Scientific Writer/Analyst
Date: March 15, 2019
Subject: Draft Safety Assessment on *Punica granatum*-Derived Ingredients

Enclosed is the Draft Report of the Safety Assessment of *Punica granatum*-Derived Ingredients as Used in Cosmetics. (It is identified as *pomegr042019DR* in the pdf document.) *Punica granatum* is the Latin nomenclature for pomegranate. According to the *Dictionary*, most of the 18 *Punica granatum*-derived ingredients detailed in this safety assessment are reported to function in cosmetics as skin conditioning agents, while some are reported to have other functions, such as abrasives and antioxidants. The Scientific Literature Review (SLR) of these botanical ingredients was issued by CIR on January 24, 2019.

The Council provided concentration of use survey data (*pomegr042019data1; pomegr042019data2*) and some composition, physical properties, genotoxicity, dermal and ocular irritation, and dermal sensitization data on Punica Granatum Pericarp Extract and Punica Granatum Fruit Extract (*pomegr042019data3; pomegr042019data4*). Some of the data were poorly detailed summaries (*pomegr042019data5*) that the Panel may want to closely consider the informational value, especially with regards to the sensitization studies. Comments on the SLR were received from the Council and addressed (*pomegr042019pcpc*). In the comments, the Council notes that Punica Granatum Extract has been incorrectly defined as an extract of "the whole plant." Companies that reported concentration of use for this safety assessment have been asked to clarify the source of the ingredient with this INCI name. An update from Council will hopefully be provided in time for a Wave 2 package.

According to 2019 VCRP data, Punica Granatum Extract has the most reported uses in cosmetic products, with a total of 312; the majority of the uses are in leave-on skin care products. Punica Granatum Fruit Extract has the second greatest number of reported uses in this safety assessment with 172 uses; the majority of these uses are also in leave-on skin care products. The results of the concentration of use survey conducted in 2018 by the Council indicated that Punica Granatum Seed Extract is used at up to 0.3% in leave-on cuticle softeners. Punica Granatum Extract and Punica Granatum Fruit Extract are used at up to 0.13% (in a moisturizing preparation) and 0.1% (in face and neck and night skin preparations), respectively. Punica Granatum Fruit Juice is used at up to 0.1% in makeup preparations.

If no further data are needed to reach a conclusion of safety, the Panel should formulate a Discussion and issue a Tentative Report. However, if additional data are required, the Panel should be prepared to identify those needs and issue an Insufficient Data Announcement.

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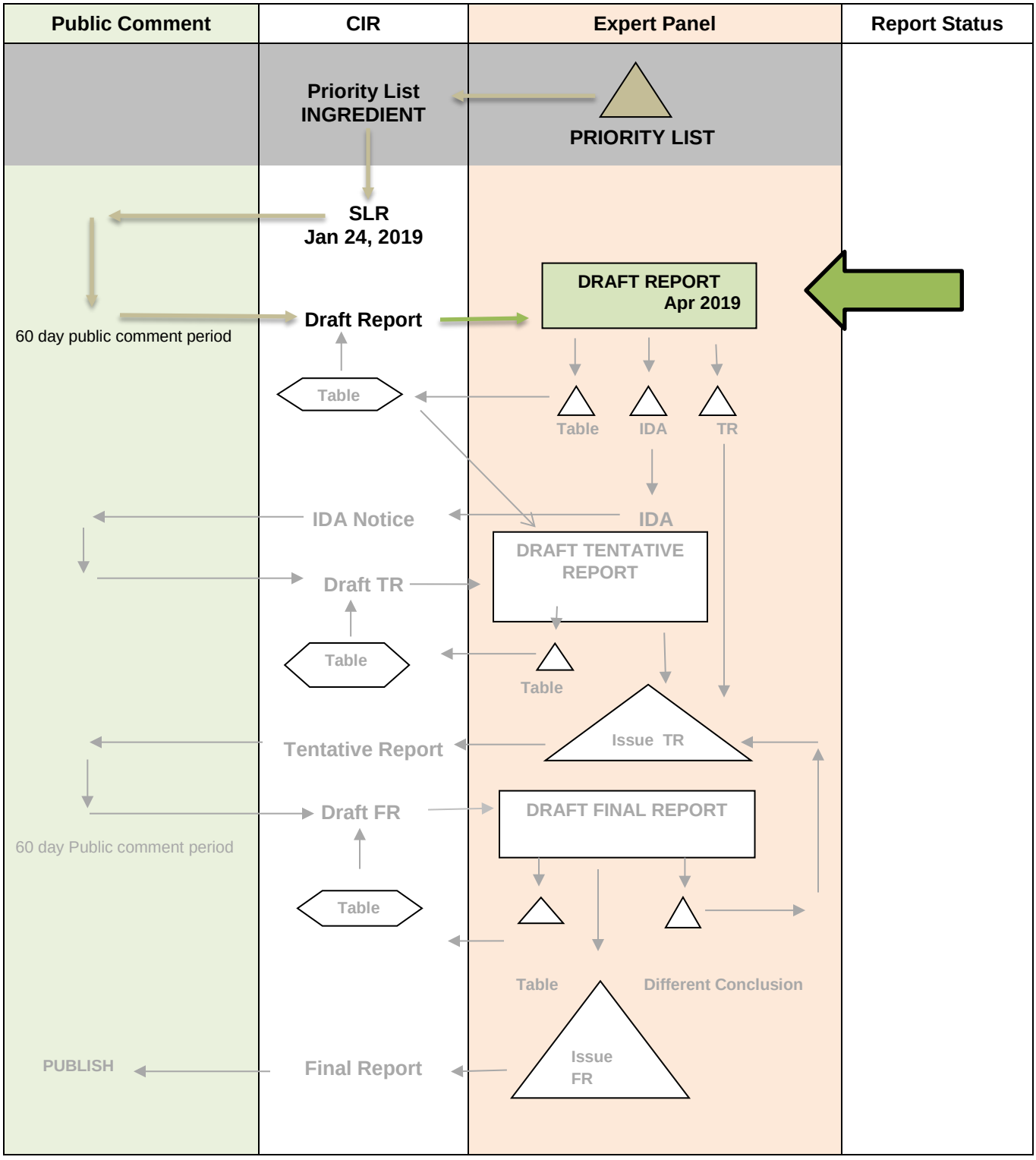
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY _____ *Punica granatum* -Derived Ingredients _____

MEETING ___April 2019 _____



***Punica granatum* (Pomegranate) History**

January 24, 2019 – Scientific Literature Review announced.

Report Name Data Profile* – Panel Date – Writer, name																															
						Toxico-kinetics		Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	GRAS	Method of Mfg	Constituents	Impurities	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
Punica Granatum Extract	X																													
Punica Granatum Bark Extract	X																													
Punica Granatum Bark/Fruit Extract																														
Punica Granatum Callus Culture Extract																														
Punica Granatum Flower Extract	X			X																										
Punica Granatum Fruit Extract	X		X	X	X				X			X	X	?	?	X	X													
Punica Granatum Fruit Juice	X														X							X								
Punica Granatum Fruit/Root/Stem Powder																														
Punica Granatum Fruit/Sucrose Ferment Filtrate																														
Punica Granatum Fruit Water	X																													
Punica Granatum Juice Extract	X			X											X															
Punica Granatum Leaf Cell Extract																														
Punica Granatum Peel Extract				X								X																		
Punica Granatum Pericarp Extract	X		X	X	X				X							X				X			X	X	X	X	X			
Punica Granatum Seed	X																													
Punica Granatum Seed Cell Culture Lysate																														
Punica Granatum Seed Extract	X			X					X						X															
Punica Granatum Seed Powder	X		X																											
Pomegranate or Punica Granatum natural extractives including distillates (plant part not defined)		X																												

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

Punica granatum-Derived Ingredients

Ingredient	CAS #	InfoB	SciFin	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Punica Granatum Extract	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Bark Extract	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Bark/Fruit Extract	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Callus Culture Extract	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Flower Extract	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Fruit Extract	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Fruit Juice	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Fruit/Root/Stem Powder	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Fruit/Sucrose Ferment Filtrate		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Fruit Water	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Juice Extract	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Leaf Cell Extract	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Peel Extract	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Pericarp Extract	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Seed	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Seed Cell Culture Lysate		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Seed Extract	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Punica Granatum Seed Powder	84961-57-9 (generic)	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Botanical and/or Fragrance Websites (if applicable)

Ingredient	CAS #	Dr. Duke's	Taxonomy	GRIN	Sigma-Aldrich	AHPA	EMA	AGRICOLA	SSA	IFRA	RIFM
Punica Granatum (generic search)		?	√	√	√	?	√	√	√	√	√

Search Strategy

SciFinder – Search utilized generic CAS No. and INCI names. Search resulted in a single entry for an "unspecified pomegranate, ext." No reference hits were associated with this entry.

PubMed
Punica Granatum Extract – 536 hits, 65 relevant
Punica Granatum Bark Extract – 14 hits, 1 relevant
Punica Granatum Bark/Fruit Extract – 5 hits, 0 relevant
Punica Granatum Callus Culture Extract – 0 hits
Punica Granatum Flower Extract – 34 hits, 6 relevant
Punica Granatum Fruit Extract – 239 hits, 34 relevant
Punica Granatum Fruit Juice – 233 hits, 14 relevant
Punica Granatum Fruit/Root/Stem Powder – 0 hits
Punica Granatum Fruit/Sucrose Ferment Filtrate – 0 hits
Punica Granatum Fruit Water – 103 hits, 7 relevant
Punica Granatum Juice Extract – 82 hits, 15 relevant
Punica Granatum Leaf Cell Extract – 13 hits, 2 relevant
Punica Granatum Peel Extract – 147 hits, 31 relevant
Punica Granatum Pericarp Extract – 16 hits, 4 relevant
Punica Granatum Seed – 229 hits; exclude "oil" = 137 hits, 33 relevant
Punica Granatum Seed Cell Culture Lysate – 0 hits
Punica Granatum Seed Extract – 73 hits; exclude "oil" = 56 hits, 24 relevant
Punica Granatum Seed Powder – 6 hits, 2 relevant

Typical Search Terms

- INCI names
- CAS numbers
- chemical/technical names
- additional terms will be used as appropriate

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)
- Scifinder (https://scifinder.cas.org/scifinder)

appropriate qualifiers are used as necessary
search results are reviewed to identify relevant documents

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://ofmext.epa.gov/hpvis/HPVISlogon
- 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) - http://rifm.org/

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INTRODUCTION

Most of the *Punica granatum*-derived ingredients detailed in this safety assessment are reported to function in cosmetics as skin conditioning agents, while some are reported to have other functions, such as abrasives and antioxidants, according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*; see Table 1).[1] This assessment of the safety of the following 18 *Punica granatum*-derived ingredients is based on the data contained in this report:

Punica Granatum Extract
Punica Granatum Bark Extract
Punica Granatum Bark/Fruit Extract
Punica Granatum Callus Culture Extract
Punica Granatum Flower Extract
Punica Granatum Fruit Extract
Punica Granatum Fruit Juice
Punica Granatum Fruit/Root/Stem Powder
Punica Granatum Fruit/Sucrose Ferment Filtrate
Punica Granatum Fruit Water
Punica Granatum Juice Extract
Punica Granatum Leaf Cell Extract
Punica Granatum Peel Extract
Punica Granatum Pericarp Extract
Punica Granatum Seed
Punica Granatum Seed Cell Culture Lysate
Punica Granatum Seed Extract
Punica Granatum Seed Powder

Punica granatum, commonly referred to as pomegranate, has been used as a source of Unani and Chinese medicines.[2] Investigations into the antioxidant activity of various extracts derived from parts of *Punica granatum* are numerous; however, CIR is not evaluating these claims as these are not related to the safety of the use of these ingredients in cosmetic products.[3-8] There are no publicly available toxicity data that corresponds to any one of these cosmetic ingredients, specifically. The CIR Expert Panel (Panel) has previously reviewed the safety of Punica Granatum Seed Oil and Hydrogenated Punica Granatum Seed Oil and concluded that these ingredients are safe in the present practices of use and concentration.[9] The Panel also previously reviewed the safety of Punica Granatum Sterols and concluded that this phytosterol ingredient is safe in the present practices of use and concentration.[10]

The pomegranate ingredients in this assessment are found in foods, and daily exposure from food use would result in much larger systemic exposures than those from use in cosmetic products. Additionally, essential oils, oleoresins (solvent free), and natural extracts (including distillates) derived from *Punica granatum* L. are generally recognized as safe (GRAS) for their intended use in foods for human and animal consumption according to the US Food and Drug Administration (FDA). The focus of this safety assessment will be on data relevant to the use of *Punica granatum*-derived ingredients in cosmetics, with specific focus on topical exposure when available.

Botanicals, such as *Punica granatum*-derived ingredients, may contain hundreds of constituents, some of which may have the potential to cause toxic effects. In this assessment, CIR is reviewing the potential toxicity of each of the *Punica granatum*-derived ingredients as a whole, complex mixture. CIR is not reviewing the potential toxicity of the individual constituents, except wherein such constituents are also ingredients under review.

This safety assessment includes relevant published and unpublished data 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 CIR typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Note: In many of the published studies, it is not known how the substance being tested compares to the cosmetic ingredient. Therefore, if it is not known whether the substance being discussed is a cosmetic ingredient, the test substance will be identified as "pomegranate…" (e.g., pomegranate seed extract or *Punica granatum* fruits); if it is known that the substance is a cosmetic ingredient, the *Dictionary* nomenclature "Punica Granatum…" (e.g., Punica Granatum Seed Extract or Punica Granatum Fruit Extract) will be used.

CHEMISTRY

Definition and Plant Identification

The definitions and functions of the *Punica granatum*-derived ingredients included in this report are provided in Table 1. The raw materials for the ingredients in this report are obtained from the deciduous shrub or small tree, *Punica granatum.*[11] These trees can grow to 6 to10 m (20 to 30 ft) tall. *Punica granatum* trees are native to Afghanistan, Iran, Iraq, Turkey, the Russian Federation, Tajikistan, Turkmenistan, and India.[12] In the US, the trees are cultivated in Arizona and California.[11]

The fruit produced by the tree are nearly round and are 2.5 to 5 inches wide with a tough, leathery skin or rind, and are light to deep pink or red in color.[11] The fruit interior is separated into compartments by membranous walls and white spongy tissue. The compartments are filled with transparent sacs containing fleshy, tart pulp, known as arils, that are red, pink, or white in color. The seeds in the arils represent approximately half of the weight of the whole fruit.

Physical Properties

Punica Granatum Fruit Extract

A supplier reported that a tradename mixture containing 20% Punica Granatum Fruit Extract was a clear to slightly hazy liquid with a specific gravity of 1.015-1.035 and a pH (direct) of 5.5 -7.5.[13]

Punica Granatum Pericarp Extract

A supplier reported that a tradename mixture containing glycerin, water, and 0.1% - 1% Punica Granatum Pericarp Extract was yellowish to red-brown with a density (at 20º C) of 1.176 - 1.232 g/ml.[14] Another supplier reported a tradename mixture containing water, butylene glycol, and Punica Granatum Pericarp Extract (concentration not reported) was a light brown to brown liquid with a pH of 3.1 – 5.1 and a specific gravity of 1.0-1.1.[15]

Methods of Manufacturing

Punica Granatum Fruit Extract

A supplier reported that a pomegranate extract is produced through the mechanical processing (grinding/milling) of whole *Punica granatum* fruits followed by aqueous extraction at a specific pH, temperature, and duration.[16] The supplier incorporates this extract into a tradename mixture for sale by dilution in butylene glycol, addition of phenoxyethanol and tetrasodium ethylenediaminetetraacetic acid (EDTA), filtration, and quality control. The final tradename mixture product contains 20% Punica Granatum Fruit Extract.

Punica Granatum Pericarp Extract

A supplier reported that a tradename mixture containing water, butylene glycol, and Punica Granatum Pericarp Extract (concentration not reported) is produced by extracting the dried raw material with a 50% ethanolic solution prior to filtering, concentrating, and incorporating 30% butylene glycolic solution.[15]

Punica Granatum Seed Powder

A supplier reported that Punica Granatum Seed Powder is produced by grinding and sieving pomegranate seeds prior to decontaminating through heat or gamma-rays.[17]

Composition/Impurities

The main classes of phytochemicals identified from pomegranate (various plant parts) are as follows: ellagitannins, gallotannins, and derivatives; flavonoids; lignans; triterpenoids and phytosterols; fatty acids and lipids; organic acids and phenolic acids; and other compounds, such as catechol and coumestrol.[18] Specifically, the triterpenes ursolic acid and oleanolic acid are reported to be constituents of pomegranate leaves, bud, fruits, flowers and seeds.[19] Gallic acid is reported to be a constituent of pomegranate peel, pomegranate juice, pomegranate fruit, and pomegranate flowers. The major constituents of pomegranate pericarp are reported to be hydrolysable ellagitannins (up to 28%) and other polyphenols.[20] The main biologically active constituents of pomegranate root and stem bark are alkaloids (0.5% to 0.9%) and tannins (up to 22% in bark).[20] Yields of constituents have been found to be dependent on solvent types, with polar solvents having a greater ability to extract antioxidants when compared to non-polar solvents.[4,5,21] Pomegranates grown in different conditions and locations may have varying composition levels in different plant parts.[6] Table 2 describes the total phytochemical contents of pomegranate extracts by plant part.

Punica Granatum Flower Extract

The tannin content of a pomegranate flower extract used in a wound healing efficacy study was 48.7%.[22] The test material was extracted with ethanol. Analyses of methanol extracts of a flower extract characterized a total of 57 phenolic compounds.[23]

The gallic acid and ellagic acid contents of an ethyl acetate soluble fraction of a methanolic extract of pomegranate flower extract were 2.00 mg/g and 68.80 mg/g, respectively.[2] A methanolic extract, and the water-soluble fraction of the methanolic extract, quantified ellagic acid content as 18.85 mg/g and 10.88 mg/g, respectively.

Punica Granatum Fruit Extract

A food-grade pomegranate fruit extract that was produced from whole pomegranate fruit was standardized to contain 70% polyphenols total, including 30% punicalagins.[24] Other constituents of the extract included not more than 5% ellagic acid and 0.3% gallic acid. Analyses of methanol extracts of a patented pomegranate fruit extract characterized a total of 71 phenolic compounds, including 64 tannins.[23]

A supplier reported that a pomegranate extract contained 20% Punica Granatum Fruit Extract, ~40% butylene glycol, ~40% water, 1% phenoxyethanol, and 0.1% tetrasodium EDTA.[25] This supplier has certified that this product does

not contain the 26 allergenic flavors or fragrances restricted by the European Union, nor does it contain pesticides exceeding US Environmental Protection Agency limits. Heavy metals, lead, arsenic, cadmium, microbial content, yeast and mold, and gram-negative bacteria were below detection limits.[13]

Punica Granatum Leaf Extract

A chromatogram of an acetyl acetate extract of pomegranate leaves identified the following constituents: punicalin, ellagic acid derivate, galloyl-hexahydroxydiphenyl-glucose, castalagin derivatives, granatin B, ellagic acid rhamnoside, kaempferol-3-*O*-glucoside, kaempferol-arbinoside, and a kaempferol derivative.[26]

Punica Granatum Peel Extract

The major constituents of aqueous pomegranate peel extract were reported as punicalagin, punicalin, ellagic acid, gallic acid, quercetin, luteolin, kaempferol, and naringenin. [27] Ellagic acid, punicalagin α, and punicalagin β contents of a methanolic pomegranate peel extract were 2.75 mg/g, 3.52 mg/g, and 5.04 mg/g, respectively.[2] A methanolic extract of pomegranate peel used in a wound healing efficacy study contained 34.03% gallic acid and 3.31% catcechin.[28]

Punica Granatum Pericarp Extract

A supplier reported that a tradename mixture containing water, butylene glycol, and Punica Granatum Pericarp Extract (concentration not reported) contains tannin and sugar.[15] The heavy metals content is not more than 20 ppm and the arsenic content is not more than 2 ppm.

Punica Granatum Seed Extract

The fatty acid composition of an ethanol extract of pomegranate seed is described in Table 3.[3]

An ethanolic extract of pomegranate seeds was found to contain triterpenoids, steroids, glycosides, saponins, tannins, alkaloids, and flavonoids.[29] No further details were provided.

Total phenolic content of pomegranate seed extracts was dependent on the solvent type used during extraction.[5] Methanol and water yielded the highest amount of phenolic compounds (27.93 and 22.61 mg/l seed extract, respectively), followed by acetone (3.41 mg/l), butanol (0.57 mg/l), ethyl acetate (0.37 mg/l), and hexane (0.29 mg/l).

USE

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the US FDA and the cosmetics industry on the expected use of these ingredients in cosmetics. 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 cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2019 VCRP survey data, Punica Granatum Extract has the most reported uses in cosmetic products, with a total of 312; the majority of the uses are in leave-on skin care products (Table 4).[30] Punica Granatum Fruit Extract has the second greatest number of reported uses in this safety assessment with 172 uses; the majority of these uses are also in leave-on skin care products. The results of the concentration of use survey conducted in 2018 by the Council indicated that Punica Granatum Seed Extract is used at up to 0.3% in leave-on cuticle softeners.[31] Punica Granatum Extract and Punica Granatum Fruit Extract are used at up to 0.13% (in a moisturizing preparation) and 0.1% (in face and neck and night skin preparations), respectively. Punica Granatum Fruit Juice is used at up to 0.1% in makeup preparations. Ingredients with no reported uses in the VCRP or by the Council are listed in Table 5.

Punica granatum-derived ingredients may be used in products that can be incidentally ingested or come into contact with mucous membranes; for example, Punica Granatum Seed Extract is reported to be used in lipstick at up to 0.11%.[31] Additionally, some ingredients have been reported to be used in products that may come into contact with the eyes; for example, Punica Granatum Extract is used at up to 0.018% in eye shadows. Moreover, some ingredients have been reported to be used in spray and powder products that could possibly be inhaled; for example, Punica Granatum Extract is used in a face and neck spray at 0.001% and Punica Granatum Fruit Juice is used in a face powder at 0.01%. 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 below 10 µm compared with pump spray.[32-35] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[32,33] 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.[36-38]

The *Punica granatum*-derived ingredients described in this report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[39]

Non-Cosmetic

In the US, according to 21CFR182.20 and 21CFR582.20, the essential oils, oleoresins (solvent-free) and natural extractives (including distillates) from *Punica granatum* L. (pomegranate) are GRAS for their use in food intended for human consumption and in animal drugs, feeds, and related products.

Because of antioxidant and anti-inflammatory properties, the extracts of various parts of *Punica granatum* have been researched for use as alternative or therapeutic treatments (as herbal medicines or dietary supplements) for burn injuries and other dermal wounds, canker sores and oral hygiene, neurodegenerative conditions, convulsions, management of diabetes and weight, acute pancreatitis, acute lung injury, myocardial infarctions and other cardiovascular protection, and various cancers.[3,4,8,19,22,26,28,29,40-51] The juice and peel extracts have also been researched for use as antifungal and antibacterial treatments.[52-56]

TOXICOKINETICS STUDIES

No relevant toxicokinetics studies on *Punica granatum*-derived ingredients were found in the published literature, and unpublished data were not submitted. In general, toxicokinetics 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

Oral

Punica Granatum Fruit Extract

In separate experiments by the same researchers, groups of 6 male and 6 female Wistar rats and Swiss albino mice received a single dose of pomegranate fruit extract (solvent not reported) at 0, 50, 500, or 5000 mg/kg body weight via gavage.[24] The oral LD_{50} was determined to be greater than 5000 mg/kg body weight for both species. No adverse effects were observed during the 14-day observation period, and no gross pathological abnormalities were observed during necropsy in both the rats and mice.

Punica Granatum Pericarp Extract

A supplier reported that the oral LD_{50} for a tradename mixture containing water, butylene glycol, and Punica Granatum Pericarp Extract (concentration not reported) was greater than 2000 mg/kg in mice.[15] No further details were provided.

Punica Granatum Seed Extract

An ethanolic extract of pomegranate seeds was administered orally to 5 groups of 6 fasted NMRI male mice.[29] Doses were 2, 3, or 5 g/kg. No mortalities were observed in any dose level. No further details were provided.

Short Term Toxicity Studies

Oral

Punica Granatum Peel Extract

In a 15-day study, groups of 7 male Wistar rats received pomegranate peel extract by gavage at 250 mg/kg/d body weight as a control and at up to 500 mg/kg/d body weight in treatment groups induced with oral candidiasis.[54] No adverse effects from the test material were observed in the rats.

Intranasal

Punica Granatum Fruit Extract

The toxic effects of an ethanolic pomegranate fruit extract was studied in a 35 day intranasal study in groups of 10 male Wistar rats.[57] The rats received 0, 0.4, 1.2, or 7 mg/kg lyophilized extract in each nasal cavity with a microsyringe. The controls received saline solution. The rats were weighed and feed consumption was measured every 7 days. At the end of the treatment period, biochemical and histopathology samples were analyzed and organs were weighed. No statistically significant differences in mean animal weight or feed consumption were observed. There were no clinical signs of toxicity. The only biochemical effect noted was an increase in creatinine values in the highest dose group (7 mg/kg), but these values were still within the normal range and there was no indication of kidney damage in the histopathology samples. No treatment-related effects were observed in any dose group.

Subchronic Toxicity Studies

Oral

Punica Granatum Fruit Extract

The toxicity of a pomegranate fruit extract was investigated in a 90-day oral toxicity study in Wistar rats in accordance with the Organization for Economic Co-operation and Development (OECD) test guideline (TG) 408.[24] Groups of 10 male and 10 female rats received 0, 60, 240, or 600 mg/kg body weight/day pomegranate fruit extract (solvent not reported) via gavage. Two additional groups of animals that received 0 and 600 mg/kg/day of the extract were recovery groups that were observed for 28 days after the initial 90-day treatment period. Clinical observations, body weight and feed consumption measurements, clinical pathology, and macroscopic and microscopic examinations of tissues from over 40 sites (including ovaries and uteri in females and testes and epididymides in males) were performed on all animals.

All animals survived until scheduled necropsies in both the 90-day study group and the recovery group. No adverse effects were observed during clinical observations. No treatment-related biologically significant effects were noted on body weight or body weight gain, feed consumption, in urinalysis parameters, in hematology parameters, in serum chemistry parameters, in absolute or relative organ weights, or in macroscopic or microscopic findings at any dose tested. No treatment-related effects were reported in the recovery groups. The no-observed-adverse-effect-level (NOAEL) for pomegranate fruit extract was determined to be 600 mg/kg/day.[24]

Chronic Toxicity Studies

No relevant chronic toxicity studies were found in the published literature, and unpublished data were not submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

Punica Granatum Fruit Extract

Abnormal sperm were observed 5 weeks after male Balb/C mice were treated with a hydroalcoholic pomegranate fruit extract in a sperm-shape abnormality assay.[58] Route of exposure was not defined. The extract was tested at doses of 0, 7, 70, or 700 mg/kg body weight in groups of 5 mice. There as a dose-dependent increase in sperm with amorphous and hookless head. The frequency of abnormal sperm was significant ($p < 0.05$) at doses $\geq$ 70 mg/kg body weight.

Oral

Punica Granatum Fruit Juice Extract and Punica Granatum Seed Extract

The potential effects of pomegranate seed extract (described as husk extract) and pomegranate juice extract on chondrogenesis and osteogenesis in developing embryos was investigated in female Balb/c mice.[59] Both test materials were extracted in water. Groups of 10 pregnant mice received the seed extract (1.0 g/kg suspended in 0.2 ml distilled water), the juice extract (3.3 ml/kg suspended in 0.2 ml distilled water), a mixture of both extracts, or distilled water daily in an oral dietary supplement between days 8 and 18 of gestation. On day 19 of gestation, the embryos were weighed and the length of the femur, tibia, and the ossification zones were measured by stereomicroscopy. The bone calcium content of the femurs of the pregnant mice was also measured.

Body weight gains of the pregnant mice were not affected by the test material. The pregnant mice that received the pomegranate extracts had an increase in bone calcium content, with a statistically significant increase ($P < 0.05$) in the group that received pomegranate juice extract. The fetuses from the mixed extract group did have significantly reduced body weights and crown-rump lengths; these effects were not observed in the pomegranate seed extract only and pomegranate juice extract only treatment groups. Significantly increased femur lengths and osteogenesis indices were observed in all extract-exposed groups. No craniofacial abnormalities or limb defects were reported during gross observations; and no pathological changes, including necrosis, abnormal cells, or congestion in longitudinal section of fetuses were observed. The liver and kidneys of the fetuses and the dams were within normal parameters.[59]

Punica Granatum Fruit Juice

The effects of pomegranate juice on sperm quality, spermatogenic cell density, antioxidant activity, and testosterone levels were studied in male Wistar rats.[7] Groups of 7 rats received 0.25 ml pomegranate juice with 0.75 ml distilled water, 0.50 ml pomegranate juice with 0.50 ml distilled water, 1 ml pomegranate juice, or 1 ml distilled water via gavage daily for 7 weeks. Body weights, reproductive organ weights, spermatogenic cell density, sperm characteristics, levels of antioxidant vitamins (A, C, and E), testosterone, lipid peroxidation, and antioxidant enzyme activities (glutathione, glutathione peroxidase, and catalase) were recorded. Analyses were done only once at the end of the study. There were no statistically significant effects on body weights in the treated groups when compared to the control group. Weights of testes, epididymides, seminal vesicles, prostate glands, and Cowper glands were higher in the treated groups when compared to the controls, but the differences were not statistically significant. A significant ($p < 0.05$) decrease in malondialdehyde level and marked increases in glutathione, glutathione peroxidase and catalase activities, and vitamin C levels were observed in rats treated with different doses of pomegranate juice. Increases in epididymal sperm concentration, sperm motility,

spermatogenic cell density, diameter of seminiferous tubules, germinal cell layer thickness, and a decreased abnormal sperm rate were observed with pomegranate juice consumption when compared to the controls.

GENOTOXICITY

In Vitro

Punica Granatum Fruit Extract

A hydroalcoholic extract of pomegranate fruit (including peel) induced significant increases of revertants (2 mg/plate $p < 0.05$; 4 mg/plate $p < 0.01$) in an Ames study using *Salmonella typhimurium* strain TA100, with and without S9 metabolic activation.[58] The extract was tested at 0, 0.45, 1, 2, and 4 mg/plate. The results of the lower doses tested were comparable with negative controls. The positive control yielded expected results.

The same pomegranate fruit extract described above did not induce gene-conversion events in *Saccharomyces cerevisiae* strain D7, but an increased frequency of reverse mutations was observed, with and without metabolic activation.[58] The yeast cells were treated with the extract at concentrations up to 18 mg/ml.

In Chinese hamster ovary (CHO) cell assays tested with and without metabolic activation, a dose-dependent and statistically significant increase in sister chromatid exchanges per cell was observed after treatment with a hydroalcoholic pomegranate fruit extract at concentrations up to 450 µg/ml in the absence of S9 metabolic activation.[58] Significant increases in the percentage of chromosomal aberrations were also observed following treatment with the extract at concentration up to 225 µg/ml without metabolic activation.

Punica Granatum Pericarp Extract

The mutagenic potential of a tradename mixture containing 10% Punica Granatum Pericarp Extract, 10% *Lactobacillus* ferment lysate, 10% *Camillia sinesis* leaf extract, 2% Lactobacillus ferment, and 1% caffeine in water was studied in an Ames test using *S. typhimurium* strains TA98, A100, TA1535, and TA1537 and *Escherichia coli* strain WP2*uvr*A, with and without metabolic activation.[60] Cells were incubated with the test material at doses of 1.5 to 5000 µg/plate in sterile deionized water. No mutagenicity was observed at any dose level. Positive and negative controls yielded expected results.

A supplier reported that a tradename mixture containing water, butylene glycol, and Punica Granatum Pericarp Extract (concentration not reported) was negative in an Ames test when tested at 5000 µg/plate.[15] No further details were provided.

In Vivo

Punica Granatum Fruit Extract

In a mouse bone marrow micronucleus assay studying the genotoxic effects of a hydroalcoholic extract of pomegranate fruit, a dose-dependent increase in the number of polychromatic erythrocytes with micronuclei was observed.[58] The extract was administered intraperitoneally at doses of 7, 70, 184, 369, or 700 mg/kg body weight in 5 Balb/C mice/sex/group. The genotoxicity index increase was statistically significant at doses $\geq$ 70 mg/kg bodyweight in both sexes. The cytotoxicity index was significantly increased at doses of $\geq$ 70 and 184 mg/kg body weight in males and females, respectively.

ANTI-GENOTOXICITY

Punica Granatum Leaf Extract

In a mouse bone marrow micronucleus assay studying antigenotoxicity effects of an aqueous pomegranate leaf extract, groups of 6 male Swiss mice received 0, 400, 600, or 800 mg/kg body weight of the extract in distilled water by gavage for 7 days before exposure to the genotoxin cyclophosphamide (CPH).[61] Another two groups of 6 mice served as genotoxin and test material (800 mg/kg extract) controls. Prior to the final treatment with the extract, the mice received 40 mg/kg CPH and all mice were killed after 24 h and the micronucleus assay was performed. Antigenotoxic effects were observed in a non-dose dependent manner in all 3 extract dose levels. The maximum reduction was observed in mice that received 800 mg/kg of the extract. There was no reduction in the percentage of polychromatic erythrocytes following treatment with the extract and CPH. No genotoxic effects were observed to the pomegranate leaf extract alone.

CARCINOGENICITY

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

OTHER RELEVANT STUDIES

Skin Lightening

In Vitro

Punica Granatum Fruit Extract

The potential for an ethanolic pomegranate fruit extract to inhibit melanin production has been studied in vitro using the Melan-a melanocyte cell culture model.[62] The Melan-a cells were treated with pomegranate fruit extract that was standardized to 20% punicalagins. The test material was produced from fruit (with peel) that was macerated and extracted with a 75% - 80% ethanol solution at a ratio of 1:4 (fruit:solvent) before filtration and vacuum processing. Melanin content was reduced by approximately 40% to 60% at test concentrations of 50 µg/ml and 100 µg/ml, respectively. Further testing with the purified punicalagins isolated from pomegranate fruit found that these constituents reduced melanin production by 60%, 70%, and 75% of control levels at test concentrations of 20 µg/ml, 60 µg/ml, and 100 µg/ml, respectively.

Punica Granatum Peel Extract

An aqueous pomegranate extract of rind containing 90% ellagic acid showed inhibitory activity against mushroom tyrosinase (IC_{50} 182.2 µg/ml) in vitro.[63] The inhibition effects were comparable to arbutin (IC_{50} 162.2 µg/ml), but was about ten times weaker than L-ascorbic acid (IC_{50} 18.4µg/ml).

Animal

Punica Granatum Peel Extract

Ultraviolet (UV) light-induced skin pigmentation was inhibited in female brownish guinea pigs after the animals received the same aqueous pomegranate extract orally for 35 days.[63] There were 6 animals per dose group that received either 100 mg/kg/day of the extract diluted in water at 100 mg/ml, 1000 mg/kg/day of the extract diluted in water at 100 mg/ml, water, or 600 mg/kg/day L-ascorbic acid diluted in water at 60 mg/ml. The animals were irradiated on days 7, 9, and 11. The number of L-3,4-dihydroxyphenylalanine (DOPA)-positive melanocytes in the epidermis of the UV-irradiated guinea pigs were reduced in the animals that received the pomegranate extract. The researchers of this in vitro and in vivo study above concluded that the skin-whitening effects were likely due to inhibition of the proliferation of melanocytes and melanin synthesis by tyrosinase in melanocytes.

Human

Punica Granatum Juice

Significant decreases (details not provided) in skin melanin content were observed in an efficacy study of a water/oil emulsion containing 4% concentrated pomegranate juice.[64] The test material was applied daily to the cheeks of 25 healthy volunteers for 60 days. A mexameter was used to measure the melanin on the cheeks of the volunteers on the day prior to application and on weeks 1, 2, 3, 4, 6, and 8.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

In Vitro

Punica Granatum Pericarp Extract

An undiluted tradename mixture containing 10% Punica Granatum Pericarp Extract, 10% *Lactobacillus* ferment lysate, 10% *Camillia sinesis* leaf extract, 2% *Lactobacillus* ferment, and 1% caffeine in water was predicted to be non-irritating in an EpiDerm™ reconstructed human epidermal model.[65] Negative and positive controls yielded expected results.

Human

Punica Granatum Juice

No dermal irritation was observed in a 60-day efficacy study of a water/oil emulsion containing 4% concentrated pomegranate juice in 25 healthy volunteers.[64] The test material was applied daily to the cheeks.

Sensitization

In Vitro

Punica Granatum Pericarp Extract

A tradename mixture containing 10% Punica Granatum Pericarp Extract, 10% *Lactobacillus* ferment lysate, 10% *Camillia sinesis* leaf extract, 2% Lactobacillus ferment, and 1% caffeine in water was not predicted to be a sensitizer in a direct peptide reactivity assay (DPRA) performed in accordance with OECD TG 442C.[66] The 100 mM product (in

acetonitrile) was tested at 5 mM with the cysteine peptide and at 25 mM with the lysine peptide. The controls yielded expected results.

The same tradename mixture containing 10% Punica Granatum Pericarp Extract was not predicted to be a sensitizer in a KeratinoSens™ ARE-Nrf2 Luciferase test performed in accordance with OECD TG 422D.[67] The test material was prepared in dimethyl sulfoxide at 0.98 to 2000 µM. The controls yielded expected results.

Animal

Punica Granatum Pericarp Extract

A supplier reported that a tradename mixture containing water, butylene glycol, and Punica Granatum Pericarp Extract (concentration not reported) tested at 20% was negative in a guinea pig skin sensitization test using 5 animals.[15] No further details were provided.

Human

Punica Granatum Pericarp Extract

A supplier reported that a tradename mixture containing water, butylene glycol, and Punica Granatum Pericarp Extract (concentration not reported) tested at 20% was negative in a human patch test using 44 subjects.[15] No further details were provided.

The same supplier reported that the tradename mixture tested at 30% was negative in a human repeat insult patch test using 52 subjects.[15] No further details were provided.

Photosensitization

Animal

Punica Granatum Pericarp Extract

A supplier reported that a tradename mixture containing water, butylene glycol, and Punica Granatum Pericarp Extract (concentration not reported) tested at 20% was negative in a photosensitization test using 5 guinea pigs.[15] No further details were provided.

OCULAR IRRITATION STUDIES

In Vitro

Punica Granatum Pericarp Extract

An undiluted tradename mixture containing 10% Punica Granatum Pericarp Extract, 10% *Lactobacillus* ferment lysate, 10% *Camillia sinesis* leaf extract, 2% Lactobacillus ferment, and 1% caffeine in water was predicted to be non-irritating in an EpiOcular™ cornea epithelial model.[65] Negative and positive controls yielded expected results.

A supplier reported that a tradename mixture containing water, butylene glycol, and Punica Granatum Pericarp Extract (concentration not reported) tested at 100% was predicted to be non-irritating in a human corneal epithelium eye irritation test.[15] No further details were provided.

SUMMARY

According to the *Dictionary*, most of the 18 *Punica granatum*-derived ingredients detailed in this safety assessment are reported to function in cosmetics as skin conditioning agents, while some are reported to have other functions, such as abrasives and antioxidants. Investigations into the antioxidant activity of various extracts derived from parts of *Punica granatum* are numerous; these studies are not detailed in this report. The available toxicity data that correspond to specific use of these ingredients in cosmetics are extremely limited. There are no publicly available toxicity data that corresponds to any one of these cosmetic ingredients, specifically. The focus of this safety assessment will be on data relevant to the use of *Punica granatum*-derived ingredients in cosmetics, with specific focus on topical exposure when available.

According to 2019 VCRP survey data, Punica Granatum Extract has the most reported uses in cosmetic products, with a total of 312; the majority of the uses are in leave-on skin care products. Punica Granatum Fruit Extract has the second greatest number of reported uses in this safety assessment with 172 uses; the majority of these uses are also in leave-on skin care products. The results of the concentration of use survey conducted in 2018 by the Council indicated that Punica Granatum Seed Extract is used at up to 0.3% in leave-on cuticle softeners. Punica Granatum Extract and Punica Granatum Fruit Extract are used at up to 0.13% (in a moisturizing preparation) and 0.1% (in face and neck and night skin preparations), respectively.

In the US, the essential oils, solvent-free oleoresins, and natural extractives from *Punica granatum* L. (pomegranate) are GRAS for their use in food intended for human consumption and in animal drugs, feeds, and related products. Extensive

research has been performed on the extracts of various parts of *Punica granatum* for use as alternative or therapeutic treatments for various conditions.

The oral LD_{50} in mice and rats for a pomegranate fruit extract was greater than 5000 mg/kg body weight. No mortalities were observed in mice that received an ethanolic extract of pomegranate seeds at up to 5000 mg/kg. The oral LD_{50} for a tradename mixture containing Punica Granatum Pericarp Extract (concentration in mixture not reported) was greater than 2000 mg/kg in mice.

In repeated dose studies, no adverse effects were reported in a 15-day oral rat study of methanolic pomegranate peel extract at up to 500 mg/kg/day. In a 90-day study, the NOAEL for an oral study of a pomegranate fruit extract in rats was 600 mg/kg/day, the maximum dose tested. No adverse effects were noted in rats that received lyophilized ethanolic pomegranate fruit extract at up to 7 mg/kg intranasally for 35 days. The only biochemical effect observed was an increase in creatinine values in the high dose group, but there was no kidney damage noted histopathologically.

Abnormal sperm were observed in male mice treated with a hydroalcoholic pomegranate fruit extract at doses up to 700 mg/kg body weight. Route of exposure was not defined. No adverse effects were observed in an oral DART study in female mice that received pomegranate seed extract (1.0 g/kg suspended in 0.2 ml distilled water) or pomegranate juice extract (3.3 ml/kg suspended in 0.2 ml distilled water) separately or as a mixture, and there was no effect on the fetuses. In a rat sperm study, increases in epididymal sperm concentration, sperm motility, spermatogenic cell density, diameter of seminiferous tubules, germinal cell layer thickness, and a decreased abnormal sperm rate were observed with pomegranate juice consumption when compared to the controls.

Positive genotoxic effects to a hydroalcoholic extract of pomegranate fruit were observed in an Ames test (at $\geq$ 2 mg/plate), a reverse mutation study in *S. cerevisiae*, and in CHO cell assays (at $\geq$ 45 µg/ml), with and without metabolic activation. The same extract was associated with a dose-dependent increase in the number of polychromatic erythrocytes in a mouse micronucleus assay with statistical significance at $\geq$ 70 mg/kg body weight. No genotoxic effects were observed to tradename mixtures containing Punica Granatum Pericarp Extract in Ames tests or to a pomegranate leaf extract in a mouse micronucleus assay.

In vitro and in vivo studies indicate that a pomegranate fruit extract and pomegranate juice, and a pomegranate peel extract may inhibit melanin production. In an in vitro human epidermal model, an undiluted tradename mixture containing 10% Punica Granatum Pericarp Extract was predicted to be non-irritating. In a 60-day efficacy study of an emulsion containing 4% concentrated pomegranate juice, no dermal irritation was observed. Sensitization was not predicted in in vitro assays of a tradename mixture containing 10% Punica Granatum Pericarp Extract. Results of a guinea pig sensitization test, a photosensitization test in guinea pigs, and sensitization tests in humans to a tradename mixture containing Punica Granatum Pericarp Extract (concentration in mixture not reported) were negative. No ocular irritation was predicted in in vitro cornea epithelial models of tradename mixtures containing Punica Granatum Pericarp Extract.

No relevant chronic toxicity or carcinogenicity studies on *Punica granatum*-derived ingredients were found in the published literature, and no unpublished data were provided. No relevant toxicokinetics studies were found in the published literature; however, in general, toxicokinetics data are not expected to be found on botanical ingredients because each botanical ingredient is a complex mixture of constituents.

DISCUSSION

To be determined.

CONCLUSION

To be determined.

TABLES

Table 1. Definitions and functions of the ingredients in this safety assessment.

Ingredient/CAS No.	Definition & Structure	Function
Punica Granatum Extract 84961-57-9 (generic)	Punica Granatum Extract is the extract of the whole plant, *Punica granatum.*	Fragrance Ingredient; Skin-Conditioning Agent – Misc.
Punica Granatum Bark Extract 84961-57-9 (generic)	Punica Granatum Bark Extract is the extract of the bark of *Punica granatum.*	Fragrance Ingredient; Skin-Conditioning Agent – Misc.
Punica Granatum Bark/Fruit Extract 84961-57-9 (generic)	Punica Granatum Bark/Fruit Extract is the extract of the bark and fruit of *Punica granatum.*	Antimicrobial Agent; Antioxidant; Cosmetic Astringent
Punica Granatum Callus Culture Extract 84961-57-9 (generic)	Punica Granatum Callus Culture Extract is the extract of a culture of the callus of *Punica granatum.*	Skin-Conditioning Agent – Misc.
Punica Granatum Flower Extract 84961-57-9 (generic)	Punica Granatum Flower Extract is the extract of the flowers of *Punica granatum.*	Skin-Conditioning Agent – Misc.
Punica Granatum Fruit Extract 84961-57-9 (generic)	Punica Granatum Fruit Extract is the extract of the fruit of *Punica granatum.*	Skin-Conditioning Agent – Misc.
Punica Granatum Fruit Juice 84961-57-9 (generic)	Punica Granatum Fruit Juice is the juice expressed from the fruit of the pomegranate, *Punica granatum.*	Flavoring Agent; Skin-Conditioning Agent – Misc.
Punica Granatum Fruit/Root/Stem Powder 84961-57-9 (generic)	Punica Granatum Fruit/Root/Stem Powder is the powder obtained from the finely ground fruit, roots, and stems of *Punica granatum.*	Antioxidants; Hair Conditioning Agent; Skin-Conditioning Agent – Misc.
Punica Granatum Fruit/Sucrose Ferment Filtrate	Punica Granatum Fruit/Sucrose Ferment Filtrate is a filtrate of the product obtained by the spontaneous fermentation of the fruit of *Punica granatum* and sucrose.	Antioxidants
Punica Granatum Fruit Water 84961-57-9 (generic)	Punica Granatum Fruit Water is an aqueous solution of the steam distillates obtained from the fruit of *Punica granatum.*	Flavoring Agent; Fragrance Ingredient; Skin-Conditioning Agent – Misc.
Punica Granatum Juice Extract 84961-57-9 (generic)	Punica Granatum Juice Extract is the extract of the juice of *Punica granatum.*	Skin-Conditioning Agent – Misc.
Punica Granatum Leaf Cell Extract 84961-57-9 (generic)	Punica Granatum Leaf Cell Extract is the extract of a culture of the leaf cells of *Punica granatum.*	Antioxidant; Skin Protectant
Punica Granatum Peel Extract 84961-57-9 (generic)	Punica Granatum Peel Extract is the extract of the peel of *Punica granatum.*	Antimicrobial Agent; Antioxidant; Cosmetic Astringent; Preservative; Skin-Conditioning Agent – Misc.
Punica Granatum Pericarp Extract 84961-57-9 (generic)	Punica Granatum Pericarp Extract is the extract of the pericarp of *Punica granatum.*	Skin-Conditioning Agent – Misc.
Punica Granatum Seed 84961-57-9 (generic)	Punica Granatum Seed is the seed of *Punica granatum.*	Abrasive; Bulking Agent; Skin-Conditioning Agent – Misc.
Punica Granatum Seed Cell Culture Lysate	Punica Granatum Seed Cell Culture Lysate is a lysate of a suspension of the cultured seed cells of *Punica granatum.*	Skin-Conditioning Agent – Misc.
Punica Granatum Seed Extract 84961-57-9 (generic)	Punica Granatum Seed Extract is the extract of the seeds of *Punica granatum.*	Skin-Conditioning Agent – Misc.
Punica Granatum Seed Powder 84961-57-9 (generic)	Punica Granatum Seed Powder is the powder obtained from the dried, ground seeds of *Punica granatum.*	Abrasive

Table 2. Phytochemical constituents of pomegranate extracts (mg/g of dry extract) [3,6,46,61,68,69]

	Flower Extract	Peel Extract	Seed Extract	Juice Extract	Leaf Extract	Stem Extract
Total phenolic content	336.51 (M)	276-413 (E) 190.27-298 (M) 185 (A)	2.57-73 (E) 0.65 (M)	12.4-23.8 (E) 0.094 (A) 0.057 (B)	87.81 (M) 70.00 (A)	52.92 (M)
Total flavonoid content	213.54 (M)	36-54 (E) 49.8-80.10 (M) 23.05 (A)	7.55-38.0 (E) 0.33 (M)	1.8-8.7 (E) 0.46 (A) 0.22 (B)	63.89 (M) 50.43 (A)	41.36 (M)
Total flavonol content		25-45 (E) 0.39-0.44 (A)	3.4-22 (E)	1.5-2.0 (E)		
Total proanthocyanidin content	1.46 (M)	2.48-14.09 (M) 9.09 (A)	0.13 (M)		0.21 (M)	0.32 (M)

Solvents: M = methanol, E = ethanol, A = water/aqueous, B = n-butanol

Table 3. Fatty acid composition (%) for pomegranate seed extract (ethanolic)[3]

Palmitic Acid	4.7
Stearic Acid	2.2
Oleic Acid	5.3
Vaccenic Acid	0.8
α-Linoleic Acid	8.8
α-Linolenic Acid	0.5
Gondoic Acid	0.5
Punicic Acid	73.7
α-Eleostearic Acid	1.6
Catalpic Acid	1.2

Table 4. 2019 frequency and concentration of use according to duration and type of exposure for *Punica granatum*-derived ingredients.[30,31]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Punica Granatum Extract		**Punica Granatum Bark Extract**		**Punica Granatum Flower Extract**		**Punica Granatum Fruit Extract**	
Totals[†]	**312**	**0.0000002-0.13**	**13**	**NR**	**5**	**0.0001**	**172**	**0.00025-0.1**
Duration of Use								
Leave-On	219	0.0000002-0.13	12	NR	4	NR	118	0.0005-0.1
Rinse Off	92	0.000005-0.1	1	NR	1	0.0001	52	0.00025-0.01
Diluted for (Bath) Use	1	0.0005	NR	NR	NR	NR	2	0.0005
Exposure Type								
Eye Area	20	0.000005-0.018	1	NR	NR	NR	20	0.0005-0.0022
Incidental Ingestion	13	0.0047-0.02	NR	NR	NR	NR	2	0.0005
Incidental Inhalation-Spray	2; 73[a]; 62[b]	0.00001-0.001; 0.00001-0.02[a]	2[a]; 8[b]	NR	2[b]	NR	33[a]; 48[b]	0.0005; 0.001-0.1[a]
Incidental Inhalation-Powder	7; 62[b]	0.005; 0.0002-0.1[c]	8[b]	NR	2[b]	NR	48[b]	0.0022-0.1[c]
Dermal Contact	238	0.0000002-0.13	10	NR	4	NR	151	0.00039-0.1
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	0.0005
Hair - Non-Coloring	53	0.00001-0.1	2	NR	1	0.0001	15	0.00025-0.0005
Hair-Coloring	8	NR	NR	NR	NR	NR	NR	NR
Nail	NR	0.00001-0.001	NR	NR	NR	NR	NR	NR
Mucous Membrane	24	0.0005-0.02	NR	NR	NR	NR	17	0.0005
Baby Products	2	0.000005	1	NR	NR	NR	NR	NR
	Punica Granatum Fruit Juice		**Punica Granatum Fruit Water**		**Punica Granatum Juice Extract**		**Punica Granatum Pericarp Extract**	
Totals[†]	**86**	**0.0001-0.1**	**15**	**NR**	**6**	**0.005**	**5**	**NR**
Duration of Use								
Leave-On	68	0.01-0.1	9	NR	3	NR	4	NR
Rinse Off	18	0.0001	6	NR	2	0.005	1	NR
Diluted for (Bath) Use	NR	NR	NR	NR	1	NR	NR	NR
Exposure Type								
Eye Area	9	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	3	NR	NR	NR	NR	NR	3	NR
Incidental Inhalation-Spray	27[a]; 23[b]	NR	9[a]	NR	1[a]; 1[b]	NR	1[b]	NR
Incidental Inhalation-Powder	23[b]	0.01	NR	NR	1[b]	NR	1[b]	NR
Dermal Contact	75	0.01-0.1	15	NR	5	0.005	2	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	8	0.0001	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	8	NR	NR	NR	2	NR	3	NR
Baby Products	NR	NR	NR	NR	1	NR	NR	NR

Table 4. 2019 frequency and concentration of use according to duration and type of exposure for *Punica granatum*-derived ingredients.[30,31]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Punica Granatum Seed		**Punica Granatum Seed Extract**		**Punica Granatum Seed Powder**			
Totals†	**3**	**NR**	**1**	**0.01-0.3**	**6**	**0.01**		
Duration of Use								
Leave-On	3	NR	1	0.01-0.3	4	NR		
Rinse Off	NR	NR	NR	NR	1	0.01		
Diluted for (Bath) Use	NR	NR	NR	NR	1	0.01		
Exposure Type								
Eye Area	NR	NR	1	NR	NR	NR		
Incidental Ingestion	NR	NR	NR	0.11	NR	NR		
Incidental Inhalation-Spray	3[a]	NR	NR	NR	2[a]; 2[b]	NR		
Incidental Inhalation-Powder	NR	NR	NR	NR	2[b]	NR		
Dermal Contact	3	NR	1	0.01	6	0.01		
Deodorant (underarm)	NR	NR	NR	NR	NR	NR		
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR		
Hair-Coloring	NR	NR	NR	NR	NR	NR		
Nail	NR	NR	NR	0.3	NR	NR		
Mucous Membrane	NR	NR	NR	0.11	1	0.01		
Baby Products	NR	NR	NR	NR	NR	NR		

NR = Not reported.

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

[a.] It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.

[b.] Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

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

Table 5. Ingredients not reported in use.

Punica Granatum Bark/Fruit Extract
Punica Granatum Callus Culture Extract
Punica Granatum Fruit/Root/Stem Powder
Punica Granatum Fruit/Sucrose Ferment Filtrate
Punica Granatum Leaf Cell Extract
Punica Granatum Peel Extract
Punica Granatum Seed Cell Culture Lysate

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2019 FDA VCRP RAW DATA

PUNICA GRANATUM (POMEGRANATE) BARK EXTRACT	01A - Baby Shampoos	1
PUNICA GRANATUM (POMEGRANATE) BARK EXTRACT	03F - Mascara	1
PUNICA GRANATUM (POMEGRANATE) BARK EXTRACT	05I - Other Hair Preparations	1
PUNICA GRANATUM (POMEGRANATE) BARK EXTRACT	12C - Face and Neck (exc shave)	7
PUNICA GRANATUM (POMEGRANATE) BARK EXTRACT	12D - Body and Hand (exc shave)	1
PUNICA GRANATUM (POMEGRANATE) BARK EXTRACT	12F - Moisturizing	1
PUNICA GRANATUM (POMEGRANATE) BARK EXTRACT	12G - Night	1
PUNICA GRANATUM (POMEGRANATE) EXTRACT	01A - Baby Shampoos	1
PUNICA GRANATUM (POMEGRANATE) EXTRACT	01C - Other Baby Products	1
PUNICA GRANATUM (POMEGRANATE) EXTRACT	02D - Other Bath Preparations	1
PUNICA GRANATUM (POMEGRANATE) EXTRACT	03C - Eye Shadow	4
PUNICA GRANATUM (POMEGRANATE) EXTRACT	03D - Eye Lotion	9
PUNICA GRANATUM (POMEGRANATE) EXTRACT	03G - Other Eye Makeup Preparations	7
PUNICA GRANATUM (POMEGRANATE) EXTRACT	04A - Cologne and Toilet waters	1
PUNICA GRANATUM (POMEGRANATE) EXTRACT	04E - Other Fragrance Preparation	1
PUNICA GRANATUM (POMEGRANATE) EXTRACT	05A - Hair Conditioner	20
PUNICA GRANATUM (POMEGRANATE) EXTRACT	05E - Rinses (non-coloring)	1
PUNICA GRANATUM (POMEGRANATE) EXTRACT	05F - Shampoos (non-coloring)	19
PUNICA GRANATUM (POMEGRANATE) EXTRACT	05G - Tonics, Dressings, and Other Hair Grooming Aids	7
PUNICA GRANATUM (POMEGRANATE) EXTRACT	05I - Other Hair Preparations	5
PUNICA GRANATUM (POMEGRANATE) EXTRACT	06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	3
PUNICA GRANATUM (POMEGRANATE) EXTRACT	06C - Hair Rinses (coloring)	1
PUNICA GRANATUM (POMEGRANATE)	06D - Hair Shampoos (coloring)	4

EXTRACT		
PUNICA GRANATUM (POMEGRANATE) EXTRACT	07A - Blushers (all types)	3
PUNICA GRANATUM (POMEGRANATE) EXTRACT	07B - Face Powders	7
PUNICA GRANATUM (POMEGRANATE) EXTRACT	07C - Foundations	3
PUNICA GRANATUM (POMEGRANATE) EXTRACT	07E - Lipstick	11
PUNICA GRANATUM (POMEGRANATE) EXTRACT	07I - Other Makeup Preparations	3
PUNICA GRANATUM (POMEGRANATE) EXTRACT	09C - Other Oral Hygiene Products	2
PUNICA GRANATUM (POMEGRANATE) EXTRACT	10A - Bath Soaps and Detergents	4
PUNICA GRANATUM (POMEGRANATE) EXTRACT	10E - Other Personal Cleanliness Products	6
PUNICA GRANATUM (POMEGRANATE) EXTRACT	12A - Cleansing	21
PUNICA GRANATUM (POMEGRANATE) EXTRACT	12C - Face and Neck (exc shave)	41
PUNICA GRANATUM (POMEGRANATE) EXTRACT	12D - Body and Hand (exc shave)	21
PUNICA GRANATUM (POMEGRANATE) EXTRACT	12F - Moisturizing	34
PUNICA GRANATUM (POMEGRANATE) EXTRACT	12G - Night	9
PUNICA GRANATUM (POMEGRANATE) EXTRACT	12H - Paste Masks (mud packs)	10
PUNICA GRANATUM (POMEGRANATE) EXTRACT	12I - Skin Fresheners	4
PUNICA GRANATUM (POMEGRANATE) EXTRACT	12J - Other Skin Care Preps	29
PUNICA GRANATUM (POMEGRANATE) EXTRACT	13A - Suntan Gels, Creams, and Liquids	1
PUNICA GRANATUM (POMEGRANATE) EXTRACT	13B - Indoor Tanning Preparations	18
PUNICA GRANATUM (POMEGRANATE) FLOWER EXTRACT	05A - Hair Conditioner	1
PUNICA GRANATUM (POMEGRANATE) FLOWER EXTRACT	12C - Face and Neck (exc shave)	2
PUNICA GRANATUM (POMEGRANATE) FLOWER EXTRACT	12J - Other Skin Care Preps	2

PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	02B - Bubble Baths	2
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	03A - Eyebrow Pencil	1
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	03C - Eye Shadow	6
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	03D - Eye Lotion	5
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	03F - Mascara	2
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	03G - Other Eye Makeup Preparations	6
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	05A - Hair Conditioner	6
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	05F - Shampoos (non-coloring)	7
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	05G - Tonics, Dressings, and Other Hair Grooming Aids	1
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	05I - Other Hair Preparations	1
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	07C - Foundations	2
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	07E - Lipstick	1
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	07F - Makeup Bases	1
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	07I - Other Makeup Preparations	2
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	09C - Other Oral Hygiene Products	1
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	10A - Bath Soaps and Detergents	7
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	10C - Douches	2
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	10E - Other Personal Cleanliness Products	4
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	11G - Other Shaving Preparation Products	1
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	12A - Cleansing	13
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	12C - Face and Neck (exc shave)	39
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	12D - Body and Hand (exc shave)	9
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	12F - Moisturizing	24
PUNICA GRANATUM (POMEGRANATE)	12G - Night	4

FRUIT EXTRACT		
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	12H - Paste Masks (mud packs)	11
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	12I - Skin Fresheners	1
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	12J - Other Skin Care Preps	10
PUNICA GRANATUM (POMEGRANATE) FRUIT EXTRACT	13B - Indoor Tanning Preparations	3
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	03D - Eye Lotion	7
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	03G - Other Eye Makeup Preparations	2
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	05A - Hair Conditioner	3
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	05E - Rinses (non-coloring)	1
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	05F - Shampoos (non-coloring)	1
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	05G - Tonics, Dressings, and Other Hair Grooming Aids	2
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	05I - Other Hair Preparations	1
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	07C - Foundations	1
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	07E - Lipstick	1
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	07F - Makeup Bases	1
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	09A - Dentifrices	1
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	09B - Mouthwashes and Breath Fresheners	1
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	10A - Bath Soaps and Detergents	5
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	12A - Cleansing	4
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	12C - Face and Neck (exc shave)	14
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	12D - Body and Hand (exc shave)	9
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	12F - Moisturizing	14
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	12G - Night	7
PUNICA GRANATUM (POMEGRANATE)	12H - Paste Masks (mud packs)	2

FRUIT JUICE		
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	12J - Other Skin Care Preps	6
PUNICA GRANATUM (POMEGRANATE) FRUIT JUICE	13B - Indoor Tanning Preparations	3
PUNICA GRANATUM (POMEGRANATE) FRUIT WATER	12A - Cleansing	6
PUNICA GRANATUM (POMEGRANATE) FRUIT WATER	12F - Moisturizing	9
PUNICA GRANATUM (POMEGRANATE) JUICE EXTRACT	01B - Baby Lotions, Oils, Powders, and Creams	1
PUNICA GRANATUM (POMEGRANATE) JUICE EXTRACT	02D - Other Bath Preparations	1
PUNICA GRANATUM (POMEGRANATE) JUICE EXTRACT	10C - Douches	1
PUNICA GRANATUM (POMEGRANATE) JUICE EXTRACT	12A - Cleansing	1
PUNICA GRANATUM (POMEGRANATE) JUICE EXTRACT	12C - Face and Neck (exc shave)	1
PUNICA GRANATUM (POMEGRANATE) JUICE EXTRACT	12J - Other Skin Care Preps	1
PUNICA GRANATUM (POMEGRANATE) PERICARP EXTRACT	07E - Lipstick	3
PUNICA GRANATUM (POMEGRANATE) PERICARP EXTRACT	12A - Cleansing	1
PUNICA GRANATUM (POMEGRANATE) PERICARP EXTRACT	12C - Face and Neck (exc shave)	1
PUNICA GRANATUM (POMEGRANATE) SEED	12J - Other Skin Care Preps	3
PUNICA GRANATUM (POMEGRANATE) SEED EXTRACT	03G - Other Eye Makeup Preparations	1
PUNICA GRANATUM (POMEGRANATE) SEED POWDER	02D - Other Bath Preparations	1
PUNICA GRANATUM (POMEGRANATE) SEED POWDER	12A - Cleansing	1
PUNICA GRANATUM (POMEGRANATE) SEED POWDER	12C - Face and Neck (exc shave)	2
PUNICA GRANATUM (POMEGRANATE) SEED POWDER	12F - Moisturizing	1
PUNICA GRANATUM (POMEGRANATE)	12J - Other Skin Care Preps	1

SEED POWDER		

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 14, 2017

SUBJECT: Concentration of Use by FDA Product Category: Pomegranate-Derived Ingredients

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

Punica Granatum Extract
Punica Granatum Bark Extract
Punica Granatum Bark/Fruit Extract
Punica Granatum Callus Culture Extract
Punica Granatum Flower Extract
Punica Granatum Fruit Extract
Punica Granatum Fruit Juice
Punica Granatum Fruit/Root/Stem Powder
Punica Granatum Fruit/Sucrose Ferment Filtrate
Punica Granatum Fruit Water
Punica Granatum Juice Extract
Punica Granatum Leaf Cell Extract
Punica Granatum Peel Extract
Punica Granatum Pericarp Extract
Punica Granatum Seed
Punica Granatum Seed Cell Culture Lysate
Punica Granatum Seed Extract
Punica Granatum Seed Powder

Ingredient	Product Category	Maximum Concentration of Use
Punica Granatum Extract	Baby lotions, oils and creams Not powder	 0.000005%
Punica Granatum Extract	Other bath preparations	0.0005%
Punica Granatum Extract	Eyeliners	0.000005%
Punica Granatum Extract	Eye shadows	0.018%
Punica Granatum Extract	Eye lotions	0.00005-0.001%
Punica Granatum Extract	Eye makeup removers	0.000005%
Punica Granatum Extract	Other eye makeup preparations	0.00005%
Punica Granatum Extract	Hair conditioners	0.00024-0.1%
Punica Granatum Extract	Hair sprays Aerosol Pump spray	 0.00001-0.0003% 0.00001-0.00002%
Punica Granatum Extract	Shampoos (noncoloring)	0.0001-0.1%
Punica Granatum Extract	Tonics, dressings and other hair grooming aids	0.00001-0.02%
Punica Granatum Extract	Other hair preparations (noncoloring)	0.1%
Punica Granatum Extract	Blushers	0.0001%
Punica Granatum Extract	Face powders	0.005%
Punica Granatum Extract	Foundations	0.001-0.0025%
Punica Granatum Extract	Lipstick	0.0047-0.02%
Punica Granatum Extract	Makeup bases	0.0005%
Punica Granatum Extract	Makeup fixatives	0.00005%
Punica Granatum Extract	Basecoats and undercoats (manicuring preparations)	0.00001-0.001%
Punica Granatum Extract	Nail polish and enamel	0.00007%
Punica Granatum Extract	Bath soaps and detergents	0.001-0.0043%
Punica Granatum Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0001-0.02%
Punica Granatum Extract	Face and neck products Not spray Spray	 0.001-0.1% 0.001%

Punica Granatum Extract	Body and hand products Not spray	0.0002-0.02%
Punica Granatum Extract	Moisturizing products Not spray	0.0002-0.13%
Punica Granatum Extract	Night products Not spray	0.0005%
Punica Granatum Extract	Paste masks and mud packs	0.00005%
Punica Granatum Extract	Skin fresheners	0.0001%
Punica Granatum Extract	Other skin care preparations	0.0000002%
Punica Granatum Extract	Indoor tanning preparations	0.005%
Punica Granatum Flower Extract	Rinses (noncoloring)	0.0001%
Punica Granatum Flower Extract	Shampoos (noncoloring)	0.0001%
Punica Granatum Fruit Extract	Other bath preparations	0.0005%
Punica Granatum Fruit Extract	Eye lotions	0.0022%
Punica Granatum Fruit Extract	Other eye makeup preparations	0.0005%
Punica Granatum Fruit Extract	Colognes and toilet waters	0.0005%
Punica Granatum Fruit Extract	Hair conditioners	0.00025-0.0005%
Punica Granatum Fruit Extract	Shampoos (noncoloring)	0.0005%
Punica Granatum Fruit Extract	Lipstick	0.0005%
Punica Granatum Fruit Extract	Bath soaps and detergents	0.0005%
Punica Granatum Fruit Extract	Deodorants Not spray	0.0005%
Punica Granatum Fruit Extract	Shaving soap	0.01%
Punica Granatum Fruit Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.00039-0.0004%
Punica Granatum Fruit Extract	Face and neck products Not spray	0.0022-0.1%
Punica Granatum Fruit Extract	Body and hand products Not spray	0.0039%
Punica Granatum Fruit Extract	Night products Not spray	0.025-0.1%
Punica Granatum Fruit Extract	Other skin care preparations	0.01%
Punica Granatum Fruit Extract	Indoor tanning preparations	0.001%
Punica Granatum Fruit Juice	Hair conditioners	0.0001%
Punica Granatum Fruit Juice	Face powders	0.01%
Punica Granatum Fruit Juice	Foundations	0.1%
Punica Granatum Fruit Juice	Makeup bases	0.1%
Punica Granatum Fruit Juice	Makeup fixatives	0.1%
Punica Granatum Juice Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.005%
Punica Granatum Seed Extract	Blushers	0.01%
Punica Granatum Seed Extract	Lipstick	0.11%
Punica Granatum Seed Extract	Cuticle softeners	0.3%
Punica Granatum Seed Powder	Other bath preparations	0.01%
Punica Granatum Seed Powder	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.01%

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

Information collected in 2017
Table prepared December 14, 2017

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: February 6, 2019

SUBJECT: Punica Granatum Fruit Extract and Punica Granatum Pericarp Extract

Active Concepts. 2018. Compositional breakdown: ABS Pomegranate Extract (contains 20% Punica Granatum Fruit Extract).

Active Concepts. 2018. Manufacturing flow chart: ABS Pomegranate Extract (contains 20% Punica Granatum Fruit Extract).

Active Concepts. 2018. Product specification: ABS Pomegranate Extract (contains 20% Punica Granatum Fruit Extract).

Active Concepts. 2013. Cellular viability assay analysis: ABS Pomegranate Extract (contains 20% Punica Granatum Fruit Extract).

Active Concepts. 2019. Composition statement Revital-eyes (contains 10% Punica Granatum Pericarp Extract).

Active Concepts. 2012. Manufacturing flow chart: Revital-eyes (contains 10% Punica Granatum Pericarp Extract).

Active Concepts. 2013. Dermal and ocular irritation tests: : Revital-eyes (contains 10% Punica Granatum Pericarp Extract).

Active Concepts. 2016. OECD TG 442C: *In chemico* skin sensitization Revital-eyes (contains 10% Punica Granatum Pericarp Extract).

Active Concepts. 2016. OECD TG 442D: *In vitro* skin sensitization Revital-eyes (contains 10% Punica Granatum Pericarp Extract).

Active Concepts. 2015. Bacterial reverse mutation test: Revital-eyes (contains 10% Punica Granatum Pericarp Extract).

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Active Concepts

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Compositional Breakdown

ABS Pomegranate Extract
Code: 10248

Compositional Breakdown:

Ingredient	%
Water	39.45
Butylene Glycol	39.45
Punica Granatum Fruit Extract	20.00
Phenoxyethanol	1.00
Tetrasodium EDTA	0.10

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Compositional Breakdown

This is to certify that ABS Pomegranate Extract does not contain, neither directly nor through cross contamination, any of the 26 allergenic flavors or fragrances (Gas Chromatography-Mass Spectrometer Coupled):

ALLERGENS listed in Annex III of EU Cosmetic Regulation(EC) No. 1223/2009 amending EU Directive 2003/15/EC		
INCI NAME	CAS NUMBER	Limit (ppm)
Alpha-IsoMethyl Ionone	127-51-5	< 0.02
Amyl Cinnamal	122-40-7	< 0.10
Anise Alcohol	105-13-5	< 0.00
Benzyl Alcohol	100-51-6	< 0.01
Benzyl Benzoate	120-51-4	< 0.09
Benzyl Cinnamate	103-41-3	< 0.30
Benzyl Salicylate	118-58-1	< 0.06
Butylphenyl Methylpropional	80-54-6	< 0.50
Cinnamal	104-55-2	< 0.01
Cinnamyl Alcohol	104-54-1	< 0.30
Citral	5392-40-5	< 1.00
Citronellol	106-22-9	< 1.00
Coumarin	91-64-5	< 0.00
Eugenol	97-53-0	< 0.70
Farnesol	4602-84-0	< 0.04
Geraniol	106-24-1	< 0.08
Hexyl Cinnamal	101-86-0	< 0.40
Hydroxycitronellal	107-75-5	< 1.00
Hydroxymethylpentyl 3-Cyclohexene carboxaldehyde	31906-04-4	< 0.00
Isoeugenol	97-54-1	< 0.06
Limonene	5989-27-5	< 0.05
Linalool	78-70-6	< 0.00
Methyl 2 Octynoate	111-12-6	< 0.20
Evernia prunastri	90028-68-5	< 0.02
Evernia furfuracea	90028-67-4	< 0.00
Amylcinnamyl Alcohol	101-85-9	< 1.00

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Compositional Breakdown

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This is to certify that ABS Pomegranate Extract does not contain pesticide levels exceeding the following (Reverse Phase High Performance Liquid Chromatography-Mass Spectrometer Coupled):

EPA Pesticide Levels	
INCI NAME	LIMIT (mg/kg)
Alachlor	< 0.02
Aldrin and Dieldrin	< 0 .05
Azinphos-methyl	< 1. 00
Bromopropylate	< 3.0 0
Chlordane(cis and trans)	< 0.05
Chlorfenvinphos	< 0.50
Chlorpyrifos	< 0.20
Chlorpyrifos-methyl	< 0.10
Cypermethrin	< 1.00
DDT	< 1.00
Deltamethrin	< 0.50
Diazinon	< 0.50
Dichlorvos	< 1.00
Dithiocarbamates	< 2.00
Endosulfan	< 3.00
Endrin	< 0.05
Ethion	< 2.00
Fenitrothion	< 0.50
Fenvalerate	< 1.50
Fonofos	< 0.05
Heptachlor	< 0.05
Hexachlorobenzene	< 0.10
Hexachlorocyclohexane	< 0.30
Lindane	< 0.60
Malathion	< 1.00
Methidathion	< 0.20
Parathion	< 0.50
Parathion-methyl	< 0.20

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Compositional Breakdown

Permethrin	< 1.00
Phosalone	< 0.10
Piperonyl butoxide	< 3.00
Pirimiphos-methyl	< 4.00
Pyrethrins	< 3.00
Quintozene(sum of 3 items)	< 1.00

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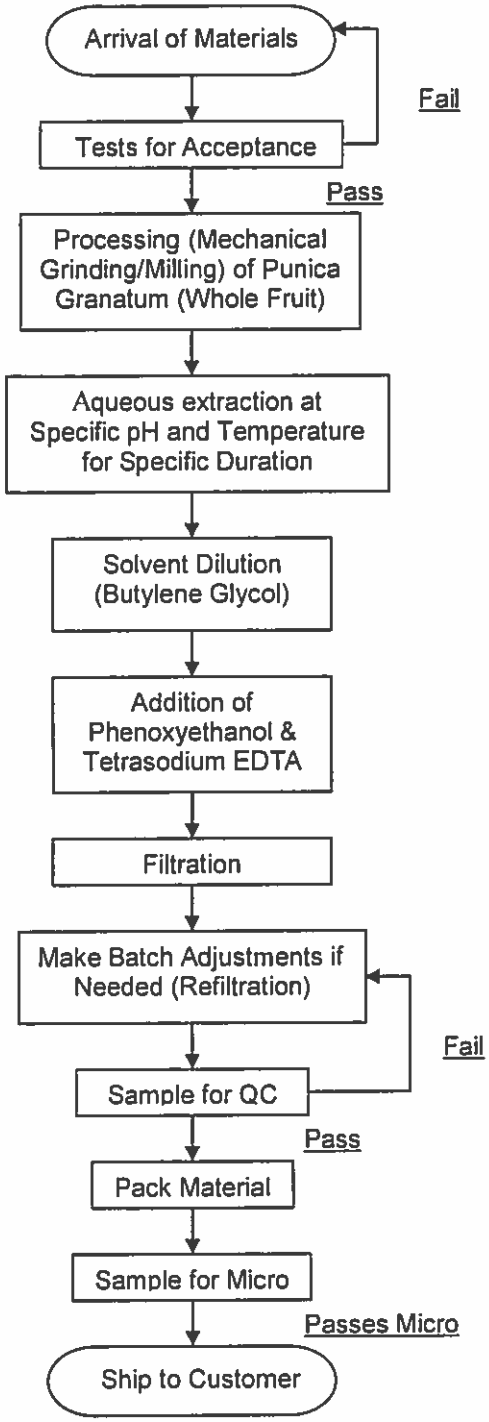
10248-ABS Pomegranate Extract- Manufacturing Flow Chart



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Product Specification

Product Name: ABS Pomegranate Extract
Code Number: 10248
CAS #'s: 7732-18-5 & 107-88-0 & 84961-57-9
EINECS #'s: 231-791-2 & 203-529-7 & 284-646-0
INCI Name: Water & Butylene Glycol & Punica Granatum Fruit Extract
Status: Approved

Specification	Parameter
Appearance	Clear to Slightly Hazy Liquid
Color (Gardner)	8 Maximum
Odor	Characteristic
Specific Gravity	1.015 – 1.035
Refractive Index	1.3915 – 1.3975
pH (Direct)	5.5 – 7.5
Heavy Metals	< 20 ppm
Lead	< 10 ppm
Arsenic	< 2 ppm
Cadmium	< 1 ppm
Microbial Content Yeast & Mold Gram Negative Bacteria	< 100 CFU/g; No pathogens < 100 CFU/g 0 CFU/g

May Sediment upon Standing; Mix Well Prior to Use

**Note: Product may change appearance if exposed to cold temperatures during shipment or storage. If this happens, please gently warm to 45-50°C and mix until normal appearance is restored

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Cellular Viability Assay Analysis

Tradename: ABS Pomegranate Extract

Code: 10248

CAS #: 7732-18-5 & 107-88-0 & 84961-57-9

Test Request Form #: 371

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Cellular Viability Assay

Introduction

The cellular viability assay is useful for quantitatively measuring cell-mediated cytotoxicity, cell proliferation and mitochondrial metabolic activity. Increased metabolism in a cell indicates ample cellular respiration and adenosine triphosphate (ATP) production. ATP is the molecular energy of cells and is required in basic cell function and signal transduction. A decrease is ATP levels indicates cytotoxicity and decreased cell function while an increase in ATP levels indicates healthy cells.

The cellular viability assay was conducted to assess the ability of **ABS Pomegranate Extract** to increase cellular metabolic activity in cultured dermal fibroblasts.

Assay Principle

The assay utilizes a nonfluorescent dye, resazurin, which is converted to a fluorescent dye, resorufin, in response to chemical reduction of growth medium from cell growth and by respiring mitochondria. Healthy cells that are in a proliferative state will be able to easily convert resazurin into resorufin without harming the cells. This method is a more sensitive assay than other commonly used mitochondrial reductase dyes such as MTT. An increase in the signal generated by resazurin-conversion is indicative of a proliferative cellular state.

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Cellular Viability Assay Analysis

Materials

A.	**Kit:**	PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)
B.	**Incubation Conditions:**	37°C at 5% CO_2 and 95% relative humidity (RH)
C.	**Equipment:**	Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes
D.	**Cell Line:**	Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E.	**Media/Buffers:**	Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS)
F.	**Culture Plate:**	Falcon flat bottom 96-well tissue culture treated plates
G.	**Reagents:**	PrestoBlue™ reagent (10X)
H.	**Other:**	Sterile disposable pipette tips

Methods

Human dermal fibroblasts were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete DMEM. A 10-fold serial dilution was performed resulting in **ABS Pomegranate Extract** concentrations on 1%, 0.1%, and 0.01% in complete DMEM and incubated with fibroblasts for 24 hours.

Ten microliters of viability reagent was added to 90µL of cell culture media in culture wells.

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Cellular Viability Assay Analysis

Results

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated.

ABS Pomegranate Extract at all concentrations is able to increase cellular metabolism compared to the control.

Cellular metabolism results are expressed as a percentage of the control.

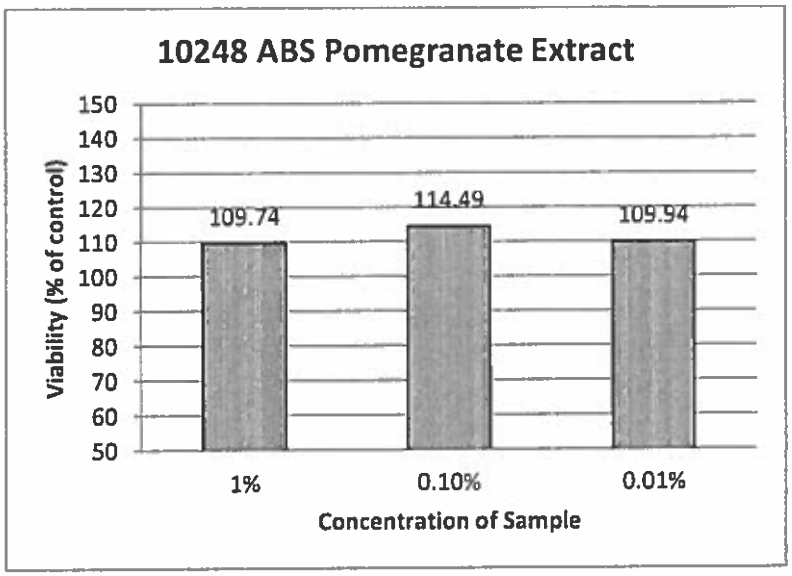

Figure 1: Cellular Metabolism of **ABS Pomegranate Extract**-treated fibroblasts expressed in terms of percent of control.

Discussion

As shown in figure 1, **ABS Pomegranate Extract** exhibited positive results by increasing cell metabolism. The increase in fluorescent signal indicates an increase in cellular metabolism and viability post **ABS Pomegranate Extract** treatment. For these reasons, we can assume **ABS Pomegranate Extract** is suitable for cosmetic applications designed to increase cell viability and metabolism.

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February 7, 2019

To Whom It May Concern,

This letter is to certify the below information regarding Revital-Eyes (16671) manufactured by Active Concepts, LLC, located at 107 Technology Drive, Lincolnton, NC 28092:

Ingredient	Percentage (%)
Water	67.00
Lactobacillus Ferment Lysate	10.00
Camellia Sinensis Leaf Extract	10.00
Punica Granatum Pericarp Extract	10.00
Lactobacillus Ferment	2.00
Caffeine	1.00

Thank you for your interest in Active Concepts products. If you have any further questions, feel free to contact us at (704) 276-7100.

Best Regards,

Heather N. Ferguson

Tomorrow's Vision... *Today!* ®

Heather Ferguson |R&D Coordinator

107 Technology Drive | Lincolnton, NC 28092

Direct: 704.276.7083|Main: 704.276.7100|Fax: 704.276.7101

Email: hferguson@activeconceptsllc.com

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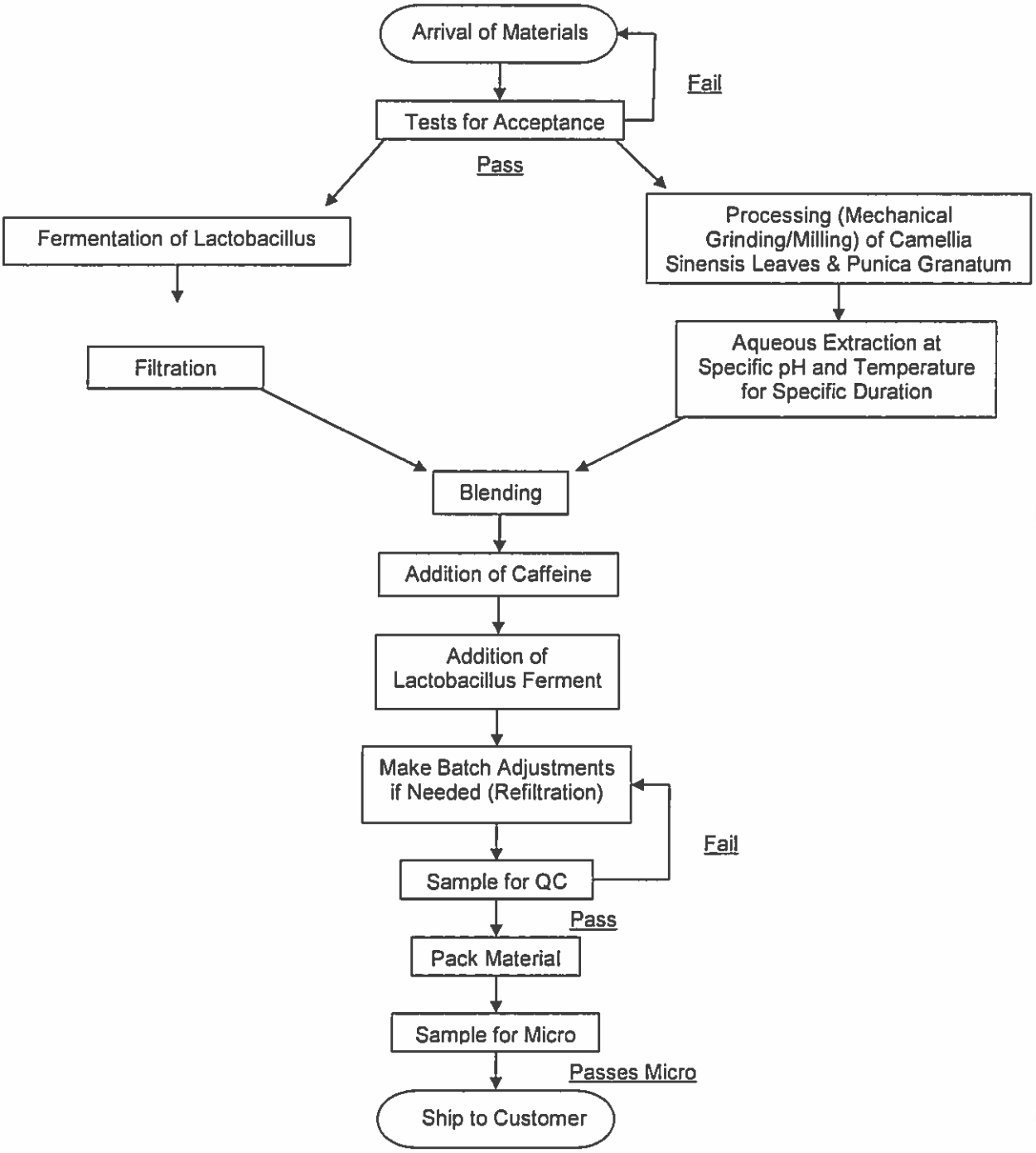
16671-Revital-Eyes- Manufacturing Flow Chart



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Dermal and Ocular Irritation Tests

Sample: Revital-Eyes

Code: 16671

CAS #: 7732-18-5 & 68333-16-4 & 84650-60-2 & 84961-57-9 & 58-08-2

Test Request Form/Submission #: 314

Lot #: 28324P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
In Vitro EpiDerm™ Dermal Irritation Test (EPI-200-SIT)
EpiOcular™ Eye Irritation Test (OCL-200-EIT)

SUMMARY

In vitro dermal and ocular irritation studies were conducted to evaluate whether **Revital-Eyes** would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays.

The product was tested according to the manufacture's protocol. The test article solution was found to be a **non-Irritant**. Reconstructed human epidermis and cornea epithelial model were incubated in growth media overnight to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substances were applied to the tissue inserts and incubated for 60 minutes for liquid and solid substances in the EpiDerm™ assay and 30 minutes for liquid substances and 90 minutes for solid substances in the EpiOcular™ assay at 37°C, 5% CO_2, and 95% relative humidity (RH). Tissue inserts were thoroughly washed and transferred to fresh plates with growth media. After post substance dosing incubation is complete, the cell viability test begins. Cell viability is measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The irritation potential of the test chemical is dictated by the reduction in tissue viability of exposed tissues compared to the negative control.

Under the conditions of this assay, the test article was considered to be **non-irritating**. The negative and positive controls performed as anticipated.

I. Introduction

A. Purpose

In vitro dermal and ocular irritation studies were conducted to evaluate whether a test article would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays. MatTek Corporation's reconstructed human epidermal and human ocular models are becoming a standard in determining the irritancy potential of test substances. They are able to discriminate between irritants and non-irritants. The EpiDerm™ assay has accuracy for the prediction of UN GHS R38 skin irritating and no-label (non-skin irritating) test substances. The EpiOcular™ assay can differentiate chemicals that have been classified as R36 or R41 from the EU classifications based on Dangerous Substances Directive (DSD) or between the UN GHS Cat 1 and Cat 2 classifications.

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II. Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity
B. Equipment:	Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes
C. Media/Buffers:	DMEM based medium; DPBS; sterile deionized H_2O
D. Preparation:	Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
E. Tissue Culture Plates:	Falcon flat bottom 96-well, 24-well, 12-well, and 6-well tissue culture plates
F. Reagents:	MTT (1.0mg/mL); Extraction Solution (Isopropanol); SDS (5%); Methyl Acetate
G. Other:	Nylon Mesh Circles (EPI-MESH); Cotton tip swabs; 1mL tuberculin syringes; Ted Pella micro-spatula; 220mL specimen containers; sterile disposable pipette tips; Parafilm

III. Test Assay

A. Test System

The reconstructed human epidermal model, EpiDerm™, and cornea epithelial model, EpiOcular™, consist of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis and cornea epithelium. These models consist of organized basal, spinous, and granular layers, and the EpiDerm™ systems also contains a multilayer stratum corneum containing intercellular lamellar lipid layers that the EpiOcular™ system is lacking. Both the EpiDerm™ and EpiOcular™ tissues are cultured on specially prepared cell culture inserts.

B. Negative Control

Sterile DPBS and sterile deionized water are used as negative controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

C. Positive Control

Known dermal and eye irritants, 5% SDS solution and Methyl Acetate, were used as positive controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

D. Data Interpretation Procedure

a. EpiDerm™

An irritant is predicted if the mean relative tissue viability of the 3 tissues exposed to the test substance is reduced by 50% of the mean viability of the negative controls and a non-irritant's viability is > 50%.

b. EpiOcular™

An irritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance is reduced by 60% of the mean viability of the negative controls and a non-irritant's viability is > 40%.

IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Concepts, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for an additional 18 to 21 hours.

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B. Test Substance Exposure

a. EpiDerm™

30µL (liquid) or 25mg (solid) of the undiluted test substance is applied to 3 tissue inserts and allowed to incubate for 60 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

b. EpiOcular™

Each tissue is dosed with 20µL DPBS prior to test substance dosing. 50µL (liquid) or 50mg (solid) of the undiluted test substance is applied to 2 tissue inserts and allowed to incubate for 90 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

C. Tissue Washing and Post Incubation

a. EpiDerm™

All tissue inserts are washed with DPBS, dried with cotton tipped swab, and transferred to fresh media and culture plates. After 24 hours the inserts are again transferred into fresh media and culture plates for an additional 18 to 20 hours.

b. EpiOcular™

Tissue inserts are washed with DPBS and immediately transferred into 5mL of assay medium for 12 to 14 minutes. After this soak the inserts are transferred into fresh media and tissue culture plates for 120 minutes for liquid substances and 18 hours for solid substances.

D. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO_2, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD_{570}) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

B. Positive Control

a. EpiDerm™

The assay meets the acceptance criterion if the mean viability of positive control tissues expressed as a % of the negative control is ≤ 20%.

b. EpiOcular™

The assay meets the acceptance criterion if the mean viability of positive control tissues is < 60% of control viability.

C. Standard Deviation

Since each irritancy potential is predicted from the mean viability of 3 tissues for EpiDerm™ and 2 tissues for EpiOcular™, the variability of the replicates should be < 18% for EpiDerm™ and < 20% EpiOcular™.

VI. Results

A. Tissue Characteristics

The tissue inserts included in the MatTek EpiDerm™ and EpiOcular™ assay kits were in good condition, intact, and viable.

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B. Tissue Viability Assay
The results are summarized in Figures 1 and 2. In no case was the tissue viability ≤ 50% for EpiDerm™ or ≤ 60% for EpiOcular™ in the presence of the test substance. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited substantial loss of tissue viability and cell death.

C. Test Validity
The data obtained from this study met criteria for a valid assay.

VII. Conclusion

Under the conditions of this assay, the test article substance was considered to be **non-irritating**. The negative and positive controls performed as anticipated.

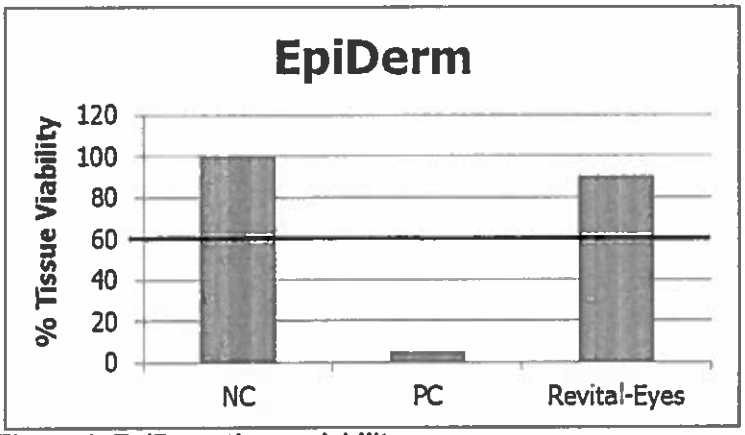

Figure 1: EpiDerm tissue viability

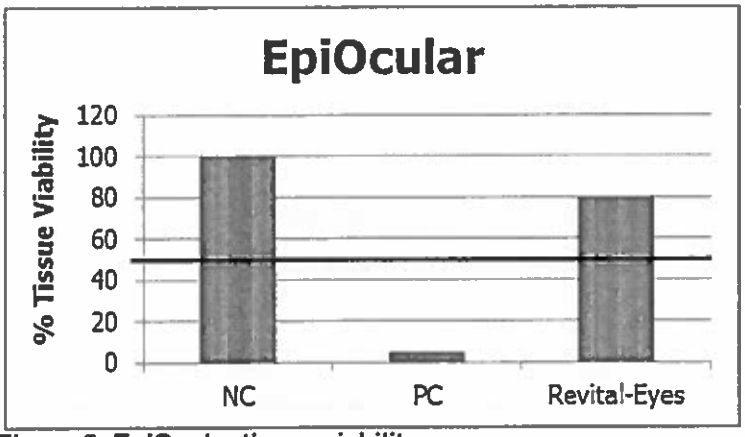

Figure 2: EpiOcular tissue viability

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OECD TG 442C: *In Chemico* Skin Sensitization

Tradename: Revital-Eyes

Code: 16671

CAS #: 7732-18-5 & 68333-16-4 & 84650-60-2 & 84961-57-9 & 58-08-2

Test Request Form #: 2261

Lot #: 47147P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
OECD TG 442C: *In Chemico* Skin Sensitization
Direct Peptide Reactivity Assay (DPRA)

Introduction

A skin sensitizer is a substance that will lead to an allergic response following skin contact[1]. Haptenation is the covalent binding of a hapten, or low-molecular weight substance or chemical, to proteins in the skin. This is considered the prominent mechanism which defines a chemical as a sensitizer. Haptenation is described as a "molecular initiating event" in the OECD Adverse Outcome Pathway (AOP) for skin sensitization which summarizes the key events known to be involved in chemically-induced allergic contact dermatitis[2]. The direct peptide reactivity assay (DPRA) is designed to mimic the covalent binding of electrophilic chemicals to nucleophilic centers in skin proteins by quantifying the reactivity of chemicals towards the model synthetic peptides containing cysteine and lysine. The DPRA is able to distinguish sensitizers from non-sensitizer with 82% accuracy (sensitivity of 76%; specificity of 92%)[3].

This assay was conducted to determine skin sensitization hazard of **Revital-Eyes** in accordance with European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) and OECD Test Guideline 442C.

Assay Principle

The DPRA is an *in chemico* method which addresses peptide reactivity by measuring depletion of synthetic heptapeptides containing either cysteine or lysine following 24 hours incubation with the test substance. The peptide is a custom material containing phenylalanine to aid in detection. Depletion of the peptide in the reaction mixture is measured by HPLC with gradient elution and UV detection at 220 nm. Cysteine and lysine peptide percent depletion values are then calculated and used in a prediction model which allows assigning the test chemical to one of four reactivity classes used to support the discrimination between sensitizers and non-sensitizers.

1. United Nations Economic Commission (UNECE) (2013) Global Harmonized System of Classification and Labelling of Chemicals (GHS) 5th Revised Edition
2. OECD (2012). The Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins. Part 1: Scientific Evidence. Series on Testing and Assessment No. 168
3. EC EURL ECVAM (2012) Direct peptide reactivity assay (DPRA) validation study report; pp 1 -74

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Materials

A. **Equipment:**	HPLC-UV (Waters Breeze - Waters 2998 Photodiode Array Detector); Pipettes; Analytical balance
B. **HPLC/Guard Columns:**	Agilent Zorbax SB-C18 2.1mm x 100mm x 3.5µm; Phenomenex Security Guard C18 4mm x 2mm
C. **Chemicals:**	Trifluoroacetic acid; Ammonium acetate; Ammonium hydroxide; Acetonitrile; Cysteine peptide (Ac-RFAACAA-COOH); Lysine peptide (Ac-RFAAKAA-COOH); Cinnamic aldehyde
D. **Reagents/Buffers:**	Sodium phosphate buffer (100mM); Ammonium acetate buffer (100mM)
E. **Other:**	Sterile disposable pipette tips

Methods

Solution Preparation:

- 0.667mM Cysteine Peptide in 100mM Phosphate Buffer (pH 7.5)
- 0.667mM Lysine Peptide in 100mM Ammonium Acetate Buffer (pH 10.2)
- 100mM Cinnamic Aldehyde in Acetonitrile
- 100mM* **Revital-Eyes** in Acetonitrile

*For mixtures and multi-constituent substances of known composition such as **Revital-Eyes**, a single purity should be determined by the sum of the proportion of its constituents (excluding water), and a single apparent molecular weight determined by considering the individual molecular weights of each component in the mixture (excluding water) and their individual proportions. The resulting purity and apparent molecular weight can then be used to calculate the weight of test chemical necessary to prepare a 100 mM solution.

Reference Controls:

- Reference Control A: For calibration curve accuracy
- Reference Control B: For peptide stability over analysis time of experiment
- Reference Control C: For verification that the solvent does not impact percent peptide depletion

Sample, Reference Control, and Co-Elution Control Preparation:

- Once these solutions have been made they should be incubated at room temperature, protected from light, for 24±2 hours before running HPLC analysis.
- Each chemical should be analyzed in triplicate.

1:10 Ratio, Cysteine Peptide 0.5mM Peptide, 5mM Test Chemical	1:50 Ratio, Lysine Peptide 0.5mM Peptide, 25mM Test Chemical
• 750µL Cysteine Peptide Solution (or 100mM Phosphate Buffer, pH 7.5, for Co-Elution Controls) • 200µL Acetonitrile • 50µL Test Chemical Solution (or Acetonitrile for Reference Controls)	• 750µL Lysine Peptide Solution (or 100mM Ammonium Acetate Buffer, pH 10.2, for Co-Elution Controls) • 250µL Test Chemical Solution (or Acetonitrile for Reference Controls)

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Calibration Curve:

- Standards are prepared in a solution of 20% Acetonitrile:Buffer
  - For the Cysteine peptide using the phosphate buffer, pH 7.5
  - For the Lysine peptide using the ammonium acetate buffer, pH 10.2

	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
mM Peptide	0.534	0.267	0.1335	0.0667	0.0334	0.0167	0.000

HPLC Analysis:

- HPLC-UV system should be equilibrated at 30°C with 50% Mobile Phase A (0.1% (v/v) trifluoroacetic acid in water) and 50% Mobile Phase B (0.085% (v/v) trifluoroacetic acid in acetonitrile) for 2 hours
- Absorbance is measured at 220nm
- Flow Conditions:

Time	Flow	%A	%B
0 minutes	0.35 mL/min	90	10
10 minutes	0.35 mL/min	75	25
11 minutes	0.35 mL/min	10	90
13 minutes	0.35 mL/min	10	90
13.5 minutes	0.35 mL/min	90	10
20 minutes	End Run		

Data and Reporting

Acceptance Criteria:

1. The following criteria must be met for a run to be considered valid:
   a. Standard calibration curve should have an $r^2 > 0.99$.
   b. Mean percent peptide depletion values of three replicates for the positive control cinnamic aldehyde should be between 60.8% and 100% for the cysteine peptide and between 40.2% and 69% for the lysine peptide and the maximum standard deviation should be <14.9 for the percent cysteine depletion and <11.6 for the percent lysine depletion.
   c. Mean peptide concentration of reference controls A should be 0.50±0.05mM and the coefficient of variable of the peptide peak areas for reference B and C in acetonitrile should be <15.0%.

2. The following criteria must be met for a test chemical's results to be considered valid:
   a. Maximum standard deviation should be <14.9 for percent cysteine depletion and <11.6 for percent lysine depletion.
   b. Mean peptide concentration of the three reference control C should be 0.50±0.05mM.

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Prediction Model:

Cysteine 1:10/Lysine 1:50 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
0% < Mean % Depletion < 6.38%	Minimal Reactivity	Non-sensitizer
6.38% < Mean % Depletion < 22.62%	Low Reactivity	Sensitizer
22.62% < Mean % Depletion < 42.47%	Moderate Reactivity	Sensitizer
42.47% < Mean % Depletion < 100%	High Reactivity	Sensitizer

If co-elution occurs with the lysine peptide, than the cysteine 1:10 prediction model can be used:

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
0% < Cys % Depletion < 13.89%	Minimal Reactivity	Non-sensitizer
13.89% < Cys % Depletion < 23.09%	Low Reactivity	Sensitizer
23.09% < Cys % Depletion < 98.24%	Moderate Reactivity	Sensitizer
98.24% < Cys % Depletion < 100%	High Reactivity	Sensitizer

Therefore the measured values of % depletion in the three separated runs for each peptide depletion assay include:

Cysteine 1:10/Lysine 1:50 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
3.32	Minimal Reactivity	Non-sensitizer
3.26	Minimal Reactivity	Non-sensitizer
3.24	Minimal Reactivity	Non-sensitizer

Cysteine 1:10 Prediction Model		
Mean of Cysteine and Lysine % Depletion	**Reactivity Class**	**Prediction**
3.14	Minimal Reactivity	Non-sensitizer
3.12	Minimal Reactivity	Non-sensitizer
3.16	Minimal Reactivity	Non-sensitizer

Results and Discussion

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated.

Percent peptide depletion is determined by the following equation:

$$Percent\ Peptide\ Depletion = \left[1 - \left(\frac{Peptide\ Peak\ Area\ in\ Replicate\ Injection}{Mean\ Peptide\ Peak\ Area\ in\ Reference\ Controls\ C}\right)\right] \times 100$$

Based on HPLC-UV analysis of **Revital-Eyes (16671)** we can determine this product is not classified as a sensitizer and is not predicted to cause allergic contact dermatitis. The Mean Percent Depletion of Cysteine and Lysine was 3.21% causing minimal reactivity in the assay giving us the prediction of a non-sensitizer.

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OECD TG 442D: *In Vitro* Skin Sensitization

Tradename: Revital-Eyes

Code: 16671

CAS #: 7732-18-5 & 68333-16-4 & 84650-60-2 & 84961-57-9 & 58-08-2

Test Request Form #: 2116

Lot #: 46276P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
OECD TG 442D: In Vitro Skin Sensitization *ARE-Nrf2 Luciferase Test Method*

Introduction

Skin sensitization refers to an allergic response following skin contact with the tested chemical, as defined by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals[1]. Substances are classified as skin sensitizers if there is evidence in humans that the substance can lead to sensitization by skin contact or positive results from appropriate tests, both *in vivo* and *in vitro*. Utilization of the KeratinoSens™ cell line allows for valid *in vitro* testing for skin sensitization.

This assay was conducted to determine skin sensitization potential of **Revital-Eyes** in accordance with the UN GHS.

Assay Principle

The ARE-Nrf2 luciferase test method addresses the induction of genes that are regulated by antioxidant response elements (ARE) by skin sensitizers. The Keap1-Nrf2-ARE pathways have been shown to be major regulator of cytoprotective responses to oxidative stress or electrophilic compounds. These pathways are also known to be involved in the cellular processes in skin sensitization. Small electrophilic substances such as skin sensitizers can act on the sensor protein Keap1 (Kelch-like ECH-associated protein 1), by covalent modification of its cysteine residue, resulting in its dissociation from the transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2). The dissociated Nrf2 can then activate ARE-dependent genes such as those coding for phase II detoxifying enzymes.

The skin sensitization assay utilizes the KeratinoSens™ method which uses an immortalized adherent human keratinocyte cell line (HaCaT cell line) that has been transfected with a selectable plasmid to quantify luciferase gene induction as a measure of activation of Keap1-Nrf2-antioxidant/electrophile response element (ARE). This test method has been validated by independent peer review by the EURL-ECVAM. The addition of a luciferin containing reagent to the cells will react with the luciferase produced in the cell resulting in luminescence which can be quantified with a luminometer.

1. United Nations (UN) (2013). Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Fifth revised edition. UN New York and Geneva. 2013

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OECD TG 442D: *In Vitro* Skin Sensitization

Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity (RH)
B. Equipment:	Humidified incubator; Biosafety laminar flow hood; Microplate Reader; Pipettes
C. Cell Line:	KeratinoSens™ by Givaudan Schweiz AG
D. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Geneticin
E. Culture Plate:	Flat bottom 96-well tissue culture treated plates
F. Reagents:	Dimethyl Sulfoxide (DMSO); Cinnamic Aldehyde; ONE-Glo Reagent; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); sodium lauryl sulfate (SLS)
G. Other:	Sterile disposable pipette tips; wash bottles

Methods

KeratinoSens™ were into seeded four 96-well tissue culture plates and allowed to grow to 80 – 90% confluency in DMEM containing 10% FBS and 500µg/mL G418 geneticin. Twelve test concentrations of **Revital-Eyes** were prepared in DMSO with a concentration range from 0.98 - 2000 µM. These 12 concentrations were assayed in triplicate in 2 independently performed experiments. The positive control was cinnamic aldehyde for which a series of 5 concentrations prepared in DMSO had final test concentrations of 4 – 64 µM. The negative control was a 1% test concentration of DMSO.

24 hour post KeratinoSens™ seeding, the culture media was removed and replaced with fresh media containing 10% FBS without G418 geneticin. 50 µL of the above described test concentrations was added to the appropriate wells. The treated plates were then incubated for 48 hours at 37°C in the presence of 5% CO_2 and 95% relative humidity. After treatment incubation was complete the media was removed and the wells were washed with PBS 3 times.

One of the four plates was used for a cytotoxicity endpoint, where MTT was added to the wells and incubated for 4 hours at 37°C in the presence of 5% CO_2. SLS was then added to the wells and incubated overnight at room temperature. A spectrometer measured the absorbance at 570 nm. The absorbance values (optical density) were then used to determine the viability of each well by comparing the optical density of each test material treated well to that of the solvent control wells to determine the IC_{50} and IC_{30} values.

The remaining 3 plates were used in the luciferase induction endpoint of the assay. 100 µL of Promega's ONE-Glo Reagent was added to 100 µL of fresh media containing 10% FBS without geneticin. Cells were incubated for 5 minutes to induce cell lysis and release luciferin into the media. Plates were read with a luminometer and $EC_{1.5}$ and maximum response (I_{max}) values were obtained.

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Data and Reporting

Acceptance Criteria:

1. Gene induction obtained with the positive control, cinnamic aldehyde, should be statistically significant above the threshold of 1.5 in at least one of the tested concentrations (from 4 to 64 µM).
2. The EC1.5 value should be within two standard deviations of the historical mean and the average induction in the three replicates for cinnamic aldehyde at 64 µM should be between 2 and 8.
3. The average coefficient of variability of the luminescence reading for the negative (solvent) control DMSO should be below 20% in each experiment.

A KeratinoSens™ prediction is considered positive if the following conditions are met:

1. The Imax is higher than 1.5-fold and statistically significantly higher as compared to the solvent (negative) control
2. The cellular viability is higher than 70% at the lowest concentration with a gene induction above 1.5 fold (i.e., at the EC1.5 determining concentration)
3. The $EC_{1.5}$ value is less than 1000 µM (or < 200 µg/ml for test chemicals with no defined MW)
4. There is an apparent overall dose-response for luciferase induction

Results

Compound	Classification	$EC_{1.5}$ (µM)	IC_{50}	I_{max}
Cinnamic aldehyde	Sensitizer	19	289.19 µM	31.43
DMSO	Non-Sensitizer	No Induction	243.24 µM	0.17
Revital-Eyes	Non-Sensitizer	No Induction	> 1000 µM	0.32

Table 1: Overview of KeratinoSens™ Assay Results (I_{max} equals the average induction values Fg.1)

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OECD TG 442D: *In Vitro* Skin Sensitization

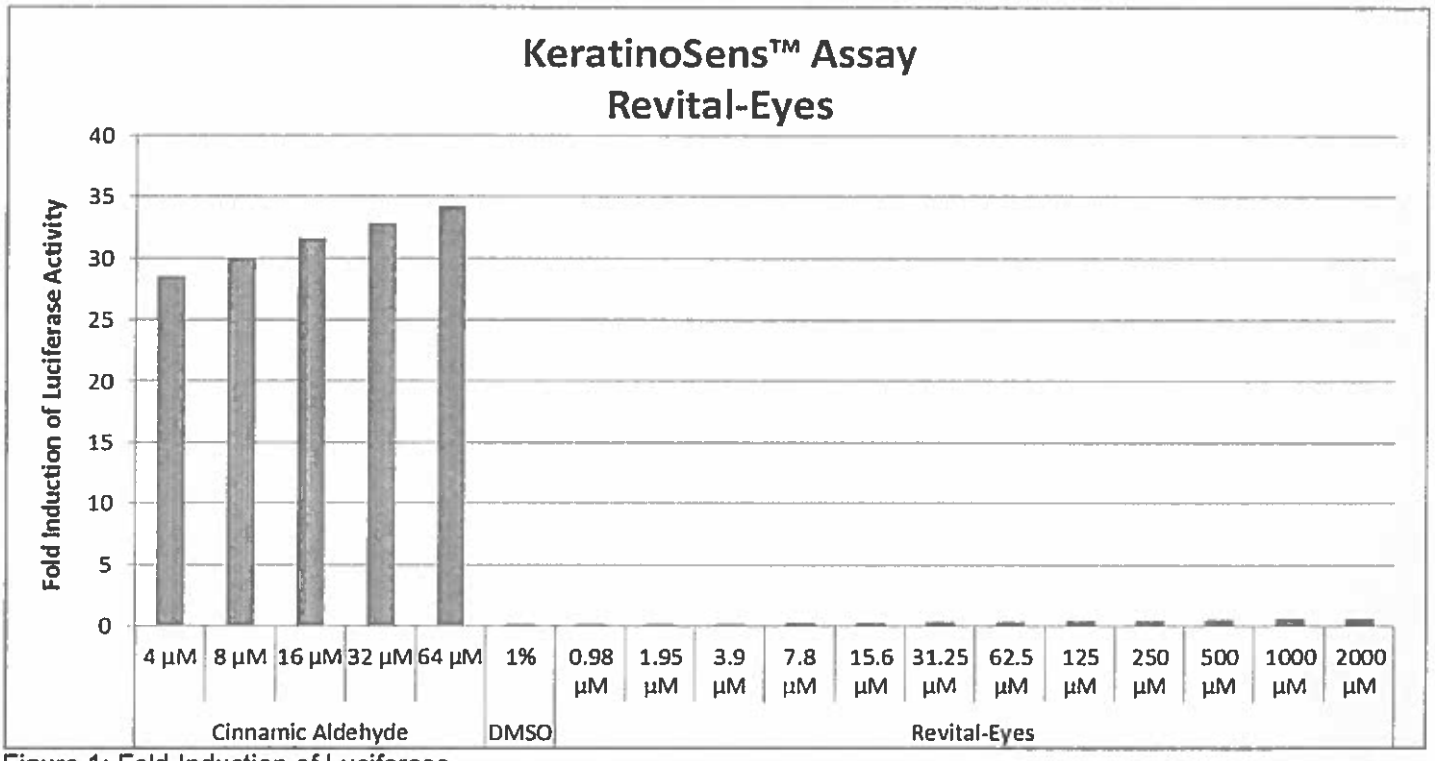

Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **Revital-Eyes (16671)** was not predicted to be a skin sensitizer based on the KeratinoSens™ ARE-Nrf2 Luciferase Test Method as there was not a significant increase in luciferase expression. It can be concluded that **Revital-Eyes** can be safely used in cosmetics and personal care products at typical use levels.

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Bacterial Reverse Mutation Test

Test Article: Revital-Eyes
Code Number: 16671
CAS #: 7732-18-5 & 68333-16-4 & 84650-60-2 & 84961-57-9 & 58-08-2

Sponsor:
Active Concepts, LLC
107 Technology Drive
Lincolnton, NC 28092

Study Director: *Erica Segura*
Principle Investigator: *Monica Beltran*

Test Performed:
Genotoxicity: Bacterial Reverse Mutation Test

Reference:
OECD471/ISO10993.Part3

Test Request Number: 644

SUMMARY

A *Salmonella typhimurium/Escherichia coli* reverse mutation standard plate incorporation study described by Ames *et al.* (1975) was conducted to evaluate whether a test article solution **Revital-Eyes** would cause mutagenic changes in the average number of reveratants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and tryptophan-dependent *Escherichia coli* strain WP2*uvr*A in the presence and absence of Aroclor-induced rat liver S9. This study was conducted to satisfy, in part, the Genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

The stock test article was tested at eight doses levels along with appropriate vehicle control and positive controls with overnight cultures of tester strains. The test article solution was found to be noninhibitory to growth of tester strain TA98, TA100, TA1537, TA1535 and WP2*uvr*A after Sport Inhibition Screen.

Separate tubes containing 2 ml of molten top agar at 45°C supplemented with histidine-biotin solution for the *Salmonella typhimurium* strains and supplemented with tryptophan for *Escherichia coli* strain were inoculated with 100 µl of tester strains, 100 µl of vehicle or test article dilution were added and 500 µl aliquot of S9 homogenate, simulating metabolic activation, was added when necessary. After vortexing, the mixture was poured across the Minimal Glucose Agar (GMA) plates. Parallel testing was also conducted with positive control correspond to each strain, replacing the test article aliquot with 50µl aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for 48 hours at 37°C. The mean numbers of revertants of the test plates were compared to the mean number of revertants of the negative control plates for each of the strains tested. The means obtained for the positive controls were used as points of reference.

Under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* tester strain WP2*uvr*A. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

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Bacterial Reverse Mutation Test

I. Introduction

A. **Purpose**

A *Salmonella typhimurium/Escherichia coli* reverse mutation standard plate incorporation study was conducted to evaluate whether a test article solution would cause mutagenic changes in the average number of revertants for *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2*uvr*A in the presence and absences of the S9 metabolic activation. Bacterial reverse mutation tests have been widely used as rapid screening procedures for the determination of mutagenic and potential carcinogenic hazards.

II. Materials

A. **Storage Conditions:** Room temperature (23-25C).
B. **Vehicle:** Sterile DI Water.
C. **Preparation:** Eight different doses level were prepared immediately before use with sterile DI water.
D. **Solubility/Stability:** 100% Soluble and Stable.
E. **Toxicity:** No significant inhibition was observed.

III. Test System

A. Test System

Each *Salmonella typhimurium* and *Escherichia coli* tester strain contains a specific deep rough mutation (*rfa*), the deletion of *uvr*B gene and the deletion in the *uvr*A gene that increase their ability to detect mutagens, respectively. These genetically altered *Salmonella typhimurium* strains (TA98, TA100, TA1537 and TA1535) and *Escherichia coli* strain (WP2*uvr*A) cannot grow in the absence of histidine and tryptophan, respectively. When placed in a histidine-tryptophan free medium, only those cells which mutate spontaneously back to their wild type states are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

Tester strain	Mutations/Genotypic Relevance
TA98	hisD3052, Dgal chlD bio *uvr*B *rfa* pKM101
TA100	hisG46, Dgal chlD BIO *uvr*B *rfa* pKM101
TA1537	hisC3076, *rfa*, Dgal chlD bio *uvrB*
TA 1535	hisG46, Dgal chlD bio *uvr*B *rfa*
WP2*uvr*A	trpE, *uvr*A

rfa	=	causes partial loss of the lip polysaccharide wall which increases permeability of the cell to large molecules.
uvrB	=	deficient DNA excision-repair system (i.e., ultraviolet sensitivity)
pKM101	=	plasmid confers ampicillin resistance (R-factor) and enhances sensitivity to mutagens.
*uvr*A	=	All possible transitions and transversions, small deletions.

B. Metabolic Activation

Aroclor induced rat liver (S9) homogenate was used as metabolic activation. The S9 homogenate is prepared from male Sprague Dawley rats. Material is supplied by MOLTOX, Molecular Toxicology, Inc.

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C. Preparation of Tester strains
Cultures of *Salmonella typhimurium* TA98, TA100,TA1537, TA1535 and *Escherichia coli* WP2uvrA were inoculated to individual flasks containing Oxoid broth No.2. The inoculated broth cultures were incubated at 37°C in an incubator shaker operating at 140-150 rpm for 12-16 hours.

D. Negative Control
Sterile DI water (vehicle without test material) was tested with each tester strain to determine the spontaneous reversion rate. Each strain was tested with and without S9 activation. These data represented a base rate to which the number of reveratants colonies that developed in each test plate were compared to determine whether the test material had significant mutagenic properties.

E. Positive Control
A known mutagen for each strain was used as a positive control to demonstrate that tester strains were sensitive to mutation to the wild type state. The positive controls are tested with and without the presence of S9 homogenate.

F. Titer of the Strain Cultures:
Fresh cultures of bacteria were grown up to the late exponential or early stationary phase of growth; to confirm this, serial dilutions from each strain were conducted, indicating that the initial population was in the range of 1 to $2x10^9$/ml.

IV. Method

A. Standard Plate Incorporation Assay:
Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution for the *Salmonella typhimurium* and tryptophan for *Escherichia coli* were inoculated with 100 µl of culture for each strain and 100 µl of testing solution or vehicle without test material. A 500 µl aliquot of S9 homogenate, simulating metabolic activation, was added when necessary. The mixture was poured across Minimal Glucose Agar plates labeled with strain number and S9 activation (+/-). When plating the positive controls, the test article aliquot was replaced by 50µl aliquot of appropriate positive control. The test was conducted per duplicate. The plates were incubated for 37°C for 2 days. Following the incubation period, the revertant colonies on each plate were recorded. The mean number of reveratnts was determined. The mean numbers of revertants of the test plates were compared to the mean number of reverants of the negative control of each strain used.

V. Evaluation

For the test solution to be evaluated as a test failure or "potential mutagen" there must have been a 2-fold or greater increase in the number of mean revertants over the means obtained from the negative control for any or all strains. Each positive control mean must have exhibited at least a 3-fold increase over the respective negative control mean of the *Salmonella* tester strain used.

VI. Results and Discussion

A. Solubility:
Water was used as a solvent. Solutions from the test article were made from 0.015 to 50mg/ml.

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B. Dose levels tested:
The maximum dose tested was 5000 µg per plate. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate.

C. Titer (Organisms/ml):
5 x 10^8 UFC/ml plate count indicates that the initial population was in the range of 1 to 2 x 10^9 UFC/ml.

C. Standard Plate Incorporation Assay
In no case was there a 2-fold or greater increase in the mean number of revertant testing strains TA98, TA100, TA1537, TA1535 and WP2*uvr*A in the presence of the test solution compared with the mean of vehicle control value. The positive controls mean exhibited at least a 3-fold increase over the respective mean of the *Salmonella typhimurium* and *Escherichia coli* tester strains used. The results are summarized in Appendix 2.

VII. Conclusion

All criteria for a valid study were mete as described in the protocol. The results of the Bacterial Reverse Mutation Assay indicate that under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2*uvr*A.The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

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Bacterial Reverse Mutation Test

Appendix 2:

Bacterial Mutation Assay
Plate Incorporation Assay Results

	Concentration µg per Plate	*TA98*		
		Revertants per plate (CFU)		Mean
	5000	26	25	26
	1500	14	29	22
	500	23	16	20
	150	16	23	20
	50	20	15	18
	15	15	12	14
	5.0	12	20	16
	1.5	11	20	11
	5000	41	31	36
	1500	28	36	32
	500	34	17	26
	150	24	20	22
	50	25	18	22
	15	19	19	19
	5.0	24	27	25
	1.5	23	37	30
DI Water w/S9		21	10	21
DI Water w/o S9		15	21	18
2-aminoanthracen w/ S9		380	347	364
2-nitrofluorene w/o S9		178	137	158
Historical Count Positive w/S9		**43-1893**		
Historical Count Positive w/o S9		**39-1871**		
Historical Count Negative w/S9		**4-69**		
Historical Count Negative w/o S9		**3-59**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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Bacterial Reverse Mutation Test

	Concentration µg per Plate	*TA100*		
		Revertants per plate (CFU)		Mean
	5000	192	332	262
	1500	236	208	222
	500	160	148	154
	150	116	204	160
	50	200	168	184
	15	144	156	150
	5.0	100	168	134
	1.5	184	148	166
	5000	112	112	112
	1500	134	135	134
	500	228	220	224
	150	180	172	176
	50	126	142	134
	15	136	138	137
	5.0	122	130	126
	1.5	124	148	136
DI Water w/S9		134	140	137
DI Water w/o S9		116	146	131
2-aminoanthracen w/ S9		812	813	813
Sodium azide w/o S9		1040	680	860
Historical Count Positive w/S9		**224-3206**		
Historical Count Positive w/o S9		**226-1837**		
Historical Count Negative w/S9		**55-268**		
Historical Count Negative w/o S9		**47-250**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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Bacterial Reverse Mutation Test

	Concentration µg per Plate	*TA1537*		
		Revertants per plate (CFU)		Mean
	5000	16	19	18
	1500	10	18	14
	500	8	18	9
	150	6	9	8
	50	11	19	15
	15	7	26	17
	5.0	13	14	14
	1.5	18	13	16
	5000	42	21	32
	1500	23	32	28
	500	16	13	15
	150	7	7	7
	50	10	16	13
	15	13	7	10
	5.0	7	10	9
	1.5	13	9	11
DI Water w/S9		14	20	17
DI Water w/o S9		10	10	10
2-aminoanthracen w/ S9		50	52	51
2-aminoacridine w/o S9		58	106	82
Historical Count Positive w/S9		**13-1934**		
Historical Count Positive w/o S9		**17-4814**		
Historical Count Negative w/S9		**0-41**		
Historical Count Negative w/o S9		**0-29**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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Bacterial Reverse Mutation Test

	Concentration µg per Plate	*TA1535*		
		Revertants per plate (CFU)		Mean
	5000	25	25	25
	1500	25	39	32
	500	23	23	23
	150	13	17	15
	50	17	22	20
	15	11	14	13
	5.0	19	8	14
	1.5	10	11	11
	5000	27	53	40
	1500	28	36	32
	500	18	21	20
	150	29	16	23
	50	15	11	13
	15	16	23	20
	5.0	17	18	18
	1.5	16	11	14
DI Water w/S9		9	20	15
DI Water w/o S9		12	13	13
2-aminoanthracen w/ S9		86	95	91
Sodium azide w/o S9		688	960	824
Historical Count Positive w/S9		**22-1216**		
Historical Count Positive w/o S9		**47-1409**		
Historical Count Negative w/S9		**1-50**		
Historical Count Negative w/o S9		**1-45**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

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Bacterial Reverse Mutation Test

	Concentration µg per Plate	*WP2uvrA*		
		Revertants per plate (CFU)		Mean
	5000	15	16	14
	1500	20	22	21
	500	13	12	13
	150	11	11	11
	50	10	9	10
	15	12	13	13
	5.0	14	16	15
	1.5	11	13	12
	5000	3	1	2
	1500	4	5	5
	500	9	8	7
	150	5	5	5
	50	2	3	3
	15	2	2	2
	5.0	5	4	5
	1.5	3	3	3
DI Water w/S9		21	23	21
DI Water w/o S9		27	25	26
2-aminoanthracen w/ S9		121	130	126
Methylmethanesulfonate w/o S9		252	271	265
Historical Count Positive w/S9		**44-1118**		
Historical Count Positive w/o S9		**42-1796**		
Historical Count Negative w/S9		**8-80**		
Historical Count Negative w/o S9		**8-84**		

*CFU = Colony Forming Units
*Mean = Average of duplicate plates

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: February 12, 2019

SUBJECT: Punica Granatum Pericarp Extract and Punica Granatum Seed Powder

Anonymous. 2019. Product specification: Pomegranate Extract (Punica Granatum Pericarp Extract).

Anonymous. 2018. Cosmetic ingredient information: Punica Granatum Seed Powder.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

2019

Product specification Pomegranate Extract

(in Glycerine/Water, not preserved (V82))

Article No.	P-00004851
Manufacturer	
INCI EU + CH	Glycerin, Aqua, Punica Granatum Pericarp Extract
INCI US	Glycerin, Water, Punica Granatum Pericarp Extract
INCI CN	Glycerin, Water, Punica Granatum Pericarp Extract
Ratio	1 kg product out of ca. 0.1 kg dried material
Extraction	At room temperature with a circulating glycerin-water mixture
Origin	Purely natural, not animal, vegan suitable, halal suitable Batch specific
Preservation System	Without preservative, pseudo preserved, therefore avoid contamination
Shelf life	To reevaluate after 18 months
Storage Conditions	Store in closed original packaging, cool, dry, and protected from light.
Recommended Application	2 - 7 % in cosmetic formulations
Note	The plant material used are natural and not standardized. Hence, there are some variations between different harvests and origins - they do not injure the quality of the product.
Specification Date	**13.07.2015. All older specifications are no longer valid.**
Remark	Slight turbidity, sedimentation or colour changes can occur in natural products and do not injure the quality of the product. Botanica neither performs nor commissions tests on animals. The raw materials used are produced neither from nor by GMO.
VOC (CH)	Not VOC-taxed in Switzerland, as product is not listed on tariff-code positive list.
Customs tariff no.	CH: 1302.1900, EU: 1302.1970

Analysis	**Method**	**Specification**
Odour		characteristic
Appearance		yellowish - red-brown
Tannin detection	V-APP-D03	positiv
Density 20°C [g/ml]	V-APP-D23	1.176 – 1.232
Refraction index 20°C	V-APP-D24	1.419 – 1.463
Solubility in Water	10%	clear - slightly turbid
Solubility in Alcohol	10%	clear - slightly turbid
Microbiology	TAMC, PhEur	<=10^2 cfu/g

Composition

Ingredient INCI (EU)	**CAS**	**EC**	**origin**	**%-range**
Glycerin	56-81-5	200-289-5	plant	> 50%
Aqua	7732-18-5	231-791-2	min	> 10% - <= 25%
Punica Granatum Pericarp Extract	84961-57-9	284-646-0	plant	> 0.1% - <= 1%

26 compounds according to EU 2009/1223, Appendix III, No. 45, 67 - 92 (> 1 ppm)

Further compounds according to EU 2009/1223, Appendix III, No. 45, 67-92 are not expected at concentrations above 1 ppm in this extract.

01/19/2018

Cosmetic Ingredient Information – Punica Granatum Seed Powder

CAS Number: 84961-47-9

EINECS/ELINCS: 284-646-0

Manufacturing Process

Grinding, sieving, decontamination of pomegranate seeds (decontaminated by heat or gamma-rays)

Heavy Metals

Based on our actual knowledge of our production process and raw materials used we hereby certify that the above-mentioned ingredient contains less that 20 ppm of heavy metals (Total = Arsenic + Antimony + Cadmium + Mercury + Nickel + Lead).

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Memorandum

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

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

DATE: March 4, 2019

SUBJECT: Punica Granatum Pericarp Extract

Anonymous. 2019. Summary information: Trade name mixture containing, Water, Butylene Glycol and Punica Granatum Pericarp Extract.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

March 2019

Summary Information

Trade Name Mixture Containing: Water, Butylene Glycol and Punica Granatum Pericarp Extract

(1) Physical properties

Trade name	Physical properties
Water, Butylene Glycol and Punica Granatum Pericap Extract	(1) The product is a light brown to brown liquid, having a slight characteristic odor. (2) pH (10vol%) : 3.1~5.1 (3) Specific gravity (d_{20}^{20}) : 1.000~1.100

(2) Method of manufacturing

Trade name	Method of manufacture
Water, Butylene Glycol and Punica Granatum Pericap Extract	Dried raw material ⇒extract with 50vol% ethanolic solution ⇒ filtrate ⇒ concentration ⇒ concentration adjustment ⇒ sedimentation ⇒filtrate ⇒concentration ⇒add 30vol% 1,3-butylen glycolic solution ⇒sedimentation ⇒filtrate ⇒packaging

(3) The composition and impurities

Trade name	The composition and impurities
Water, Butylene Glycol and Punica Granatum Pericap Extract	<Composition> Tannin and sugar <Solid content) 0.5% <Impurities> Heavy metals: not more than 20ppm Arsenic: not more than 2ppm

(4) Toxicological data, specifically dermal irritation and sensitization data on these cosmetic ingredients at use concentrations

Trade name	Toxicological data
Water, Butylene Glycol and Punica Granatum Pericap Extract	Acute toxicity (oral) : LD50>2000mg/kg (mouse) Skin sensitization test : Negative (20% of the trade name mixture) (guinea pig)

	(5 guinea pigs tested) Photosensitization test : Negative (20% of the trade name mixture) (guinea pig) (5 guinea pigs tested) Human patch test : Negative (20% of the trade name mixture) (44 subjects tested) Ames test : Negative (5000μg/plate) Eye irritation test (HCE method) : Non irritant (100%) HRIPT : Negative (30% of the trade name mixture) (52 subjects tested)

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Memorandum

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

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

DATE: February 15, 2019

SUBJECT: Scientific Literature Review: Safety Assessment of *Punica granatum*-Derived Ingredients as Used in Cosmetics (release date January 24, 2019)

The Council respectfully submits the following comments on the scientific literature review, Safety Assessment of *Punica granatum*-Derived Ingredients as Used in Cosmetics.

The Council has no suppliers listed for Punica Granatum Fruit Water and Punica Granatum Seed.

Key Issues

In the *International Cosmetic Ingredient Dictionary*, Punica Granatum Extract has been incorrectly defined as an extract of "the whole plant". These extracts are actually extracts of the fruit or various parts of the fruit. e.g., pericarp. Joanne Nikitakis of the Council is in the process of reassigning the trade names associated with Punica Granatum Extract with the appropriate INCI name. Companies reporting concentration of use information associated with the name Punica Granatum Extract have been asked to clarify the source of the ingredient.

Studies on antioxidant activity should be included in the CIR report (and a number of these studies are in the report) as antioxidant activity may influence whether or not a substance is genotoxic or has other activity. It would also be helpful for the report to state which compounds found in pomegranates are associated with antioxidant activity.

The Introduction should note that Punica Granatum Seed Oil has been reviewed by CIR (in the 2017 published report on plant oils) and found safe for use. It would be helpful if studies on pomegranate seed oil in the 2017 report were summarized in a table.

Volume 4 of *WHO Monographs on Selected Medicinal Plants* (http://apps.who.int/medicinedocs/en/m/abstract/Js16713e/) contains monographs on Cortex Granati (bark) and Pericarp Granati that include useful information on the plant part composition and safety studies of preparations from these plant parts of *Punica granatum*.

A 2008 review by Julie Jurenka at http://www.altmedrev.com/archive/publications/13/2/128.pdf on therapeutic applications of pomegranate may also be helpful in summarizing composition information and history of use.

Additional Considerations

Cosmetic Use - Please identify the product categories in which Punica Granatum Extract and Punica Granatum Fruit Extract are used at the highest concentrations.

Toxicological Studies - If available, the extraction solvent should be identified for each extract tested, e.g., it is stated for the acute study of Punica Granatum Seed Extract, but not for the acute study of Punica Granatum Fruit Extract.

DART - In the study on sperm effects in mice, it is not clear if 70 mg/kg/day was the lowest dose tested. Were there any doses that did not result in an effect?

DART, Oral - Which antioxidant vitamins and enzymes were measured (reference 7)? Please correct "Coper" gland to "Cowper" gland.

Genotoxicity, In Vitro - Were the lower doses tested negative in the Ames assay? Was the study in Chinese hamster ovary cells (reference 49) also completed with S9 metabolic activation (results are give "in the absence of S9 metabolic activation" and "without S9 metabolic activation"). Were there any concentrations tested that were not genotoxic?

Genotoxicity, In Vivo - Were any doses less than 70 mg/kg tested in the mouse bone marrow micronucleus assay of Punica Granatum Fruit Extract (reference 49)?

Summary - Units of mg/kg body weight should be called doses rather than concentrations.

Summary - The Summary indicates that the study in Chinese hamster ovaries was completed with and without metabolic activation, but as stated above, the Genotoxicity section does not clearly state that metabolic activation was included. Were there any concentrations tested that were not associated with genotoxic effects? In general, antioxidants tend to be negative in genotoxicity assays at low concentrations and positive at high concentrations.