Safety Assessment of Radish Root – Derived Ingredients as Used in Cosmetics

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

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

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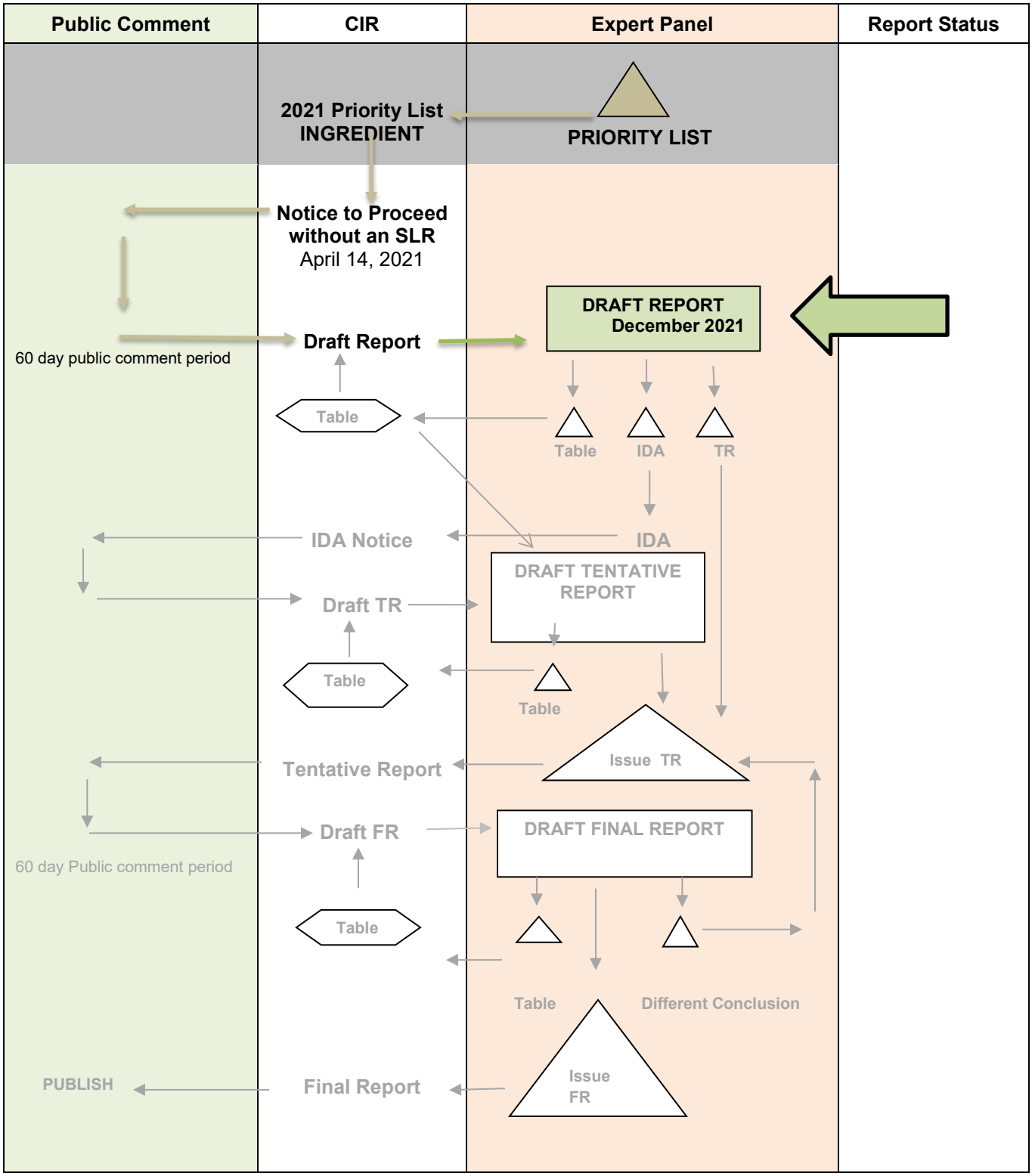
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Radish Root-Derived Ingredients

MEETING December 2021



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons

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

Date: November 10, 2021

Subject: Safety Assessment of Radish Root-Derived Ingredients as Used in Cosmetics

Enclosed is the Draft Report of the Safety Assessment of Radish Root-Derived Ingredients as Used in Cosmetics (identified as *report_RadishRoot_122021* in the pdf). This is the first time the Panel is seeing a safety assessment of these 7 cosmetic ingredients. Due to a dearth of published data found upon search of these ingredients, a Scientific Literature Review Notice to Proceed (SLR NTP) was announced on April 14, 2021. Information sought included chemistry, manufacturing, composition, impurities, toxicity, and dermal irritation/sensitization data.

Concentration of use data and 2021 VCRP data were received from the Council and the FDA, respectively, in January 2021 (*data1_RadishRoot_122021; VCRP_RadishRoot_122021*). The following unpublished data were also received, and have been incorporated in the report:

data2_RadishRoot_122021

- Anonymous. (2019) Repeated insult patch test (eyebrow gel containing 0.04% Leuconostoc/Radish Root Ferment Filtrate)

data3_RadishRoot_122021 (all for Leuconostoc/Radish Root Ferment Filtrate)

- Active Micro Technologies. (2018) Bacterial reverse mutation test
- Active Micro Technologies. (2017) Dermal and ocular irritation tests
- Active Micro Technologies. (2017). K_{ow} statement
- Active Micro Technologies. (2017). OECD TG 442C: *In chemico* skin sensitization
- Active Micro Technologies. (2017). OECD TG 442D: In vitro skin sensitization
- Active Micro Technologies. (2017). Phototoxicity assay analysis
- AMA Laboratories. (2008). HRIPT in 50 subjects (was cited to Active Micro Technologies on the memo)

data4_RadishRoot_122021

- Active Micro Technologies. (2021). Composition for Leuconostoc/Radish Root Ferment Filtrate
- Active Micro Technologies. (2020). Specifications for Leuconostoc/Radish Root Ferment Filtrate

Also included in this package, for your review, are a flow chart (*flow_RadishRoot_122021*), literature search strategy (*search_RadishRoot_122021*), ingredient data profile (*dataprofile_RadishRoot_122021*), and ingredient history (*history_RadishRoot_122021*).

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

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CIR History of:

Radish Root-Derived Ingredients

January 2021

-Frequency of use data obtained

-Concentration of use data submitted by Council

April 2021

-SLR Notice to Proceed was issued

Data received (for Leuconostoc/Radish Root Ferment Filtrate):

- April 29, 2021: HRIPT of eyebrow gel containing 0.04% Leuconostoc/Radish Root Ferment Filtrate
- May 6, 2021: K_{ow} statement, bacterial reverse mutation assay, dermal and ocular irritation tests, *In Chemico* skin sensitization, in vitro skin sensitization, phototoxicity, and HRIPT data for Leuconostoc/Radish Root Ferment Filtrate
- May 7, 2021: Composition and Specifications information for Leuconostoc/Radish Root Ferment Filtrate
-

December 2021

-A Draft Report is being presented to the Panel.

Radish Root -Derived Ingredients Profile* – December 6-7, 2021 – Writer, Preethi Raj

				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Lactobacillus/Radish Root Ferment Filtrate		X																											
Lactobacillus/Radish Root Ferment Extract Filtrate	X	X																											
Leuconostoc/Radish Root Ferment Filtrate	X	X	X	X											X				X			X		X	X	X			
Leuconostoc/Radish Root Ferment Lysate Filtrate		X																											
Raphanus Sativus (Radish) Root Extract	X	X									X																		
Raphanus Sativus (Radish) Root Juice		X													X														X
Raphanus Sativus (Radish) Root Powder		X																											

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

[Radish Root-Derived Ingredients]

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Lactobacillus/Radish Root Ferment Extract Filtrate	NR	✓	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	✓
Lactobacillus/Radish Root Ferment Filtrate	NR	✓	NR	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	NR	
Leuconostoc/Radish Root Ferment Filtrate	NR	NR	NR	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	NR	
Leuconostoc/Radish Root Ferment Lysate Filtrate	1686112-10-6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	
Raphanus Sativus (Radish) Root Extract	84775-94-0	✓*	NR	NR	NR	✓*	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	
Raphanus Sativus (Radish) Root Juice	NR	✓*	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	
Raphanus Sativus (Radish) Root Powder	NR	✓	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	

Botanical and/or Fragrance Websites (if applicable)

Ingredient	CAS #	Dr. Duke's	Taxonomy	GRIN	Sigma-Aldrich	AHPA	AGRICOLA	IFRA	RIFM
Lactobacillus/Radish Root Ferment Extract Filtrate	NR	NR	NA	NA	NA	NR	NA	NR	NR
Lactobacillus/Radish Root Ferment Filtrate	NR	NR	NA	NA	NA	NR	NA	NR	NR
Leuconostoc/Radish Root Ferment Filtrate	NR	NR	NA	NA	NA	NR	NA	NR	NR
Leuconostoc/Radish Root Ferment Lystate Filtrate	1686112-10-6	NR	NA	NA	NA	NR	NA	NR	NR
Raphanus Sativus (Radish) Root Extract	84775-94-0	✓	✓	30857	✓*	NR	✓*	NR	NR
Raphanus Sativus (Radish) Root Juice	NR	✓	✓	30857	✓*	NR	✓*	NR	NR
Raphanus Sativus (Radish) Root Powder	NR	✓	✓	30857	✓*	NR	✓*	NR	NR

✓ - data is available
✓* - data is available, but not relevant

Search Strategy (PubMed)

Fermentation cosmetics – 2738 hits/

Lactobacillus/Radish Root Ferment Extract Filtrate -1 hit/1 useful
((Lactobacillus Ferment Extract Filtrate) OR (Radish Root Ferment Extract Filtrate)) OR (Lactobacillus/Radish Root Ferment Extract Filtrate) – 22 hits/1 useful

General Web Search (Google)

Fermented radish atopic dermatitis – 11,000,000/2

Fermented radish dermal safety – 3,460,000/2
Probiotic radish dermal safety – 193,000/4

Lactobacillus/Radish Root Ferment Extract Filtrate – 74,300 hits/0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate cosmetics – 59,500 hits/4 useful
Lactobacillus/Radish Root Ferment Extract Filtrate chemical properties - 63,000 hits/ 2 useful
Lactobacillus/Radish Root Ferment Extract Filtrate method of manufacture – 396,00 hits/ 1 useful
Lactobacillus/Radish Root Ferment Extract Filtrate composition – 80 hits/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate impurities – 25,300 hits/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate dermal penetration – 262,000 hits/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate penetration enhancement – 244,000 hits/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate toxicokinetics – 10 hits/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate acute toxicity – 22 hits/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate short term toxicity – 34 hits/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate subchronic toxicity - 18 hits/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate chronic toxicity – 24 hits/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate developmental toxicity – 26 results/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate reproductive toxicity- 25 results/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate genotoxicity – 19 results/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate carcinogenicity – 24 results/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate mutagenicity – 19 results/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate dermal irritation – 259,000 results/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate dermal sensitization – 120,000 results/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate phototoxicity – 25 results/ 0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate ocular irritation – 250,000 results/0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate mucous membrane irritation – 32 results/0 useful
Lactobacillus/Radish Root Ferment Extract Filtrate clinical studies – 255,000 results/ 0 useful

Lactobacillus/Radish Root Ferment Filtrate chemical properties – 9 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate method of manufacture – 109 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate composition – 258,000 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate impurities – 25, 900 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate dermal penetration – 259,000 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate penetration enhancement – 246,000 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate toxicokinetics- 11 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate acute toxicity – 23 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate short term toxicity – 40 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate subchronic toxicity – 20 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate chronic toxicity – 28 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate developmental toxicity – 29 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate reproductive toxicity – 33 results/ 0 useful

Lactobacillus/Radish Root Ferment Filtrate genotoxicity – 30 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate carcinogenicity- 36 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate mutagenicity – 40 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate dermal irritation – 86 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate dermal sensitization – 122,000 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate phototoxicity – 23 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate ocular irritation – 130,000 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate mucous membrane irritation – 25 results/ 0 useful
Lactobacillus/Radish Root Ferment Filtrate clinical studies - 187,700 results/ 0 useful

Leuconostoc/Radish Root Ferment Filtrate– 289, 000 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate chemical properties – 12,100 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate method of manufacture – 40,000 results/0 useful
Leuconostoc/Radish Root Ferment Filtrate composition – 19,800 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate impurities – 74,500 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate dermal penetration – 64,900 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate penetration enhancement – 26,900 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate toxicokinetics – 10 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate acute toxicity – 23 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate short term toxicity – 234,000 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate subchronic toxicity – 11 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate chronic toxicity – 27 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate developmental toxicity – 30,700 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate reproductive toxicity – 44 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate genotoxicity – 37 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate carcinogenicity – 47 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate mutagenicity – 35 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate dermal irritation – 36,900 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate dermal sensitization – 7, 080 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate phototoxicity – 12 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate ocular irritation – 29, 200 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate mucous membrane irritation – 32 results/ 0 useful
Leuconostoc/Radish Root Ferment Filtrate clinical studies – 10,700 results/ 0 useful

Leuconostoc/Radish Root Ferment Lysate Filtrate – 25, 900 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate chemical properties – 26 results/0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate method of manufacture – 35 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate composition – 38 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate impurities – 28 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate dermal penetration – 9,320 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate penetration enhancement – 4,880 results/ 0 useful

Leuconostoc/Radish Root Ferment Lysate Filtrate toxicokinetics – 8 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate acute toxicity – 24 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate short term toxicity – 15 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate subchronic toxicity – 11 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate chronic toxicity – 20 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate developmental toxicity – 17 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate reproductive toxicity – 15 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate genotoxicity – 11 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate carcinogenicity – 11 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate mutagenicity – 12 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate dermal irritation – 34 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate dermal sensitization – 29 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate phototoxicity – 11 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate ocular irritation – 24 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate mucous membrane irritation – 9 results/ 0 useful
Leuconostoc/Radish Root Ferment Lysate Filtrate clinical studies – 27 results/ 0 useful

Raphanus Sativus (Radish) Extract – 200,000 results/ 5 useful
Raphanus Sativus (Radish) Extract chemical properties – 132, 000 results/ 6 useful
Raphanus Sativus (Radish) Extract method of manufacture – 117 results/ 0 useful
Raphanus Sativus (Radish) Extract composition – 122, 000 results/ 4 useful
Raphanus Sativus (Radish) Extract impurities – 53,200 results/ 2 useful
Raphanus Sativus (Radish) Extract dermal penetration – 84, 700 results/ 0 useful
Raphanus Sativus (Radish) Extract penetration enhancement – 57,100 results/ 0 useful
Raphanus Sativus (Radish) Extract toxicokinetics – 12 results/ 0 useful
Raphanus Sativus (Radish) Extract acute toxicity – 46 results/ 0 useful
Raphanus Sativus (Radish) Extract short term toxicity – 102,000 results/ 0 useful
Raphanus Sativus (Radish) Extract subchronic toxicity – 39 results/ 0 useful
Raphanus Sativus (Radish) Extract chronic toxicity – 41,700 results/ 0 useful
Raphanus Sativus (Radish) Extract developmental toxicity – 96,200 results/ 0 useful
Raphanus Sativus (Radish) Extract reproductive toxicity – 102,000 results/ 0 useful
Raphanus Sativus (Radish) Extract genotoxicity – 51 results/ 0 useful
Raphanus Sativus (Radish) Extract carcinogenicity – 59 results/ 0 useful
Raphanus Sativus (Radish) Extract mutagenicity – 60 results/ 0 useful
Raphanus Sativus (Radish) Extract dermal irritation – 18,800 results/ 2 useful
Raphanus Sativus (Radish) Extract dermal sensitization – 46 results/ 0 useful
Raphanus Sativus (Radish) Extract phototoxicity – 23 results/ 0 useful
Raphanus Sativus (Radish) Extract ocular irritation – 24 results/ 0 useful
Raphanus Sativus (Radish) Extract mucous membrane irritation – 35 results/ 0 useful
Raphanus Satibus (Radish) Extract clinical studies – 78 results/ 1 useful

Raphanus Sativus (Radish) Root Juice – 67,100 results/ 4 useful
Raphanus Sativus (Radish) Root Juice chemical properties – 50,2000 results/8 useful
Raphanus Sativus (Radish) Root Juice method of manufacture – 43,900 results/ 6 useful
Raphanus Sativus (Radish) Root Juice composition – 78,400 results/ 3 useful
Raphanus Sativus (Radish) Root Juice impurities – 39 results/ 0 useful
Raphanus Sativus (Radish) Root Juice dermal penetration – 42,500 results/ 0 useful
Raphanus Sativus (Radish) Root Juice penetration enhancement – 24, 700 results/ 0 useful
Raphanus Sativus (Radish) Root Juice toxicokinetics – 21 results/ 0 useful
Raphanus Sativus (Radish) Root Juice acute toxicity – 24,100 results/ 0 useful
Raphanus Sativus (Radish) Root Juice short term toxicity – 69 results/ 0 useful
Raphanus Sativus (Radish) Root Juice subchronic toxicity – 12,200 results/ 0 useful
Raphanus Sativus (Radish) Root Juice chronic toxicity – 22,100 results/ 0 useful
Raphanus Sativus (Radish) Root Juice developmental toxicity – 29,100 results/ 0 useful
Raphanus Sativus (Radish) Root Juice reproductive toxicity – 47,100 results/ 0 useful
Raphanus Sativus (Radish) Root Juice genotoxicity – 39 results/ 0 useful
Raphanus Sativus (Radish) Root Juice carcinogenicity – 45 results/ 0 useful
Raphanus Sativus (Radish) Root Juice mutagenicity – 41 results/ 1 useful
Raphanus Sativus (Radish) Root Juice dermal irritation – 7,540 results/ 0 useful
Raphanus Sativus (Radish) Root Juice dermal sensitization – 13,300 results/ 0 useful
Raphanus Sativus (Radish) Root Juice phototoxicity – 2,150 results/ 0 useful
Raphanus Sativus (Radish) Root Juice ocular irritation – 60 results/ 0 useful
Raphanus Sativus (Radish) Root Juice mucous membrane irritation – 50 results/ 0 useful
Raphanus Sativus (Radish) Root Juice clinical studies – 30,100 results/ 6 useful
NIOSH Raphanus Sativus (Radish) Root Juice – 8 results/5 useful

Raphanus Sativus (Radish) Root Powder – 81,800 results/ 4 useful
Raphanus Sativus (Radish) Root Powder chemical properties – 98,900 results/ 0 useful
Raphanus Sativus (Radish) Root Powder composition – 98,700 results// 5 useful
Raphanus Sativus (Radish) Root Powder impurities – 40,300 results/ 0 useful
Raphanus Sativus (Radish) Root Powder method of manufacture – 194,000 results/ 1 useful
Raphanus Sativus (Radish) Root Powder dermal penetration – 98 results/ 0 useful
Raphanus Sativus (Radish) Root Powder penetration enhancement – 69,200 results/ 0 useful
Raphanus Sativus (Radish) Root Powder toxicokinetics – 10 results/ 0 useful
Raphanus Sativus (Radish) Root Powder acute toxicity – 32 results/ 0 useful
Raphanus Sativus (Radish) Root Powder short term toxicity – 98,000 results/ 0 useful
Raphanus Sativus (Radish) Root Powder subchronic toxicity – 36 results/ 0 useful
Raphanus Sativus (Radish) Root Powder chronic toxicity - 33 results/ 0 useful
Raphanus Sativus (Radish) Root Powder developmental toxicity – 57 results/ 0 useful
Raphanus Sativus (Radish) Root Powder reproductive toxicity – 85 results/ 0 useful
Raphanus Sativus (Radish) Root Powder genotoxicity – 33 results/ 1 useful

Raphanus Sativus (Radish) Root Powder carcinogenicity – 42 results/ 0 useful
Raphanus Sativus (Radish) Root Powder mutagenicity – 48 results/ 1 useful
Raphanus Sativus (Radish) Root Powder dermal irritation – 55 results/ 0 useful
Raphanus Sativus (Radish) Root Powder dermal sensitization – 24 results/ 0 useful
Raphanus Sativus (Radish) Root Powder phototoxicity – 33 results/ 0 useful
Raphanus Sativus (Radish) Root Powder ocular irritation – 41 results/ 0 useful
Raphanus Sativus (Radish) Root Powder mucous membrane irritation – 22 results/ 0 useful
Raphanus Sativus (Radish) Root Powder clinical studies – 50 results/ 0 useful

LINKS

Search Engines

- Pubmed - http://www.ncbi.nlm.nih.gov/pubmed
- Connected Papers - https://www.connectedpapers.com/

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

- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/

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

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
- 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) – https://ifrafragrance.org/
- Research Institute for Fragrance Materials (RIFM) - https://www.rifm.org/#gsc.tab=0

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ABBREVIATIONS

ACP	acid phosphatase
AD	atopic dermatitis
ALT	alanine transaminase
ARE-Nrf2	antioxidants response elements – transcription factor Nrf2
AST	aspartate aminotransferase
CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
Dictionary	*International Cosmetic Ingredient Dictionary and Handbook*
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DPRA	direct reactivity peptide assay
EC	European Commission
ECHA	European Chemicals Agency
FDA	Food and Drug Administration
GRAS	generally recognized as safe
HaCaT	human keratinocyte cell line
HRIPT	human repeated insult patch test
IC_{50}	half-maximal inhibitory concentration
IgE	immunoglobulin E
LD	lethal dose
LDH	lactate dehydrogenase
MIC	minimum inhibitory concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide
N/A	not applicable
NR	not reported/none reported
OD	odds ratio
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate buffer solution
TG	test guideline
US	United States
VCRP	Voluntary Cosmetic Registration Program

INTRODUCTION

This assessment reviews the safety of the following 7 radish root-derived ingredients as used in cosmetic formulations:

Lactobacillus/Radish Root Ferment Extract Filtrate
Lactobacillus/Radish Root Ferment Filtrate
Leuconostoc/Radish Root Ferment Filtrate
Leuconostoc/Radish Root Ferment Lysate Filtrate
Raphanus Sativus (Radish) Root Extract
Raphanus Sativus (Radish) Root Juice
Raphanus Sativus (Radish) Root Powder

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), these ingredients are mostly reported to function in cosmetics as hair and skin conditioning agents (Table 1).[1] These ingredients are also reported to function as antimicrobial agents, a preservative, an anti-dandruff agent, an antifungal agent, and an antioxidant. It should be noted that most of these other functions are not considered cosmetic functions in the United States (US), and therefore, use as such does not fall under the purview of the Expert Panel for Cosmetic Ingredient Safety (Panel).

Botanicals, such as radish root-derived ingredients, may contain hundreds of constituents. Thus, in this assessment, the Panel will assess the safety of each of the *Raphanus sativus*-derived ingredients as a whole, complex mixture; toxicity from single components may not predict the potential toxicity of botanical ingredients. Some of the ingredients reviewed in this safety assessment may be consumed as food, and daily exposure from food use would result in much larger systemic exposures than those from use in cosmetic products. Therefore, the primary focus in this assessment of these ingredients is to evaluate the potential for effects from topical exposures.

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

The cosmetic ingredient names, according to the *Dictionary*, are written as listed above, without italics. In many of the published studies, it is not known how the substance being tested compares to the ingredient as used in cosmetics. Therefore, if it is not known whether the ingredients being discussed are cosmetic ingredients, the test substances will be identified by the standard taxonomic practice of using italics to identify genus and species (i.e., " *Lactobacillus/*radish root*…", "Leuconostoc/*radish root*…", or* "*Raphanus sativus (*radish*)….*" However, if it is known that the substance is a cosmetic ingredient, the terminology the International Nomenclature Committee (INC) terminology will be used (e.g., Raphanus Sativus (Radish) Root Extract).

CHEMISTRY

Definition and Plant Identification

The ingredients in this report are related as derivatives from the same species, *Raphanus sativus*. Additionally, only ingredients made from the root portion of the *Raphanus sativus* plant are being reviewed. The definitions of these radish root-derived ingredients are presented in Table 1.[1] Leuconostoc/Radish Root Ferment Lysate Filtrate and Raphanus Sativus (Radish) Root Extract have the CAS Nos. 1686112-10-6 and 84775-94-0, respectively.

Raphanus sativus is a tap root from the Brassicaceae family, which has been historically cultivated in Asia and Europe.[2] It grows in temperate climates at altitudes between 190 and 1240 m, is 30 - 90 cm high, and has thick edible roots which have a pungent taste and are of various sizes, forms, and colors.[3] Generically, the root is defined as the organ of a plant that absorbs and transports water and nutrients, lacks leaves and nodes, and is usually underground.[1]

Four of these ingredients are filtrates of *Raphanus sativus* fermented with either the *Lactobacillus* or *Leuconostoc* microorganism. Both strains are gram-positive and anaerobic, occurring as non-spore forming rods and cocci, and are considered lactic acid bacteria because they consume carbohydrates to produce lactic acid.[4] A lysate is obtained by breaking down cell outer membranes via chemical or physical processes.[5] The filtrate ingredients in this report are made by removing the bacterial cells (alive or dead), potentially along with other molecules (like peptides), from the fermented products.[5]

Chemical Properties

Leuconostoc/Radish Root Ferment Filtrate

A supplier has indicated that 1 g of Leuconostoc/Radish Root Ferment Filtrate is specified to contain 48 – 52% solids (when observed for 1 h at 105° C).[6] The log K_{ow} of Leuconostoc/Radish Root Ferment Filtrate is -1.92.[7] Additional physical and chemical properties are presented in Table 2.

Method of Manufacture

The methods below are general to the processing of the radish root-derived ingredients, and it is unknown if they apply to cosmetic ingredient manufacturing. In some cases, the definition of the ingredients, as given in the *Dictionary*, provides insight as to the method of manufacture.

Lactobacillus/Radish Root Ferment Extract Filtrate

Lactobacillus/Radish Root Ferment Extract Filtrate is a filtrate of the extract of the product obtained by the fermentation of the roots of *Raphanus sativus* (radish) by the microorganism, *Lactobacillus*.[1]

Lactobacillus/Radish Root Ferment Filtrate

Lactobacillus/Radish Root Ferment Filtrate is a filtrate of the product obtained by the fermentation of the roots of *Raphanus sativus* (radish) by the microorganism, *Lactobacillus*.[1]

Leuconostoc/Radish Root Ferment Filtrate

Leuconostoc/Radish Root Ferment Filtrate is a filtrate of the product obtained by the fermentation of *Raphanus sativus* roots by the microorganism, *Leuconostoc*.[1]

Leuconostoc/Radish Root Ferment Lysate Filtrate

Leuconostoc/Radish Root Ferment Lysate Filtrate is a filtrate of a lysate of the product obtained by the fermentation of the roots of *Raphanus sativus* (radish) by the microorganism, *Leuconostoc*.[1]

Raphanus Sativus (Radish) Root Extract

Radish roots, sized 30 cm each, were made into powder by washing, cutting into ~ 3 mm pieces, being dried at 60 °C for 21 h, and then being blended and sieved with a 60 mesh sifter.[8] The resulting powder was macerated at a 1:10 ratio at ~24 °C using 3 different solvents (hexane, ethyl acetate, and ethanol) for 8, 16, and 24 h. The resulting suspensions were filtered and evaporated at 45 °C.

One gram of powdered black *Raphanus sativus* roots was used to make an ethanolic radish root extract.[9] Aqueous ethanol, 50 ml, 50% (v/v) was used to extract the powder on a magnetic stirrer for 120 min at room temperature, and then centrifuged at 5000 rpm for 10 min at 4 °C.

Raphanus Sativus (Radish) Root Juice

Fresh *Raphanus sativus* roots were washed well and processed in an electric blender to obtain 2 l of fresh root juice.[10] The *Raphanus sativus* root juice was then filtered and concentrated in a rotary evaporator at 35 ± 5 °C under reduced pressure. The resulting material was freeze dried to obtain a semisolid mass of 40 g, 11.3% w/w, which was then dissolved in distilled water.

Raphanus Sativus (Radish) Root Powder

White radish roots were washed with water, sliced, and dried at 50 °C.[11] The dried slices of white radish were ground to a powder and sieved through a 40 mesh sifter. The resulting product was stored in a sealed bag and frozen at -20 °C until further extraction. In another study, peeled and unpeeled black radish roots were sliced and freeze-dried before being ground to a fine powder and sifted through a 0.5 mm mesh sieve; the powdered samples were stored in air-tight containers at 4 °C.[9]

Composition and Impurities

Leuconostoc/Radish Root Ferment Filtrate

A supplier has reported that a sample of Leuconostoc/Radish Root Ferment Filtrate, with a pH 4.0 – 6.0, comprises 48.80% water, 30.60% protein, 20.10% phenolics (tested as salicylic acid), and 0.50% polysaccharide content.[12] Specifications for this ingredient provide the following parameters: < 20 ppm heavy metals, < 10 ppm lead, < 2 ppm arsenic, and < 1 ppm cadmium.[6] Additionally, the ingredient was specified to be positive to ninhydrin, and potentially contain 18-22% phenolics (tested as salicylic acid), and 0.10 - 0.50% bacteriocins (quantified via high-performance liquid chromatography).

Raphanus Sativus (Radish) Root Extract

In one study, a 16-h, crude ethyl acetate *Raphanus sativus* root extract contained the highest total phenolic and flavonoid content at 37.37 mg gallic acid equivalents (GAE)/g, and 5.74 mg quercetin equivalents (QE)/g, respectively.[8] A compositional analysis of fresh radish root extracts yielded a flavonoid content of 267.47 ± 6.38 mg quercetin/100 g, total phenolic content of 371.59 mg/100 g, and 380 ± 0.87 g/100g potassium (highest mineral content).[13] Silica gel chromatography of a dichloromethane extract of *Raphanus sativus* roots yielded the following constituents: 3-(E)-(methylthio)methylene-2-pyrrolidinethione, a mixture of 4-methylthio-3-butenyl isothiocyanate and 4-(methylthio)butyl isothiocyanate, β-sitosterol, β-sitosteryl-3β-glucopyranoside-6'-O-palmitate, monoacylglycerols, and a mixture of α-linolenic acid and linoleic acid.[14] A methanolic extract of *Daikon* (vegetable; a *Raphanus sativus* var.) was the most constituent-rich,

compared to extracts made with water, petrolatum, ethanol, and chloroform; phytochemical screening showed the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, and glycosides.[15]

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2021 VCRP survey data, Leuconostoc/Radish Root Ferment Filtrate is reported to be used in 255 formulations, 104 of which are leave-on moisturizing products; Lactobacillus/Radish Root Ferment Filtrate and Raphanus Sativus (Radish) Root Extract are reported to have 2 uses each, in moisturizing and face and neck products, respectively (Table 3).[16] The results of the concentration of use survey conducted by the Council indicate that Raphanus Sativus (Radish) Root Extract has the highest reported maximum concentration of use in leave-on products, at up to 6% in lipstick; Leuconostoc/Radish Root Ferment Filtrate is used at up to 1.1% in skin cleansing products.[17] The highest concentration of use reported for products resulting in leave-on dermal exposure is 0.03% Leuconostoc/Radish Root Ferment Filtrate in face and neck spray formulations. Use concentration data were not reported for Lactobacillus/Radish Root Ferment Filtrate, but uses were reported in the VCRP. Lactobacillus/Radish Root Ferment Extract Filtrate, Leuconostoc/Radish Root Ferment Lysate Filtrate, Raphanus Sativus (Radish) Root Juice, and Raphanus Sativus (Radish) Root Powder are not reported to be in use in the VCRP or industry survey (Table 4).

Raphanus sativus- derived ingredients have been reported to be used in products that may lead to incidental ingestion and exposure to mucous membranes; for example, Raphanus Sativus (Radish) Root Extract is reported to be used in a lipstick at up to 6%,[17] and Leuconostoc/Radish Root Ferment Filtrate is reported to be used at up to 0.01% in other eye makeup preparations.[17] Additionally, Leuconostoc/Radish Root Ferment Filtrate is reported to be used in products that could be potentially inhaled, e.g., Leuconostoc/Radish Root Ferment Filtrate is used in spray face and neck products at up to 0.03%.[17] In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 µm, with propellant sprays yielding a greater fraction of droplets/particles < 10 µm compared with pump sprays.[18,19] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[20,21]

All of the radish root-derived ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[22]

Non-Cosmetic

According to the US FDA, commercially-produced products of carbohydrates, such as glucose, sucrose, or lactose, which undergo lactic acid fermentation, are generally recognized as safe (GRAS) for their intended use in foods [21CFR § 184.1016]. *Leuconostoc* is an approved bacterial strain used to produce a butter starter distillate [21CFR § 184.1848].

Furthermore, *Raphanus sativus* roots are consumed as cruciferous vegetables worldwide, both raw and cooked, in pickles, salads, and curries.[23] Of note, *Raphanus sativus* fermented with *Lactobacillus* strains is consumed as a non-salted dish called Sinki in South Asia.[24] The Korean dish, kimchi, comprises variations of a mixed vegetable brine fermentation (achieved with lactic acid bacteria, such as *Lactobacillus* or *Leuconostoc*), and often includes radish roots.[25] Generally, *Lactobacillus* and *Leuconostoc* strains are used in the lactic acid fermentation of dairy, sauerkraut, and various food products.[26,27]

TOXICOKINETIC STUDIES

No relevant toxicokinetics studies on radish root-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

Subchronic Toxicity Studies

Oral

Raphanus Sativus (Radish) Root Extract

Groups of albino rats were dosed with 0, 150, 250, 350, 450, or 550 mg/kg bw of methanolic *Daikon* (vegetable; a *Raphanus sativus* var.) extract, in the diet, for 90 d.[15] Body weight, as well as various hematological parameters and enzymes, including red blood cell count, hemoglobin, white blood cell count, aspartate aminotransferase (AST), alanine

transaminase (ALT), acid phosphatase (ACP), urea, uric acid, and protein were measured and compared at 30 and 90 d of treatment. Upon sacrifice, heart, kidney, liver, spleen, and brain weights were also measured, and those of treated animals were compared to controls. No statistically significant differences were observed between the mean body weights, organ weights, and measured hematological parameters in treated animals, compared to controls, throughout the experiment.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

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

GENOTOXICITY STUDIES

Leuconostoc/Radish Root Ferment Filtrate

The genotoxicity potential of Leuconostoc/Radish Root Ferment Filtrate was evaluated in a bacterial reverse mutation assay (Ames test) at concentrations of 1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg/plate, in distilled water, using the following strains: *Salmonella typhimurium* TA98, TA100, TA1535, TA 1537, and *Escherichia coli* WP2 *uvr*A.[28] Distilled water served as the negative control and appropriate positive controls were used. The test substance did not induce a mutagenic effect in the presence or absence of metabolic activation.

Raphanus Sativus (Radish) Root Juice

In a Comet assay, the genotoxic potential of radish juice made from white, red, and large red *Raphanus sativus* tubers, as well as dichloromethane extracts of hydrolyzed *Raphanus* sativus white and cherry belle, red tubers, was tested in breast adenocarcinoma (MCF-7), chronic myelogenous leukemia (K562), and colorectal cancer (HT-29) cell lines.[29] Each cell line was incubated with 500 µl of the root juice and 50 µg/ml of the dichloromethane juice extract; porcine aortic endothelial (PAE) cell lines were used as the negative control and immortalized cell lines exposed to 0.01% hydrogen peroxide for 20 min were used as positive controls. Tail length, percent deoxyribonucleic acid (DNA), and tail moment measurements were used to evaluate the extent of DNA damage. Juices from all 3 tubers exhibited significantly lower DNA damage in the porcine aortic endothelial cells, compared to positive controls. The breast adenocarcinoma cell line, MCF-7, showed the greatest amount of genetic fragmentation among all cancer cells, and the white tuber root juice was the most genotoxic towards aberrant cell lines.

CARCINOGENICITY STUDIES

Carcinogenicity studies were not found in the published literature, and unpublished data were not submitted.

OTHER RELEVANT STUDIES

Radical Scavenging and Skin Lightening Potential

Raphanus Sativus (Radish) Root Extract and Raphanus Sativus (Radish) Root Juice

A freeze-dried juice and methanolic extract of white *Raphanus sativus* roots were evaluated for tyrosinase inhibition, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability, cytotoxicity, and L-ascorbic acid content.[30] The ability of *Raphanus sativus* root extract and root juice to scavenge DPPH, superoxide anion, and singlet oxygen was measured in triplicates, which were used to calculate average half-maximal inhibitory concentration (IC_{50}) values, compared to L-ascorbic acid and Trolox©, a water soluble analog of Vitamin E. Various concentrations of the *Raphanus sativus* root extract and root juice in 20% v/v propylene glycol (in water) were tested, in tandem with L-ascorbic acid and licorice extract as reference tyrosinase inhibitors, using the DOPAchrome method. Lactate dehydrogenase (LDH) activity in fibroblasts treated with the root extract and root juice, compared to L-ascorbic acid and sinapic acid, was used to measure cytotoxic activity. Five replicates of the root extract and root juice were titrated with 0.1 N iodine to determine the L-ascorbic acid or vitamin C content. The freeze-dried juice showed higher potential for tyrosinase inhibition compared to the methanolic extract (IC_{50} = 3.09 mg/ml vs. IC_{50} = 9.62 mg/ml). The radical scavenging activity of the freeze-dried juice on DPPH radical, superoxide anion radical, and singlet oxygen were also greater compared to the methanolic extract (IC_{50} = 0.64, 4.20, 1.42 mg/ml vs. IC_{50} = 1.25, 6.28, 2.40 mg/ml). Although a dose-dependent release of LDH was observed for both the root extract and root juice, the observed cytotoxicity was relatively lower than in the reference antioxidants. The authors surmised that the higher L-ascorbic content of 1 mg of freeze-dried *Raphanus sativus* root juice compared to the root extract (24.11 µg vs. 8.28 µg), as well as higher phenolic content, may be responsible for greater anti-tyrosinase and radical scavenging activity, possibly lending to skin lightening.

Raphanus Sativus (Radish) Root Extract

Raphanus sativus radish root extracts were eluted using 3 solvents with varying polarities (hexane (non-polar), ethyl acetate (semi-polar), and ethanol (polar)) for 8, 16, and 24 h each, to determine which extract would have the highest phenolic or flavonoid content.[8] The radish root extract extracted with ethyl acetate for 16 h was found to have the highest

flavonoid content, and was used for further testing. The ethyl acetate radish root extract was tested for phenolic and flavonoid content stability based on changes in pH (4, 5, 6, and 7) and heating temperature (70, 80, 90 °C). In conjunction, the IC_{50} value of the ethyl acetate root extract was measured in a DPPH assay. Overall, decreases in total phenolic and flavonoid content, as well as antioxidant activity, were observed when the radish root extract was exposed to increasing heat and pH. Statistically significant interactions between change in pH and heating temperature with antioxidant activity were observed. The radish root extract with a pH of 4 at a temperature of 70° C had an IC_{50} value (1071.93±45.71 mg/l) closest to that of the control extract (770.78±99.91 mg/l) which was not exposed to pH or temperature changes).

Antimicrobial Activity

Leuconostoc/Radish Root Ferment Filtrate

According to specifications provided by a supplier, a sample of Leuconostoc/Radish Root Ferment Filtrate is expected to have a minimum inhibitory concentration (MIC) of 1- 4% against *Pseudomonas aeruginosa* , 0.50 – 4% against *Escherichia coli*, and 0.25 – 2% against *Aspergillus brasiliensis*, *Candida albicans*, and *Staphylococcus aeruginosa*.[6]

Raphanus Sativus (Radish) Root Juice

The antimicrobial potential of *Raphanus sativus* root juice was compared to that of ampicillin in strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pnuemoniae*, and *Enterococcus faecalis*.[10] Upon incubation with 0.078 - 2.5 mg/ml of the root juice for 24 h, the highest MIC values were against *P. aeruginosa* at 0.625 ± 0.4 mg/ml and *S.aureus* at 0.312 ± 0.2 mg/ml (significantly greater than the corresponding ampicillin MIC values of 0.156 ± 0.8 mg/ml and 0.156 ± 0.07 mg/ml) and the *Raphanus sativus* root juice MIC values against *E.coli* and *E. faecalis* were equivalent to ampicillin MIC values.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

In Vitro

Leuconostoc/Radish Root Ferment Filtrate

The dermal irritancy of Leuconostoc/Radish Root Ferment Filtrate was evaluated in a reconstructed three-dimensional human epidermis model (EpiDerm™).[31] A single application of 30 µl of the test article was applied to the epidermis model (3 tissue samples). Sterile phosphate-buffered saline (PBS) was used as the negative control and chloropromazine, ranging from concentrations of 0.001-0.1%, were used as positive controls. The tissues were washed with sterile PBS 1 h after the application, post-incubated under normal medium and culture conditions for 2 h, and then measured for cell viability via a 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) assay. Under the conditions of this study, the test article was considered non-irritating.

Sensitization

In Chemico/In Vitro

Leuconostoc/Radish Root Ferment Filtrate

The sensitization potential of Leuconostoc/Radish Root Ferment Filtrate (in acetonitrile) was evaluated in a direct peptide reactivity assay (DPRA), in accordance with Organisation for Economic Cooperation and Development (OECD) test guideline (TG) 442C.[32] This assay is designed to mimic the covalent binding of electrophilic chemicals to nucleophilic centers in skin proteins by quantifying the reactivity of test chemicals towards the model synthetic peptides containing cysteine and lysine. The mean percent depletion of cysteine and lysine was 2.89%, which was interpreted as minimal reactivity in the assay, with a prediction of no sensitization.

Leuconostoc/Radish Root Ferment Filtrate was also evaluated for sensitization potential in an antioxidants response elements - transcription factor Nrf2 (ARE-Nrf2) luciferase assay utilizing the KeratinoSens™ cell line, in accordance with OECD TG 442D.[33] In this assay, transfection with the luciferase gene allows for measurement of the activation of the Keap1-Nrf2-ARE complex, a proxy for sensitization. Fifty µl, each, of 12 concentrations of the test article prepared in DMSO (ranging from 0.98 – 2000 µM) were added to human keratinocyte (HaCaT) cell lines seeded for 24 h, as per the KeratinoSens™ method, and were incubated for 48 h. No significant increases in luciferase expression were observed; the test article was predicted to be a non-sensitizer.

Human

Leuconostoc/Radish Root Ferment Filtrate

In a human repeated insult patch test (HRIPT), Leuconostoc/Radish Root Ferment Filtrate, as a 10% dilution in water, was tested in 50 subjects.[34] The test material was applied to the back via 9, occlusive, 24- h induction applications (0.2 g applied to an unspecified area), made over a 3-wk induction period. Induction sites were scored 24 or 48 h after patch removal. After a 2-wk non-treatment period, a 24-h challenge application was made to a previously untreated site in the same manner as the induction applications, and the reactions were scored on a scale of 0 - 4 at 24 and 48 h after application.

No adverse reactions occurred during the course of the study; the researchers concluded that the test material was not an irritant or sensitizer.

An eyebrow gel formulation containing 0.04% Leuconostoc/Radish Root Ferment Filtrate was tested neat in an HRIPT in 105 subjects.[35] The test material was applied to the back via 9, occlusive, 24- h induction applications (0.2 g applied to approximately 0.75 in^2), made over a 3-wk induction period. Induction sites were scored 24 or 48 h after patch removal. After a 2-wk non-treatment period, a 24-h challenge application was made to a previously untreated site in the same manner as the induction applications, and the reactions were scored on a scale of 0 - 4, at 24 and 72 h after application. No signs of irritation or sensitization were observed during induction or challenge; the researchers concluded that the test material did not cause dermal sensitization.

Photosensitization/Phototoxicity

In Vitro

Leuconostoc/Radish Root Ferment Filtrate

The phototoxicity of Leuconostoc/Radish Root Ferment Filtrate was tested using a reconstructed three-dimensional human epidermis model (EpiDerm™).[36] Five concentrations of the test article 0, 0.4%, 1.2%, 3.7%, and 11%, diluted in Dulbecco's modified Eagle medium were used. Sterile deionized water and 0.001-0.1% chloropromazine were used as negative and positive controls, respectively. After the EpiDerm™ model was incubated in growth media for 1 h, 50 µl of each test article concentration was applied to tissue inserts and allowed to incubate overnight at 37 °C. The tissue inserts were either irradiated with 6 J/cm^2 UVA(ultraviolet), or incubated without irradiation, for 1 h at room temperature and were tested in an MTT assay. As per the definition of a potential photoirritant reducing cell viability by ≥ 20%, when comparing irradiated to non-irradiated controls, significant reduction was only seen in the 11% concentration, with and without radiation (51.1% and 72.6%, respectively). Leuconostoc/Radish Root Ferment Filtrate was therefore not considered a photosensitizer at the 0.4, 1.2, or 3.7% concentrations.

OCULAR IRRITATION STUDIES

In Vitro

Leuconostoc/Radish Root Ferment Filtrate

The ocular irritation potential of Leuconostoc/ Radish Root Ferment Filtrate to cause eye irritation was evaluated in a reconstructed human cornea-like epithelium test, using an EpiOcular™ three-dimensional human cornea model.[31] Fifty µl of the undiluted test article were applied to 2 tissue samples. The treated tissues were incubated for 90 min, washed out with PBS, post-incubated under normal medium and culture conditions for 2 h, and then measured for cell viability via an MTT assay. The negative control tissues received applications of de-ionized water. The test article was considered to be non-irritating.

CLINICAL STUDIES

Occupational Exposures

A 46-yr-old kitchen porter, with metal allergy and no prior food allergies, presented to the emergency room with dizziness, generalized eruptions on the skin, and gastrointestinal upset.[37] During recent employment in a Korean kitchen, she had been exposed to *Raphanus sativus* roots while chopping fresh young radish, 3 and 1 d prior to her hospital treatment. Upon initial exposure, she experienced immediate urticaria with pruritus and burning sensation (which spontaneously disappeared); however, upon second exposure, the pruritus presented more severely with generalized erythematous eruption and dizziness. Systemic anaphylatic symptoms manifested within 12 h. Upon hospital admission, total serum immunoglobulin E (IgE) level was measured at 30 IU/l; she received subcutaneous epinephrine (0.3 ml) followed by intravenous saline and antihistamine. Three wk post-recovery, she tested positive to a skin prick test with young radish extract; 5 controls tested with a skin prick test using young radish extract and 55 common allergens did not exhibit positive reactions. The allergic reaction was attributed to biphasic, IgE-mediated anaphylaxis to physical contact with young radish.

A 38-yr-old waitress, with no prior history of dermatological illness, developed an acute vesiculo-bullous dermatitis of both palms, 3 wk after chopping tomatoes, cabbage, endive, and radishes for the salad bar.[38] She sought medical attention 2 wk after the dermatitis appeared; findings were normal, with the exception of the sides of her fingers, which were more severely affected. Patch tests were performed with the neat application of *Raphanus sativus* root juice, cabbage leaf, tomato fruit, and endive leaf. Additionally, patch tests were performed with 0.1% allyl isothiocyanate, 0.1% benzyl isothiocyanate, 0.05% phenyl isothiocyanate, 1% sinigrin, and 1% myrosinase (all in petrolatum). Samples of the thioglucoside, sinigrin, which yields allyl isothiocyanate, and of the enzyme, myrosinase, were mixed together and either applied to the skin immediately after mixture or 1 wk later; a positive reaction to the previously mixed test article was observed. Positive reactions to allyl isothiocyanate, and benzyl isothiocyanate were also observed. There was no reaction to freshly mixed sinigrin and myrosinase. No further details were provided.

SUMMARY

This assessment reviews the safety of the following 7 radish root-derived ingredients. According to the *Dictionary*, various functions are reported for these ingredients, with hair and skin conditioning agents being the most common. Most of the other reported are not considered cosmetic functions in the US, and therefore, use as such does not fall under the purview of the Panel. Leuconostoc/Radish Root Ferment Filtrate is reported to have the greatest frequency of use, in 255 formulations, 104 of which are in leave-on moisturizing products. The highest reported concentration of use amongst these ingredients is for Raphanus Sativus (Radish) Root Extract, at up to 6% in lipstick formulations. *Raphanus sativus* roots are widely consumed in raw, cooked, and fermented forms; in the US, foods that are commercially produced using lactic acid fermentation are considered to have GRAS status.

Groups of albino rats were administered up to 550 mg/kg bw/d of methanolic *Daikon* (vegetable) extract, in the diet, for 90 d. Throughout the course of the experiment, no statistically significant differences were seen between controls and treated animals for mean body weights, organ weights, and hematological parameters such as red blood cell count, hemoglobin, white blood cell count, AST, ALT, ACP, urea, uric acid, and protein levels.

Leuconostoc/Radish Root Ferment Filtrate was not genotoxic when tested at concentrations up to 5000 µg/plate in an Ames test. In a study evaluating the genotoxic potential of several *Raphanus sativus* root juices against cancerous cell lines, 500 µl of the white tuber root juice caused the most DNA damage in all aberrant cell lines; the breast cancer adenoma cell line was the most highly affected.

Raphanus sativus root juice exhibited a higher potential for tyrosinase inhibition (IC_{50}=3.09 mg/ml vs. 9.62 mg/ml), radical scavenging, and had a higher content of L-ascorbic acid than a methanolic *Raphanus sativus* root extract. In another study, ethyl acetate *Raphanus sativus* root extract exposed to pH and temperature changes exhibited an IC_{50} value that was closest to an unexposed control extract at a pH of 4 and temperature of 70 °C. A sample of Leuconostoc/Radish Root Ferment Filtrate exhibited MIC values of 1 - 4% against *P. aeruginosa*, 0.50 - 4% against *E.coli*, and 0.25 - 2% against *A.brasiliensis*, *C.albicans*, and *S.aeruginosa*. The highest MIC values for a *Raphanus sativus* root juice, which were greater than the corresponding ampicillin MIC values, were against *P.aeruginosa* and *S.aureus* at 0.625± 0.4 mg/ml and 0.312±0.2 mg/ml, respectively.

A single 30 µl application of Leuconostoc/Radish Root Ferment Filtrate did not cause irritation in a triplicate series of EpiDerm™ model epidermis tests. In a DPRA assay testing the sensitizing potential of 100 mM Leuconostoc Ferment Filtrate, the mean percent depletion for cysteine and lysine was 2.89%; the test article was predicted to be a non-sensitizer. Leuconostoc/Radish Root Ferment Filtrate, tested at concentrations of up to 2000 µM in DMSO (50 µl), was found to be non-sensitizing in an ARE-Nrf2 luciferase assay. Leuconostoc/Radish Root Ferment Filtrate, as a 10% dilution in water, did not cause sensitization in an occlusive HRIPT using 50 subjects. An eyebrow gel formulation containing 0.04% Leuconostoc/Radish Root Ferment Filtrate also was found to be non-sensitizing in an occlusive HRIPT using 105 subjects.

Leuconostoc/Radish Root Ferment Filtrate was tested at 0, 0.4, 1.2, 3.7, and 11% (in Dulbecco's modified Eagle medium) for phototoxicity in an irradiated EpiDerm™ reconstructed epidermis model. Significant reduction in cell viability (≥20 %) when compared to non-radiated controls, was seen at the 11% concentration, both with and without radiation; the test article was not considered a photosensitizer. Leuconostoc/Radish Root Ferment Filtrate was not considered an ocular irritant when tested in 2 EpiOcular™ human cornea-like epithelium tissue samples.

A 46-yr old female kitchen porter, with pre-existing metal allergy, presented to the emergency room with dizziness, generalized eruptions on the skin, and gastrointestinal upset after chopping fresh young radish 3 and 1 d prior to hospitalization. Systemic anaphylactic symptoms manifested within 12 h. Three wk post-recovery the subject tested positive to a skin prick test with young radish extract, which was attributed to biphasic, IgE-mediated anaphylaxis upon physical contact. A 38-yr old female waitress developed an acute vesiculo-bullous dermatitis of both palms 3 wk after chopping tomatoes, cabbage, endive, and radishes for the salad bar. Patch tests were performed with the neat application of all plant substances, plus, 0.1% each of allyl isothiocyanate, benzyl isothiocyanate, sinigrin, myrosinase, and 1% sinigrin, either mixed with 1% myrosinase 1 wk prior to application, or mixed with 1% myrosinase immediately prior to application. Positive reactions were observed for *Raphanus sativus* root juice, allyl isothiocyanate, benzyl isothiocyanate, and to the sinigrin previously mixed with myrosinase.

DISCUSSION

To be developed.

CONCLUSION

To be determined.

TABLES

Table 1. INCI names, definitions, and functions of *Raphanus sativus* (root)-derived ingredients in this safety assessment[1]

Ingredient/ CAS Number	Definition	Function(s)
Lactobacillus/Radish Root Ferment Extract Filtrate	is a filtrate of the extract of the product obtained by the fermentation of the roots of *Raphanus sativus* (radish) by the microorganism, *Lactobacillus*.	Preservative
Lactobacillus/Radish Root Ferment Filtrate	is a filtrate of the product obtained by the fermentation of the roots of *Raphanus sativus* (radish) by the microorganism, *Lactobacillus*.	Antimicrobial agent; hair conditioning agent; skin- conditioning agent - miscellaneous
Leuconostoc/Radish Root Ferment Filtrate	is a filtrate of the product obtained by the fermentation of *Raphanus sativus* roots by the microorganism, *Leuconostoc.*	Anti-dandruff agent; antifungal agent; antimicrobial agent; hair conditioning agent; skin-conditioning agent - miscellaneous
Leuconostoc/Radish Root Ferment Lysate Filtrate 1686112-10-6	is a filtrate of a lysate of the product obtained by the fermentation of the roots of *Raphanus sativus* (radish) by the microorganism, *Leuconostoc*.	Hair conditioning agent; skin-conditioning agent- miscellaneous
Raphanus Sativus (Radish) Root Extract 84775-94-0 (generic)	is the extract of the roots of *Raphanus sativus*.	Antioxidant; skin-conditioning agents - miscellaneous
Raphanus Sativus (Radish) Root Juice	is the juice expressed from the roots of *Raphanus sativus*.	Skin-conditioning agents - miscellaneous
Raphanus Sativus (Radish) Root Powder	is the powder obtained from the dried, ground roots of *Raphanus sativus*.	Skin – conditioning agents – emollient; skin – conditioning agents – humectant

Table 2. Chemical properties of Leuconostoc/Radish Root Ferment Filtrate

Property	Value	Reference
	Leuconostoc/Radish Root Ferment Filtrate	
Physical Form	Clear to slightly hazy liquid	6
Color	Yellow to light amber	6
Odor	Characteristic	6
Specific Gravity (@ 25 ºC)	1.140 – 1.180	6
pH	4.0 - 6.0	6
log K_{ow}; K_{ow}	-1.92; 0.013	7

Table 3. Frequency (2021)[16] and concentration (2020)[17] of use according to duration and exposure

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Lactobacillus/Radish Root Ferment Filtrate		**Leuconostoc/Radish Root Ferment Filtrate**		**Raphanus Sativus (Radish) Root Extract**	
Totals*	**2**	**NR**	**255**	**0.0001 – 1.1**	**2**	**6**
Duration of Use						
Leave-On	*2*	*NR*	*185*	*0.0001 - 0.03*	*2*	*6*
Rinse-Off	*NR*	*NR*	*70*	*0.0001 – 1.1*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	3	0-0.002 – 0.01	NR	NR
Incidental Ingestion	NR	NR	9	0.0002	NR	6
Incidental Inhalation-Spray	2[a]	NR	5; 111[a]; 40[b]	0.0001-0.03; 0.001[a]	NR	NR
Incidental Inhalation-Powder	NR	NR	40[b]; 1[c]	0.0002 - 0.002[c]	2[b]	NR
Dermal Contact	2	NR	220	0.0002 – 1.1	2	NR
Deodorant (underarm)	NR	NR	1[a]	NR	NR	NR
Hair - Non-Coloring	NR	NR	26	0.0001 – 0.002	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	0.0022 – 0.01	NR	NR
Mucous Membrane	NR	NR	39	0.0002	NR	6
Baby Products	NR	NR	1	NR	NR	NR

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[c] It is possible these products are powders, but it is not specified whether the reported uses are powders
NR – not reported

Table 4. Raphanus sativus – derived ingredients not reported to be in use[16,17]

Lactobacillus/Radish Root Ferment Extract Filtrate
Leuconostoc/Radish Root Ferment Lysate Filtrate
Raphanus Sativus (Radish) Root Juice
Raphanus Sativus (Root) Powder

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Concentration of Use by FDA Product Category - Leuconostoc/Radish Root Ferment Filtrate and Related Ingredients*

Leuconostoc/Radish Root Ferment Filtrate
Leuconostoc/Radish Root Ferment Lysate Filtrate
Lactobacillus/Radish Root Ferment Filtrate
Lactobacillus/Radish Root Ferment Extract Filtrate
Raphanus Sativus (Radish) Root Extract
Raphanus Sativus (Radish) Root Juice
Raphanus Sativus (Radish) Root Powder

Ingredient	Product Category	Maximum Concentration of Use
Leuconostoc/Radish Root Ferment Filtrate	Eye makeup removers	0.002%
Leuconostoc/Radish Root Ferment Filtrate	Mascaras	0.002%
Leuconostoc/Radish Root Ferment Filtrate	Other eye makeup preparations	0.01%
Leuconostoc/Radish Root Ferment Filtrate	Hair conditioners	0.0001%
Leuconostoc/Radish Root Ferment Filtrate	Hair sprays Pump spray	 0.0001%
Leuconostoc/Radish Root Ferment Filtrate	Shampoos (noncoloring)	0.002%
Leuconostoc/Radish Root Ferment Filtrate	Tonics, dressings, and other hair grooming aids	0.001%
Leuconostoc/Radish Root Ferment Filtrate	Lipstick	0.0002%
Leuconostoc/Radish Root Ferment Filtrate	Other makeup preparations	0.024%
Leuconostoc/Radish Root Ferment Filtrate	Basecoats and undercoats	0.0022%
Leuconostoc/Radish Root Ferment Filtrate	Nail creams and lotions	0.01%
Leuconostoc/Radish Root Ferment Filtrate	Other manicuring preparations	0.0022%
Leuconostoc/Radish Root Ferment Filtrate	Bath soaps and detergents	0.0002%
Leuconostoc/Radish Root Ferment Filtrate	Other personal cleanliness products	0.0002%
Leuconostoc/Radish Root Ferment Filtrate	Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	0.04-1.1%
Leuconostoc/Radish Root Ferment Filtrate	Face and neck products Not spray Spray	 0.0002% 0.03%
Leuconostoc/Radish Root Ferment Filtrate	Body and hand products Not spray	 0.002%
Leuconostoc/Radish Root Ferment Filtrate	Moisturizing products Not spray	 0.01%
Leuconostoc/Radish Root Ferment Filtrate	Other skin care preparations	0.001%
Raphanus Sativus (Radish) Root Extract	Lipstick	6%

*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 2020
Table prepared: January 5, 2021

Memorandum

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

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

DATE: April 29, 2021

SUBJECT: Leuconostoc/Radish Root Ferment Filtrate

Anonymous. 2019. Repeated insult patch test (eyebrow gel containing 0.04% Leuconostoc/Radish Root Ferment Filtrate).

Distributed for Comment Only -- Do Not Cite or Quote

[redacted]

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

eyebrow gel containing 0.04% Leuconostoc/Radish Root Ferment Filtrate

TEST: Repeated Insult Patch Test
Protocol No.: [redacted]
Protocol Date: [redacted]

TEST MATERIAL: Project Name: [redacted]

STUDY NUMBER: [redacted]

Reviewed by: [redacted]
Medical Director
Board Certified Dermatologist

Approved by: [redacted]
Vice President, Clinical Evaluations

Approved by: [redacted]
Executive Vice President, Clinical Evaluations

[redacted]

[redacted]

QUALITY ASSURANCE UNIT STATEMENT

Study Number: [redacted]

The [redacted] is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at CPTC.

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, [redacted] Standard Operating Procedures, and the approved protocol.

The [redacted] has reviewed all data, records, and documents relating to this trial and also this Final Report. The following [redacted] representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the [redacted] archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the [redacted] to obtain custody of trial records once the [redacted] archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, trial-related records shall be destroyed at the end of the [redacted] archive period with no further notice in a manner that renders them useless.

[redacted]

Quality Assurance Representative

11/27/19

Date

[redacted]

 [redacted]

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Participants: One hundred twelve (112) qualified subjects, male and female, ranging in age from 18 to 70 years, were selected for this evaluation. One hundred five (105) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16[a] to 79 years.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History Form and the understanding and signing of an Informed Consent Form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: Project Name: [redacted]

Study Schedule:

Panel #	Initiation Date	Completion Date
[redacted]	September 16, 2019	October 25, 2019
	September 18, 2019	October 25, 2019

[a]With parental or guardian consent

[redacted]

Methodology:

The upper back between the scapulae served as the treatment area. Approximately 0.2 g of the test material, or an amount sufficient to cover the contact surface, was applied to the 3/4" x 3/4" absorbent pad portion of an adhesive dressing. This was then applied to the appropriate treatment site to form an occlusive patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, one day after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of one day following each Tuesday and Thursday removal, and two days following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic Day 1 and Day 3 post-application.

[redacted]

Methodology (continued):

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	=	No visible skin reaction	E	=	Edema
0.5	=	Barely perceptible	D	=	Dryness
1	=	Mild	S	=	Staining
2	=	Moderate	P	=	Papules
3	=	Marked	V	=	Vesicles
4	=	Severe	B	=	Bullae
			U	=	Ulceration
			Sp	=	Spreading

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Adverse Events: There were no adverse events.

Amendments: There were no amendments.

Deviations: There were no deviations.

Results: The results of each participant are appended (Table 1).

Observations remained negative throughout the test interval.

Subject demographics are presented in Table 2.

Photograph of sample, sample receipt form and protocol are appended.

Summary: Under the conditions of this study, test material, Project Name: [redacted] indicated no potential for dermal irritation or allergic contact sensitization.

[redacted]

[redacted]

Table 1

[redacted]

Individual Results

Project Name: [redacted]

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day 1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	WITHDREW CONSENT									
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	WITHDREW CONSENT									
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	WITHDREW CONSENT					
24	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	WITHDREW CONSENT								

Day 1* = Supervised removal

[redacted]

 [redacted]

Table 1
(continued)
Panel [redacted]

Individual Results

Project Name: [redacted]

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day 1*	Day 3
30	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0[m]	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	WC
44	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0[m]	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	WITHDREW CONSENT									
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* – Supervised removal
m = Additional makeup day granted at the discretion of the clinic supervisor
WC = Withdrew Consent

[redacted]

[redacted]

Table 1
(continued)
Panel [redacted]

Individual Results

Project Name: [redacted]

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day 1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	---WITHDREW CONSENT---			

Day 1* = Supervised removal

[redacted]

[redacted]

Table 1
(continued)
Panel [redacted]

Individual Results

Project Name: [redacted]

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day 1*	Day 3
30	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal

[redacted]

[redacted]

Table 2
Panel [redacted]

Subject Demographics

Subject Number	ID#	Age	Gender
1	88987	65	F
2	82620	37	F
3	80734	22	M
4	85948	70	M
5	34229	68	F
6	77172	44	F
7	73941	61	F
8	79832	60	F
9	18727	66	F
10	73844	33	F
11	73845	47	M
12	88543	56	M
13	89162	39	F
14	53820	31	F
15	21	59	F
16	84430	55	F
17	53505	48	F
18	18245	52	M
19	87208	28	F
20	87562	24	F
21	89012	39	M
22	60204	70	F
23	46142	52	M
24	3673	43	F
25	82350	40	F
26	48723	45	M
27	86906	41	F
28	22564	58	F
29	66000	42	F

[redacted]

[redacted]

Table 2
(continued)
Panel [redacted]

Subject Demographics

Subject Number	ID#	Age	Gender
30	89666	41	M
31	82764	32	F
32	78666	21	F
33	89700	59	M
34	21806	39	F
35	62982	26	F
36	84695	39	F
37	80130	50	F
38	44945	34	F
39	89701	38	F
40	13439	24	F
41	78224	59	F
42	82491	21	F
43	87488	24	F
44	74716	57	F
45	89602	46	F
46	50004	68	F
47	89702	28	F
48	89703	57	F
49	87272	20	M
50	78402	52	F
51	86989	46	M
52	89704	34	F
53	84697	18	F
54	88869	52	F
55	66296	32	F
56	74068	57	F

[redacted]

[redacted]

Table 2
(continued)
Panel [redacted]

Subject Demographics

Subject Number	ID#	Age	Gender
1	82456	52	F
2	81433	30	F
3	84543	70	F
4	57358	66	F
5	85553	59	F
6	57991	69	M
7	68115	25	F
8	12891	58	F
9	1129	66	F
10	47442	45	F
11	37324	43	F
12	88031	34	M
13	88052	35	F
14	87149	57	F
15	66170	58	M
16	66174	59	F
17	82138	52	F
18	87363	62	M
19	15064	52	F
20	51093	67	M
21	28794	68	F
22	76255	54	F
23	11535	70	F
24	84801	20	F
25	88971	64	F
26	50670	69	F
27	79466	55	M
28	65680	49	F
29	81936	25	F

[redacted]

Table 2
(continued)
Panel [redacted]

Subject Demographics

Subject Number	ID#	Age	Gender
30	88791	20	F
31	23454	49	F
32	76318	39	F
33	44953	65	F
34	89412	37	F
35	86433	19	F
36	71175	48	M
37	89413	44	F
38	77774	62	F
39	29339	59	F
40	89425	44	F
41	76246	53	F
42	72301	27	M
43	54964	41	F
44	69337	55	F
45	49665	43	F
46	89714	61	F
47	55166	46	F
48	1221	66	F
49	71343	65	F
50	89446	37	F
51	1121	69	F
52	72790	43	F
53	84994	37	F
54	82849	35	M
55	79420	68	F
56	86670	61	F

Memorandum

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

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

DATE: May 6, 2021

SUBJECT: Leuconostoc/Radish Root Ferment Filtrate

Active Micro Technologies. 2018. Bacterial reverse mutation test Leucidal® Liquid (Leuconostoc/Radish Root Ferment Filtrate).

Active Micro Technologies. 2017. Dermal and ocular irritation tests Leucidal® Liquid (Leuconostoc/Radish Root Ferment Filtrate).

Active Micro Technologies. 2017. Kow statement Leucidal® Liquid (Leuconostoc/Radish Root Ferment Filtrate).

Active Micro Technologies. 2017. OECD TG 442C: *In Chemico* skin sensitization Leucidal® Liquid (Leuconostoc/Radish Root Ferment Filtrate).

Active Micro Technologies. 2017. OECD TG 442D: In Vitro skin sensitization Leucidal® Liquid (Leuconostoc/Radish Root Ferment Filtrate).

Active Micro Technologies. 2017. Phototoxicity Assay Analysis Leucidal® Liquid (Leuconostoc/Radish Root Ferment Filtrate).

Active Micro Technologies. 2008. 50 Human subject repeat insult patch test skin irritation /sensitization evaluation (occlusive patch) Leucidal® Liquid (Leuconostoc/Radish Root Ferment Filtrate).

Bacterial Reverse Mutation Test

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

Test Article: Leucidal® Liquid
Code Number: M15008
CAS #: 84775-94-0

Sponsor:
Active Micro Technologies, 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.Part 3

Test Request Number: 1004

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 **Leucidal® Liquid** 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^{0}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^{0}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.

All *Salmonella* tester strain cultures demonstrated the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvr*A cultures demonstrated the deletion in the *uvr*A gene. All cultures demonstrated the characteristic mean number of spontaneous revertants in the vehicle controls as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21, WP2*uvr*A, 10-60.

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Bacterial Reverse Mutation Test

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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.

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.

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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. Criteria for a Valid Test

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* and *Escherichia coli* tester strains used.

All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21, WP2*uvr*A, 10-60. To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to $0.3x10^9$ cells/ml. The mean of each positive control must exhibit at least 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control. A minimum of three non-toxicdose levels is required to evaluate assay data. A dose level is considered toxic if one of both of the following criteria are met: (1). A >50% reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count. (2). At least a moderate reduction in the background lawn.

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.

D. 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.

All *Salmonella* tester strain cultures demonstrated the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 demonstrated the presence of the Pkm101 plasmid R-factor. All WP2 *uvr*A cultures demonstrated the deletion in the *uvr*A gene. All cultures demonstrated the characteristic mean number of spontaneous revertants in the vehicle controls as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21, WP2*uvr*A, 10-60.

VII. Conclusion

All criteria for a valid study were met 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

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Appendix 2:

Bacterial Mutation Assay
Plate Incorporation Assay Results

	Concentration µg per Plate	***TA98***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	32	34	33
	1500	15	17	16
	500	28	32	30
	150	26	36	31
	50	28	18	23
	15	14	20	17
	5.0	24	21	23
	1.5	26	26	26
Test Solution w/o S9	5000	18	16	17
	1500	33	45	39
	500	15	19	17
	150	21	35	28
	50	18	23	21
	15	25	27	26
	5.0	21	21	21
	1.5	25	15	20
DI Water w/S9		36	36	36
DI Water w/o S9		28	32	30
2-aminoanthracen w/ S9		410	398	404
2-nitrofluorene w/o S9		257	225	241
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

ACTIVE MICRO TECHNOLOGIES
107 Technology Drive · Lincolnton, NC 28092
(704) 276-7100 · Fax (704) 276-7101

	Concentration µg per Plate	***TA100***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	112	110	111
	1500	108	144	126
	500	115	117	116
	150	114	132	123
	50	128	156	142
	15	144	162	153
	5.0	132	146	139
	1.5	168	134	151
Test Solution w/o S9	5000	132	148	140
	1500	112	124	118
	500	152	126	139
	150	112	68	90
	50	102	44	73
	15	116	125	121
	5.0	136	112	124
	1.5	126	124	125
DI Water w/S9		154	185	170
DI Water w/o S9		194	210	202
2-aminoanthracen w/ S9		425	368	397
Sodium azide w/o S9		398	410	404
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**		

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*Mean = Average of duplicate plates

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	Concentration µg per Plate	***TA1537***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	10	8	9
	1500	16	22	19
	500	14	12	13
	150	24	16	20
	50	22	24	23
	15	14	14	14
	5.0	12	32	22
	1.5	19	25	22
Test Solution w/o S9	5000	42	22	32
	1500	12	12	12
	500	10	8	9
	150	10	12	11
	50	14	18	16
	15	22	14	18
	5.0	16	22	19
	1.5	16	11	14
DI Water w/S9		10	5	8
DI Water w/o S9		15	16	16
2-aminoanthracen w/ S9		355	347	351
2-aminoacridine w/o S9		348	306	327
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

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	Concentration µg per Plate	***TA1535***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	24	35	25
	1500	25	28	27
	500	42	31	37
	150	22	16	19
	50	21	24	23
	15	18	15	17
	5.0	17	17	17
	1.5	14	22	18
Test Solution w/o S9	5000	45	61	53
	1500	48	33	41
	500	82	81	82
	150	65	42	54
	50	15	28	22
	15	12	25	19
	5.0	44	36	40
	1.5	22	24	23
DI Water w/S9		15	18	17
DI Water w/o S9		25	33	29
2-aminoanthracen w/ S9		224	256	240
Sodium azide w/o S9		416	475	446
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

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	Concentration µg per Plate	***WP2uvrA***		
		Revertants per plate (CFU)		Mean
Test Solution w/ S9	5000	20	32	26
	1500	21	11	16
	500	26	24	25
	150	25	42	34
	50	29	36	33
	15	20	12	16
	5.0	45	47	46
	1.5	51	55	53
Test Solution w/o S9	5000	62	36	49
	1500	44	62	53
	500	26	38	32
	150	16	16	16
	50	35	52	44
	15	61	47	54
	5.0	52	37	45
	1.5	40	60	50
DI Water w/S9		44	42	43
DI Water w/o S9		62	51	56
2-aminoanthracen w/ S9		482	502	492
Methylmethanesulfonate w/o S9		385	363	374
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**		

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*Mean = Average of duplicate plates

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Dermal and Ocular Irritation Tests

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6 (or) 84775-94-0

Test Request Form #: 23

Lot #: 26051

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 **Leucidal® Liquid** 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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ACTIVE MICRO TECHNOLOGIES

Dermal and Ocular Irritation Tests

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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 Micro Technologies, 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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ACTIVE MICRO TECHNOLOGIES

Dermal and Ocular Irritation Tests

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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.

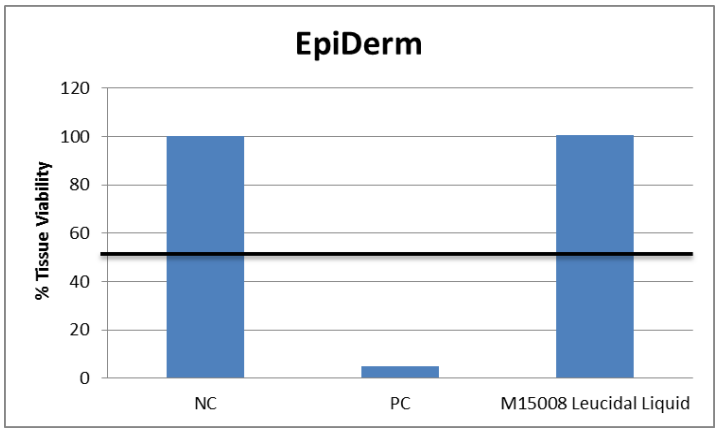

Figure 1: EpiDerm tissue viability

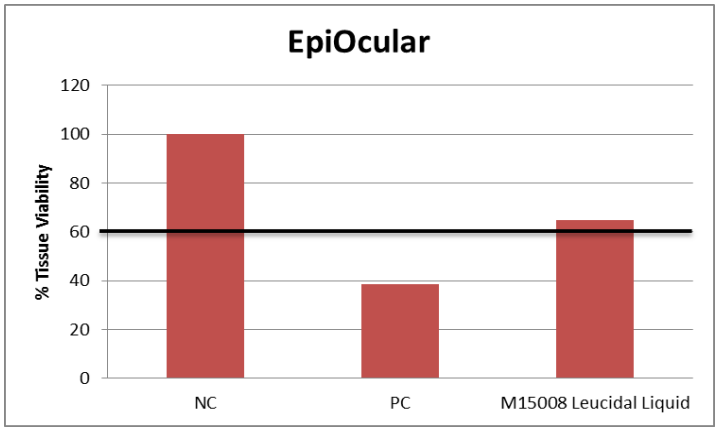

Figure 2: EpiOcular tissue viability

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December 21, 2017

To whom it may concern,

This letter is to certify that Leucidal® Liquid (M15008) manufactured by Active Micro Technologies, LLC has an average octanol/water partition coefficient (K_{ow}) of 0.013 and Log(K_{ow}) of -1.92.

The aforementioned results were analyzed via EPA OPPTS 830.7570 testing at Jordi Labs, LLC.

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

Best Regards,

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
www.activeconceptsllc.com

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OECD TG 442C: *In Chemico* Skin Sensitization

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6 (or) 84775-94-0

Test Request Form #: 1237

Lot #: 4786P

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

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 **Leucidal® Liquid** 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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ACTIVE MICRO TECHNOLOGIES

OECD TG 442C: *In Chemico* Skin Sensitization

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Materials

A. Equipment:	HPLC-UV (Waters Alliance 2695 - Waters 996 Photodiode Array); 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-RFAA**C**AA-COOH); Lysine peptide (Ac-RFAA**K**AA-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 **Leucidal® Liquid** in Acetonitrile

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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ACTIVE MICRO TECHNOLOGIES

OECD TG 442C:
In Chemico Skin Sensitization

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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

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 **Leucidal® Liquid (code M15008)** we can determine that this product is not a sensitizer and will not cause allergic contact dermatitis. The Mean Percent Depletion of Cysteine and Lysine was 2.89% 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

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6 (or) 84775-94-0

Test Request Form #: 1192

Lot #: 4752P

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

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 **Leucidal® Liquid** 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 **Leucidal® Liquid** 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.

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.

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***In Vitro* Skin Sensitization**

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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	31.6
DMSO	Non-Sensitizer	No Induction	243.24 µM	1.2
Leucidal® Liquid	Non-Sensitizer	No Induction	> 1000 µM	0.5

Table 1: Overview of KeratinoSens™ Assay Results

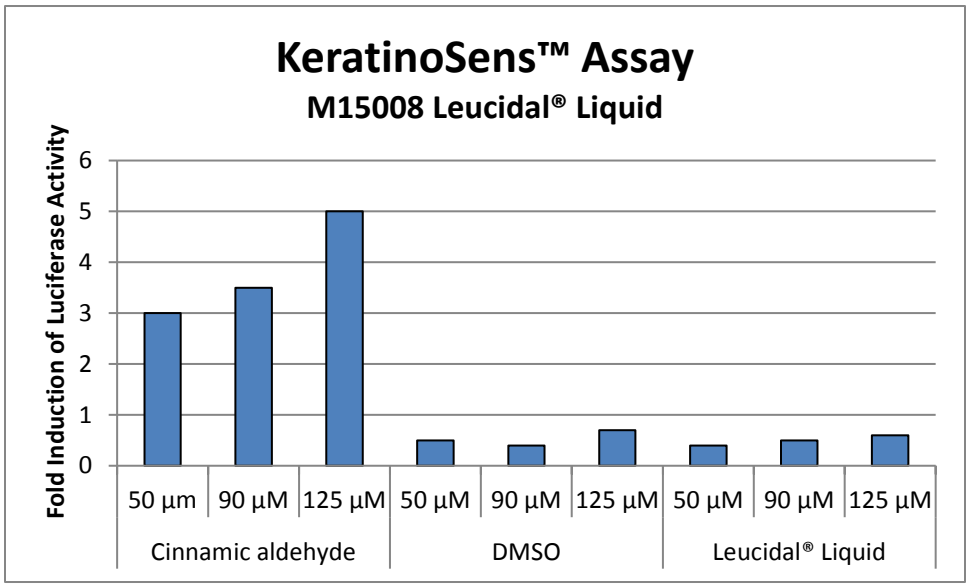

Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **Leucidal® Liquid (code M15008)** 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 **Leucidal® Liquid** can be safely used in cosmetics and personal care products at typical use levels.

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Phototoxicity Assay Analysis

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Tradename: Leucidal® Liquid

Code: M15008

CAS #: 1686112-10-6 (or) 84775-94-0

Test Request Form #: 23

Lot #: 24723

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

Test Performed:
In Vitro EpiDerm™ Model (EPI-200-SIT) Phototoxicity

SUMMARY

In vitro phototoxicity irritation studies were conducted to evaluate whether **Leucidal® Liquid** would induce phototoxic irritation in the EpiDerm™ model assay.

The product was tested according to the manufacturer's protocol. The test article solution was found to be a **non-photoirritant** at concentrations of 0.4%, 1.2%, and 3.7%. Reconstructed human epidermis was incubated in growth media for one hour to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substance was applied to the tissue inserts in five varying concentrations and incubated overnight at 37°C, 5% CO_2, and 95% relative humidity (RH). The following day, the appropriate tissue inserts were irradiated (UVA) for 60 minutes with 1.7 mW/cm^2 (=6 J/cm^2). After substance incubation, irradiation, and washing was completed, the cell viability test was conducted. Cell viability was measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that was measured after extraction from the tissue. The photoirritation potential of the test chemical was dictated by the reduction in tissue viability of UVA exposed tissues compared to non-UVA exposed tissues.

Under the conditions of this assay, the test article was considered to be **non-phototoxic** at concentrations of 0.4%, 1.2%, and 3.7%. The negative and positive controls performed as anticipated.

I. Introduction

A. Purpose

In vitro dermal phototoxicity study was conducted to evaluate whether a test article would induce photoirritation in the EpiDerm™ model assay. MatTek Corporation's reconstructed human epidermal model is becoming a standard in determining the phototoxicity potential of a test substance. This assay is able to discriminate between photoirritants and non-photoirritants at varying concentrations.

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Phototoxicity Assay Analysis

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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; UVA-vis Irradiation Equipment; UVA meter; Pipettes
C. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM) based medium; Dulbecco's Phosphate-Buffered Saline (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, and 6-well tissue culture plates
F. Reagents:	MTT (*3-4,5-dimethyl thiazole 2-yl*) (1.0mg/mL); Extraction Solution (Isopropanol); Chlorpromazine; Triton X-100 (1%)
G. Other:	Wash bottle; sterile disposable pipette tips; Parafilm; forceps

III. Test Assay

A. Test System

The reconstructed human epidermal model, EpiDerm™ consists of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis. This model consists of organized basal, spinous, and granular layers, and contains a multilayer stratum corneum containing intercellular lamellar lipid layers. The EpiDerm™ tissues are cultured on specially prepared cell culture inserts.

B. Negative Control

Sterile deionized water is used as the negative controls for the EpiDerm™ Phototoxicity assay.

C. Positive Control

Concentrations of chloropromazine, ranging from 0.001% to 0.1%, were used as positive controls for the EpiDerm™ Phototoxicity assay.

D. Data Interpretation Procedure

A photoirritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance and 60 minutes of 6 J/cm^2 is reduced by 20% compared to the non-irradiated control tissues.

IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Micro Technologies, 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 tissue insert dosing begins.

B. Test Substance Exposure

50µL of the diluted test substance in their respective concentrations are applied to 2 tissue inserts and allowed to incubate for overnight in a humidified incubator (37°C, 5% CO_2, 95% RH).

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C. Tissue Irradiation

Tissue inserts in their 6-well plates are UVA-irradiated for 60 minutes with 6 J/cm^2 at room temperature. The non-irradiated tissue inserts are incubated at room temperature in the dark.

D. Tissue Washing and Post Incubation

After UVA-irradiation and dark incubation is complete the tissue inserts are washed using sterile DPBS and transferred to fresh 6-well plates and media for overnight incubation at 37°C, 5% CO_2, 95% RH.

E. 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 ≥ 0.8.

B. Positive Control

The assay meets the acceptance criterion if a dose dependent reduction in cell viability in the UVA-irradiated tissues is between 0.00316% and 0.0316%.

C. Standard Deviation

Since the phototoxicity potential is predicted from the mean viability of 2 tissues for the EpiDerm™ Phototoxicity Protocol, the variability of the replicates should not exceed 30%.

VI. Results

A. Tissue Characteristics

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

B. Tissue Viability Assay

The results are summarized in Figure 1. Cell viability is calculated for each tissue as a percentage of the corresponding vehicle control either irradiated or non-irradiated. Tissue viability was not reduced by 20% in the presence of the test substance and UVA-irradiation at concentrations of 0.4%, 1.23%, and 3.7%. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited dose dependent loss of tissue viability and cell death.

C. Test Validity

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

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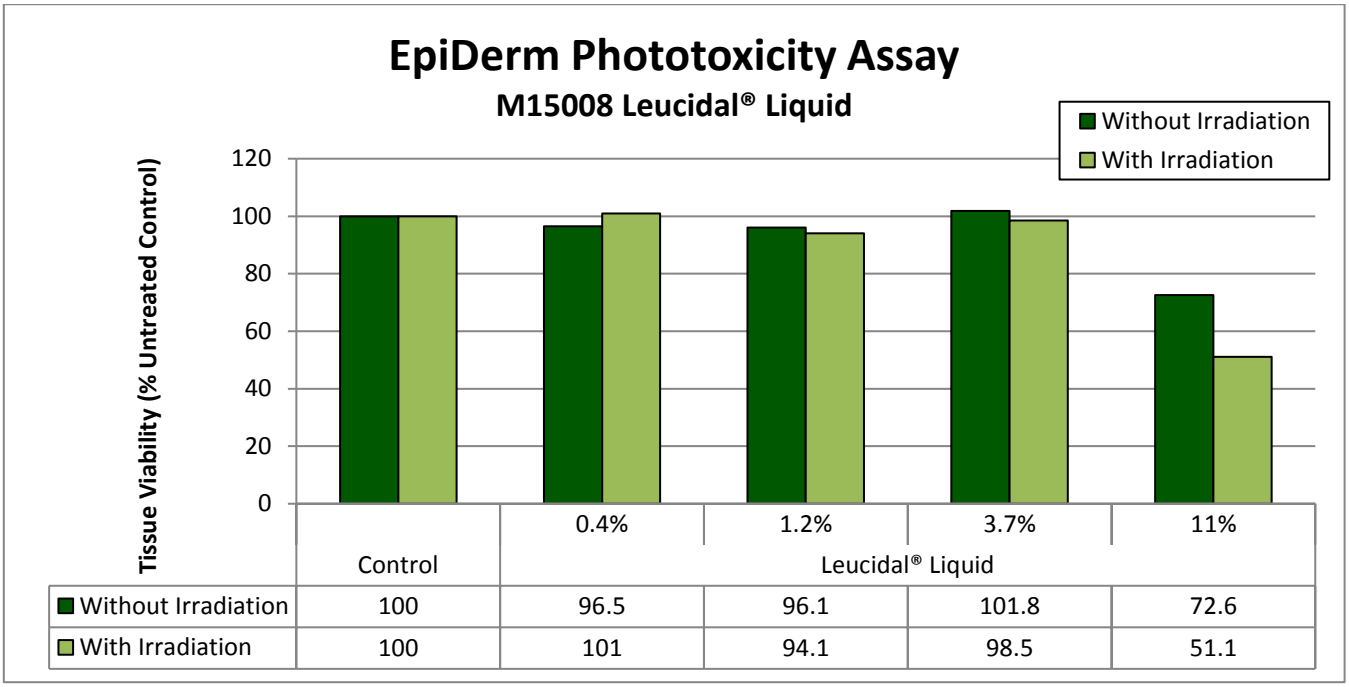
VII. Conclusion

Phototoxicity (photoirritation) is defined as an acute toxic response that is elicited after exposure of the skin to certain chemicals and subsequent exposure to light. Under the conditions of this assay, the test article substance was considered to be **non-phototoxic** at concentrations of 0.4%, 1.2%, and 3.7%. There is a decrease in viability at the 11% test concentration with and without irradiation but this concentration is significantly higher than the suggested use levels. We can safely say that **Leucidal® Liquid** is not a photoirritant when used at the suggested use levels of 2 – 4%.



	Control	0.4%	1.2%	3.7%	11%
		Leucidal® Liquid			
Without Irradiation	100	96.5	96.1	101.8	72.6
With Irradiation	100	101	94.1	98.5	51.1

Figure 1: EpiDerm Phototoxicity Graph

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The following report evaluates a sample of

Leucidal® Liquid (M15008)* – AMA Lab No. L-2090

Provided by Active Concepts, LLC to AMA Laboratories, Inc.

Utilizing the Repeat Insult Patch Test
Skin Irritation / Sensitization Evaluation (Occlusive Patch)

February 18, 2008

*The test material was received as a 10% Dilution in Water

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AMA LABORATORIES, INC.
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New City, NY 10956 USA
(845) 634-4330
FAX: (845) 634-5565
www.amalabs.com

50 HUMAN SUBJECT REPEAT INSULT PATCH TEST SKIN IRRITATION/SENSITIZATION EVALUATION (Occlusive Patch)

AMA Ref. No.: MS08.RIPT.L2090O.50.ACTC

Date: February 18, 2008

Sponsor: Active Concepts, LLC
121 Ethel Road West, Suite 3
Piscataway, New Jersey 08854

1.0 Objective:

Consumer products or raw materials designed for consistent reapplication to areas of the skin may, under proper conditions, prove to be contact sensitizers or irritants in certain individuals. It is the intention of a Repeat Insult Patch Test (RIPT) to provide a basis for evaluation of this irritation/sensitization potential if such exists.

2.0 Test Material:

2.1 Test Material Description:

On January 11, 2008 one test sample labeled EN080110-E was received from Active Concepts, LLC and assigned AMA Lab No. L-2090.

2.2 Handling:

Upon arrival at AMA Laboratories, Inc., the test material is assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date received and tests requested.

Samples are retained for a period of three months beyond submission of final report unless otherwise specified by the sponsor or, if sample is known to be in support of governmental applications, representative retained samples are kept two years beyond final report submission.

Sample disposition is conducted in compliance with appropriate federal, state and local ordinances.

2.3 Test Material Evaluation Prerequisite:

Prior to induction of a human test panel, toxicology, microbiology or in-vitro performance spectra may be required to assess the feasibility of commencement as dictated by an Institutional Review Board (IRB) described in Section 3.0.

Sponsor purports that prior to sample submission the following tests were conducted with no adverse results and that the test data are on file on their premises and have not been made available to AMA personnel:

- USP or CTFA Preservative Efficacy Test or equivalent
- 90 Day Accelerated Stability and Container Compatibility Study

3.0 Institutional Review Board:

Reference: CFR Title 21 Part 56, Subparts A, B, C, and D. The IRB of AMA Laboratories, Inc., consists of five or more individuals, chosen from within the company for technical expertise and from the local community for lay interaction. The list of IRB members is kept on file at AMA Laboratories, Inc. and is available for inspection during the hours of operation.

4.0 Panel Selection:

4.1 Standards for Inclusion in a Study:

- Individuals who are not currently under a doctor's care.
- Individuals free of any dermatological or systemic disorder which would interfere with the results, at the discretion of the Investigator.
- Individuals free of any acute or chronic disease that might interfere with or increase the risk of study participation.
- Individuals who will complete a preliminary medical history form mandated by AMA Laboratories, Inc. and are in general good health.
- Individuals, who will read, understand and sign an informed consent document relating to the specific type of study they are subscribing. Consent forms are kept on file and are available for examination on the premises of AMA Laboratories, Inc. only.
- Individuals able to cooperate with the Investigator and research staff, willing to have test materials applied according to the protocol, and complete the full course of the study.

4.2 Standards for Exclusion from a Study:

- Individuals under 18 years of age.
- Individuals who are currently under a doctor's care.
- Individuals who are currently taking any medication (topical or systemic) that may mask or interfere with the test results.
- Subjects with a history of any acute or chronic disease that might interfere with or increase the risk associated with study participation.
- Individuals diagnosed with chronic skin allergies.
- Female volunteers who indicate that they are pregnant or lactating.

4.3 Recruitment:

Panel selection is accomplished by advertisements in local periodicals, community bulletin boards, phone solicitation, electronic media or any combination thereof.

4.4 Informed Consent and Medical History Forms:

An informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Panelists signed and dated the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each subject was assigned a permanent identification number and completed an extensive medical history form. These forms along with the signed consent forms, are available for inspection on the premises of AMA Laboratories, Inc. only. Reference 21 CFR Ch. 1 Part 50, Subpart B.

The parties agree to comply with applicable state and federal privacy laws for the use and disclosure of a subject's personal health information by taking reasonable steps to protect the confidentiality of this information. This obligation shall survive the termination or expiration of this Agreement.

5.0 Population Demographics:

Number of subjects enrolled		52
Number of subjects completing study		50
Age Range		26-64
Sex	Male	7
	Female	45
Race	Caucasian	42
	Hispanic	9
	Asian	1

6.0 Equipment:

- Patch Description: Parke-Davis Hypoallergenic Readi Bandages or the equivalent.
- 1ml volumetric syringe without a needle.

7.0 Procedure:

- Subjects are requested to bathe or wash as usual before arrival at the facility.
- 0.2 ml or 0.2g of the test material is dispensed onto the occlusive, hypoallergenic patch.
- The patch is then applied directly to the skin of the infrascapular regions of the back, to the right or left of the midline and the subject is dismissed with instructions not to wet or expose the test area to direct sunlight.
- After 24 hours the patch is removed by the panelist at home.
- This procedure is repeated until a series of nine consecutive 24 hour exposures have been made for every Monday, Wednesday, and Friday for three consecutive weeks.
- In the event of an adverse reaction, the area of erythema and edema is measured. The edema is estimated by the evaluation of the skin with respect to the contour of the unaffected normal skin. Reactions are scored just before applications two through nine and the next test date following application nine. In most instances this is approximately 24 hours after patch removal. Clients are notified immediately in the case of adverse reaction and determination is made as to treatment program if necessary.
- Subjects are then given a 10 - 14 day rest period after which a challenge or retest dose is applied once to a previously unexposed test site. The retest dose is equivalent to any one of the original nine exposures. Reactions are scored 24 and 48 hours after application.
- Comparison is made between the nine inductive responses and the retest dose.

8.0 Results:

Please refer to attached Table.

9.0 Observations:

No adverse reactions of any kind were noted during the course of this study.

10.0 Archiving:

All original samples, raw data sheets, technician's notebooks, correspondence files and copies of final reports and remaining specimens are maintained on premises of AMA Laboratories, Inc. in limited access storage files marked "Archive". A duplicate disk copy of final reports is separately archived in a bank safe deposit vault.

11.0 Reference:

Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics, published by The Association of Food and Drug Officials of The United States, 1965 (modified).

12.0 Conclusions:

The test material (AMA Lab. No.: L-2090; Client No.: EN080110-E) when tested under occlusion as described herein, may be considered:
a **NON-PRIMARY IRRITANT** and **NON-PRIMARY SENSITIZER** to the skin according to the reference.

Mayya Tatsene, M.D.
Study Director

Patrycja Bienias, M.S.
Technician

David R. Winne, B.S.
Technical Director

2/18/08
Date

TABLE
SUMMARY OF RESULTS
(Occlusive Patch)

AMA Lab No.: L-2090
Client No.: EN080110-E

No.	Subject ID	RACE	SEX	Response 1	2	3	4	5	6	7	8	9	Chall. 24 HR	48 HR	Score
1	25 0215	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
2	28 0971	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
3	34 4672	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
4	36 2168	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
5	36 7304	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
6	36 7970	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
7	36 8248	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
8	40 6489	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
9	42 1835	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
10	42 1837	C	F	0	0	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	N/A
11	44 9258	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
12	46 4172	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
13	48 4004	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
14	50 1699	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
15	50 1729	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
16	50 3800	A	M	0	0	0	0	0	0	0	0	0	0	0	0.0
17	50 5772	C	M	0	0	0	0	0	0	0	0	0	0	?	0.0
18	50 8253	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
19	52 4898	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
20	52 5000	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
21	54 0763	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
22	54 1935	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
23	54 2951	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
24	54 4408	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
25	54 6357	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
26	56 0719	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
27	56 3659	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
28	56 4962	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
29	56 5529	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0

TABLE (CONT'D)
SUMMARY OF RESULTS
(Occlusive Patch)

AMA Lab No.: L-2090
Client No.: EN080110-E

No.	Subject ID	RACE	SEX	Response 1	2	3	4	5	6	7	8	9	Chall. 24 HR	48 HR	Score
30	58 3087	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
31	58 3965	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
32	58 7412	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
33	58 9750	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
34	60 0082	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
35	60 1825	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
36	60 2888	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
37	60 3135	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
38	60 6328	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
39	60 9336	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
40	62 3596	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
41	62 5624	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
42	62 8070	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
43	64 2464	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
44	64 4340	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
45	64 6653	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
46	64 8003	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
47	66 1927	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
48	70 5391	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
49	72 2318	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
50	76 2719	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
51	82 4417	H	M	0	0	0	0	0	0	0	0	0	0	0	0.0
52	90 3845	H	F	0	0	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	N/A

Evaluation Period:

This study was conducted from January 14, 2008 through February 15, 2008.

Scoring Scale and Definition of Symbols Shown in Table:

0 - No evidence of any effect

? - (Barely perceptible) minimal faint (light pink) uniform or spotty erythema

1 - (Mild) pink uniform erythema covering most of contact site

2 - (Moderate) pink\red erythema visibly uniform in entire contact area

3 - (Marked) bright red erythema with accompanying edema, petechiae or papules

4 - (Severe) deep red erythema with vesiculation or weeping with or without edema

D - Patch eliminated due to reaction

Dc - Discontinued due to absence of subject on application date

M - Patch applied to an adjacent site after strong test reaction

N/A - Score is not calculated for subjects discontinued before challenge

S - Skin stained from pigment in product

T - Tan

NOTE: All technical employees of AMA LABORATORIES, INC. are required to take and pass a visual discrimination examination conducted by a Board Certified Ophthalmologist using the Farnsworth-Munsell 100 Hue Test as published; which determines a person's ability to discern color against a black background. This test was additionally modified to include a flesh tone background more nearly approaching actual use conditions, wherein erythematous skin is graded according to intensity.

13.0 Quality Assurance Statement:

This study was inspected in accordance with the Standard Operating Procedures of AMA Laboratories, Inc. To assure compliance with the study protocol, the Quality Assurance Unit completed an audit of the study records and report.

Report reviewed by:

Kamil Wojtowicz

Kamil Wojtowicz, M.S.
Quality Assurance Supervisor

2/18/08
Date

Memorandum

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

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

DATE: May 7, 2021

SUBJECT: Leuconostoc/Radish Root Ferment Filtrate

Active Microtechnologies. 2021. Composition Leucidal® Liquid (Leuconostoc/Radish Root Ferment Filtrate).

Active Microtechnologies. 2020. Specifications Leucidal® Liquid (Leuconostoc/Radish Root Ferment Filtrate).

Distributed for Comment Only -- Do Not Cite or Quote

ACTIVE MICRO TECHNOLOGIES

107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

May 6, 2021

To whom it may concern,

This letter is to certify that Leucidal® Liquid (M15008), manufactured by Active Micro Technologies, LLC, located at 107 Technology Drive, Lincolnton, NC 28092, is standardized for 0.10 – 0.50% Bacteriocins, 18.0 – 22.0% Phenolics and 48.0 -52.0% Solids as noted on the product Specification.

Solids often refers to the amount of dry extract within a tested liquid. The typical chemical composition of the solids and water content within Leucidal® Liquid is as follows:

Content	**Percentage (%)**
Water Content	48.80%
Protein Content	30.60%
Phenolics Content*	20.10%
Polysaccharide Content	0.50%
Total	**100.00%**

*tested as salicylic acid

We further certify that Leucidal® Liquid does not contain detectable amounts of the five organic acids tested for via HPLC analysis, such as Citric Acid, Malic Acid, Tartaric Acid, Lactic Acid, and Glycolic Acid.

Please note that the values above are based on testing of one particular lot. All other lots may not be an exact match to these particular values. Leucidal® Liquid is not standardized for protein or polysaccharide content at this time.

Thank you for your interest in Active Micro Technologies' 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

www.activeconceptsllc.com

This information is presented in good faith but is not warranted as to accuracy of results. Also, freedom from patent infringement is not implied.
This information is offered solely for your investigation, verification, and consideration.

ACTIVE MICRO TECHNOLOGIES
107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

US Patent Number 10,159,708
US Patent Application Number 16/205,883

Specification

Product Name: Leucidal® Liquid
Code Number: M15008
CAS #'s: 1686112-10-6 (or) 84775-94-0
EINECS #'s: N/A (or) 283-918-6
INCI Name: Leuconostoc/Radish Root Ferment Filtrate

Specification	Parameter
Appearance	Clear to Slightly Hazy Liquid
Color	Yellow to Light Amber
Odor	Characteristic
Solids (1g-105°C-1hr)	48.0 – 52.0%
pH	4.0 – 6.0
Specific Gravity (25°C)	1.140 – 1.180
Ninhydrin	Positive
Phenolics (tested as Salicylic Acid)[1]	18.0 – 22.0%
Heavy Metals	< 20 ppm
Lead	< 10 ppm
Arsenic	< 2 ppm
Cadmium	< 1 ppm
Bacteriocins (HPLC)	0.10 – 0.50%
Minimum Inhibitory Concentration[2]	
Organism (ATCC#)	
E. coli (#8739)	0.50 – 4.00%
S. aureus (#6538)	0.25 – 2.00%
P. aeruginosa (#9027)	1.00 – 4.00%
C. albicans (#10231)	0.25 – 2.00%
A. brasiliensis (#16404)	0.25 – 2.00%

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107 Technology Drive • Lincolnton, NC 28092
(704) 276-7100 • Fax (704) 276-7101

US Patent Number 10,159,708
US Patent Application Number 16/205,883

DO NOT FREEZE; Store at or near room temperature;
Mix well prior to use; May Sediment upon Standing

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.

Note:

1) Phenolic compounds of natural origin, tested as Salicylic acid via USP HPLC method.
2) Refer to Inhibition Activity Data

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2021 VCRP Frequency of Use Data – Radish Root-Derived Ingredients

Ingredient Name	Category code and Description	CPIS count
Lactobacillus/Radish Root Ferment Filtrate Total: 2		
Lactobacillus/Radish Root Ferment Filtrate	12F- Moisturizing	2
Leuconostoc/Radish Root Ferment Filtrate Total: 255		
Leuconostoc/Radish Root Ferment Filtrate	01B - Baby Lotions, Oils, Powders, and Creams	1
Leuconostoc/Radish Root Ferment Filtrate	03G - Other Eye Makeup Preparations	3
Leuconostoc/Radish Root Ferment Filtrate	04E - Other Fragrance Preparation	5
Leuconostoc/Radish Root Ferment Filtrate	05A - Hair Conditioner	14
Leuconostoc/Radish Root Ferment Filtrate	05E - Rinses (non-coloring)	1
Leuconostoc/Radish Root Ferment Filtrate	05F - Shampoos (non-coloring)	8
Leuconostoc/Radish Root Ferment Filtrate	05G - Tonics, Dressings, and Other Hair Grooming Aids	3
Leuconostoc/Radish Root Ferment Filtrate	07A - Blushers (all types)	3
Leuconostoc/Radish Root Ferment Filtrate	07E - Lipstick	9
Leuconostoc/Radish Root Ferment Filtrate	07I - Other Makeup Preparations	7
Leuconostoc/Radish Root Ferment Filtrate	10A - Bath Soaps and Detergents	30
Leuconostoc/Radish Root Ferment Filtrate	10B - Deodorants (underarm)	1
Leuconostoc/Radish Root Ferment Filtrate	11F - Shaving Soap	3
Leuconostoc/Radish Root Ferment Filtrate	12A - Cleansing	11
Leuconostoc/Radish Root Ferment Filtrate	12C - Face and Neck (exc shave)	39
Leuconostoc/Radish Root Ferment Filtrate	12D - Body and Hand (exc shave)	1
Leuconostoc/Radish Root Ferment Filtrate	12F - Moisturizing	104
Leuconostoc/Radish Root Ferment Filtrate	12G - Night	2
Leuconostoc/Radish Root Ferment Filtrate	12H - Paste Masks (mud packs)	3
Leuconostoc/Radish Root Ferment Filtrate	12I - Skin Fresheners	2
Leuconostoc/Radish Root Ferment Filtrate	12J - Other Skin Care Preps	5
Raphanus Sativus (Radish) Root Extract Total: 2		
Raphanus Sativus (Garden Radish) Root Extract	12C - Face and Neck (exc shave)	2