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

Status:	Draft Final Report for Panel Review
Release Date:	May 23, 2022
Panel Meeting Date:	June 16-17, 2022

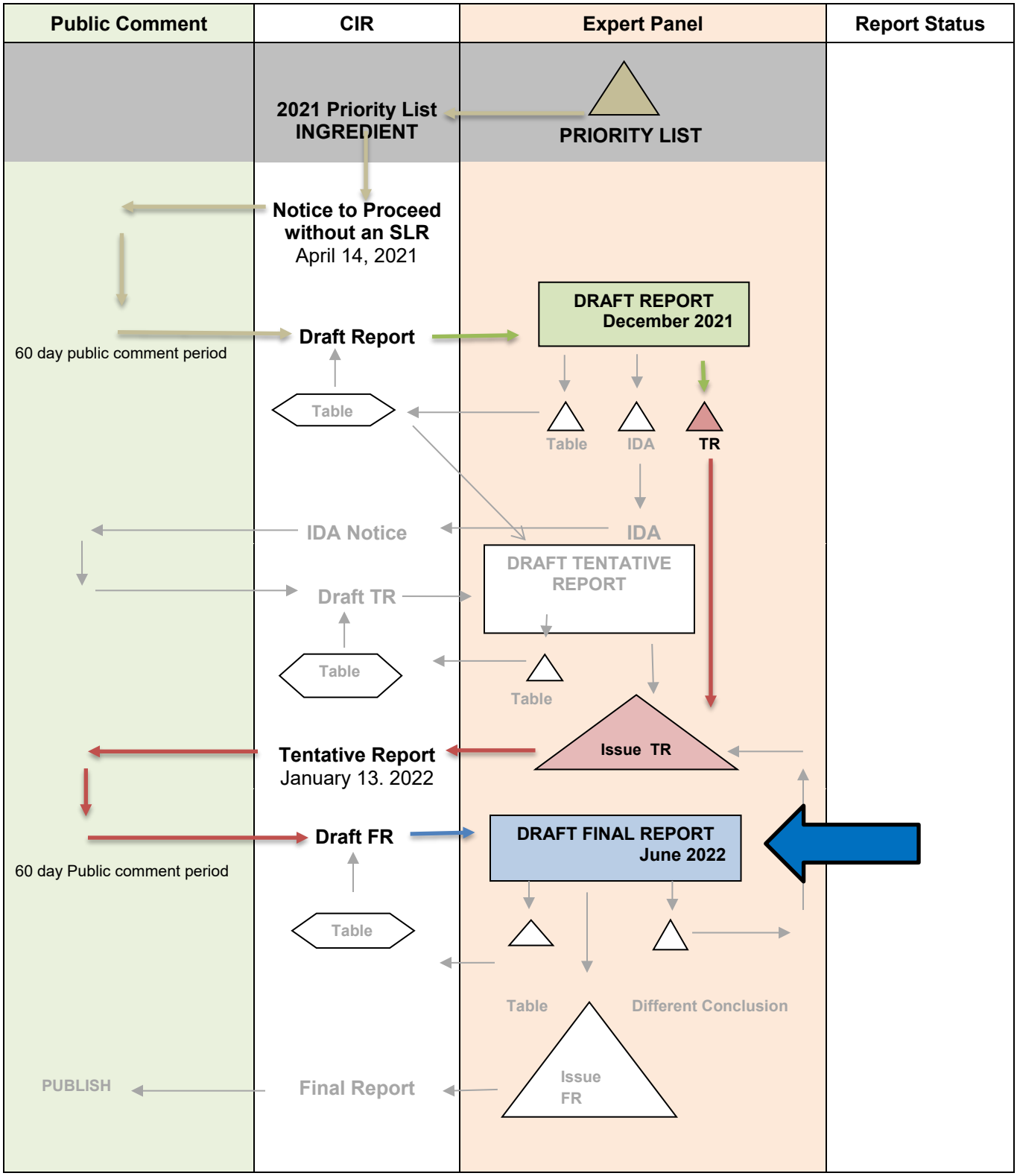
The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D., and Susan C. Tilton, Ph.D. Previous Panel member involved in this assessment: Lisa A. Peterson, 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.

1620 L Street, NW, Suite 1200 ◇ Washington, DC 20036-4702 ◇ ph 202.331.0651 ◇ fax 202.331.0088 ◇
cirinfo@cir-safety.org

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Radish Root-Derived Ingredients

MEETING June 2022



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons

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

Date: May 23, 2022

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

Enclosed is the Draft Final Report of the Safety Assessment of Radish Root-Derived Ingredients as Used in Cosmetics (identified as *report_RadishRoot_062022* in the pdf). This is the second time the Panel is seeing a safety assessment of these 7 cosmetic ingredients. At the December 2021 Panel meeting, the Panel issued a Tentative Report for public comment with the conclusion that these ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment when formulated to be non-sensitizing.

Updated VCRP data were received and have been incorporated (*VCRP_RadishRoot_062022*). Reported use categories and number of uses did not change significantly. Changes reflecting updated VCRP data are highlighted in yellow. Also, changes to the language involving the inhalation exposure boilerplate and use in airbrush delivery systems have been highlighted to aid the Panel's review.

Comments on the Tentative Report that were received from the Council (*PCPCcomments_RadishRoot_062022*) have been addressed. A comments response checklist is included (*response-PCPC*comments*_RadishRoot_062022*).
Also included in this package, for your review, are a flow chart (*flow_RadishRoot_062022*), minutes from the previous meeting (*transcripts_RadishRoot_062022*), literature search strategy (*search_RadishRoot_062022*), ingredient data profile (*dataprofile_RadishRoot_062022*), and ingredient history (*history_RadishRoot_062022*).

The Panel should carefully consider the updated data, the Abstract, Discussion, and Conclusion, and be prepared to issue a Final Report.

1620 L Street, NW Suite 1200, Washington, DC 20036
(Main) 202-331-0651 (Fax) 202-331-0088
(email) cirinfo@cir-safety.org (website) www.cir-safety.org

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: January 27, 2022

SUBJECT: Tentative Report: Safety Assessment of Radish Root-Derived Ingredients as Used in Cosmetics (release date January 13, 2022)

The Personal Care Products Council respectfully submits the following comments on the tentative report, Safety Assessment of Radish Root-Derived Ingredients as Used in Cosmetics.

Key Issue

Definition and Plant Identification; Composition and Impurities – These two sections do not agree. The Definition and Plant Identification section suggests that peptides are removed from filtrates (cited to reference 5) while the Composition and impurities section states that Leuconostoc/Radish Root Ferment Filtrate is comprised of 30.6% protein (cited to reference 12). The Definition and Plant Identification section should be revised so that it does not suggest that peptides are removed from filtrates.

Additional Considerations

Introduction; Summary – The Introduction and Summary should clearly state which functions are considered drug functions (antimicrobial, anti-dandruff, antifungal) and are not considered under the purview of the Expert Panel for Cosmetic Ingredient Safety.

Genotoxicity, Raphanus Sativus (Radish) Root Juice – It should be clearly stated that the radish ingredients tested were not genotoxic in the normal cell line (PAE).

Discussion – Where is the evidence that the ingredients included in this report are eaten in food? It would be better to state that the radishes from which the ingredients are derived are eaten as food. Use of the word “including” when referring to Lactobacillus and Leuconostoc bacteria suggest other bacteria may be used to make these ingredients. Please delete the word “including”.

Radish Root-derived Ingredients - June 16-17, 2022 Panel Meeting – Preethi Raj			
Comment Submitter: Personal Care Products Council **Date of Submission: January 27, 2022 (Comments on TR from December 2021 meeting)**			
#	**Report section/Comment**	**Response/Action**	**Needs Panel Input**
1	Definition & Plant Identification-- Correct statement that peptides are removed from filtrates (ref 5)	Revised – replaced with 'larger weight molecules' as stated in ref 5	
2	Introduction: Summary – in both sections, clearly state which functions are considered drug functions (antimicrobial, anti-dandruff, antifungal) and are not considered to be under the Panel's purview	Revised	
3	Genotoxicity, Root Juice – clearly state that the radish ingredients tested were not genotoxic in the normal (PAE) cell line	Revised	
4	Discussion – better to state that the radishes from which the ingredients are derived are eaten as food (there is no evidence in report that the ingredients are eaten in food) -delete 'including' because that suggests that other bacteria may be used to make these ingredients (only *Lactobacillus* & *Leuconostoc* are relevant)	Revised	

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: Kow 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 was presented to the Panel. The Panel considered that the root portion of the Raphanus sativus plant is consumed as food, and that foods fermented with lactic acid and the Leuconostoc bacterial strains have GRAS status, mitigating any systemic or dermal toxicity concerns. Although some data suggested the potential for a root juice and a methanolic root extract of Raphanus sativus to cause skin-lightening, the Panel concluded that the use concentrations of these ingredients in cosmetics are too low, and not purified or potent enough to produce a skin-lightening effect, which is considered to be a drug effect.

The Panel issued a Tentative Report for public comment with a conclusion that these 7 radish root-derived ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment when formulated to be non-sensitizing.

No additional data were found or received.

June 2022

A Draft Final Report is being presented to the Panel.

Radish Root -derived Ingredients Profile* – June 16-17, 2022 – 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
Lactobacills/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																	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 (Updated 4/18/2022)

PubMed

((((((((((lactobacillus/radish root ferment extract filtrate) OR (lactobacillus/radish root ferment filtrate)) OR (leuconostoc/radish root ferment filtrate)) OR (leuconostoc/radish root ferment lysate filtrate)) OR (1686112-10-6)) OR (84775-94-0)) OR (radish root extract)) OR (raphanus sativus root extract)) OR (raphanus sativus root juice)) OR (radish root juice)) OR (raphanus sativus root powder)) OR (radish root root powder)) AND (toxicity)- 25/0

General Web Search (Google)
Lactobacillus/radish root ferment extract filtrate toxicity – 58/0
Lactobacillus/radish root ferment filtrate toxicity – 68/0
Leuconostoc/radish root ferment filtrate toxicity – 71/0
Leuconostoc/radish root ferment lysate filtrate toxicity – 30/0
Raphanus Sativus (radish) root extract toxicity –83/2
Raphanus Sativus (radish) root juice toxicity – 81/0
Raphanus Sativus (radish) root powder toxicity – 84/0
Fermented radish dermal safety – 61/0
Probiotic radish dermal safety – 40/0

LINKS

Search Engines

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

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

DECEMBER 2021 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Belsito Team – December 6, 2021

DR. BELSITO: You got a lot of first-timers here, Preethi. This is, again, the first time that we're looking at this. We've got a whole lot of material there. We're looking at primarily all of the ingredients are root, and the root is a food, is GRAS, right? So that should allay our systemic toxicity, no?

DR. SNYDER: Right.

DR. LIEBLER: Correct.

DR. KLAASSEN: Yes.

DR. BELSITO: We have the fermentation, and it's *Lactobacillus* and another bacillus that both of which are used to ferment -- *Leuconostoc*, both are used to ferment food. It did mention that, for some reason under non-cosmetic use, that one the *Raphanus sativus* derived ingredients, but also the *Lactobacillus* are also used in foods, right?

DR. LIEBLER: Yep.

DR. BELSITO: So I wasn't concerned with the bacterial use. We do have the issue of the skin lightening potential again with this one, so we need to come up with that boilerplate or, Dan, you thought this wasn't an issue. I think you emailed me yesterday.

DR. LIEBLER: Right. Yeah, Paul and I both felt that radical scavenging might be appropriate. I'm not sure why the term skin lightening was even in the subhead for this because I don't think there's any evidence that these are skin lighteners. Now the point of these two paragraphs and the data cited is that these roots are chock full of antioxidant-like molecules: flavonoids and phenolics. They were evaluated for tyrosinase inhibition. These are all in vitro. I'm talking about PDF 18.

DR. BELSITO: Yeah, I'm with you.

DR. LIEBLER: The DPPH radical scavenging assay. So they can scavenge these model oxidants. Many chemicals that can do this kind of radical scavenging in vitro can also inhibit tyrosinase reaction in vitro. Of course, tyrosinase is a key enzyme initiating the synthesis of melanin from tyrosine. But just because in an in vitro system tyrosinase can be inhibited by an extract from a plant. It doesn't mean that extract is going to produce any skin lightening. That's a big jump, and without some kind of biological system or in vivo evidence for skin lightening potential, I would tend to discount this.

So I think the use of skin lightening potential in the subheading is really not justified. I think this is just a representation of the fact that these radish root extracts are chock full of antioxidant-like molecules, and they can do the antioxidant reactions that are listed in these couple paragraphs.

DR. BELSITO: So does that go into the discussion as well since we do mention that it has a potential effect on tyrosinase?

DR. LIEBLER: Correct, yeah.

DR. BELSITO: Okay, so you sort of got the gist of that, Preethi, for the discussion?

MS. RAJ: Yeah, so it looks like I would, I guess, have in the discussion the potential effect on tyrosinase inhibition, but not necessarily mention the skin lightening potential. Is that right?

DR. BELSITO: Well, what we're doing is, first of all, in the title here, we're getting rid of the skin lightening and just saying radical scavenging, right?

DR. SNYDER: Antioxidant activity.

DR. LIEBLER: Yeah. Radical scavenging and antioxidant reactions.

DR. SNYDER: Yeah.

MS. RAJ: Okay.

DR. SNYDER: Even though this, the last sentence is what the authors surmise, it's a very crappy scientific summary to say it may be responsible, possibly, and so there's two wishy-washy things in there of which I didn't think they had a leg to stand on. That was quite the stretch for me.

DR. LIEBLER: Yeah, that was unjustified speculation.

DR. SNYDER: Yeah, exactly. They definitely are antioxidants, that's well-known, but the relationship to the melanin and anti-tyrosinase is -- I just echo what Dan said, that was quite a stretch.

MS. RAJ: So would you want that sentence deleted?

DR. SNYDER: I would. I just don't think it's a valid summary. I mean, if you want to address it, it has to be addressed in the discussion to say that it was not a valid scientifically justified summation.

MS. RAJ: Okay.

DR. BELSITO: Are we striking it from this paragraph or keeping it and then putting it into the discussion?

DR. SNYDER: I would prefer to take it -- since we took out the skin lightening potential subheading and just said radical scavenging activity, I would just leave it at that.

DR. LIEBLER: Yeah, I would delete it.

DR. SNYDER: This is just you can make stuff up. I mean, you can say that something else may be or possibly.

DR. BELSITO: Okay. There are a couple of places here where that's discussed. In the middle of the paragraph, it says, "the freeze-dried juice showed higher potential for tyrosinase inhibition." Does that stay?

DR. LIEBLER: Oh, yeah, that's fine. That's true. That was demonstrated.

DR. BELSITO: Okay. So we're just striking the last sentence, "surmise that the higher ascorbic acid root juice compared to root extract, as well as the higher phenolic content may be responsible for greater anti-tyrosinase." We getting rid of anti-tyrosinase and just leave radical scavenging? What are we doing with that sentence?

DR. LIEBLER: Well, most of the sentence is commenting on the data that they actually generated. It's that last clause, "possibly leading," or it says "lending," but "possibly leading to skin lightening." That's the part we have a problem with.

DR. BELSITO: So we delete that portion. We end the sentence at scavenging activity, period?

DR. LIEBLER: Yeah. So my question here for Preethi, are you literally quoting them in this sentence, or did you derive this language yourself?

MS. RAJ: I believe that I wrote it as it was in the paper, Dr. Liebler, but I can go back and check.

DR. SNYDER: They're really saying it could have been due to the root juice or it could have been the higher phenolic content. I mean -- as well as the higher content that may be responsible. So it wasn't -- it's just I don't think that they have the data to --

DR. BELSITO: They don't because they used the word "lending." I presume that should be leading, but --

DR. LIEBLER: So this is why I asked Preethi if she wrote this or if they wrote this. I think, if they wrote this and they made this assertion and it got published in their paper even if it's not supportable, then I think we need to keep this in. Then I think we can briefly deal with it in the discussion because we don't want to make it look like we're editing parts of the paper out that we don't want to report even if we don't agree. We can point out in the discussion that we don't agree that this is not a skin-lightening potential. I think we can take out skin-lightening potential out of the subhead title as we talked about.

MS. RAJ: Okay.

DR. LIEBLER: Are you all okay with that?

MS. RAJ: Yes.

DR. BELSITO: Yeah.

DR. SNYDER: All right.

DR. BELSITO: All right, Preethi, you're going to check if they used the words, that stays, and then we need to discuss it. If they don't use the words, you're striking it and we don't need to discuss anything about skin lightening, is that correct?

MS. RAJ: Yes. Thank you.

DR. SNYDER: Yep.

DR. BELSITO: Okay.

DR. LIEBLER: But, Don, I would prefer not to have the skin lightening in that subheading. We can talk about --

DR. BELSITO: Yeah, we deleted the skin thing.

DR. LIEBLER: Okay. Okay. Okay.

DR. BELSITO: No, no, the heading is radical scavenging and antioxidant activity.

DR. LIEBLER: Okay. Thank you.

DR. BELSITO: Yeah. So, in terms of skin sensitization, we have two negative in vitros that clears our sensitizing. I'm fine with the skin. Even though the HRIPT was not done at the max, which is six percent I believe, but we have the in vitro and the

HRIPT that we do have. One is on 0.04 in 105 subjects, and the other is over 10 percent but on a small number. I thought was fine.

I guess, my concern was they did an in vitro phototox study. Does this raise any concern for photosensitization? We really didn't have the components, although they are very readily available in the literature. I did a search and got radish composition, and I really didn't see anything that struck me, although there were a lot of cyclic molecules. Dan, did you get a chance to look at any of those articles? Did anything pop at you as photosensitizing?

DR. LIEBLER: Yeah. There's nothing photosensitizing in there. There's a couple of papers you had on purification of enzymes involved in electron transfer biochemistry. They're not relevant to photosensitization. They are part of non-photosynthetic electron transport as is appropriate for a root. Then, I sort of think the third article was something else. I think it was just components of --

DR. BELSITO: One was on iso peroxidases, the other one was on iso proteins, and the third actually looked at composition.

DR. LIEBLER: Yeah. None of those have anything to do with photosensitization at all.

DR. BELSITO: Okay. But should we include those to say that we're not concerned?

DR. SNYDER: No, but this was negative, right? It's a negative photosensitizer.

DR. BELSITO: No, the phototox was negative; we don't know about photosensitization.

DR. LIEBLER: Those articles have nothing to do with photosensitization or --

DR. SNYDER: No, but this one that's in the report right now says it was not considered a photosensitizer at 0.4, 1.2, or 3.7 percent.

DR. BELSITO: That's not correct. The conclusion is not correct. That's not a photosensitization test; it's a phototoxicity test.

DR. SNYDER: Okay. Okay, okay, okay.

DR. BELSITO: There's no in vitro test for photosensitization.

DR. SNYDER: Okay.

MS. RAJ: I think the council comment had said to correct that to say it's not a photo irritant, is that right?

DR. BELSITO: Right. That's correct.

MS. RAJ: Okay.

DR. BELSITO: Yeah, so we don't have photosensitization data, and we don't have composition.

MS. RAJ: If I may clarify, the articles Dr. Liebler was talking about, are they in the report already or is --

DR. BELSITO: No, they're not. They're articles that I looked up trying to figure out composition.

MS. RAJ: I see.

DR. BELSITO: Dan, did you not feel that the Hanlon and Barnes article, "The Phytochemical Composition and Biological Activity of The Radish Sprouts and Mature Taproots," that should have covered everything, no?

DR. LIEBLER: Yeah, I just reopened it to take a look at the abstract again. Hang on, okay. Oh, yeah, this is the one with the isethionates and glucosinolates.

DR. BELSITO: Yeah.

DR. LIEBLER: Yeah, these are not photosensitizing-involved chemicals.

DR. BELSITO: If Preethi includes this article, she doesn't need the other two. We can clear the photosensitization?

DR. LIEBLER: No, no, this doesn't have anything to do with photosensitization. I mean, these are compounds that are present in a radish. They don't absorb. I don't think they are significant UV absorbers.

DR. BELSITO: Right.

DR. LIEBLER: The whole point of these is that they, well, they're phenolic or flavonoid -- anthocyanins, excuse me; they do absorb light. Then the glucoraphanin or glucoraphasatin are the glucosinolates. They break down isothiocyanates, which induce antioxidant cell reactions.

DR. SNYDER: Where's the photosensitization issue coming from?

DR. BELSITO: The fact that we don't have any data on composition. I mean, why did they study it as a photo irritant unless they were concerned about a photo effect? That's why I was trying to get composition. Dan, are you saying that some of the components in this Hanlon report are potentially photosensitizers?

DR. LIEBLER: I don't know if these anthocyanins are photosensitizers, Don, off the top of my head. At the very least, this paper can go into the composition and impurities.

DR. SNYDER: Yeah, I thought it was a really good paper. Had a lot of good data in there.

DR. BELSITO: Okay, so I can send that. I don't think that I have your email address, Preethi, but I can send it to Bart, and he can forward it to you. The other two papers, Dan, you didn't feel were helpful?

DR. LIEBLER: No.

DR. BELSITO: Okay.

MS. RAJ: I do have a question though. I mean, typically we're looking at things as mixtures. So, I mean, I know in searching for these ingredients, I had come across papers discussing iso- -- I'm not pronouncing it right -- isothiocyanates, but again, we're not trying to look at the individual components, right?

DR. BELSITO: Right. I understand, but they can trigger some tox endpoints, right?

DR. LIEBLER: Yeah, what I don't know is whether the anthocyanins or the anthocyanidins are photosensitizers or photo anything. Okay? They're flavonoid-like structures, flavonoid family type structures. They certainly absorb UV light, but, whether or not that leads to any significant excitation that has an adverse outcome pathway associated with it, I don't know.

DR. BELSITO: Yeah.

DR. LIEBLER: Off the top of my head. Then, the isothiocyanates don't have chromophores that absorb light, at least in the phototox, photosensitization, photo irritation range. The story with those is they are inducers of the expression of protective antioxidant enzymes.

DR. BELSITO: Right.

DR. LIEBLER: It's like, the same thing's in broccoli, right? They induce protective antioxidant enzymes through this antioxidant response element, ARE.

DR. BELSITO: Right. Are we --

MS. RAJ: Thank you.

DR. BELSITO: Are we dealing with photosensitization at all?

DR. LIEBLER: I don't think so.

DR. SNYDER: I didn't think so.

DR. BELSITO: Okay. But, in answer to your question, Preethi, this article is looking at the whole root, so it is the ingredient that we're looking at, and it has composition data, which I think is helpful.

MS. RAJ: Okay. Well, I'll be happy to add whatever you found, so, yes, please send it my way.

DR. BELSITO: Yeah. Then, where are we with these? Safe as used?

DR. LIEBLER: Yes.

DR. KLAASSEN: I agree.

DR. BELSITO: Okay. So, in the discussion, we have the botanical boilerplate if it can be modified once everyone looks at the composition data that I'm going to send. Respiratory -- we'll address skin lightening only if the authors actually had that in their conclusion statement that the GRAS obviates lack of the systemic data, and that the conclusion is safe as used. Correct?

DR. LIEBLER: Correct.

DR. BELSITO: Anything else on these?

MS. RAJ: I guess, inhalation boilerplate?

DR. BELSITO: Yes. I mentioned that. So botanical, respiratory boilerplate, and then address skin lightening only if the author's concluded it. The GRAS status for these obviates the relative lack of systematic absorption.

MS. RAJ: Okay. Thank you.

DR. LIEBLER: In the inhalation boilerplate, note the low toxicity systemic safety and low concentration of use in spray products.

MS. RAJ: Sorry, say that again, Dr. Liebler.

DR. LIEBLER: So, for the inhalation boilerplate, add to it that there's no evidence of systemic toxicity and very low use concentrations in spray products.

MS. RAJ: Okay. Great, thank you. I got it.

DR. LIEBLER: Great.

DR. BELSITO: Okay. Anything else?

DR. LIEBLER: That's it.

DR. BELSITO: Okay. We will see you tomorrow at 8:30.

MS. BURNETT: One more thing before we leave.

DR. BELSITO: Oh, what?

Cohen Team – December 6, 2021

DR. COHEN: Okay. So a radish root, Preethi this is yours as well. This is a draft report, first time we're reviewing it. There are seven ingredients we're looking at. It's used as a hair and skin conditioning agent. We have a frequency of use reported. We have max use of the root extract at 6 percent in a lipstick and the root ferment filtrate at 1.1 percent in some skin cleansers. I was going to ask the group to comment. We see the root ferment filtrate was not considered a photosensitizer at 0.41, 1.2, and 3.7 percent concentration but possibly at 11 percent.

And the max use of the root ferment filtrate is at 1.1 percent, but the max use for the root extract is higher. I think we need irritation and sensitization at max use because we have an eyebrow gel formulation at 0.04. This also discusses some skin lightening qualities. I'll stop there. There's one or two more comments I'll make later. Lisa?

DR. PETERSON: So, we have method of manufacturing for all the ingredients. We have composition and impurities for two, so we could probably do some read across there. And then, I guess, the impurities that -- again, here are the impurities to worry about are the pesticides, heavy metals, so the boilerplate would need to be incorporated. I also thought it's inhalation similar to the sage. So, I was on the fence as whether to say there are needs from a chemistry perspective -- having some more information on composition but basically the ferment, we have some information on it.

I guess you can go a little bit more that I don't know the big difference between the lactobacillus and the leuconostoc, but they're not big concerns about reading across between those. And then for the root extract or root powder, there's no impurities, but they're coming from foods. And so that's where I thought the boilerplates would probably work for those.

DR. COHEN: In this case all of them are -- am I right? These are all part of the food part of the radish.

DR. SLAGA: Right.

DR. PETERSON: Yeah, and I think even the ferment is a food.

DR. COHEN: It's part of the root. It's a fermented root, but we're not dealing with the greens on the top, the aerial components --

DR. PETERSON: No, it's all the root. It's all the part that we eat. And actually people eat the -- you can make salads out of the greens on the top too --

DR. COHEN: Radish greens.

DR. PETERSON: -- so --

DR. SHANK: There are many cultivars for this radish, and I think we need clarification on the relationship between the ingredients in this report and the vegetable that's eaten by humans. One place it says that the radish had a 30-centimeter radish, and when I first read that --

DR. COHEN: That's a big radish.

DR. SHANK: -- that's a radish that's over a foot.

DR. PETERSON: It's like horseradish.

DR. SHANK: Right. It's probably a different radish that is a long one, more like a carrot, than the red radish that you buy at the farmer's market. So, if we can be assured that all of these are human foods, that eliminates a lot of the systemic tox data requirements. If not, then we need a whole lot of data. So, at square one is what is the relationship between these ingredients and the human food? And then for skin and sensitization data on the root extract, would that apply also to the juice and the powder?

And what about the fermented one? I would think those would be quite different from a freshly expressed juice.

DR. COHEN: So, Ron, you're asking for sensitization and irritation?

DR. SHANK: Well, Raphanus sativus root extract and can that apply -- I think the first thing is we have to know what we're dealing with, and I was intrigued by a 30-centimeter radish. So I went, and I looked up radishes. And they're all kinds of different radishes, and they're listed in the report. Are all of these human foods? That I could not find out.

DR. COHEN: We'll ask for that.

DR. SHANK: Because if the answer's yes, that changes data needs greatly.

DR. COHEN: So just playing that out, if they're not, we'll need further information. If they are, then we're going to need sensitization and irritation at max use of what, though? Because we're going to try to read across on some of these things. I suspect the ferment filtrate is a bit different than just the powdered juice and extracts, right? So, what would satisfy us for sensitization and irritation, assuming they're all foods? Ferment, any of the fermented products at max use and the non-fermented products at max use?

DR. SHANK: The only sensitization data we have is on one --

DR. SLAGA: Ingredient, luco.

DR. SHANK: -- ferment.

DR. COHEN: The ferment.

DR. SHANK: One of them.

DR. COHEN: At 0.04 percent. Which is much less than -- I shouldn't say half. It's like a tenth the max use.

MS. FIUME: On PDF page 20, it looks as if it was tested as a 10 percent dilution.

DR. COHEN: Yeah, so, Monice, I have that as my final comment. Is that 10 percent of the lucidol solution, or is that 10 percent of the ferment filtrate? I couldn't tell. I didn't understand it.

MS. FIUME: Preethi, can you give some input?

MS. RAJ: Yes, so I believe that's the trade mixture. I'm trying to look back at the data file now, but I think that was a trade mixture, which includes the ferment filtrate in it.

DR. COHEN: Right, so if it's 10 percent -- it seemed to be 10 percent of the lucidol solution, which is the trade name for this. But I didn't know how that related to the max use of the filtrate in the final product. So, is it one tenth, or is it full -- I don't know what the concentration of that HRIPT is?

MS. RAJ: Yeah, but correct me if I'm wrong, Monice, but isn't it the percentages that they were referring to -- wouldn't it be the percentage of the trade mixture as the mixture? Sorry.

DR. COHEN: You're on mute, Monice.

MS. FIUME: The test material was received as a 10 percent dilution in water, so it would be a 10 percent of the lucidol. Do we have the -- how much of the leuconostoc is actually in lucidol?

DR. COHEN: I didn't find that.

MS. RAJ: I think it was the 10 percent.

DR. COHEN: And in the max use reporting is it only this product that we're talking about? Is this the only one?

MS. RAJ: This was definitely a main ingredient that we had a lot of data for.

MS. FIUME: So according to PDF page 79 --

DR. COHEN: 79.

MS. FIUME: So that's composition. Page 80, so solids are 48 to 52 percent, but I don't know -- it doesn't -- unless I'm missing something it's not clearly saying how much of this leucocyte -- or the radish root is in the material. It just says it's 48 to 52 percent solids, so that may be the cosmetic ingredient. Jay or Linda, if you're on, can you provide any more clarification for the 48 to 52 percent solids is actually the cosmetic ingredient percentage?

DR. ANSELL: No, I mean, just -- we'll take that back and try to see whether we can put more information concerning that.

DR. COHEN: Preethi, yeah, you make a lot of sense though because of this 255 formulations with the ferment filtrate in it and two with the root extract, right? And we have a 0.04 HRIPT on the root extract, which is way off from the 6 percent on the lipstick, but if this HRIPT is actually 10 percent, we're way over for the ferment filtrate, which is in the majority of the products.

DR. SLAGA: Yeah.

DR. COHEN: So, we're going to go out with an IDA on this, right?

DR. SLAGA: Right.

DR. COHEN: And we're going to ask for the relationship between the ingredients in the report and its relationship to food.

DR. SLAGA: Yes, please.

DR. COHEN: Yes. And sensitization and irritation for the root extract and ferment filtrate at max use but we may learn we have that; we're just not sure.

MS. RAJ: And, Dr. Cohen, I'll ask you to repeat all this again if you don't mind. But would you be asking for ferment filtrate data for both ingredients, like, not just the leuconostoc but also the lactobacillus which we don't really have anything on?

DR. SHANK: I'd say yes.

DR. SLAGA: Yes.

DR. SHANK: I don't think we can assume they're the same.

DR. SLAGA: Right now, we can't. Yeah.

DR. COHEN: So, all of the -- basically, all of the fermentation products. Okay.

DR. BERGFELD: I have that you need to put notes in your discussion to think about inhalation again and (audio skip) just to make sure that those are addressed there.

MS. FIUME: Okay. And then can I just ask one more question, and hopefully it'll clarify for you as well, Preethi. On PDF page 17, it says that Raphanus sativus roots are consumed as cruciferous vegetables. So, is that enough information, or do you want a true relationship to what type of radish it is versus what's used in cosmetics?

DR. COHEN: Monice, what was the question?

MS. FIUME: So, Ron was asking for is this truly a food, or is it a different type of radish. So I didn't know what was needed additionally beyond what's in the second paragraph of the non-cosmetic use section so that we can make sure that we convey the request clearly.

DR. COHEN: Ron, that's on you, that question, I think.

DR. SHANK: Okay. The Raphanus sativus roots it says are, I suppose, worldwide. There are all types of cultivars of that, and they're very, very different if you look at pictures of them. So I need to have that clarified. Are all of these ingredients for cosmetics derived from human food radishes? And I couldn't find that out.

MS. FIUME: Thank you. I was missing the part about the cultivars. That helps, I think, for what we need to request.

DR. SHANK: Thank you.

DR. COHEN: Should we also be asking for phototox data? I mean, there's some signal here in the ferment filtrate. I can only see that possibly being worse in just fresh material coming and being used.

MS. RAJ: I thought it was considered a negative signal, Dr. Cohen.

DR. COHEN: Well, it said it was negative at 0.4, 1.2, and 3.7. But 11 percent had possible photosensitizing effects. Did I get that right?

DR. BERGFELD: I see that.

MS. RAJ: Yeah, I see that.

DR. SHANK: That's right.

MS. RAJ: From a request standpoint, if there is concern being that it's going out in the IDA, I think it'd be better to ask now than maybe have a concern resurface later.

DR. COHEN: Yeah, we'll ask for it, and we can see what comes through.

MS. RAJ: And this is, again, for the ferment ingredients phototox?

DR. COHEN: Well, I might ask for it for whatever we may be able to get from the rest of the plant.

DR. SHANK: Okay.

DR. COHEN: I'll do a little more background on that.

MS. RAJ: Dr. Cohen, if you could please repeat the needs again.

DR. COHEN: Okay. So, it's an IDA. We want sensitization and irritation of the root extract and all the fermentation products at max use. We'd like clarification on the relationship between the ingredients presented in the report and whether they relate to all cultivars of radishes as being human food.

DR. SHANK: Yes.

DR. COHEN: Just for us, we would in the discussion have comments about the skin lightening products, the skin lightening qualities. We wanted information about the actual concentration of the ferment filtrate in the HRIPT that used 10 percent lucidol solution and photosensitivity at max use of the root. I'm going to look into that a little more tonight, though, on photosensitivity.

MS. RAJ: Okay.

DR. COHEN: Does that work for everyone?

DR. SHANK: Yup.

DR. BERGFELD: Dr. Cohen, can you just say inhalation in the discussion?

DR. COHEN: Yes.

MS. FIUME: That's just discussion, not a data request of the inhalation.

DR. COHEN: Right.

MS. FIUME: Discussion.

DR. COHEN: Preethi, does that seem reasonable the way we have it lined up?

MS. RAJ: Yes, yes. I guess we'll see what we get as far as information on the cultivars. I'm not sure how much we've received in the past but --

DR. COHEN: I guess Ron's point is is there a radish that is grown for cosmetic use that people won't eat? So, if it's just meant for that.

DR. SLAGA: I think radishes are -- almost all of them that are grown are eaten. I mean, that's -- if you go to Japan, you go to India, you go to Thailand, any place, China, there's different ones, and I eat them all. They're all edible.

MS. RAJ: That's definitely the impression I got, Dr. Slaga.

DR. SLAGA: Yeah, that's the impression -- I can't give you a reference for that other than I've eaten most of them and I'm still alive.

DR. COHEN: Tom has not met a radish he wouldn't eat.

DR. SLAGA: I like them fermented -- however.

DR. SHANK: Would you eat a one-foot diameter radish?

DR. SLAGA: Well, that would be for a couple meals.

DR. COHEN: Got to slice that up, Ron. You don't just go at that like an apple.

DR. SHANK: It's okay if it's sliced, huh?

MS. FIUME: David and Ron, can I ask a question then? Because, as Preethi said, sometimes we don't have good luck in getting information on cultivars, is this a case where maybe asking method of manufacture of these ingredients as specific to use in cosmetics would be a good option? Because right now what we have tends to be general method of manufacture.

DR. SHANK: Before we get to that, I would say at least for the toxicology. If all of these are human foods, that eliminates a lot of toxicological data needs. If they're not, some are foods and some are not, and we're going to have a lot of toxicology needs. And this is in addition to methods of manufacture/impurities.

DR. COHEN: So, let's assume we put that question out, and we don't get anything back. Would we just in the discussion indicate that we're under the impression that this report is for radish food?

DR. SHANK: We would have to, yeah. It would be totally insufficient, or the Panel assumes all of these radishes are human foods.

DR. COHEN: Got it. Okay. You want to try to get through silicates?

DR. SHANK: Yeah.

DR. COHEN: Yeah, you know, tomorrow I have silicates and zeolites. I don't think I'm going to make it.

DR. SHANK: That's not fair.

DR. SLAGA: Well, let's play this hand and have fun.

Full Panel – December 7, 2021

DR. BELSITO: Yeah. I was already on radish. So it's getting to lunch time, we have more of the antioxidants. So this is the first time we're looking at this ingredient. We've got lots of data. It is a food. There's no question about it, including the fermented filtrates, the bacteria used are used in food uses. And having looked at all of the data, we thought we could go with a safe as used.

DR. BERGFELD: Is that a motion?

DR. BELSITO: Yes.

DR. COHEN: So, Don, again, before we verify that, did your team feel that you had clarity that the radishes that were discussed in the report represent the universe of food radishes out there? And the question came up are there radishes that are grown just for cosmetic use that are not foods? Because some of these were pretty big radishes, like a foot long radish. So what was your thought on that?

DR. SNYDER: The report defines which radish we're looking at with the genus and species, so that wouldn't include universally all radishes; correct?

DR. BELSITO: Right.

DR. COHEN: So Paul, all of those are food radishes.

DR. SNYDER: Well, they're all Raphanus sativus, and so I think it falls under that -- so that would be defined as reported in this report. If there's a different genus and species of radish, we didn't evaluate that.

DR. COHEN: Okay. And Don, just for my own clarity, this would go out as safe as used when formulated not to be sensitizing because it's a botanical?

DR. BELSITO: No. There are no -- so I provided Preethi with the composition information on this, which is lacking in this report. And there are no obvious sensitizers in this. In addition, we have two in vitro OECD approved tests for sensitization that are negative, so I didn't think we needed the botanical boilerplate here. And for the typical boilerplate for pesticides, yada, yada, yada.

DR. COHEN: Yeah. We had that. We were going to ask for sensitization and irritation on the root ferment filtrate and the root extract at max use. We only had, I think 0.04 percent for an eyebrow gel study, and the max use was 6 percent. So there's a big gap between there, I guess notwithstanding your previous comments.

DR. BELSITO: If you look on PDF page 19 --

DR. SNYDER: Well, I think the 6 percent comes from lipstick. The max leave on is 0.03 percent, and we have a 10 percent HRIPT of the root ferment filtrate with 50 subjects and a 0.04 percent root ferment filtrate with 105 subjects all negative. So I think the 6 percent comes from the lipstick. That's not a leave on.

DR. BELSITO: Right. But in addition, David, on PDF page 19 we have a negative DPRA which means it's not going to bind to protein, and we have a negative keratinases which means it's not going to active the keratinocyte portion. So in the adverse outcome pathway for sensitization, we have two negative tests. And sort of the global rule is two out of three makes this a non-sensitizer, so by definition from the in vitro test, this would be non-sensitizing.

DR. COHEN: I accept that information. I view lipsticks as a pretty vulnerable body site. That's not a wash off. I mean, 6 percent on a lipstick is a pretty high exposure, so I sort of set that as my max use when I was looking at this.

DR. BELSITO: But, again, we have -- I mean, if you look at the composition data, which I know you don't have, and you have the negative in vitro test, I don't think there's anything there that causes alerts. But, I mean, if you want to go formulate to be non-sensitizing and keep the whole botanical boilerplate, I mean, I'm okay with it. I just don't -- manufacturers aren't going to have a problem because it doesn't appear to be sensitizing.

DR. COHEN: Well, that's a little more comforting. One other thing, what did you think of the photosensitivity data? It sort of was that the ferment filtrate was fine until 3.7 percent and 11 percent maybe demonstrated some phototox. I couldn't really find much in the way of reports on radish photosensitive or phytophotodermatitis from it.

DR. BELSITO: Yeah. I mean, again, that's why I looked up the composition of this. I was actually concerned why would someone do an in vitro phototox test unless they thought that it could absorb light. And, again, there're really no components of the radish root that would be UV absorbing. So yeah, I mean, the problem with the higher percentages, I think, is not so pert. A significant reduction was in viability at 11 percent, but that was with and without radiation which suggested to me that there was some cytotoxicity, not phototoxicity. So if you look and --

DR. COHEN: Yeah, no. I saw it. Look, I suppose if we ask for sensitization irritated to max use or we had safe as used not to be sensitizing, they're kind of equivalent in my mind.

DR. BELSITO: Right. I would just go with safe as used to be non-sensitizing, and obviously we'd have the full botanical boilerplate. For the respiratory boilerplate in the discussion, we would mention the food use, so not concerned about systemic toxicity, and the low use in spray products. The skin lightening effect was clarified because Preethi had just sort of added that in. The actual paper had just looked at an effect on tyrosinase, and our group felt that that was really -- we looked at that paper in detail. It was due to the antioxidant effects, and if you have questions on that, Dan could answer that.

DR. LIEBLER: Yeah, Don. Preethi sent the paper around last night. I looked at it this morning before our meeting. Paul and I both looked at it.

DR. BELSITO: I did, too.

DR. LIEBLER: Yeah. Okay. And I think the point of the paper was attempting to demonstrate the utility of these extracts as possible skin lighteners. And they showed very high concentrations of the radish extracts inhibiting tyrosinase at, like, mg per mil concentrations of the extracts. And they went on to say that this might be explored with better purification as a strategy for skin lightening from radish extracts. That's what they said, so they're pushing the skin lightening thing.

So yesterday in our discussion, we wanted to make sure that Preethi wasn't putting in this as an overinterpretation of what they did. But no, this is what their message was. This is the paper we're going to cite, and I think that rather than to represent it as not being about skin lightening, we might as well leave the language that Preethi had in there because it does accurately describe what they were doing and then treat it in the discussion, which I think is not a relevant indicator of skin lightening.

In fact, the paper itself demonstrates very low potency for inhibiting tyrosinase. So it's a little different, Don, than our discussion yesterday afternoon, but yesterday afternoon I hadn't had a chance to read the paper. Paul and Don, let me know if you concur with this assessment.

DR. BELSITO: Right. I mean, they started the paper with the idea of looking to see if there would be tyrosinase activity that might allow this ingredient to be used as a skin lightener and also as an antiaging antioxidant. That was their two purposes, to look at tyrosinase and antioxidant properties. And what they found was that the levels currently are too low, and their suggestion was that they needed further purification methods to make this viable. Right, Dan?

DR. LIEBLER: Yeah.

DR. BELSITO: Yeah. And so all of that could go in our discussion, that this material would not cause skin lightening as presently formulated.

DR. LIEBLER: Right.

DR. COHEN: We're satisfied with that in the discussion point. It was one of our discussion points here and also that the DART was performed with intraperitoneal administration that was not consistent with cosmetic exposure.

DR. BELSITO: Right. Very high level.

DR. LIEBLER: Yeah. Very, very high.

DR. BERGFELD: So we have a motion; right?

DR. COHEN: Seconded.

DR. BERGFELD: Are you seconding the motion of Don? Okay. That it's safe?

DR. COHEN: Wait. No, Wilma. It was a second with a provision.

DR. BELSITO: When formulated to be non-sensitizing.

DR. BERGFELD: With a provision. Okay. So we have a non-sensitizing end on this. Okay. So this was Don's, and David is supporting it with the non-sensitizing statement to the conclusion. Preethi, do we have any discussion that we need to answer with you?

MS. RAJ: No, Dr. Bergfeld. I think the Panel has sufficiently described everything, and I have discussion points.

DR. BERGFELD: Okay. Thank you. I'm going to call the question, then. Those opposing? Abstaining? Unanimous agreement to move forward with a safe non-sensitizing. Yes, David?

DR. COHEN: Wilma, can I just retract my comment about the intraperitoneal for the radish? It was for the next one. I had moved on to that, so, Preethi, just strike that from the radish discussion.

DR. BERGFELD: Okay. All right.

MS. RAJ: Thank you.

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

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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.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D., and Susan C. Tilton, Ph.D. Previous Panel member involved in this assessment: Lisa A. Peterson, 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.

1620 L Street, NW, Suite 1200 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651 ♢ fax 202.331.0088 ♢
cirinfo@cir-safety.org

ABBREVIATIONS

ACP	acid phosphatase
AD	atopic dermatitis
ALT	alanine transaminase
ARE	antioxidant response element
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
HPLC	high performance liquid chromatography
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

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 7 radish root-derived ingredients, most of which are reported to function as hair and skin conditioning agents in cosmetic products. Industry should use current good manufacturing practices to minimize impurities that could be present in botanical ingredients. The Panel reviewed the available data and concluded that these ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-sensitizing.

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] Lactobacillus/Radish Root Ferment Extract Filtrate is reported to additionally function as an antimicrobial agent, while Leuconostoc/Radish Root Ferment Filtrate is reported to also function as an anti-dandruff agent and an antifungal agent; all 3 of these uses are considered drug functions in the United States (US); therefore, use as such does not fall under the purview of the Expert Panel for Cosmetic Ingredient Safety (Panel). Lactobacillus/Radish Root Ferment Filtrate is exclusively reported to function as a preservative, and Raphanus Sativus (Radish) Root Extract is also reported to function as an antioxidant.

The ingredients reviewed in this safety assessment are derived from radish roots, which are consumed as food; 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.

Botanicals, such as radish root-derived ingredients, may contain hundreds of constituents. However, in this assessment, the Panel will assess the safety of each of the radish root-derived ingredients as a whole, complex mixture; toxicity from single components may not predict the potential toxicity of botanical ingredients.

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 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. The other ingredients do not have CAS numbers assigned.

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 larger weight molecules, 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

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

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]

High performance liquid chromatography (HPLC) analyses were used to compare glucosinolate, anthocyanin, and total isothiocyanate concentrations in 8 varieties of radish sprouts and 8-wk old radish tap roots.[16] No anthocyanins were found in the mature tap roots; glucosinolate and isothiocyanate concentrations were significantly greater in the sprouts than in the mature tap roots.

Varying amino acid compositions were observed in anionic and cationic isoperoxidases isolated from crude *Raphanus sativus* enzyme extracts.[17] In another amino acid sequence analysis, 3 isoferredoxin isoproteins were purified from white radish roots, while 2 isoferredoxin isoproteins were obtained from the leaves.[18] Although the amino acid sequence of the root and leaf-derived isoferredoxin isoproteins differed, no significant physiological differences in the coupling activities of these ferredoxin isoproteins were measured in the NADP+-photoreduction system of radish chloroplasts and glutamate synthase.

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, and does not cover their use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety. Therefore, airbrush application of cosmetic products is not assessed by the Panel.

According to 2022 VCRP survey data, Leuconostoc/Radish Root Ferment Filtrate is reported to be used in 254 formulations, 190 of which are leave-on products (Table 3).[19] 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.[20] The 4 ingredients not reported to be in use are listed in Table 4.

Radish root-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%.[20] Leuconostoc/Radish Root Ferment Filtrate is reported to be used in products that may come into contact with the eyes; for example, at up to 0.01% in other eye makeup preparations.[20] Leuconostoc/Radish Root Ferment Filtrate has 1 reported use in baby lotions, oils, powders, and creams. 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%.[20] In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetics would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.

Although products containing some of these ingredients may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

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.[21]

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.[22] Of note, *Raphanus sativus* fermented with *Lactobacillus* strains is consumed as a non-salted dish called Sinki in South Asia.[23] 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.[24] Generally, *Lactobacillus* and *Leuconostoc* strains are used in the lactic acid fermentation of dairy, sauerkraut, and various food products.[25,26]

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.[27] 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.[28] 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 juice extracts were not considered genotoxic towards normal PAE cells. 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

Antioxidant and Radical Scavenging 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.[29] The ability of *Raphanus sativus* root extract and root juice to scavenge DPPH, superoxide anion, and singlet oxygen was measured in triplicate and 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

Details regarding the irritation and sensitization studies summarized below can be found in Table 5.

In a dermal irritancy test, a single application of 30 µl Leuconostoc/Radish Root Ferment Filtrate to 3 tissue samples of a reconstructed three-dimensional human epidermis model (EpiDerm™) was considered non-irritating.[30] The mean percent depletion of cysteine and lysine in response to Leuconostoc/Radish Root Ferment Filtrate was 2.89%, in a direct peptide reactivity assay (DPRA), performed according to Organisation of Economic Cooperation and Development (OECD) test guideline (TG 422C); the test article was predicted to not cause sensitization.[31] 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.[32] In this assay, transfection with the luciferase gene allows for measurement of the activation of the Keap1-Nrf2-ARE complex, a proxy for sensitization. No significant increases in luciferase expression were observed; the test article was predicted to be a non-sensitizer.

Leuconostoc/Radish Root Ferment Filtrate, tested at 10% in water and applied neat at 0.04%, was determined to be neither an irritant or a sensitizer in two separate human repeated insult patch tests, using 50 subjects and 105 subjects, respectively.[33,34]

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™).[35] 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 (significantly higher than use levels in cosmetics), with and without radiation (51.1% and 72.6%, respectively). Leuconostoc/Radish Root Ferment Filtrate was therefore not considered a photoirritant 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.[30] 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.[36] During recent employment in a Korean kitchen, she had been exposed to *Raphanus sativus* roots while chopping fresh young radish, 1 and 3 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.[37] 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. Reported functions for 2 of these ingredients, including use as an antimicrobial agent, an anti-dandruff agent, and an antifungal agent are not considered cosmetic in the US, and therefore, use as such does not fall under the purview of the Panel. Commercially-produced products of carbohydrates, such as glucose, sucrose, or lactose, which undergo lactic acid fermentation (fermentation organism not identified), are GRAS for their intended use in foods; Leuconostoc is an approved strain used as a butter starter

distillate. Leuconostoc/Radish Root Ferment Filtrate is reported to have the greatest frequency of use, in 254 formulations, 190 of which are in leave-on 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 photoirritant. 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 1 and 3 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

The Panel reviewed the safety of 7 ingredients obtained from radish roots, all of which are derived from the *Raphanus sativus* species. The Panel concluded that the available data are sufficient for determining the safety of all 7 ingredients, as reportedly used in cosmetics. The Panel noted that the radish roots, from which the ingredients included in this safety assessment are derived, are consumed regularly as food, and, therefore, these food exposures would likely result in much larger systemic exposure compared to that resulting from use in cosmetic products. Likewise, the fermentation of a few of these ingredients with lactic acid bacteria, *Lactobacillus* and *Leuconostoc* strains, which have GRAS status, was not concerning to the Panel. The Panel discussed that although data from a wide variety of radishes is included in this report (i.e., various colors, sizes, etc.), most of these radishes are indicated to be consumed as food, mitigating any concerns for systemic toxicity. Additionally, the potential for systemic exposure from the absorption of these ingredients through the skin is expected to be much less than the potential for systemic exposure from absorption through oral exposures. These considerations, coupled with low reported use concentrations and negative findings in human dermal irritation and sensitization studies, led the Panel to determine that the radish root-derived ingredients are safe as used in cosmetic products.

An in vitro study investigated the potential for a freeze-dried juice and methanolic extract of white *Raphanus sativus* root to have an inhibitory effect on tyrosinase activity, which can be associated with skin-lightening. Upon review of the paper by the Panel, it was noted that very low potency for inhibiting tyrosinase was actually demonstrated in the study. Nevertheless, the Panel stated that skin lightening is considered to be a drug effect and should not occur during the use of cosmetic products.

Because final product formulations may contain multiple botanicals, each possibly containing the same constituents of concern, formulators are advised to be aware of anthocyanins and isothiocyanates,and to avoid reaching levels that may be hazardous to consumers. The Panel also expressed concern about pesticide residues, heavy metals, and other plant species that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities.

Some radish root-derived ingredients were reported to be used in spray products that could possibly be inhaled. For example, Leuconostoc/Radish Root Ferment Filtrate is reported to be used at up to 0.03% in spray face and neck products. Inhalation toxicity data were not available; the Panel reiterated that radish root-derived ingredients are used as foods, mitigating concerns of systemic toxicity. However, the Panel noted that in aerosol products, the majority of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which these ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. As indicated in the respiratory exposure resource document and in the Cosmetic Use section of this report, airbrush application of cosmetic products is not assessed by the Panel. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the following 7 radish root-derived ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment when formulated to be non-sensitizing:

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*

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

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 (2022)[19] and concentration (2020)[20] 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*	**3**	**NR**	**254**	**0.0001 – 1.1**	**6**	**6**
Duration of Use						
Leave-On	*3*	*NR*	*190*	*0.0001 - 0.03*	*6*	*6*
Rinse-Off	*NR*	*NR*	*64*	*0.0001 – 1.1*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	2	0-0.002 – 0.01	NR	NR
Incidental Ingestion	NR	NR	3	0.0002	NR	6
Incidental Inhalation-Spray	2[a]; 1[b]	NR	1; 116[a]; 42[b]	0.0001-0.03; 0.001[a]	2[b]	NR
Incidental Inhalation-Powder	1[b]	NR	42[b]; 1[c]	0.0002 - 0.002[c]	2[b]	NR
Dermal Contact	3	NR	228	0.0002 – 1.1	6	NR
Deodorant (underarm)	NR	NR	1[a]	NR	4[a]	NR
Hair - Non-Coloring	NR	NR	19	0.0001 – 0.002	NR	NR
Hair-Coloring	NR	NR	4	NR	NR	NR
Nail	NR	NR	NR	0.0022 – 0.01	NR	NR
Mucous Membrane	NR	NR	34	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[19,20]

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

Table 5. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			IN CHEMICO/IN VITRO STUDIES		
Leuconostoc/Radish Root Ferment Filtrate	30 µl	EpiDerm™	A single application of the test article was applied to the epidermis model (3 tissue samples). 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 an MTT assay.	Not irritating	[30]
Leuconostoc/Radish Root Ferment Filtrate	100 mM, in acetonitrile	NA	A DPRA was performed in accordance with OECD TG 442C. 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.	Prediction to be non-sensitizing. The mean percent depletion of cysteine and lysine was 2.89%, which was interpreted as minimal reactivity in the assay.	[31]
Leuconostoc/Radish Root Ferment Filtrate	50 µl, in DMSO	HaCaT cell line	OECD TG 442D. Fifty µl, each, of 12 concentrations of the test article (ranging from 0.98 – 2000 µM) were added to the human keratinocyte cell lines, were seeded for 24 h, as per the KeratinoSens™ method, and were incubated for 48 h.	Predicted to be non-sensitizing	[32]
			HUMAN		
Leuconostoc/Radish Root Ferment Filtrate	10%, in water	50 subjects	HRIPT; nine, occlusive, 24- h induction applications (0.2 g applied to an unspecified area), of the test article were 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.	Not irritating or sensitizing	[33]
Leuconostoc/Radish Root Ferment Filtrate	0.04%, applied neat	105 subjects	HRIPT; nine, occlusive, 24- h induction applications (0.2 g applied to approximately 0.75 in^2), of the test article were 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.	Not irritating or sensitizing	[34]

DMSO- dimethyl sulfoxide; DPRA- direct peptide reactivity assay; HaCaT cell line- human keratinocyte; HRIPT- human repeated insult patch test; MTT- 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide; OECD- Organisation for Economic Cooperation and Development; NA – not applicable; PBS- phosphate buffered solution; TG- test guideline

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2022 VCRP Frequency of Use Data – Radish Root-Derived Ingredients

Ingredient Name	Category code and Description	CPIS count
Lactobacillus/Radish Root Ferment Filtrate Total: 3		
Lactobacillus/Radish Root Ferment Filtrate	12C- Face and Neck (exc shave)	1
Lactobacillus/Radish Root Ferment Filtrate	12F- Moisturizing	2
Leuconostoc/Radish Root Ferment Filtrate Total: 254		
Leuconostoc/Radish Root Ferment Filtrate	01B - Baby Lotions, Oils, Powders, and Creams	1
Leuconostoc/Radish Root Ferment Filtrate	03D – Eye Lotion	1
Leuconostoc/Radish Root Ferment Filtrate	03G - Other Eye Makeup Preparations	1
Leuconostoc/Radish Root Ferment Filtrate	04E - Other Fragrance Preparation	1
Leuconostoc/Radish Root Ferment Filtrate	05A - Hair Conditioner	10
Leuconostoc/Radish Root Ferment Filtrate	05E - Rinses (non-coloring)	1
Leuconostoc/Radish Root Ferment Filtrate	05F - Shampoos (non-coloring)	5
Leuconostoc/Radish Root Ferment Filtrate	05G - Tonics, Dressings, and Other Hair Grooming Aids	3
Leuconostoc/Radish Root Ferment Filtrate	05I – Other Hair Preparations	4
Leuconostoc/Radish Root Ferment Filtrate	07A - Blushers (all types)	3
Leuconostoc/Radish Root Ferment Filtrate	07E - Lipstick	3
Leuconostoc/Radish Root Ferment Filtrate	07H – Makeup Fixatives	1
Leuconostoc/Radish Root Ferment Filtrate	07I - Other Makeup Preparations	11
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	10E – Other Personal Cleanliness Products	1
Leuconostoc/Radish Root Ferment Filtrate	11F - Shaving Soap	3
Leuconostoc/Radish Root Ferment Filtrate	12A - Cleansing	8
Leuconostoc/Radish Root Ferment Filtrate	12C - Face and Neck (exc shave)	40
Leuconostoc/Radish Root Ferment Filtrate	12D - Body and Hand (exc shave)	2
Leuconostoc/Radish Root Ferment Filtrate	12F - Moisturizing	110
Leuconostoc/Radish Root Ferment Filtrate	12G – Night	1
Leuconostoc/Radish Root Ferment Filtrate	12H - Paste Masks (mud packs)	2
Leuconostoc/Radish Root Ferment Filtrate	12I - Skin Fresheners	2
Leuconostoc/Radish Root Ferment Filtrate	12J - Other Skin Care Preps	9
Raphanus Sativus (Radish) Root Extract Total: 6		
Raphanus Sativus (Garden Radish) Root	10B – Deodorants (underarm)	4
Raphanus Sativus (Garden Radish) Root Extract	12C - Face and Neck (exc shave)	2