
Safety Assessment of *Portulaca oleracea*- Derived Ingredients as Used in Cosmetics

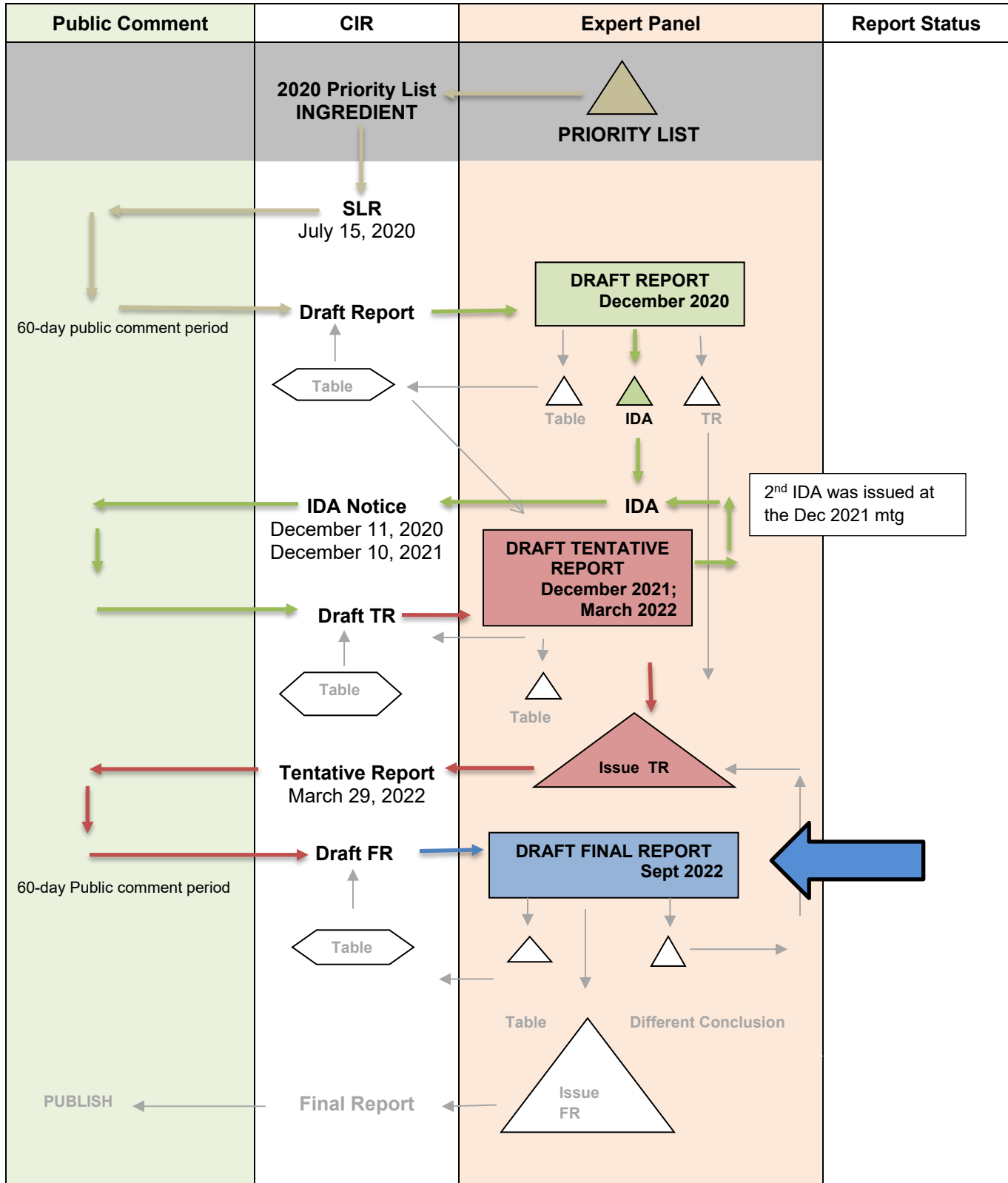
Status: Draft Final Report for Panel Review
Release Date: September 1, 2022
Panel Meeting Date: September 26-27, 2022

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.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D., and Susan C. Tilton, Ph.D. Previous Panel members involved in this assessment: Lisa A. Peterson, Ph.D. and Ronald C. Shank, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Preethi S. Raj, M.Sc., Senior Scientific Analyst/Writer, CIR.

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Portulaca oleracea-derived ingredients

MEETING September 2022





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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: September 1, 2022
Subject: Safety Assessment of *Portulaca oleracea*-Derived Ingredients as Used in Cosmetics

Enclosed is a Draft Final Report of the Safety Assessment of *Portulaca oleracea*-Derived Ingredients as Used in Cosmetics (identified as *report_PortulacaOleracea_092022* in the pdf). This is the fourth time the Panel is seeing a safety assessment of these 4 cosmetic ingredients. At the March 2022 meeting, a revised Draft Tentative Report was presented to the Panel. The Panel acknowledged the confirmed use of whole *Portulaca oleracea* as a food, and that fact, coupled with negative findings in human dermal irritation and sensitization studies on the whole plant extract, mitigated systemic toxicity concerns. The Panel also acknowledged the presence of potentially sensitizing constituents in these ingredients (namely, terpenes). Accordingly, the Panel issued a Tentative Report for public comment with the conclusion that the following 4 *Portulaca oleracea*-derived ingredients are safe in cosmetic in the present practices of use and concentration described in the safety assessment when formulated to be non-sensitizing.

Comments on the Tentative Report that were received from the Council (*PCPCcomments_PortulacaOleracea_092022*) have been addressed. A comments response checklist is included (*response-PCPCcomments_PortulacaOleracea_092022*).

Also included in this package, for your review, are a flow chart (*flow_PortulacaOleracea_092022*), literature search strategy (*search_PortulacaOleracea_092022*), ingredient data profile (*datapofile_PortulacaOleracea_092022*), ingredient history (*history_PortulacaOleracea_092022*), 2022 FDA VCRP data (*VCRP_PortulacaOleracea_092022*), and transcripts from previous meetings (*transcripts_PortulacaOleracea_092022*).

The Panel should carefully consider the Abstract, Discussion, and Conclusion presented in this report. If these are satisfactory, the Panel should issue a Final Report.



Memorandum

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

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

DATE: April 4, 2022

SUBJECT: Tentative Report: Safety Assessment of *Portulaca oleracea*-Derived Ingredients as Used in Cosmetics (release date: March 29, 2022)

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

Composition and Impurities – Please indicate what the percentage soluble oxalate (33% in leaves, 67% stems) represents. Is this the percentage of total oxalate (accounts for about 1.3% of the plants composition)?

Developmental and Reproductive Toxicity; Summary – Table 7 indicates that some of the studies (reference 57) were completed on a “total flavonoid extract”. The text of the developmental and reproductive toxicity section should state which studies were done on “the total flavonoid extract.” Please indicate when the dams were dosed in relationship to gestation in the study in which the abortion rate was 30%, and in the teratology study.

Cytotoxicity; Summary – The description of the study from reference 62 states that the positive control was not specified. Please check this reference again. It states that PHA (phytohemagglutinin) was the control. PHA is a mitogen, often used as mitotic stimulant of human lymphocytes.

Cytotoxicity - Units of µg/ml should be called concentration rather than dose (in the last paragraph of this section).

Summary – To be consistent with the information in the Short-Term section, please delete “ethanolic” and the last “extract” in the following: “aqueous or methanolic extract of whole ethanolic *Portulaca oleracea* extract”.

Table 2 – When there is nothing in a column for a class of constituents, does this mean that constituent was not measured, or was not found?

Table 7 – Please revise the Animals/Group column for the first study. As only male rats were treated, it is not necessary to include the untreated females in this column. As stated in the Procedure column, there were 3 male rats per dose group (not 1 as currently stated in the Animals/Group column).

Table 7 – If the same extract (“Total flavonoid extract”) was used in all the studies from reference 57, it should be called the same thing in the first column for all of the studies from this reference.

Table 7 – In the Procedure column of the third study from the end of Table 7, it states: “Same mating strategy and female selection as above study.” The study “above” is a female only study that did not include mating. The study below does describe a mating procedure. Perhaps the order of the studies needs to be revised.

<i>Portulaca oleracea</i>-derived Ingredients - September 26-27th, 2022 Panel Meeting – Preethi Raj			
Comment Submitter: Personal Care Products Council			
Date of Submission: April 4, 2022 (comments received on TR, posted March 29, 2022)			
#	Report section/Comment	Response/Action	Needs Panel Input
1	Composition and Impurities:- indicate what %age of soluble oxalate (33% in leaves, 67% in stems) indicates, and whether it is ‘total oxalate’	clarified	
2	DART; Summary; -state which studies were done on the “total flavonoid extract. -Indicate when dams were dosed in relationship to gestation for study in which abortion rate = 30%	-stated -indicated	
3	Cytotoxicity, Summary; Check if positive control in ref 62 (Al-Rubai 2014) is phytohemagglutinin, and revise	Yes, it is, have included	
4	Cytotoxicity – units of µg/ml called concentration	revised	
5	Summary – delete ‘ethanolic’ and the last ‘extract in the following phrase: “aqueous or methanolic extract of whole ethanolic <i>Portulaca oleracea</i> extract”	deleted	
6	Table 2 – clarify whether blank entry is not found or not measured (Zhou 2015 is cited)	Components are presented as listed in the Zhou 2015 reference (i.e. chemical components found in various parts of the plant – aggregated from multiple sources) A blank entry likely means nothing was found.	
7	Table 7 – revise animals/group column for first study – no females – 3 male rats/dose group is correct	revised	
8	Table 7 – check if ref 57 (Nayaka 2014) used the same extract – if so, it should be called the same thing across studies	revised	
9	Table 7- rearrange studies to ensure Procedure section referencing above study is correct (or revise)	revised	

CIR History of:
***Portulaca oleracea*-derived Ingredients**

January 2019

-Concentration of use data submitted by Council

January 2020

-FDA frequency of use data obtained

July 2020

- SLR posted on the CIR website

Data received (Portulaca Oleracea Extract):

- July 29, 2020: Certificates of origin, method of manufacture, and ingredient source information for water and water/butylene glycol extracts of *Portulaca oleracea*
- August 12, 2020: Human patch test, clinical test summary, contact sensitization study results, all testing products containing 0.1% Portulaca Oleracea Extract
- November 16, 2020: Correction for previously received method of manufacture of a phenoxyethanol and water extract of Portulaca Oleracea Extract (made with the whole plant)

December 2020

-A Draft Report was presented to the Panel. The Panel issued an IDA with the following data needs:

- Clarification on the current maximum concentration of use
- A 28-d dermal toxicity study at the maximum concentration of use (preferably with the ingredient in an hydroalcoholic solvent)
- If these data are positive, further systemic toxicity data may be needed
- An Ames test (preferably with the ingredient in an hydroalcoholic solvent)

January 2021

- New VCRP data were received

Data received (Portulaca Oleracea Extract):

- January 4, 2021: Two maximization studies in a facial lotion and face treatment product, both containing 0.5% Portulaca Oleracea Extract
- January 4, 2021: Revisions/clarifications to concentration of use data for *Portulaca oleracea*-derived ingredients

December 2021

-Comments on the Draft Tentative Report were received from Council

-A Draft Tentative Report was presented for Panel review. The Panel received additional sensitization data for Portulaca Oleracea Extract, which was not concerning, as well as clarification that the maximum reported concentration of use for Portulaca Oleracea Extract is 0.5%. However, the Panel still found the available data insufficient to make a determination of safety in light of there being no genotoxicity data and varied developmental and reproductive effects observed with Portulaca oleracea extracts with aerial parts, using various solvents. The Panel also expressed that having clarity on which part(s) of the plant (or whole plant) are used in foods would further mitigate any systemic toxicity concerns.

Accordingly, the Panel issued an Insufficient Data Announcement (IDA), with the following data needs:

- 28-day dermal toxicity data at the maximum reported concentration of use for Portulaca Oleracea Extract, preferably in an hydroalcoholic solvent
 - if positive additional toxicity data, such as developmental and reproductive toxicity and genotoxicity data, may be needed
- Clarification on which part(s) of the plant are consumed as a food, and which plant part(s) are used in cosmetics

January 2022

- New VCRP data were received

March 2022

-A Draft Tentative Report was presented to the Panel for review. Confirmed use of the whole Portulaca oleracea plant as a food, coupled with negative findings in human dermal irritation and sensitization studies on the whole plant extract mitigated the Panel's previous concerns regarding systemic toxicity. The Panel identified the presence of potentially sensitizing constituents in the composition of these ingredients. Accordingly, the Panel issued a Tentative Report with the conclusion that these ingredients are safe in cosmetics in the present practices of use and concentration, when formulated to be non-sensitizing.

April 2022

-Comments on the Tentative Report were received from Council.

September 2022

A Draft Final Report is being presented to the Panel.

Portulaca oleracea-derived Ingredients Data Profile* – September 26-27, 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
Portulaca Oleracea Extract	X	X	X					X			X										X			X					
Portulaca Oleracea Flower/Leaf/Stem Extract		X	X					X			X			X															
Portulaca Oleracea Juice		X									X																		
Portulaca Oleracea Water		X																											

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

Portulaca oleracea – derived ingredients (4 ingredients- September 2022 Panel Mtg)

Ingredient	CAS #	InfoB	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Portulaca Oleracea Extract	90083-07-1	✓	✓	✓	NR	✓*	✓*	NR	NR	NR	NR	NR	✓*	NR	✓	NR	NR	NR	✓
Portulaca Oleracea Flower/Leaf/Stem Extract	90083-07-1	✓	✓	✓	NR	NR	NR	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	✓
Portulaca Oleracea Juice	NR	✓	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	✓
Portulaca Oleracea Water	90083-07-1	✓	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	IFRA	RIFM
Portulaca Oleracea Extract	90083-07-1	✓	NR	#29453	NR	NR	NR
Portulaca Oleracea Flower/Leaf/Stem Extract	90083-07-1	NR	NR	NR	NR	NR	NR
Portulaca Oleracea Juice	NR	NR	NR	NR	NR	NR	NR
Portulaca Oleracea Water	90083-07-1	NR	NR	NR	NR	NR	NR

✓ - found in database, or, data was available

✓* - found in database, but data was either irrelevant or not accessible

NR – not reported

Search Strategy

[document search strategy used for PubMed and/or Toxnet]: - [total # of hits/#hits that were useful]

((((physical chemical properties) AND portulaca oleracea extract) OR portulaca oleracea whole extract) OR portulaca oleracea juice) OR portulaca oleracea water – 97/3

Updated search as of 07/28/2022:

(((((((portulaca oleracea extract) OR (90083-07-1)) OR (portulaca oleracea flower extract)) OR (portulaca oleracea leaf extract)) OR (portulaca oleracea stem extract)) OR (portulaca oleracea juice)) OR (portulaca oleracea water)) OR (portulaca extract) – 415/11 – nothing new or useful

Whole extract

Portulaca oleracea Persian medicine – 4/2

Chinese traditional medicine ma chi xian – 4/2

Alkaloids from Portulaca oleracea – 44/10

Portulaca oleracea pharmacokinetics – 18/2

portulaca oleracea toxicokinetics humans – 4/0

Portulaca oleracea dermal toxicity – 0/0

Portulaca oleracea extract dermatology – 2/0

Portulaca oleracea skin irritation -0/0

Portulaca oleracea dermal sensitization – 0/0

Portulaca oleracea skin sensitization -0/0

Portulaca oleracea genotoxicity -1/0

Portulaca oleracea reproductive toxicity OR pregnancy OR fetal development -0/0

Portulaca oleracea inhalation toxicity

Purslane cosmetics – 3/0

Purslane topical – 5/1

Portulaca oleracea clinical study – 13/6

Stem/Flower/Leaf

Portulaca oleracea flower cosmetic toxicity – 182,000/0

Juice

Portulaca oleracea juice – 3/0

Portulaca oleracea juice toxicity -0/0

((((Portulaca Oleracea Juice) AND toxicokinetics) OR acute dermal toxicity) OR acute oral toxicity) OR acute inhalation toxicity – 9798/0

Water

Portulaca oleracea water toxicity – 0/0

Portulaca oleracea steam distillate toxicity – 0/0

General Web Search

portulaca oleracea dermal toxicity - 717,000/2

portulaca oleracea dermal sensitization – 16/1

portulaca oleracea folk medicine dosage – 111,000/6

portulaca oleracea animal toxicity – 112,000/4

LINKS

Search Engines

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

appropriate qualifiers are used as necessary

search results are reviewed to identify relevant documents

Pertinent Websites

- wINCI - <http://webdictionary.personalcarecouncil.org>
- FDA databases <http://www.ecfr.gov/cgi-bin/ECFR?page=browse>
- FDA search databases: <http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm>;
- EAFUS: <http://www.accessdata.fda.gov/scripts/fcn/fcnavigation.cfm?rpt=eafuslisting&displayall=true>
- GRAS listing: <http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm>
- SCOGS database: <http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm>
- Indirect Food Additives: <http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives>
- Drug Approvals and Database: <http://www.fda.gov/Drugs/InformationOnDrugs/default.htm>

- <http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf>
- FDA Orange Book: <https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm>
- OTC ingredient list: <https://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm135688.pdf>
- (inactive ingredients approved for drugs: <http://www.accessdata.fda.gov/scripts/cder/iig/>)

- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/opthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - <http://www.cdc.gov/niosh/>
- NTIS (National Technical Information Service) - <http://www.ntis.gov/>
- NTP (National Toxicology Program) - <http://ntp.niehs.nih.gov/>
- Office of Dietary Supplements <https://ods.od.nih.gov/>
- FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/

- EU CosIng database: <http://ec.europa.eu/growth/tools-databases/cosing/>
- ECHA (European Chemicals Agency – REACH dossiers) – <http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1>
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - <http://www.ecetoc.org>
- European Medicines Agency (EMA) - <http://www.ema.europa.eu/ema/>
- IUCLID (International Uniform Chemical Information Database) - <https://iuclid6.echa.europa.eu/search>
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- <http://webnet.oecd.org/hpv/ui/Search.aspx>
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- <https://www.nicnas.gov.au/>

- International Programme on Chemical Safety <http://www.inchem.org/>
- FAO (Food and Agriculture Organization of the United Nations) - <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/>
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/

- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

Botanical Websites, if applicable

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

Fragrance Websites, if applicable

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

DECEMBER 2020 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT**Belsito Team – December 7, 2020**

DR. BELSITO: Okay. Okey-doke. So, we're moving on to *Portulaca oleracea*. This is the first time that we're seeing these four ingredients. And, I guess, in a prior data submission it was just pointed out that the extract was said to be derived from the leaf and stem only, but that now we've been told the extract is from the whole plant.

Let me save this last one. Okay. Yes. Okay. So, comments. For developmental and reproductive toxicity, I had a note that there were lots of effects and no NOAEL. How do we handle that? The doses were high. Use concentration is 0.08 percent, but still we have these effects. Paul, what did you think of that?

DR. SNYDER: I'm sorry, Don. I was printing a paper that Preethi just sent me. Can you repeat that, please?

DR. BELSITO: So, if you look at PDF page 14 on the DART studies, there were lots of different, little effects for which we don't have an NOAEL. And the highest concentration of use is 0.008 percent, but we still don't have an NOAEL for DART. I just was wondering what you thought, or maybe you thought these effects were not --?

DR. SNYDER: I did not ping them to be honest. And I'll have another look here, but I did not ping them at all.

DR. KLAASSEN: My thoughts were that there definitely are effects, but those were mainly at pretty high doses at 250 and 500 milligrams per kilogram. And I noted that there were no effects at 75 milligram per kilogram, and this compound is used at 0.008 percent. So, I considered this not to be a problem.

DR. BELSITO: Well, but Curt, if you look, it says a statistically significant decrease in testosterone levels were observed in rats in the aqueous 75 milligram per kilogram dose, and in all methanolic extracts. That's the one, two, three, four, five, fifth and sixth line down in the first paragraph.

DR. KLAASSEN: Okay. I'm going to see if I can find that.

DR. BELSITO: It's PDF page 14. It's the first paragraph under where it says refer to table six, the fifth and sixth line down.

MS. RAJ: Yeah. I'm looking at these papers, and I don't think they provided that information, the NOEL or the NOAEL.

DR. BELSITO: Yeah. I mean, what they do say is that 75 -- 50 would appear to be a NOAEL on this study for aqueous. But in the methanolic, 25 percent also had that effect. So, it looks like it's vehicle dependent.

DR. LIEBLER: If you read through the rest of that whole paragraph, there are other endpoints that compare aqueous and methanolic extracts and show no effects. So, it's not as if there is a systematic problem with the methanolic extract versus an aqueous extract.

DR. BELSITO: Right. I agree except for this specific endpoint.

DR. LIEBLER: Yeah. Yeah.

DR. BELSITO: The other issue that I have about the 0.008 percent, as we look at it, is if it's used at 0.008 percent, why did we get a sensitization and irritation study with its use at 0.1? This is PDF page 15.

DR. EISENMANN: It could have been a study on a -- I have to look at the date of the -- it could have been an older product. They go back in their files sometimes and give me studies that they have done previously on older products. I don't know if that's for sure, because I haven't checked on the data on that study.

DR. BELSITO: It's reference 62.

MS. FIUME: It's 2006.

DR. BELSITO: 2006?

MS. FIUME: Yes.

MS. RAJ: There also seems to be a 2017, right?

DR. BELSITO: It's reference 63 and 62. Sixty-two is irritation, sixty-three is sensitization.

MS. FIUME: Sixty-three is 2007. A clinical use test was done in 2017. It was 0.1 percent.

DR. BELSITO: Yeah. Anonymous. And yeah, it's odd to me that we're looking at data that would at least suggest that this could be used in leave-on products to 0.1 percent not 0.008 percent.

MS. RAJ: If anything, does receiving data at a higher concentration than what's reported, I guess, confirm more surety of safety or safety?

DR. BELSITO: Well, yeah. Unless you had other endpoint concerns. In this case, looking at the doses that caused effects in the DART study that's still significantly higher than 0.1 percent, I agree. The major issue is I don't see that we have an NOAEL for the DART studies on this. So, I'm just curious as to what other people think about that.

DR. LIEBLER: I mean it's the one endpoint. It's the testosterone levels in the methanolic extract groups. It says in all methanolic extract groups. Presumably, that does not include the vehicle control, but that would be worth taking a look at, because we got 0, 25, 50, and 75. For the aqueous, only the 75 had an effect, so we have an NOAEL for the aqueous. But for the methanolic, it's ambiguous. It looks like we don't.

DR. BELSITO: And are we comfortable with the composition and impurities that we have on this material? They're very vague.

DR. LIEBLER: So, method of manufacture I thought was quite good.

DR. BELSITO: Yes.

DR. LIEBLER: And composition and impurities, let's see, I didn't flag anything.

DR. BELSITO: No. Nothing pops out, but it's not, you know, they're just sort of vague chemical categories.

DR. SNYDER: It appears, from the title of the references for the DART studies, that they were concerned about the flavonoids, in the fertility effects of the impurities in the botanical.

DR. LIEBLER: I think that the data profile for the organic components of these is not that different from what we get from a lot of other botanicals. (Audio skip) summarizes these. I think that this is adequate, so I don't have any concern. And the one interesting thing was the oxalate bubbles; and oxalate, of course, can be a toxicant, but only at high concentrations. I think we could deal with that in the discussion.

DR. KLAASSEN: Right.

DR. BELSITO: Okay. We don't have an absorption distribution metabolism. Do we need a 28-day dermal in light of the DART studies or not?

DR. LIEBLER: Some of the constituents of these are going to be absorbed because they're small organics.

DR. BELSITO: And we have no genotoxicity data.

DR. LIEBLER: Yeah. I'd like to see an Ames on this. There's no genotox, no carcinogenicity. A negative Ames would put this away for me. But we don't -- yeah.

DR. KLAASSEN: Yeah.

DR. BELSITO: Okay. So, we need a 28-day dermal and an Ames test, or just an Ames test?

DR. LIEBLER: Yes.

DR. BELSITO: Okay. So, we need the 28-day dermal and we need an Ames test. And in the discussion the -- assuming we get those and they're clear -- the discussion will go along with the DART studies at very high doses. Hopefully, relevant --

DR. EISENMANN: You do recognize that this material, that this plant is eaten in many places of the world?

DR. BELSITO: But it's not GRAS.

DR. EISENMANN: No. But for other plant materials you have accepted use for systemic toxicity. And, so far, we haven't heard that a methanol extract is being used in cosmetics.

DR. BELSITO: Well, I think, Carol, this is the first time we're looking at it. If it doesn't (audio skip).

DR. EISENMANN: Correct. And I'm not arguing with you on toxicity. I'm only arguing the 28-day dermal.

DR. BELSITO: Again, I think it's the first time we're looking at it. It doesn't hurt to ask for it and see if we get it or get additional data. I mean I don't know. What do you guys think?

DR. LIEBLER: Well, the method of manufacture describes a hydroalcoholic extract. And we got this one flag for methanolic extracts and the effect on testosterone. So, I think that the -- it's not as if the method of manufacture says only aqueous extracts are used, and therefore the alcoholic extract is not relevant. I don't think we can say that. So, I'm still in the camp of requiring the 28-day dermal.

DR. BELSITO: Okay. So, we're going to go insufficient for 28-day dermal at concentration of use and genotox data. And you're not asking for mammalian, Dan, you're just asking for Ames?

DR. LIEBLER: I think that's fine unless my colleagues disagree?

DR. KLAASSEN: I agree with you.

DR. SNYDER: I'm fine.

DR. LIEBLER: And, Don, you're okay with this sensitization?

DR. BELSITO: Yeah. The sensitization and irritation is fine. It just raised the sort of a flag for me that they were doing it at a concentration ten times higher than what we're told it was used at, which makes me think that there's a manufacturer out there who submitted data, who's using it at 0.1 percent, who didn't report to Carol the concentration of use. I think it's still fine at 0.1. I mean, I'm not concerned about that level of use.

I just, again, there were just -- I guess there were several -- the methicones are an example of several reports where it just seems that there may be information that we're not getting for various reasons. And that bothers me. But I think 0.1 percent would be fine. I don't have an issue with that.

DR. LIEBLER: Okay.

DR. BELSITO: Okay. So insufficient, 28-day dermal, and an Ames genotox assay. Okay?

MS. FIUME: Don, I was just --

DR. BELSITO: Yeah.

MS. FIUME: -- can I ask? So, for the IDA, because the vehicle may or may not be a concern, would you like us to specify in the IDA that the solvent is needed with the data submission? Would that make a difference?

DR. BELSITO: Yeah. I think it should be hydroalcoholic.

MS. FIUME: Thank you.

MS. RAJ: Thank you.

Cohen Team – December 7, 2020

DR. COHEN: This is a draft report. Preethi has this. This is the first time we're reviewing it. The safety assessment is for four derived ingredients: the extract, the juice, the water, and the flower, leaf, and stem extract.

This is apparently used as a food well. It has a max use of 0.008 on leave-on products, and 0.002 in rinse-off products. Frequency of use is reported in 2020, for products of the face, neck, and moisturizers. We have method of manufacturing for the extract, which is the whole plant. (Audio skip) is to include the whole plant, and can we read across on that? So, I'll open it up to the Panel.

MS. RAJ: I do --

DR. COHEN: Lisa, you want to start on the chemistry?

MS. RAJ: Sorry, I do want to bring up that you'll notice that the method of manufacture that was originally received, the second one, you'll see in the diagram they had called it a leaf and stem extract, but later, when we asked them to clarify, it was actually from the whole plant.

DR. COHEN: Ah, okay. That's very helpful.

MS. RAJ: Yeah.

DR. COHEN: So, then we could use that data for the flower/leaf/stem. It's the whole plant.

MS. RAJ: Yeah.

DR. COHEN: The extract. So, flower/leaf/stem -- so what's missing between the extract and the flower/leaf/stem extract? What am I missing?

MS. RAJ: Well, I guess, it needs -- the ingredient that we use needs to fit the dictionary definition, which would need to be either the whole plant or the other ingredient is the flower, leaf, and stem extract.

DR. COHEN: Okay.

DR. PETERSON: So, you're saying we don't have the method of manufacturing for the flower/leaf/stem extract unless you extrapolate?

MS. RAJ: No. Yeah, I guess, what I was clarifying, Dr. Peterson, is what they had sent us first. Like, I think, it's in this binder on, let's see, PDF Page -- what is this -- PDF Page 34. You'll see that they put the used plant part as leaf and stem only.

And when we got back to Council and asked them to clarify, is it just the leaf and stem, or is the whole plant. They got back to us and said they used the whole plant. So, we have that file which says now that the used plant part was the whole plant.

DR. COHEN: Okay. So, Lisa, what are your thoughts?

DR. PETERSON: Well, I thought that, you know, it's -- I guess, now then it will be insufficient for method of manufacturing of the leaf/stem/flower and then impurities on the juice and the water. But, for the extract, which is the reported use, I mean, we have everything for the chemistry part of it.

DR. COHEN: Tom, thoughts, comments?

DR. SLAGA: Yeah. Well, the whole plant has some data. It doesn't have irritation or sensitization like the extract, but what's the difference of the extract from the whole plant or the one up above? Are they the same?

MS. RAJ: I'm sorry if I confused you all. Yes, the extract is using the whole plant.

DR. SLAGA: The extract -- the first one, is that the whole plant too?

DR. PETERSON: Yeah.

MS. RAJ: Yes.

DR. SLAGA: Does the extract in the second one, when it says flower, leaf, and stem, that's the whole plant. So, are the first two very -- are similar, right? We can use the data.

MS. RAJ: Yeah. The flower, leaf, and stem meaning everything above the ground. I guess the whole plant could possibly include roots.

DR. COHEN: The aerial.

MS. RAJ: Yeah.

DR. SLAGA: Yeah. But isn't that true? In the first one listed where it has the -- it just says extract. It doesn't give you flower and stem in that, but I interpret that it would be the whole plant too.

DR. BERGFELD: Hmm. Yeah, I mean --

DR. COHEN: Well, the second one is at least the aerial components, and the whole plant may include the fruit.

DR. SLAGA: Huh?

DR. COHEN: The whole plant may include the root.

DR. SLAGA: What's the difference between the first -- but it doesn't say the root.

DR. BERGFELD: Well, let's see.

DR. COHEN: Tom.

DR. BERGFELD: No roots.

DR. SLAGA: No root. They're the same then. So, I -- you know, there is sufficient sensitivity and irritation data. Also, it's an anti-inflammatory or anti-irritant. It's anti-genotoxic, anti-cancer. So, even though we don't have certain others, the fact that it has anti activity-related genotoxicity, and carcinogenicity, and it's an anti-inflammatory, I think it's safe.

DR. COHEN: Ron?

DR. SHANK: I'm trying to find the genotox. I thought we needed genotox?

DR. SLAGA: There's no genotox. It's called anti-genotox. There's some data.

DR. SHANK: We don't have genotox data.

DR. PETERSON: There is no genotox.

DR. SHANK: We don't have genotox data.

DR. SLAGA: I know there isn't, just the anti-genotox. So, if it's anti-genotox, to me, it's not genotoxic. And it's anti-carcinogenic. So, in a way, then, you don't need genotoxicity. Hello.

DR. COHEN: Yeah. No, I was just giving Ron some time to digest the comments.

DR. SHANK: Right. Okay. Yeah, if the extract and the flower and leaf/stem extract are the same, we have enough data there.

DR. SLAGA: Right.

DR. SHANK: The leave-on concentration is very, very low, 0.008 percent. So that takes care of the data needs.

DR. PETERSON: That's right.

DR. SLAGA: Right.

DR. COHEN: Yeah, this -- I don't have, again, the PDF, but on Table 2, it says, "constituents found by plant part," and for flower, leaf, and stem, it says, "defined as the aerial parts." And for leaf and stem, it says, "sometimes includes root or seed." So, Preethi, do you have any concern with us assuming one and two are very similar to each other, and if there is, is it the root?

MS. RAJ: Yeah, Dr. Cohen. And, actually, this particular table you mentioned, I used a publication that kind of arranged it this way. And that was, I guess, the closest I could get it to match our purposes for this report.

But you're right. I mean, looking at the INCI dictionary definition, it does seem like the flower, leaf, and stem extract is referring to the aerial parts, whereas the whole extract would, you know, be the whole plant, possibly including roots. And I'm obviously not an expert in this, but, from what I understand, the roots could possibly have a very different constituent profile and possible -- I don't know -- effects.

DR. SLAGA: But we don't know though.

DR. COHEN: Yeah, so --

DR. PETERSON: Yeah.

DR. COHEN: -- we need impurities on the leaf, stem, the water. We still need more information on that.

MS. RAJ: Okay.

DR. COHEN: Is that right, Lisa?

DR. PETERSON: Um --

DR. SLAGA: We don't. We have impurities for (audio skip).

DR. SHANK: We have a lot of impurity.

DR. PETERSON: But I don't know what the impurities are.

DR. COHEN: Do we have enough?

DR. PETERSON: But, you know what? I think we have a lot of information about the composition, but I was having trouble identifying, in the text, what the impurities were. Because everything -- it's more composition than impurities. So, I would say that it's actually -- there's no information about impurities, like, heavy metals or anything else.

The whole -- on PDF Pages 12 and 13, even though the subject line is composition and impurities, there's really no -- it's all discussion of composition. And there's no impurities, so I would say that they're all insufficient for impurities.

DR. BERGFELD: Lisa, we can put into the discussion --

DR. COHEN: What was that, Wilma?

DR. BERGFELD: Lisa, we can put into the discussion the boiler- -- we can put into the discussion the boilerplate of the acceptable impurities for heavy metals and control it that way.

DR. PETERSON: Okay.

DR. SHANK : Good.

DR. PETERSON: All right. I mean, I just think there's no information --

DR. BERGFELD: We need something other than that.

DR. PETERSON: Yeah. I mean, this --

DR. BERGFELD: Is that what you're concerned with mainly, the heavy metals?

DR. PETERSON: Well, you know, it's the heavy metals and the pesticides and --

DR. BERGFELD: Yeah, we can put that -- we have boilerplates for those.

DR. PETERSON: Okay.

DR. BERGFELD: Bart, are you on? Bart?

DR. PETERSON: But, I think, otherwise you have a pretty good description of the composition of the -- at least of the plant extracts and the plant leaf extract. There's not much information about the water and the juice. So, I would say that the water and the juice are insufficient but, you know, they're not used.

DR. SHANK: You mean the water and juice? Wouldn't the water and juice be covered by the plant extract?

DR. SLAGA: Yeah, it's a food.

DR. BERGFELD: Mainly water. It's a succulent.

DR. SLAGA: Yeah.

DR. PETERSON: But I think you have lots and lots on the compositions, so the question is then what would the impurities be? And, you know, the impurities would be, you know, heavy metals and pesticides, which if you've got the boilerplate on that, it would suffice.

DR. COHEN: We have that covered.

DR. PETERSON: And that would be acceptable? Then I say it's fine.

DR. COHEN: As long as the plant -- as long as the composition -- the properties between the plant, the whole plant, and the flowers, leaf, and stem were similar to each other, and it sounds like we're agreeing on that, then we could read across on what we have here.

We have some irritation and sensitization data that looks pretty good. There's a comment about the spectral absorption at 200 to 400 nanometers. Do we need phototox on that?

DR. SHANK: It's used at a pretty low concentration.

DR. COHEN: It is. Would that sway your concerns then on that (audio skip) concentration?

DR. SHANK: No, I think -- yeah, it's used as an herbal -- a folk medicine with a topical application. So, I don't think --

DR. COHEN: Okay. So?

DR. SHANK: I don't see a need to ask for the phototox.

MS. RAJ: And this is --

DR. SLAGA: I agree. I don't think it's needed either.

MS. RAJ: Sorry to interrupt. This is colloquially known as Purslane, even though it doesn't have GRAS, and it's consumed.

DR. COHEN: Okay.

DR. SHANK: Yeah, it's a food.

DR. SLAGA: It's a food, yeah.

DR. COHEN: So, I don't want to be redundant, I just want to make sure we have the insufficient data. It is impurities. Do we need the impurities, or are we covering everything with the boilerplates?

DR. BERGFELD: Boilerplates.

DR. SLAGA: Boiler -- yeah.

DR. COHEN: Okay. So, we need method of manufacturing for the leaf, stem, and flower?

DR. SLAGA: We have that, don't we?

DR. COHEN: Sorry. I'm just -- let's see.

DR. PETERSON: Well, technically we don't have that because it was retracted, right? But is there coverage somewhere else, like in that Reference 20-- the reference used in Table 2?

MS. RAJ: Yeah, I don't know if it was --

DR. PETERSON: What reference --

MS. RAJ: Yeah, let me just check.

DR. PETERSON: Well, I mean, they did the analysis. Somebody must have reported how they got the different parts. It might be buried.

MS. RAJ: Are you referring to Reference 22, Dr Peterson?

DR. PETERSON: No, Reference 29 is the reference that's used for the Table 2.

MS. RAJ: Yes.

DR. HELDRETH: Yeah, that's a very general -- it's a review of phytochemistry and --

DR. PETERSON: Oh, it's a review (audio skip). But there's --

MS. RAJ: Yeah.

DR. HELDRETH: So, it may not be relevant to cosmetic ingredient methods.

MS. RAJ: Yeah.

DR. COHEN: So, we do need -- so the method of manufacturing we have is for the whole plant, correct?

MS. RAJ: Yes.

DR. SHANK: Correct.

DR. COHEN: Right. So, we need the flower/leaf/stem. Let me just see here.

MS. FIUME: Hi. This is Monice. I'm coming in a little late to the discussion, so, in case this is already deemed as not sufficient, but on PDF Page 12, is that method of manufacture acceptable for the flower, leaf, and stem extract?

DR. COHEN: Yeah, I'm just looking at that right now. I think it is.

DR. PETERSON: Yeah, it's fine with me.

DR. COHEN: Do we have any insufficiencies after -- because we sort of created a list, and then we walked back many of the issues here. Are there any remaining insufficiencies right now?

DR. SLAGA: I don't have any.

DR. PETERSON: No. I don't have any.

DR. COHEN: Ron?

DR. SHANK: It looks okay.

DR. COHEN: Lisa?

DR. PETERSON: It looks okay.

DR. COHEN: All right. So, we move on as safe as used?

DR. SLAGA: Yes.

DR. PETERSON: Uh-huh.

DR. BERGFELD: The only thing I see is that, in your discussion, you need to speak about the photoactivation, why it's not a problem.

DR. COHEN: Preethi, do you have enough from our discussion just now to do that?

MS. RAJ: Um, I'm just trying to see what would I be referencing in the report for the phototox concerns? Was there something in there that signaled phototox?

DR. COHEN: The absorption.

DR. BERGFELD: The (inaudible) of 200.

DR. COHEN: I thought it was absorption.

DR. BERGFELD: Two hundred.

DR. COHEN: A comment of it being absorbed between -- the phenolics being absorbed between 200 and 400 nanometers.

MS. RAJ: Okay. This is in the ADME section?

DR. COHEN: It's in Chemical Properties. It said, "In an UV spectral analysis of crude, and methanol-soluble fractions of whole *Portulaca oleracea* extract, optical spectra maxima were recorded between 200 and 400 nanometers, in which phenolic compounds showed maximum absorbance."

MS. RAJ: Okay.

DR. COHEN: Am I overreading into that?

DR. PETERSON: I think you might be.

MS. RAJ: Yeah.

DR. COHEN: Okay.

MS. RAJ: I remember taking that from a paper, but I'm not sure if it was -- yeah. I'll leave it to you all to decide on whether it (overtalking) --

DR. PETERSON: So, the way I --

DR. COHEN: No, we're not going to ask for phototox.

DR. PETERSON: The way I read that part was that they used the UV --

DR. COHEN: Just one second. So, Lisa, do you think that was a method of analysis for the product using that --

DR. PETERSON: So, it's a method of chemical characterization because the wavelength of absorbance would tell you something about the chemical structures. I mean, I thought it was sort of not useful because it's going to be this complicated mixture. So, you know, I think what they came away with it is that there were some alcohols and some carbonyls and blah, blah, blah.

DR. COHEN: Got it.

DR. PETERSON: I mean, I'm not a photo toxicity expert person, but to me that reference was more for a chemical characterization attempts.

DR. COHEN: Understood.

DR. PETERSON: But two crudes, they really amount to anything?

DR. COHEN: I completely get it. It was an analytical chemistry analysis, not anything more than that. So, I'm fine withdrawing the discussion on phototox based on that.

DR. PETERSON: Okay.

DR. COHEN: Wilma, the absorption material in 200 to 400 nanometers was more an analytical chemistry thing then a phototox issue. So, we got through the whole deck.

Full Panel – December 8, 2020

DR. BELSITO: Okay, so, *Portulaca oleracea*. This is the first time looking at these four ingredients. And, we're now told that previously the extract was derived from leaf and stem only. But now we're told that it is derived from the whole plant. So, we looked at all of this information and felt that we had no absorption distribution metabolism. And we need a 28-day dermal at concentration of use in a hydroalcoholic solvent.

And, the other interesting thing is we were told that the maximum concentration of use is .008 percent. But it was curious, at least to me, that in the sensitization and irritation studies it was done at 0.1 percent, including a study that was done in 2017. So, we would like some clarification as to really what the maximum concentration of use is.

And then we also felt that the results in the DART section needed to be explained; they were very (audio skip). (Inaudible) particularly absorption through the skin (audio skip). And, finally, there was no genotox data. We felt we needed that. Dan felt we needed -- we only had Ames test, we did not need mammalian. But I'm sure that Dr. Shank will want mammalian as well.

So, we found this insufficient, 28-day dermal at concentration of use, specifying the vehicle was hydroalcoholic. That may help us explain the DART data. Further clarification of maximum concentration of use as to why someone studied it at 0.1 percent if the maximum is .008, and genotox data.

DR. BERGFELD: So that's a motion to go out as an IDA with all those needs?

DR. BELSITO: Correct.

DR. BERGFELD: Is there a second? Is there a comment?

DR. COHEN: Yeah, Don, we considered the very low concentrations and those signals for irritation and sensitization. Same issue, the lotion used in the sensitization study was .1 percent. So, we had a safer consideration for it, but I think in light of your issues we would concur.

DR. BERGFELD: So, you're seconding it? You're seconding the motion?

DR. COHEN: Yes.

DR. BERGFELD: Any other discussion?

DR. BELSITO: David, I have no problem with the sensitization and irritation. I'm just wondering why someone would do it at .1 if in fact the maximum concentration is .008. So, what I'm asking is maybe a referral could go out to whoever did that study, if she's aware of it, and find out whether they are in fact marketing products at .1 percent. I don't think it'll change our conclusion.

DR. COHEN: It's the same as the Acetyl Hexapeptide discussion.

DR. BELSITO: Right.

DR. BERGFELD: Okay, any other comments before we move this question of an IDA on this ingredient?

MS. RAJ: Dr. Bergfeld?

DR. BERGFELD: Yes.

MS. RAJ: I quickly wanted to ask this -- I'm glad this is going as an IDA, but I know -- I think the Belsito team had discussed dealing with the oxalates in the -- personally in the discussion section. So, I was just curious, I guess, how the Panel would like to see that. And also, discussion regarding the effects seen in the DART study for testosterone effects?

DR. BELSITO: Yeah, so, we actually -- that's, Preethi, why we wanted the ADME. That may get rid of all of the issues with the DART effects that we're seeing.

MS. RAJ: Okay.

DR. BELSITO: And, in terms of the oxalates we really weren't -- I mean, the concern with oxalates is irritation.

MS. RAJ: Okay.

DR. BELSITO: We weren't really seeing irritation. So, you know, we could mention it that oxalates can be irritating, but at the concentrations that these are being used there's no evidence of irritation in cosmetic products.

MS. RAJ: Okay, thank you.

DR. BERGFELD: All right. So, I'm going to call the question now. And those that are opposed to moving forward with an IDA please indicate by stating your name. Hearing and seeing none, I will say unanimously this is approved to go out as an IDA.

DECEMBER 2021 PANEL MEETING – SECOND REVIEW/DRAFT TENTATIVE REPORT

Belsito Team – December 6, 2021

DR. BELSITO: This is the second time we're seeing the safety assessment of four cosmetic ingredients. First was in December of 2020, and we issued an IDA for clarification on the current maximum concentration of use of these ingredients, and a 28-day dermal tox at the maximum concentration use, an Ames test, both preferably with the ingredients in a hydro-alcoholic solvent.

We received some data on dermal sensitization for the extract. We got some revised concentration of use and updated VCRP. The maximum concentration is 0.5 percent in non-spray face, neck product. So, we're looking at this to see if we need more stuff. So, we haven't gotten the 28-day dermal, so I'm presuming that it remains insufficient at least for this.

DR. LIEBLER: I think, Don, that we're okay on method of manufacture and composition and impurities for all of them.

DR. BELSITO: Right.

DR. LIEBLER: The extract is the only ingredients that's used that covers them all. Then the food use mitigates concern about carcinogenicity. My only note to myself here was we need to determine whether the DART data are sufficient for safety. That I would just direct to Paul.

DR. BELSITO: So, we don't need 28-day dermal?

DR. LIEBLER: Well, I guess, do we need that too? That's another one I'd direct to Paul.

DR. SNYDER: Yeah, the DART data's not a concern. It was at very high levels, particularly at 0.5 percent. The DART effects were great at 125 milligrams per kilogram or greater. We had a small, short-term study where the lowest dose was 200. Then it looks like we had either, I guess I need to go to Table 5. It's easier to see in a table because that paragraph has a lot of studies in it.

MS. RAJ: Did you mean Table 6, Dr. Snyder?

DR. SNYDER: Well, the Table 5 is the short-term studies with the different extracts and extract, 14-day, 30-day. I don't understand why we came up with that 28-day. The repro effects were at high levels even in human studies.

DR. LIEBLER: I think the issue was, as Preethi put in the discussion, was that that they had somewhat inconsistent results in the tox studies. And now this is coming back to me. There were differences in the extraction method used. There's, you know, hydro-alcoholic extracts and I think, like, pet-ether extracts are very different, and the results were somewhat inconsistent. The request was for a 28-day dermal from an extract at maximum use concentration in a hydro-alcoholic solvent.

DR. BELSITO: Correct.

DR. LIEBLER: That's what we were asking for. I think that came from the other team as I recall. I don't think we have that. Do we have a tox study, 28-day dermal of a hydro-alcoholic extract with the whole --

DR. BELSITO: We do not.

DR. LIEBLER: Yeah, so we still have that, so I guess the question is, do they feel differently than they did last time about the body of data that we have?

DR. BELSITO: Well, and do we -- I mean, the question for us is, do we feel differently?

DR. LIEBLER: Yeah.

DR. SNYDER: Yeah, I think that's not going to go away then if that was a concern because that was when we thought the max concentration of use was 0.008 percent, and now we know it's quite a bit higher at 0.5 percent. The request would have to be a 28-day dermal at 0.5 percent of a hydro-alcoholic solvent extract.

MS. RAJ: We did receive two HRIPTs or sensitization studies at the max concentration of use.

DR. SNYDER: Yeah, those are sensitization, but not the dermal.

DR. BELSITO: Right.

DR. LIEBLER: So, we got a dermal, acute, and rapid at ten percent in ethanol, and LD₅₀ of 1800 and 65 mgs per kg.

DR. SNYDER: We really got to get to the short term, Dan, or longer --

DR. LIEBLER: Yeah.

DR. SNYDER: -- to take away the 28-day dermal. We just don't have it.

DR. LIEBLER: Right.

DR. BELSITO: Also ask for an Ames test on the hydro-alcoholic, which we --

DR. SNYDER: So, we got the clarification of maximum concentration of use, but we did not get the 28-day dermal at a maximum concentration of use or the Ames test with the hydro-alcoholic solvent. We're still deficient.

DR. BELSITO: Right.

DR. SNYDER: I think that's where we're at.

DR. LIEBLER: Yep. Still there. I don't disagree.

DR. BELSITO: Okay. In the discussion, Paul, do we need to discuss these developmental effects at all? The dose level versus the concentration of use in cosmetics?

DR. SNYDER: Yeah, I think that's exactly right, Don. The doses where there were effects where -- would never be reached with the cosmetic use.

DR. BELSITO: Okay. Okay. We're going -- delete this and create a new discussion point. So, we're back to insufficient for a 28-day dermal and Ames in a hydro-alcoholic.

DR. LIEBLER: Correct.

DR. SNYDER: Extract.

DR. BELSITO: So far, for the discussion, we have DART data and inhalation boilerplate, mucus membrane.

DR. LIEBLER: Right.

MS. RAJ: Since there isn't much, I guess, or any, inhalation language in the discussion right now, would the Panel be okay for that to be modeled after maybe what was seen in the sage report?

DR. BELSITO: Sorry. Yeah, I mean the concentrations of use here --

DR. SNYDER: Very low.

DR. BELSITO: We have low reports in deodorants. We have --

DR. SNYDER: Yeah, the spray is not reported concentration of use, and the powder is pretty low.

DR. BELSITO: Point five, right?

DR. SNYDER: Yeah.

DR. BELSITO: Yeah, so similar to what we did for sage. Anything else on this *Portulaca*? Hearing nothing we'll move to Dicaprylyl Ether.

MS. RAJ: Dr. Belsito, sorry, just to clarify, so is it going as a tentative report as safe but insufficient for --

DR. BELSITO: No, it's insufficient.

MS. RAJ: Oh, insufficient.

DR. BELSITO: Insufficient for 28-day dermal and Ames in a hydro-alcoholic solution.

MS. RAJ: Okay. Thank you.

DR. BELSITO: Then I just had a couple of questions on spelling, et cetera that you can see in the document. Okay, let me save this.

Cohen Team – December 6, 2021

DR. COHEN: We'll go to *Portulaca oleracea*. Preethi, this is yours. too. This is a draft tentative report. In December, we issued an IDA for current max use, an Ames test. We've got some updated VCRP, a memo clarifying the method of manufacturing for the extract that was using the whole plant. We have new max use data for the extract of 0.05 percent in a non-spray face and neck product. And we have HRIPT on two products, and we have 28-day dermal tox in rabbits and new DART. Wave III we had comments from the council that seemed spot-on.

So, I'll open it up. Tom, you want to start? Or, Lisa, go ahead.

DR. PETERSON: I just want to say I didn't think there were any issues with the chemistry endpoints. And then I had my own opinion about the toxicology, but I would let Ron and Tom talk first.

DR. COHEN: All right. Okay.

DR. SLAGA: I'm pulling it up right now, sorry.

DR. COHEN: All right. Ron, why don't you go ahead?

DR. SHANK: Okay. If we're still with an insufficient report, we need 28-day dermal tox on whole plant extract. And the discussion should include a statement that this plant is used as a food. So, additional systemic toxicity data are not needed. Under the section on the DART studies, I guess on the discussion we should say that the DART studies used high doses by oral gavage which would produce blood concentrations of the extract components much higher than would occur from topical application, so that makes it easier to interpret the results of the DART study.

So, I came up with basically still insufficient. It's used as food, so more systemic toxicity data are not needed. But we need more skin data, and I put that as a 28-day dermal.

DR. PETERSON: I'm just curious about why you're asking for that when it's systemically not toxic.

DR. COHEN: And we have HRIPT on it.

DR. SHANK: Let me check.

DR. PETERSON: I mean, the big question is whether it gets absorbed or not, but I mean if it's safe systemically, then it doesn't really matter. If it gets absorbed, it's going to be safe.

DR. SLAGA: Right. I agree with Lisa.

MS. RAJ: Yeah, and the sensitization studies are at the maximum concentration of use.

DR. COHEN: Yeah, because we got that updated max use data and it seemed to cover the HRIPT's that we saw.

DR. BERGFELD: So, if we get rid of the 28-day tox, can we go safe with a discussion expansion?

DR. PETERSON: Yup, that's what I thought.

DR. SLAGA: Yeah, I agree.

DR. COHEN: What would we need in the -- what additional information --

DR. SHANK: Is it the whole plant? Is the whole plant a food?

DR. SLAGA: The whole plant is a food.

DR. SHANK: Okay. I don't know why I kept 28 dermal. I'll take that out.

DR. SLAGA: Yeah.

DR. COHEN: So, we can come out as a safe as used?

DR. SLAGA: Safe as used. That's what I had.

DR. COHEN: That's what I had.

DR. PETERSON: That's what I had, too.

MS. FIUME: For the purposes of the discussion, we had asked for the Ames test so is there anything specific that should be said? We would need to include some type of statement saying we don't have genotox or carcinogenicity data and why those aren't needed. So, could some input and background be provided for the writer so they can address that in the discussion?

DR. PETERSON: I thought it was because it was food and --

DR. COHEN: Why did we ask for it last time?

MS. RAJ: I think it was because of the effects seen in the DART studies, and the Panel wanted to verify if it was solvent driven or actually because of the ingredient.

DR. COHEN: So, what are we doing -- I think the point is important. We asked for that. We don't have any, well, so --

DR. PETERSON: It was the other team that asked for that.

DR. COHEN: I know. I saw.

DR. SLAGA: Yeah.

DR. PETERSON: And I'm not sure we had a problem with it.

DR. SLAGA: Last time we disagreed with them, but we went with what they wanted.

DR. PETERSON: Yeah, and I think we can disagree again.

DR. COHEN: And we're going to disagree on the basis of it being a food?

DR. SLAGA: Right. The whole plant is a food.

DR. COHEN: Okay.

DR. BERGFELD: But I had a question. It says on Table 3 that it is in a powder. Do we need inhalation if it has -- I guess it's a possible powder.

DR. COHEN: Well, the ingredient that may be a powder, is it used as a powder?

DR. BERGFELD: I'm trying to see if that's true. I'm just looking at the use -- frequency of the used concentration table.

MS. RAJ: It's reported to be used in two face powder formulation, *Portulaca oleracea* extract.

DR. COHEN: There is something on the incidental inhalation in powders and sprays.

DR. BERGFELD: Excuse me.

MS. FIUME: So, this is one of the ingredients where the VCRP indicates the definite powder used, but there's no concentration of use for that powder reported.

DR. COHEN: So, Wilma, you're suggesting that we put an inhalation boilerplate in here?

DR. BERGFELD: I think so. If it is to be used as a powder, then we need to control it.

MS. RAJ: And could I possibly, I guess, follow the template of the sage report as far as how that's written?

DR. PETERSON: Yeah. Yeah, that would great.

MS. RAJ: Okay. Thank you.

DR. COHEN: That's great.

DR. BERGFELD: That would go on the discussion.

DR. COHEN: So, we're going as safe as used. We'll add the discussion about inhalation, and we're going as that this whole plant is a food. So, we're going to nullify on our side the request for the Ames if it comes up.

MS. FIUME: Then can I just ask one more question? Lisa, you may be able to give information. Again, this is one of the botanicals where the concern is the solvent may be causing the problems. So, does anything need to be addressed in the discussion regarding that, or would you like the Belsito team to answer that question since they raised it? Because I think --

DR. PETERSON: I think -- yeah. And I guess what is different about the solvent in this extract that has never been raised before in any of the other extracts because I'm not picking up on what solvent they're concerned about?

MS. FIUME: I think they were concerned that it was, I believe, the ethanolic solvent caused more issues than the aqueous solvent, and we have had botanicals before where it was safe except in one type of solvent. So, that has happened in the past. I'd have to look back and see what report it is.

DR. PETERSON: Okay. All right. I missed -- I think I picked up on it, but I sort of thought about it as the methanol and ethanol are going to extract different things. And, so, then it was just a different component. And so, I don't know that it's the solvent itself but rather a different chemical that the solvent is extracting. And so, the problem was in which toxic endpoint, I'm sorry?

MS. FIUME: So, I think -- and Preethi correct me if I'm wrong -- so the issues they were wondering, DART studies, the methanolic solvent resulted in some adverse effects where, if it was an extract in a different solvent, those adverse effects weren't seen.

MS. RAJ: Correct.

DR. ANSELL: Yeah.

DR. PETERSON: Right, and what is the dose at which those adverse effects are seen? I guess this just gets into their request for the genotoxicity data, right, because they're trying -- I mean, one of the things is, is if there's something different between the two extracts, one would pick up -- one would be more DNA. One of the ways of causing birth defects is to react covalently with DNA and cause toxicity that way. I guess that's where they were headed, and I mean how concerned are the toxicologists of the DART --

DR. SHANK: No concern. Those were high doses by oral gavage.

MS. RAJ: It looks like up to 800 milligrams per kilogram, Dr. Peterson.

DR. PETERSON: Yeah, and so in the concentration that humans are going to be exposed to is orders of magnitude less than that.

DR. SLAGA: Right.

DR. SHANK: And it is a food.

DR. PETERSON: And it is a food. And I think that the chemical of, whatever it is, is going to be in the food. The extracts going to pull out different things which is why you're seeing probably different biological responses to the two different extracts. But given the high concentration and the fact that this is used in food is something that -- this food would be associated with some kind of cancer. There would be some suspicious -- yeah, I don't know. I guess you see what you look for, but it's not a big concern on my part.

DR. SLAGA: I can't see it pulling up something in a large enough concentration to be the mutagenic or carcinogenic, for example.

DR. COHEN: Monice, when you -- I know you're going to have to go back, but in the circumstances where the methanolic component was not safe as used but in ethanol a component was, was that a food?

MS. FIUME: I'd have to go back and look because it was actually one of my reports. So, I need to figure out which one. I don't think it was a food, but I will have to look for sure. And so, yeah, it was safe as used except insufficient if extracted in whatever solvent. So, I will go back and try and locate that, and I will let you know. And I will find the discussion for that report because, like I said, I remember talking about it, but it's been a while. So I can't recall exactly which report it was in.

DR. COHEN: Yeah, I mean, I could see that when it's not a food how that might be very different. It's just you keep coming back to that you're going to eat it.

DR. SLAGA: You going to get more that way.

DR. COHEN: Yeah.

DR. SLAGA: You don't have to extract it.

DR. COHEN: Yes. You're going to chew it.

DR. SLAGA: And extract it from your own stomach.

DR. COHEN: Yeah, okay.

MS. FIUME: Thank you.

DR. COHEN: I think that'll be a good one tomorrow. Okay. Are we okay moving onto fatty ethers?

DR. SHANK: Yeah.

Full Panel – December 7, 2021

DR. COHEN: Okay. We have a draft tentative report for *Portulaca oleracea*. In December of 2020 the Panel issued an IDA for clarification on the current maximum concentrations of use, as well as 28 day dermal tox at maximum concentration of use and an Ames test. We have since learned that the whole plant is used as a food. We received clarifying information about method of manufacturing using the whole plant. We have new data on max use, and we have an HRIPT for two products, which look okay. Our motion is to have safe as used.

DR. BERGFELD: Is there a second or a discussion? Don?

DR. BELSITO: We had a different motion. We thought it was still insufficient for 28 day dermal and Ames test in an hydroalcoholic solvent. And I'm not sure, David, where you got the full plant was a food.

DR. COHEN: Lisa, do you -- I'm going to try to pull that up now. Do you remember?

DR. BELSITO: Under non-cosmetic uses --

MS. RAJ: That's on PDF page 22, I believe.

DR. PETERSON: It's on 22.

DR. BELSITO: Raw salads where it's used as a pot herb and cooked sauces, yada, yada, yada, but it doesn't say what part of the plant. It doesn't say the whole plant. It's traditionally used in folk medicine. I mean, you don't know what parts of the plant they're referring to here.

DR. COHEN: I think we made an assumption it was the whole plant. I'm just trying to see if we had any other corroborating information.

DR. EISENMANN: I read it's the stems, leaves, and flower buds.

DR. COHEN: Stems, leaves, and flower buds. Okay.

DR. BELSITO: Where do you see that, Carol?

DR. PETERSON: You know, if you go on the internet and you Google, there's all kinds of recipes for it because it's purslane.

DR. COHEN: So, it's the whole thing?

DR. BERGFELD: Doesn't include the root it sounds.

DR. PETERSON: All they say is you take the plant, and you cut it up.

DR. BELSITO: Yeah. But if you go on the internet, Lisa, you find a lot of stuff, and, I mean --

DR. PETERSON: Okay. They're talking about the leaves. I mean, honestly, if there's recipes for using it -- but it's probably just the leaves. Actually, apparently, they're very good sautéed in garlic.

DR. COHEN: The derived ingredients, though, are flower/leaf/stem extract and juice. Don, could you articulate what your team was looking for?

DR. BELSITO: Yeah, sure. Insufficient for a 28 day dermal and Ames test in an hydroalcoholic solution, and, Dan, that had to do with certain components of the plant that were present in the hydroalcoholic. And I didn't write that down. Can you refresh my memory? You had some concerns from the genotoxicity from the hydroalcoholic solution.

DR. LIEBLER: Actually, I think it was just the tox studies that were --

MS. RAJ: Yeah. It was the DART section -- effects from the DART studies.

DR. BELSITO: Yes.

DR. LIEBLER: And with the different extracts -- this is why this issue of which hydroalcoholic extract -- or which extract form was in our draft discussion from our previous meeting because I had not remembered why we had talked about that. And then I looked at table 5, "Short Term Toxicity Studies" and also table 6, and there's quite a variation in the kinds of extracts that are used. And for example, in table 5 on PDF 32 water ethanol, you've got a -- let's see. Go back to PDF 31.

DR. BELSITO: Dan, am I at table 6?

DR. LIEBLER: Table 4.

DR. BELSITO: Table 4. Okay.

DR. LIEBLER: That's the one I noted where table 4 under "Oral Acute Tox" it's a water ethanol one to one extract with an LD₅₀ less than 500 mg per kg bodyweight. And then with a pet ether extract LD₅₀ greater than 2,000. I think that's where our - I think that's where the question about which extract is relevant came from. It doesn't sound like something I would have come up with, but I was just trying to interpret why we had that in the insufficiency last time.

DR. BELSITO: I think as was said it had to do partially also with the DART effects.

DR. LIEBLER: Paul?

DR. SNYDER: Yeah. I'm not worried about any DART effects.

DR. BELSITO: No, we weren't worried. We were just concerned about differences, I think, in the different extracts.

DR. SNYDER: Did we have any genotox data at all that we got some kind of a weird signal or something? I was just looking for that, but why are we looking for an Ames test in that regard?

DR. LIEBLER: There's no genotox data. I think we're chasing our tails here. I did not have this objection. I was just trying to figure out why it was there from the previous meeting.

DR. BERGFELD: I think Tom had a --

DR. COHEN: We thought it can from your team.

DR. PETERSON: And I will say, you know, on the internet there's -- and I know you don't trust necessarily maybe the internet, but there's recipes for using the flower, stem, and leaf. I mean, these are commonly eaten. They're good for you kind of plants. Just because it's a weed --

DR. BELSITO: Lisa, how are they good for you? Because the internet claims it? I mean --

DR. PETERSON: Well, it says it's rich in Omega-3 fatty acids. I mean, somebody has measured these things. I mean, I understand that you're not probably going to accept this, but I just wanted to point out that there's a lot of people that eat this. And it doesn't seem to be a problem.

DR. BELSITO: I don't know. There's a lot of problems in our country.

DR. COHEN: I don't think Don's team was questioning it being used as a food. I think one of the questions that came up is, is it the whole plant or not, and we assumed it was because we had stems, leaves, and flower. And the entire plant is used in the preparation of the extract. We went forward and assumed it was the whole plant. We still kind of have that feeling. The question is are those insufficiencies that came out of Don's team still relevant and necessary, or can we pass this as safe?

DR. SNYDER: This didn't come from our team. This was from the December 2020 IDA in which both teams voted to issue an insufficient data announcement with these three requirements: maximum concentration of use, 28 day dermal at maximum concentration of use, an Ames test with the hydroalcoholic solvent extract. And, so, we looked at it and --

DR. COHEN: Paul, you're right. I'm sorry, I didn't mean to characterize.

DR. SNYDER: -- we got the clarification with the max. Yeah, we got the clarification with the max concentration of use, but the other two data needs weren't met according to the data we received. So that's why we were asking if you received some data that we missed or something. So, I think we're still in the same place.

DR. BERGFELD: I'd like Tom Slaga to talk about the Ames test.

DR. SLAGA: I don't think we need to the Ames test. I usually don't use the term specifically. I think genotox to cover both Ames as well as mammalian. The Ames test is very accurate, though. I'll emphasize that again.

DR. LIEBLER: So, I think the thing that's new here is the data information that there's widespread food use of this. I don't think that was discussed last time as far as I remember. Otherwise, we wouldn't have had those kinds of insufficiencies. So now we do have this element, and this is coming up more and more where, you know, I'm not arguing with what Lisa found, that widespread food consumption should influence us. It's just that we're kind of coming across this on an ad hoc basis. Sometimes I google them. Sometimes Lisa does. Sometimes somebody else does, and then we've got, you know, broad anecdotal evidence.

And so how do we standardize that so that we have a better look at food uses? I think it ought to be something that the authors of the reports need to do in addition to searching the usual tox databases and so forth, and perhaps you do. I don't mean to

prejudge if you're already doing it and I'm missing it. But that's the new thing here in this discussion, and that seems to be what's changing our discussion here.

DR. BERGFELD: So, you're suggesting that the scientific writers when preparing the original document search the internet for food use in some of these botanicals?

DR. LIEBLER: Yeah. I mean, what's an appropriate way to assess food use? What's a standard -- we have a nice, standardized approach to data gathering, and then this is a little less so. And, so, I'm not arguing with it, but I would like this to be something that's not so ad hoc.

DR. PETERSON: Yeah. And I agree with that. I'm just going to point out that I have been moved in this direction based on conversations that have been held, like half the algae that we accepted we accepted because people use them as food. And I don't remember whether in those documents what the documentation was for food, but it seems fairly inconsistent to me. Sometimes using it as food is acceptable. Sometimes it's not when it comes to these kind of issues.

So as a person that doesn't have the history that you all have, I'm very confused by why there's -- and it goes to an earlier conversation we had today. There was this huge discussion about barley, and, you know, in the past with other compounds or other botanicals there wouldn't have been quite this kind of strength of argument against, it's on the internet, so, therefore, we don't use it kind of thing. So, I agree with Dan that there needs to be systematic approach to how we deal with the issues going forward.

DR. BELSITO: Well, I thought that we previously said we don't use internet just postings per se. However, if you look at the non-cosmetic, systemic uses are referenced. They're reference 3, 4, 17, 44 and 46. So reference 3 is a review in the *Journal of Pharmacologic Research*. Reference 4 is an article in *Personal Care* magazine about the global panacea of this material. Reference 17 doesn't necessarily look like it's a food use. Reference 44 is from *Ethnobiology*. Again, it doesn't necessarily look like they're food use, but there may be (audio skip).

DR. PETERSON: Are reviews sufficient for this purpose because --

DR. BELSITO: No. I mean, certainly a review would hopefully go back to some primary literature, and then there's the 46, the World Health Organization regional office on medicinal plants in Vietnam, which was a World Health --

DR. COHEN: Don?

DR. BELSITO: Yeah, David?

DR. COHEN: I thought Dan's explanation was just a perfect summary of what we're up against, and I'm just wondering, Wilma and Bart, is there any way for the CIR to have an ad hoc consultant on human food where we can ask them, is this considered food? Because there's not going to be a PubMed article on people eating celery as food, but there's probably professors that do this that could say, this is a food, and by the way this is the part of the plant that people are eating. And that's somehow acceptable. I don't know if we'll have references the way we want them.

DR. PETERSON: I mean, if we found a recipe for it in a published magazine like one of the cooking magazines, to me that's where you're going to see these sorts of things. And then does that mean it's acceptable? I guess as a -- you know, one of the recipes I found was on Epicurious, which is actually a fairly well-curated recipe. I mean, I'm just throwing -- because I agree, you're not going to probably find a scientific article on (audio skip).

DR. BERGFELD: Well, I think that we can close this discussion a little bit and turn this over to Bart to come up with a plan of how we're going to do this. We may have to ask that -- our consulting group at the council to help to support our activities and also FDA. There may be a few specialists, as you mentioned the professors, but I don't think we can solve it today. But it is an issue, and it needs to be resolved. And we need to come up with some standardization. So, Bart, can I give you that job to start to develop whatever this might be?

DR. HELDRETH: Yes, absolutely.

DR. BERGFELD: And who might be involved with making a decision on it?

DR. HELDRETH: That's right. I will reach out to a number of the people that you just suggested from FDA, from industry, from even academic institutions. I suspect that, you know, we won't find one person that will answer all our questions about foods. You may have somebody like when we first started looking at algae. Dr. Rex Lowe was an expert in algae and could tell us what was food and what wasn't, so it may be a case by case situation. But yes, I will take it and contact a number of individuals and see where we can go with that.

DR. LIEBLER: Perhaps a group of maybe three academics who are on the food science, nutrition area. There may even be academic centers on plant foods -- available plant foods or whatever. There may be something like that. I'm sure that they exist. Now, to come back to this particular report, it seems to me that if we get to the point where we can accept bona fide food uses, our concerns are essentially gone.

DR. BERGFELD: Yes.

DR. LIEBLER: And we just need to verify that indeed we can be comfortable with food uses of this ingredient. If we are, then my concerns go away.

DR. BERGFELD: Well, then we have to take action on this particular ingredient was to how we're going to deal with it until we get that information. Bart, can you give us some advice?

DR. HELDRETH: Since we're at the tentative stage, if you feel like this is a data need that you've had all along, then you could come up with a tentative conclusion that's insufficient for that need. If you feel this is a new need, a need that you haven't expressed publicly that was needed for this report previously, then I would suggest going with an IDA.

DR. BERGFELD: Dr. Cohen?

DR. COHEN: I think we have to go with -- me?

DR. BERGFELD: Yeah. Dr. Cohen.

DR. COHEN: No, I didn't hear. I couldn't hear. Look, we went as a safe as used. I think to Don's point earlier as well we didn't ask for information regarding its use as food, although one might suggest that its use as food would neutralize the need for the tox and Ames, et cetera. So, we could interpret it different ways. The easiest way is probably going out with an IDA asking for further information about its use as food and specifically what part of the plants are eaten.

DR. SNYDER: I think Don made the point earlier on botanicals that it's not just the food. It's that what's the method of manufacture and composition because we need to know those extracts -- how are they extracted, and what kind of impurities are in there? So, isn't that correct, Don, and I'm quoting you right from the previous one?

DR. BELSITO: Yes.

DR. COHEN: So, you don't think we have enough composition and impurities and method of manufacturing?

DR. SNYDER: Your team's data request was for the method of manufacturing for the whole plant just now.

DR. BELSITO: No, they were clearing the whole thing.

DR. BERGFELD: They were clearing. They were clearing. They were going safe with all.

DR. COHEN: We went safe as used.

DR. SNYDER: Oh. Because I wrote down whole plant is food? Method of manufacture whole plant and HRIPT negative. Okay. Sorry.

DR. LIEBLER: Yeah. We're very good on the whole plant method of manufacture, so we're okay on that.

DR. BERGFELD: So, we have an action that's being developed. I need to know if Dr. Cohen's going to rescind his original motion as safe.

DR. COHEN: I'll rescind my original motion. I'll make a motion as an IDA. I'd actually reaffirm the IDA that we had before, 28 day dermal tox or its use as a food with clarification on what part of the plant is eaten because it's an "or"; right? Where if we get plant information we satisfied our tox, and we just want to know a little bit more is the whole plant -- if we have the whole plant eaten, I think we're done, and it's a safe as used.

DR. BELSITO: Yeah. I would agree, David. And Preethi, if I could ask you to include the entire papers and all of the references that are given for the food use and the non-cosmetic use. There are five papers that I obviously didn't review because I was focused on our prior data needs.

DR. BERGFELD: Okay. So, we appear to have a second. We appear to have a list of needs. We asked Preethi to give some added information about the references. I'm going to call for the vote, then. All those that are opposing the IDA, please indicate by stating your name? Abstaining? A unanimous decision to move forward in this direction with an IDA. Anything else that we need to say? David?

DR. COHEN: Wilma, Ron suggested in the discussion that the DART studies were carried out using oral gavage which are an exposure much higher than one would expect from a cosmetic exposure. Is that right, Ron? Did I represent that correct?

DR. SHANK: Yes, that's correct.

DR. BERGFELD: Any other comments? Okay

MARCH 2022 PANEL MEETING – THIRD REVIEW/REVISED DRAFT TENTATIVE REPORT

Belsito Team – March 7, 2022

DR. DONALD BELSITO: On to *Portulaca oleracea*. This is the third time we're looking at this safety report for cosmetic ingredients. In December, we issued a tentative report with the Panel. It was an IDA -- we asked for a 28 day dermal tox for the extract at maximum concentration of use, preferably in a hydroalcoholic solvent, and, if positive, additional tox data and clarification on which part of the plants are used as food and which parts are used as cosmetics. And, in Wave 3, is this the one that we got to wait three here last minute reprieve for this one. Is that right? Go to *Portulaca oleracea* here. Yeah, Wave 3. We received data. I believe that the whole plant is actually used as a food. Is that not correct? You know, Wave 3.

DR. DAN LIEBLER: Yeah, PDF, PDF page 9 in the Wave 3 supplement the memo from Alex.

MONICE FIUME (CIR): That's correct.

DR. DONALD BELSITO: Yeah. And, so, it looks like the Kumar report, which I actually reviewed, does illustrate that this is used as the whole plant is used as a food, which I think got rid of our 28 day dermal tox needs. So that puts us back to. [inaudible] Relooking at the documents. Which are not opening.

DR. DAN LIEBLER: K.

DR. DONALD BELSITO: Alright. So, we need to add that food reference that all plant parts are met and so I thought all our needs were met and we could now go with the safe as used.

DR. DAN LIEBLER: Good luck.

DR. DONALD BELSITO: When formulated to be non-sensitizing, it has terpenes, so that was a question to Dan. We don't have this specific terpenes.

DR. DAN LIEBLER: That's OK. I think that you know we got sort of into chasing our tails on the last meeting on this because we had originally I think intended to come in safe as used and then there was a question that arose about whether the whole plant had food uses and this led us to react to what we anticipated the other team wanted and that's why we came back with 28 day dermal and you know, in a hydroalcoholic solvent extract and I think our, you know ours I think our teams were kind of out of sync. So, we went back to a sort of a maximalist request for these data. When it turns out that the food uses were at least marginally compelling in the last meeting, when I looked at the report this time before I even saw the Wave 3 reference you've just mentioned, I was convinced that the that the evidence for food use for the whole plant was sufficient, and that made the concerns about that, you know, that need for the 28 day dermal just completely. Go away, and the Wave 3 Kumar reference that cited further supports that. So, I agree we basically can go safe is used.

DR. DONALD BELSITO: Now Dave, it does contain terpenes, we don't know which terpenes do we need to use the botanical boilerplate of when formulated to be non-sensitizing?

DR. DAN LIEBLER: I would support it if you felt that we want it. You wanted to do that. Because it's true that we don't have specific terpene chemistry info on these. So, I would support it if you if you wanted to do it, but I wouldn't initiate that myself.

DR. DONALD BELSITO: Yeah. Well, Paul and Curt?

DR. CURTIS KLAASSEN: I'm fine with it.

DR. PAUL SNYDER: Or. They would.

DR. DONALD BELSITO: I'm trying to, because I think it just says in composition terpenes, I mean it. Can't remember. Yeah, I think just to be on the safe side, since it has terpenes and we don't know which ones probably formulated to be non-sensitizing, wouldn't really cover us.

DR. PAUL SNYDER: And it would be consistent with what we've done historically, so.

DR. DONALD BELSITO: Yeah. I mean, historically, we've had data that shows that there is clearly a sensitizing terpene in there here. We don't know what the terpenes are, but I think to air on the side of conservatism, it's best to just put that in.

DR. DAN LIEBLER: Yeah, take Table 2 on PDF page 39 does list. In the flower leaf and stem, a few terpenoids that were found in that one study. Then that's all we have.

DR. DONALD BELSITO: 39 Dan.

DR. DAN LIEBLER: PDF 39.

DR. DONALD BELSITO: Yeah, that's probably why.

DR. DAN LIEBLER: The middle of the table or it says terpenoids.

DR. DONALD BELSITO: Right. Yeah. So, let's just formulated to be non-sensitizing. You're OK with that current call, Dan?

DR. DAN LIEBLER: Sure.

DR. PAUL SNYDER: Yes.

DR. DONALD BELSITO: Kurt.

DR. CURTIS KLAASSEN: Yes.

DR. DONALD BELSITO: OK.

DR. CURTIS KLAASSEN: Can you hear me?

DR. DONALD BELSITO: Yeah, we hear you loud and clear. Anything else on *Portulaca* before we move on?

DR. DAN LIEBLER: Yep.

DR. DONALD BELSITO: K.

DR. DAN LIEBLER: And the other Wave 3 comments, we agree they're sufficiently editorial.

DR. DONALD BELSITO: Yes.

DR. CURTIS KLAASSEN: Yes.

Cohen Team – March 7, 2022

DR. DAVID COHEN: But we've seen max uses vary greatly. In constituents that are studied in the same report. Right? And, so, I don't know what it means. You know, comparable if they're not really comparable necessarily and we have data on 0.01% -- should we not again these are not just for ubiquinone and but do we become more resolute and say like this is we only have data on this far --of course the counter is we read across all the time without having that information. Yeah. OK. I didn't -- I think -- I think the video commentary says it all. Which won't translate, won't translate well in the dictation, but OK. So we're going to go with safe as used. OK, to move on to *Portulaca*. OK Let me just pull it up. OK, so at the December meeting we issued an IDA requesting 28 day dermal tox -- if positive, we'd want further information and clarification on which parts are consumed as food. And yeah. And that-- that was-- that was where we left it off and we didn't didn't get any data on this. There are even more articles on *Portulaca* in peer-reviewed literature that talk about the various parts of the plants that are eaten. The stem, the leaf, the flower of the seedlings, the fruit. It's out there.

CAROL EISENMANN (PCPC): The roots are also eaten.

DR. DAVID COHEN: Yeah.

PREETHI RAJ (CIR): Dr. Cohen, did the Panel have a look at the paper that Council brought up -- the Kumar paper.

DR. DAVID COHEN: Yeah, that wasn't I. I did see. I did see that. Did you guys see that?

DR. RON SHANK: Ah, Yes.

Dr. David Cohen: I found one also-- the Scientific World Journal. It was a review article on Purslane weed. As a nutritious-- A prospective plant, source of nutrition, omega-3 fatty acids and antioxidants. So, what's the group well-- What's your take on it?

DR. RON SHANK: Um, I don't have that Wave 2 in front of me right now, but.

DR. WILMA BERGFELD: Yes.

DR. RON SHANK: It's just the one where it said the whole plant is eaten--

DR. WILMA BERGFELD: I think it's important. 3.

DR. RON SHANK: --in Sri Lanka, and the roots's eaten in Australia.

DR. WILMA BERGFELD: Yeah, that's in three dot [inaudible].

DR. RON SHANK: If we take that as if we take that as evidence as to human food--

DR. WILMA BERGFELD: Wave 3 [inaudible].

DR. RON SHANK: --then we can say these are safe.

DR. THOMAS SLAGA: Sure.

DR. DAVID COHEN: Wow, that's like an air raid siren.

DR. RON SHANK: I don't know what that is. Yeah. I didn't do it. If it is whole food. Then it would be a whole food. If the whole plant is a food, then our data needs are satisfied.

DR. WILMA BERGFELD: I have to ask your Ron --who declares it's a food? Or, is it just that we notice it's food or someone has to declare this is a GRAS? This is a food. This is a--

DR. RON SHANK: That's a very good question. I think we've been saying I don't think there is any official body, is there? Defining a food.

DR. THOMAS SLAGA: No.

DR. RON SHANK: There's a Food Chemical Codex-- but well, I guess that does define what the food is. Such as the difference between milk and an oak powder mix. But it doesn't say what is not a food, and what is a food. So that's a good question.

DR. THOMAS SLAGA: Yeah.

DR. RON SHANK: I think we've been relying on the fact that if we have data showing that it is eaten by humans. Then that's the food.

DR. WILMA BERGFELD: I think we should write that down somewhere -- what we're considering food.

DR. RON SHANK: Yep. Good point.

DR. WILMA BERGFELD: I mean. I think we should have a sort of, maybe not an official policy, but--

DR. DAVID COHEN: Why wouldn't we have a resource document on something like that? I mean I find myself, you know, remember when, you know, we had Lisa on and she was bringing up recipes and I decided to do it myself, but with peer review journals on using it as food-- but where we're looking around for it and it might be nice for us to codify how we do this.

DR. RON SHANK: I think they're going to be a lot of mixed confusion between when they say people eat it --do they eat it as a food, or as a medicine?

DR. THOMAS SLAGA: That's right.

DR. RON SHANK: You know you eat it because you have intestinal worms or something like that. That's not a food- OK? I'd like a graphic example.

DR. WILMA BERGFELD: I'd like to know if they die or not.

DR. THOMAS SLAGA: Yeah.

DR. DAVID COHEN: Yeah, that might. Yeah, that's--

DR. RON SHANK: That's another good question. They eat it, yes, once. Sorry.

DR. DAVID COHEN: So, are we going to go out as safe as used based on the information that we have now?

DR. WILMA BERGFELD: Well, you have a new reference. And you have, you said, a reference?

DR. DAVID COHEN: I have a reference to-- I'll send it.

DR. WILMA BERGFELD: So, I think it goes safe. I think you're better discuss it.

DR. RON SHANK: Yeah, that would have to be handled in the discussion that we're relying on those references to establish it is a human food.

PREETHI RAJ (CIR): And would the Panel have any language to explain why you're going with this conclusion without having received the IDA data you asked for?

DR. DAVID COHEN: Well--

DR. WILMA BERGFELD: New references have come to light. Utilizing it as a food.

DR. DAVID COHEN: Yeah, I think this was in lieu of those.

DR. WILMA BERGFELD: Yeah.

DR. DAVID COHEN: Right. Would that be fair? You know, we we've done this a few times where we say we want dermal tox and reproductive tox-- or show us it's a food-- and we can move on from there.

DR. RON SHANK: Yeah, that was a systemic tox data request. But if it's the food, then we don't ask for systemic tox.

PREETHI RAJ (CIR): Thank you.

DR. DAVID COHEN: Alright, so we're going—we'll go with safe as used--and in the discussion will point to the references we're using.

DR. RON SHANK: Yes. And that we're not asking for systemic tox-- because of--it's used as a food now. What about sensitization data do we have that? I don't remember.

DR. DAVID COHEN: I believe--I believe we do. I believe it's at a point 1% and max use is below that-- substantially below that.

DR. RON SHANK: OK. OK. OK.

DR. DAVID COHEN: Let me just go back in.

DR. WILMA BERGFELD: We have irritation too.

DR. DAVID COHEN: Yeah. Yeah. I really mulled over that one-- just I thought there was there was a lot of information online about it and in peer reviewed articles about it-- it's just wasn't, it wasn't something trivial, OK? If we may. Go on. Alright, hydroxyacetophenone, this is the first time we're reviewing this. It's used as an antioxidant. And skin conditioning agent and we're evaluating one ingredient. We have reported use, which is considerable. And we have a max, you said 5% in a non-spray night product. It's also used in paste masks and mud masks. It's used also around the eye in an eye lotion. And we saw a case of contact dermatitis from a face cream, perhaps, from that. And we have a lot of data on this. I was going to ask for some comments about the clastogenic effects in the mouse lymphoma model --and some irritancy in rabbit eyes. So, I'll just open it up for you--

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DR. WILMA BERGFELD: All right. Any other comments before we call the question? I'll call the question all those opposed? Abstaining? It's approved, so it unanimously approved, moving on to reports advancing. Dr. Belsito is up for *Portulaca*, I think you pronounce it that way.

DR. DON BELSITO: OK.

DR. DON BELSITO: *Portulaca oleracea* --So, this is the third time we're looking at these four ingredients and at the last time in December that we looked at them, I'll cut to the chase-- we either wanted data on lack of systemic absorption or knowledge that they were used in foods. We have now received information that the whole plant is in fact considered food in many countries, and thought we could go ahead with the safe is used conclusion for these ingredients. It has- they have terpenes, so we were suggesting safe as used when formulated to be non-sensitizing.

DR. DAVID COHEN: Second.

DR. WILMA BERGFELD: Any further discussion or comment on this ingredient, this botanical? David.

DR. DAVID COHEN: Well, I think I think in general it brings up the issue of --what is food? and how do we corroborate data that suggests it's food? I think on our team we were, we were not looking at Google anymore to see, or look for say it's mentioned a food or a recipe. But there were ample peer-reviewed journal articles on its use in food. And it's something that's going to come up again and again when we have a lot of botanicals or plants. As far as the terpenes, yeah, fine with when formulated to be non-sensitizing.

DR. WILMA BERGFELD: Any other comments?

PREETHI RAJ (CIR): So, just to make sure, we're relying mainly on the use in food for safety to cover--

DR. WILMA BERGFELD: (inaudible)

PREETHI RAJ (CIR): No genotox or carci data in this report. Is there any additional language you would want in the discussion?

DR. WILMA BERGFELD: Ron, do you want to make a statement on that.

DR. RON SHANK: No, that's enough. That's enough.

DR. WILMA BERGFELD: OK. Any further comments or questions?

PREETHI RAJ (CIR): Thanks.

DR. WILMA BERGFELD: Called the question, then all those opposed? Abstaining? The ingredient is approved as safe, with non-sensitizing, and moving on to the next ingredient, which is Dr Cohen hydroxyacetophenone, I guess.

Safety Assessment of *Portulaca oleracea*- Derived Ingredients as Used in Cosmetics

Status: Draft Final Report for Panel Review
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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.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D., and Susan C. Tilton, Ph.D. Previous Panel members involved in this assessment: Lisa A. Peterson, Ph.D. and Ronald C. Shank, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Preethi S. Raj, M.Sc., Senior Scientific Analyst/Writer, CIR.

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 4 *Portulaca oleracea*-derived ingredients as used in cosmetic formulations. These ingredients are mostly reported to function as skin-conditioning agents. Because final product formulations may contain multiple botanicals, each containing the same constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers; with *Portulaca oleracea*-derived ingredients, the Panel was concerned about the presence of terpenes as potential sensitizers in cosmetics. Industry should use current good manufacturing practices to minimize impurities. The Panel reviewed data relevant to the safety of these ingredients in cosmetic formulations, 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 is a safety assessment of the following 4 *Portulaca oleracea*-derived ingredients, as used in cosmetic formulations:

Portulaca Oleracea Extract

Portulaca Oleracea Juice

Portulaca Oleracea Flower/Leaf/Stem Extract

Portulaca Oleracea Water

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), all of these ingredients are reported to function as skin-conditioning agents in cosmetics (Table 1).¹ Additionally, Portulaca Oleracea Flower/Leaf/Stem Extract is reported to function as an antioxidant.

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

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

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 cosmetic ingredient. Therefore, if it is not known whether the test substance is the same as the cosmetic ingredient, the test substances will be identified by the standard scientific practice of using italics to identify genus and species (i.e., a *Portulaca oleracea* extract). However, if it is known that the substance is a cosmetic ingredient, the International Nomenclature Committee (INC) terminology "Portulaca Oleracea..." (e.g. Portulaca Oleracea Extract) will be used.

CHEMISTRY

Definition and Plant Identification

The definitions and functions for the 4 *Portulaca oleracea*-derived ingredients reviewed in this safety assessment are provided in Table 1.¹ The flower is the reproductive shoot in flowering plants, usually with sepals, petals, stamens, and pistil(s). The stem is defined as a slender or elongated structure which supports the plant, plant part, or plant organ, while the leaves are defined as the flattened photosynthetic organs, attached to the stems.

Portulaca oleracea is an annual herbaceous weed of the Portulacaceae family.² The genus *Portulaca* is thought to be derived from Latin 'porto,' to carry, and 'lac,' meaning milk, owing to the milky juice obtained upon expressing the plant.³ It is commonly referred to as purslane, pigweed, Ma-Chi-Xian, and many other regionally specific names.⁴ Although it is thought to originate from tropical and subtropical countries in Eastern Asia, it currently grows throughout the world, in unshaded areas. In spite of growing optimally in temperate climates, *Portulaca oleracea* also thrives under diverse geographic and climactic conditions due to its relatively low water and soil nutrient requirements, and tolerance to salt and drought.^{5,6} As a dicotyledonous, C4 photosynthesis plant, displaying Kranz anatomy structure, *Portulaca oleracea* has high water efficiencies in conditions that promote carbon loss through photorespiration, such as high temperatures, high light intensities, and decreased water availability.^{7,8}

The plant is a succulent, which usually grows close to the ground, and is up to 30 cm in height, with a cylindrical stem of 2 - 3 mm in diameter.⁹ The leaves are oblong and grow in an alternate arrangement, broad at the apex and tapered at the base. The leaf apex is obtuse and smooth, with no teeth or lobes. The flowers are terminal in cluster, with 2 - 6 foliar involucre, and five bright yellow petals enclosed by two subequal lanceolate sepals. The fruit is a shell and the seed is kidney-shaped and flaky.¹⁰ The stem is smooth, red, and circular, and consists of a distinct ~ 60 µm epidermis, 800 µm broad

cortex, and a pith consisting of cells similar to cortical parenchyma. The xylem elements are thick-walled and angular, and possess dense calcium oxalate crystals.

Chemical Properties

In an ultraviolet (UV) spectral analysis of crude, and methanol-soluble fractions of whole *Portulaca oleracea* extract, optical spectra maxima were recorded between 200 and 400 nm, in which phenolic compounds showed maximum absorbance.¹¹ The Fourier transform infrared spectroscopy (FTIR) spectrum of a chloroform extract of whole *Portulaca oleracea* showed peaks at 1019.52 and 1396.21/cm, corresponding to the wavenumber ranges for alcohols and phenols, amines, organic, and, possibly, nitrogen or oxygen-containing compounds.¹²

Method of Manufacture

An overview of 2 supplier-provided methods of manufacture for *Portulaca Oleracea* Extract, both using the whole plant,¹³⁻¹⁵ is outlined in Figure 1.

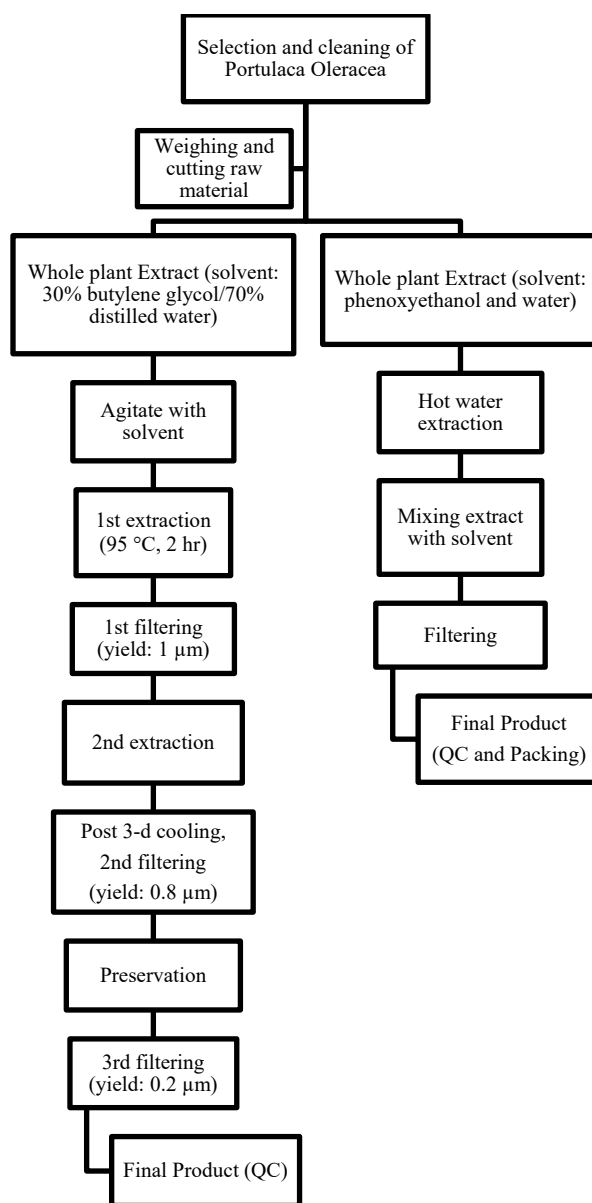


Figure 1. Overview of methods of manufacture for *Portulaca Oleracea* Extracts.¹³⁻¹⁵

Most of the methods below are general to the processing of *Portulaca oleracea*, and it is unknown if they apply to cosmetic ingredient manufacturing.

Portulaca Oleracea Extract

Extracts of *Portulaca oleracea* may be obtained through maceration of the fresh or dried plant in an alcoholic or aqueous solvent.¹⁶ Most *Portulaca oleracea* extracts are obtained using ethanol or methanol solvents.¹⁷ Methanol is preferred as a polar solvent which elutes the highest level of constituents from *Portulaca oleracea*, in turn affecting phenolic

compound content and potential antioxidant activity.¹⁸⁻²¹ Levels of individual compounds detected in crude *Portulaca oleracea* extracts may be low (e.g., phenols), but may be isolated via various techniques such as reversed-phased separation.¹¹

A method of preparing the aqueous extract of *Portulaca oleracea* (whole plant) is described as follows: distilled water (1500 ml) was added to 300 g of dried plant powder in a sealed glass container, set aside for 72 h, and then the filtrated extract was concentrated in a rotary evaporator under reduced pressure at 55 °C.¹⁶ The resulting extract was dried in a warm water bath.

An alcoholic extract of *Portulaca oleracea* seeds was obtained by refluxing 500 g of powdered seeds with 2 l of rectified spirit for 10 h on a 100 °C water bath.²² The initial filtrate was collected while hot, and the residual seeds were refluxed thrice more with 2 l of rectified spirit. Filtrates from the successive extractions were mixed and the rectified spirit was distilled off under reduced pressure, resulting in 50 g of an oily brown syrup. This syrup extract was suspended in 250 ml of sterile olive oil.

Portulaca Oleracea Flower/Leaf/Stem Extract

The aerial parts of *Portulaca oleracea* were used to prepare several extracts.²⁰ Four solvents (300 ml, each) of increasing polarity, namely, hexane, ethyl acetate, methanol, and water, were placed in the cartridge of separate Soxhlet extractors with 30 mg powdered aerial parts of *Portulaca oleracea*. The extractions took place over 24 h, after which the recovered extracts were conserved at 4 °C.

Aerial parts of the plant were washed with water, and the leaves along with the stems were stripped from the plant and divided into three equal batches.²³ The first batch was cut into small pieces and air dried at 45 °C. The second batch was boiled in water at 100 °C for 15 min in the ratio of 1:10 (w/v). The third batch was blanched in boiling water (at 100 °C) for 10 min in the ratio of 1:10 (w/v). After boiling and blanching, the remaining water was discarded and the three processed samples were cut into small pieces and dried at 45 °C. After drying, the samples were ground to a fine powder and extracted in aqueous acetone.

Portulaca Oleracea Juice

In another study, the aerial parts of *Portulaca oleracea* were washed with water, cut into small pieces, and blended.²⁴ The juice was obtained from the resultant puree by centrifugation (10,000 x g, 20 min, 4 °C) and was sterilized by filtration on 0.22 µm membrane filters.

Portulaca Oleracea Water

Portulaca Oleracea Water is the steam distillate obtained from the whole plant.¹

Composition and Impurities

Water content is high in *Portulaca oleracea* (up to 92.32%).^{10,11,25} Moisture migrates from the leaves to the stems as the plant matures.

Portulaca oleracea contains nutrients which are also found in major cultivated vegetables, and it contains a high amount of α -linolenic acid, an essential omega-3 fatty acid, compared to other leafy vegetables.^{11,26} In a study comparing nutrients found in chamber and wild-grown *Portulaca oleracea* and spinach, although β -carotene levels were lower, ascorbic acid and glutathione levels were higher, and α -linolenic acid content and α -tocopherol levels were 7 times higher in both chamber and wild-grown *Portulaca oleracea*, than those found in spinach.²⁷ One serving of fresh chamber-grown *Portulaca oleracea* (100 g) was reported to contain 300 - 400 mg α -linolenic acid, 26.6 mg ascorbic acid, 12.2 mg α -tocopherol, 14.8 mg glutathione, and 1.9 mg β -carotene.

As a weed plant, the roots of *Portulaca oleracea* draw minerals from deeper layers of the soil, by degrading and absorbing residual solid parts of other plants.¹⁰ The dry weight (mmol/kg DW) concentrations of calcium, magnesium, sodium, potassium, iron, and zinc monitored on day 15, 30, 45, and 60 of growth, were highest in the leaves of 60-d old *Portulaca oleracea* plants.¹⁸ Varying climate and soil conditions among *Portulaca oleracea* plants grown in different locations also affect mineral composition, flavonoid, and carotenoid content.^{28,29} Additionally, the composition and determination of individual constituents found in *Portulaca oleracea*-derived ingredients varies considerably depending on extraction solvent and method,^{10,17} part of the plant,^{25,30} and growth stage or time of harvest.^{18,25} A list of constituents, isolated across different studies, by plant part, is presented in Table 2.

Oxalic acid, or oxalate, is found in a variety of plants, and is generally present in *Portulaca oleracea* at 1.3%.³¹ Of the oxalate present in the *Portulaca oleracea* plant, it has been shown to be found in soluble (bound to potassium, sodium, and magnesium) and insoluble (bound to calcium and iron) forms; in one study, mean soluble oxalate values were 33% in the leaves, and 67% in the stems (each relative to the total mass balance of 1.3%).³² Upon chemical analysis of oxalate content in *Portulaca oleracea*, the highest total concentration of soluble and insoluble oxalate was found in the leaves (23.45 g/kg fresh weight (fw)), and in lesser amounts in the buds (9.09 g/kg fw) and stems (5.58 g/kg fw).³² In the same study, cooking the whole plant resulted in a 49% reduction of soluble oxalate content in plant buds, 33.5% reduction in the leaves, and 18%

reduction in the stems, while pickling the plant in white vinegar resulted in a 67% overall oxalate reduction. *Portulaca oleracea* is mentioned in the US FDA Poisonous Plant Database.³³ The potential for nitrate/nitrite poisoning in sheep and goats that consume *Portulaca oleracea*³⁴ and caution regarding the oxalate content in *Portulaca oleracea*³⁵ for dog, cat, and horse consumption has been documented.

Portulaca Oleracea Extract

Portulaca oleracea extract is composed of a wide range of constituents, of which flavonoids, alkaloids, terpenes, phenolic acids, and coumarins are preeminent.^{2,19} Other notable constituents are omega-3-fatty acids, polysaccharides, vitamins, and amino acids.³⁰

The phenolic and flavonoid content of hydrothermally processed *Portulaca oleracea* was evaluated.²³ The gallic acid equivalents of boiled, blanched, and raw *Portulaca oleracea* were determined to be 19.25, and 10.02, and 22.94 g/extract, respectively. Boiling and blanching significantly increased the rutin equivalent to 85.14 and 81.57, respectively, compared to 64.99 mg/g extract in raw *Portulaca oleracea*.

Portulaca Oleracea Flower/Leaf/Stem Extract

The chemical composition and nutritional value of *Portulaca oleracea* plants was assessed, by plant part (leaves and stems) and stage of harvest, for up to 52 d after sowing.²⁵ The moisture content of leaves was the highest at day 29, while stems contained the most water on day 43. Higher macronutrient content and protein values were observed in the leaves at the last harvest, while the carbohydrate and α -linolenic acid content of leaves was highest at day 29. In a study of total flavonoid and total phenolic content in *Portulaca oleracea* flowers, leaves, and stems, total phenolic content was significantly higher in stems compared to leaves and flowers (1008.6 vs. 441.8 - 455.6 gallic acid equivalents), in spite of total flavonoid content not differing significantly.³⁶

The impact of the dehydration method (100 W microwave, tray, vacuum, or low temperature, low humidity infrared) upon the retention of bioactive compounds in extracts made from dried *Portulaca oleracea* leaves and stems was evaluated.²¹ Flavonoid content and fatty acid composition was highest in the extract of vacuum-dried leaves.

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.

Portulaca Oleracea Extract is the only ingredient included in this report that is reported to be used in cosmetic formulations. According to 2022 VCRP survey data, Portulaca Oleracea Extract is reported to be used in 541 formulations (Table 3), of which 195 uses are in face and neck products, and 139 uses are in moisturizing products.³⁷ The results of the concentration of use survey, conducted by the Council in 2018 and updated in 2021, indicate that the reported maximum concentration of use for Portulaca Oleracea Extract is 0.5%, in non-spray face and neck formulations.^{38,39} According to VCRP and Council survey data, the other 3 *Portulaca oleracea*-derived ingredients are not reported to be in use in cosmetic products (Table 4).

Portulaca Oleracea Extract is reported to be used in products which may allow exposure near the eye or mucous membranes. Concentration of use data were not reported for these categories of use. According to VCRP data, Portulaca Oleracea Extract is reportedly used in 2 face powder formulations,³⁷ and could possibly be inhaled; concentration of use data were not reported for this use.³⁸ In practice, as stated in the Panel's respiratory exposure resource document (<https://www.cir-safety.org/cir-findings>), most particles incidentally inhaled 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. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

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 ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.⁴⁰

Non-Cosmetic

Portulaca oleracea is consumed raw in salads, or is used as a potherb in cooked sauces, soups, and pickled dishes across many cultures.^{4,41} Furthermore, the whole plant, and plant parts, of *Portulaca oleracea* are used as a food in various cultures.^{26,42} Uses as an apotropaic agent and as a source of violet and gray dye for wool are also noted.⁴¹

Historically, *Portulaca oleracea* is reported to be widely used in traditional folk medicine. In Chinese traditional medicine, the plant is used for the treatment of dysentery with bloody stools, as a topical emollient, collyrium, and as an external muscle relaxant.^{3,4} Native Americans use the plant to treat gout and headaches, and as a febrifuge.⁴ In Africa, the *Portulaca oleracea* plant is considered to exhibit anti-inflammatory, analgesic, and antifungal activity; fresh juice is used in the treatment of dysuria, coughs, and as an anti-diabetic agent.^{4,43} Additionally, it is used in religious ceremonies for purification, as an antiphlogistic substance, and for the treatment of skin diseases, erysipelas, insect and snake bites, abscesses, and eczema.^{4,17} The World Health Organization (WHO) describes *Portulaca oleracea* as a medicinal plant, with antibacterial, anti-inflammatory and antihelminth properties; poultices of fresh leaves are used to treat mastitis, boils, and impetigo.⁴⁴

TOXICOKINETIC STUDIES

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

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The acute toxicity studies summarized below are described in Table 5.

The dermal LD₅₀ of an ethanolic extract of *Portulaca oleracea* (10%), in rabbits, was determined to be 1865 mg/kg bw.⁴⁵ The oral LD₅₀ of an extract of whole *Portulaca oleracea* (water: ethanol; 1:1), in Swiss albino mice, was determined to be ≤ 500 mg/kg bw.^{46,47} The oral LD₅₀ of a petroleum ether *Portulaca oleracea* leaf extract, in Sprague-Dawley rats, was determined to be > 2000 mg/kg bw.⁴⁸ Maximum oral doses of 5000 mg/kg chloroform and methanolic *Portulaca oleracea* leaf extracts were well tolerated in rats.^{49,50}

Short-Term Toxicity Studies

The short-term oral toxicity studies summarized below are described in Table 6.

Groups of 6 Swiss albino mice were administered an oral dose of 0, 200, or 400 mg/kg bw/d, ethanolic extract of whole *Portulaca oleracea* (water: ethanol; 1:1), via gavage, for 14 d.^{46,47} No mortality occurred during observation; a statistically significant increase in hypoglycemic activity was observed in both treated groups, and to a greater extent in the 400 mg/kg bw group. Groups of 5 albino rats orally dosed at up to 75 mg/kg bw/d of aqueous or methanolic extract of whole *Portulaca oleracea* for 30 d showed a statistically significant decrease in white blood cell and neutrophil counts, and increase in lymphocyte counts in the 25 and 50 mg/kg bw/d aqueous extract groups.⁵¹ Rats in the 25 mg/kg bw/d methanolic extract group showed a significant increase in mean corpuscular volume and mean corpuscular hemoglobin, while rats in the 75 mg/kg bw/d methanolic extract group had a significant decrease in total plasma protein and albumin levels. In a 14-d study, groups of 6 Sprague-Dawley rats were orally dosed with 0, 500, 1000, or 2000 mg/kg/d petroleum ether extract of *Portulaca oleracea* leaves.⁴⁸ No mortality occurred during observation and there was a non-significant increase in body weights. Hematological parameters were examined, and a significant, dose-dependent increase in hemoglobin, red blood cell count, packed cell volume, mean corpuscular volume, and total cholesterol levels was observed in all treated rats, compared to controls. Groups of 16 male albino Wistar rats were administered 0, 125, 250, or 500 mg/kg bw/d methanolic or chloroform extract of *Portulaca oleracea* leaves, via gavage, for 60 d.^{49,50} The 500 mg/kg group showed a significant decrease in the mean hematocrit on day 28, which was considered incidental, and a significant increase in white blood cell count on day 42. Platelet count was increased in all treatment groups, and was significantly higher in the 125 and 500 mg/kg treated rats on day 60. Groups of 6 male albino Wistar rats were dosed with either distilled water or 1.5 ml/kg/d *Portulaca oleracea* juice extract, via gavage, for 12 d.⁵² Blood samples in these rats showed a statistically significant increase in uric acid, and in glutathione, glutathione reductase, glutathione peroxidase, and glutathione-S-transferase in the liver, kidney, and testes. A significant decrease in urea and creatine, reduction in malondialdehyde levels in the liver and kidney, and a significant reduction in aspartate aminotransferase (AST), γ-glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), and bilirubin was observed; changes in ALT (alanine aminotransferase) were not significant.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Details of the oral developmental and reproductive studies summarized below are described in Table 7.

Male albino rats, that were orally administered either 75 mg/kg bw aqueous or methanolic *Portulaca oleracea* leaf and stem extract for 50 d, were cohabited with 3 female rats each for 4 wk.⁵³ No pregnancy (or sterile mating) occurred between males from either extract group and the untreated female rats. Body weight changes, blood samples, sperm, testes, and epididymis were analyzed in groups of 5 male Wistar rats orally dosed with 0, 400, or 800 mg/kg bw methanolic *Portulaca oleracea* leaf and stem extracts for 14 d.⁵⁴ Although no significant differences in luteinizing hormone and testosterone levels were seen in the animals treated with the methanolic extracts, significant increases in follicle-stimulating hormone and reduction in sperm count occurred in the 800 mg/kg group and a significant reduction in sperm motility was seen in both treatment groups, compared to controls. Groups of 5 male albino rats were orally dosed with 0, 25, 50, or 75 mg/kg/d aqueous, or methanolic, *Portulaca oleracea* leaf and stem extracts for 50 d, and had blood samples from day 51 analyzed for testosterone levels; the animals were sacrificed for semen and histological analyses of the testes.⁵⁵ A statistically significant decrease in testosterone levels was observed in rats in the aqueous 75 mg/kg group, and in all methanolic extract groups. Animals in all dose groups had significantly reduced sperm motility, sperm count, and increased sperm abnormalities. In another study, groups of 16 male albino rats were orally dosed with 0, 125, 250, or 500 mg/kg chloroform, or 80% aqueous methanolic extract, for 60 d; blood samples, testes, and epididymis were harvested from 4 animals in each treatment group on days 14, 28, 42, and 60.⁵⁶ A significant increase in sperm count was observed in the animals treated with both extracts in the 250 mg/kg groups on day 28 and a significant decrease in testosterone levels was observed in the animals treated with 125 and 500 mg/kg methanolic extract on days 28 and 60. Groups of 5 - 6 female Wistar albino rats were orally dosed with 0, 250, or 500 mg/kg bw/d, flavonoid-rich, *Portulaca oleracea* stem and leaf extract and were examined for potential effects on reproductive organ weight, estrous cycles, uterine characteristics, abortifacient activity, and implantation; significant uterine changes included larger diameter and endometrial thickness.⁵⁷ In two similarly completed, but separate studies, ovary and uterine weights were significantly lower in immature, bilaterally ovariectomized rats orally dosed with 250 or 500 mg/kg bw/d *Portulaca oleracea* stem and leaf extract (indicated as “total flavonoid extract”) for 7 d, and, significantly higher in the mature rats orally dosed with 250 and 500 mg/kg bw/d of the same extract for 10 d; both effects were associated with significantly reduced protein and cholesterol uterine content, and suppression of follicular stimulating hormone, respectively.⁵⁷ The effect of orally administered 400 or 800 mg/kg bw/d *Portulaca oleracea* leaf and stem extract upon bilaterally ovariectomized rats, compared to control rats which only received distilled water, with or without ovariectomizing, was examined in a 14-d study.⁵⁸ Estrous cycle dysregulation and a statistically significant decrease in estradiol and testosterone was observed in all 3 ovariectomized groups, compared to non-ovariectomized controls, while a statistically significant increase in progesterone was only observed in the ovariectomized rats given 400 mg/kg bw and 800 mg/kg bw/d. In a 21-d study, female albino rats were first orally dosed with 75 mg/kg/d aqueous or methanolic *Portulaca oleracea* leaf and stem extracts, and then served as their own controls after an additional 21 d of no dosing to observe changes in estrous cycles.⁵⁹ Treatment for 21 d with either extract did not produce any significant changes in duration of estrous cycle phases. However, during the 21-day withdrawal of treatment, a significant decrease in the proestrus phase of both treated groups, increase in the estrous phase of the aqueous extract-treated rats, and increase in the metestrus phase of the methanolic extract group was observed. The effects of 0, 125, 250, or 500 mg/kg chloroform, or 80% aqueous methanolic, *Portulaca oleracea* leaf extracts upon estrous cycle, ovarian and uterine histology, and luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone, and estrogen serum levels were examined in groups of 5 female albino rats for 21 d.⁶⁰ Significant decreases in luteinizing hormone levels in the 250 mg/kg chloroform extract group, and in follicle-stimulating hormone levels in the 250 and 500 mg/kg chloroform extract groups were observed. Hypertrophied ovarian follicles were observed in the 125 mg/kg methanolic extract group; no other significant effects were exhibited in estrous phase, hormone levels, or histology. Groups of 5 female albino rats were orally dosed with either 0.5 ml distilled water, or 75 mg/kg/d of aqueous or methanolic *Portulaca oleracea* leaf and stem extract for 25 d, to examine ovarian and uterine histopathology.⁵⁹ No significant pathological effects or changes in ovarian or uterine weights were observed. In another study, dams dosed with up to 500 mg/kg bw/d *Portulaca oleracea* leaf and stem extract (indicated as “total flavonoid extract”) from day 7 to day 14 of gestation, via gavage, showed a statistically significant 30% abortion rate and 50% inhibition in implantation in the 250 mg/kg bw/d group, while animals in the 500 mg/kg bw/d group had a statistically significant 50% abortion rate and 70% inhibition in implantation, compared to controls (these dams were dosed from day 1 to day 7 of gestation).⁵⁷ In a teratology study of albino rats, animals were dosed with 0.5 ml distilled water or 75 mg/kg/d aqueous or methanolic *Portulaca oleracea* leaf and stem extract at three different time frames during 21 d of gestation.⁵³ No significant differences related to pregnancy stage, fetal development, or delivery were observed.

GENOTOXICITY STUDIES

Genotoxicity data on *Portulaca oleracea*-derived ingredients were not found in the published literature, and unpublished data were not submitted.

CARCINOGENICITY STUDIES

Carcinogenicity data on *Portulaca oleracea*-derived ingredients were not found in the published literature, and unpublished data were not submitted.

OTHER RELEVANT STUDIES

Anti-Inflammatory and Antioxidant Studies

Portulaca oleracea extracts were shown to significantly reduce lipopolysaccharide (LPS)-induced synthesis of nitric oxide, the production of tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and the expression levels of various transcription factors, in murine macrophage cells.⁶¹ Luteolin, kaempferol, and quercitrin components identified in the extracts were postulated to account for these anti-inflammatory effects.

Three aqueous extracts of *Portulaca oleracea* flowers, leaves, and stems were prepared using distilled water.³⁶ *Escherichia coli* DNA interjected with pBR322 plasmid, exposed to hydrogen peroxide in a DNA nicking assay, was incubated with 5 μ l (80 μ g/ml) of each extract for 10 min and measured for plasmid DNA damage. Aqueous extracts from each plant part showed a protective effect against DNA damage, through the inhibition of Fenton reaction free radicals; the highest effect was observed with the stem extract, and the lowest effect was observed in the flower extract.

Cytotoxicity

Portulaca Oleracea Extract

A 70% ethanolic crude extract of whole *Portulaca oleracea* was tested at concentrations of 0.2, 0.4, 0.8, 1.6, 3.2, or 6.4 mg/ml on human peripheral lymphocytes for the effect on mitotic index (MI) and blast index (BI).⁶² Increased MI and BI values were observed, but were not significantly different when compared with those in the positive control group, which were treated with phytohemagglutinin.

The cytotoxic potential of the chloroform extract of whole *Portulaca oleracea* against human colon adenocarcinoma (HCT-15) and normal (Vero) cell lines was examined in a (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, with doxorubicin as a reference.¹² The 50% cell growth inhibition concentration (IC₅₀) for the chloroform extract was 1132.02 μ g/ml in HCT-15 cells and 767.60 μ g/ml in Vero cells, while the IC₅₀ for doxorubicin was 460.13 μ g/ml in HCT-15 cells and 2392.71 μ g/ml in Vero cells. The chloroform extract was not considered cytotoxic to HCT-15 cells, but was considered possibly toxic to Vero cells. Cell viability was recorded to be 67%, 31%, 21%, and 17% in human hepatocellular carcinoma cells (HepG2) exposed to 50, 100, 250, and 500 μ g/ml *Portulaca oleracea* seed extracts, respectively.⁶³

An aqueous extract of whole *Portulaca oleracea* was tested in an MTT assay for its cytotoxic potential against pancreatic carcinoma cell line (PANC-1), with human umbilical vein endothelial cell (HUVEC) as a reference cell line.⁶⁴ The *Portulaca oleracea* extract was administered at concentrations of up to 1000 μ g/ml. The IC₅₀ for the aqueous extract was 174.5 μ g/ml in HUVEC and 500 μ g/ml in PANC-1 cells. Additionally, treatment with the aqueous *Portulaca oleracea* extract at the highest IC₅₀ level of 500 μ g/ml for 24 h showed an increase in the percentage of PANC-1 cells in late apoptosis, compared to untreated controls.

Portulaca Oleracea Flower/Leaf/Stem Extract

The antiproliferative potential of aqueous and methanolic extracts of *Portulaca oleracea* leaves was examined in murine mammary adenocarcinoma (AMN3) cells, human rhabdomyosarcoma (RD) cells, and normal kidney epithelium cells of the African green monkey, at concentrations up to 10,000 μ g/ml, over 72 h.⁶⁵ Both extracts exhibited time-dependent antiproliferative effects against both cancer cell lines, with more sensitivity in the AMN3 cells. The normal cells showed resistance towards all concentrations of both extracts, except the 10,000 μ g/ml dose. Similarly, significantly reduced cell viability was seen in HeLa cervical cancer cells exposed to concentrations of 0, 300, 500, 700, 1000, 1200, or 1500 μ g/ml of *Portulaca oleracea* stems and leaf extracts for up to 48 h.⁶⁶

DERMAL IRRITATION AND SENSITIZATION STUDIES

The dermal irritation and sensitization studies are summarized below and described in Table 8.

A 24-h, single-insult occlusive patch test (SIOPT) was performed in 22 subjects with a body lotion containing 0.1% *Portulaca Oleracea* Extract.⁶⁷ Another lotion was used as the reference control. No significant differences were observed in the irritation response of subjects exposed to the test material and the reference control, and the primary irritation index (PII) was 0.0 for both materials. The skin sensitization potential of a body moisturizer containing 0.1% *Portulaca Oleracea* Extract was evaluated in a maximization study completed in 26 subjects.⁶⁸ A total of 5, 48-h occlusive induction applications (0.05 ml of the test material, applied neat) were made, after inducing irritation with a 1-h, occlusive application of 0.05 ml 0.25% aqueous sodium lauryl sulfate. After a 10-d non-treatment period, an occlusive 48-h challenge application was made to an induced, but previously untreated, site. The challenge site was graded 15 - 30 min and 24 h after patch removal. Scores for all 26 subjects who completed the study were 0 at both readings (on a 0 - 3 scoring scale). The test substance was considered non-sensitizing. Two additional maximization studies, performed in an identical fashion, tested the sensitization potential of a face lotion and face treatment product, both containing 0.5% *Portulaca Oleracea* Extract, and were completed in 27 and 26 subjects, respectively.^{69,70} No signs of sensitization were observed for either product during the induction or challenge phase; both test articles were considered non-sensitizing.

OCULAR IRRITATION STUDIES

Data on the ocular irritation potential of *Portulaca oleracea*-derived ingredients reviewed in this safety assessment were neither found in the published literature, nor were they submitted.

CLINICAL STUDIES

Clinical Use

A 3-wk use study of a formulation containing 0.1% *Portulaca Oleracea* Extract was performed in 46 subjects.⁷¹ Dermatologist-assessed facial exams were conducted at the test center during the initial and final visit. Thirty-three (72%) of subjects were assessed as having sensitive skin, based on test center results for various skin conditions, as well as self-reported sensitivity to sun, allergies, and eczema at the end of the 3-wk use period. Subjects were instructed to apply the test product over their entire face (including the eye area, but avoiding contact with the eyes), at least twice a day. Subjects were also allowed to apply their own moisturizer following use of the test material, if desired. No product-related irritation was observed. Changes in scaling/flaking and conditions of acne, including papules and pustules which occurred, were determined to be within expected fluctuation in the general population. No irritation was observed.

SUMMARY

The safety of the following 4 *Portulaca oleracea*-derived ingredients, as used in cosmetics, is reviewed in this safety assessment: *Portulaca Oleracea* Extract, *Portulaca Oleracea* Flower/Leaf/Stem Extract, *Portulaca Oleracea* Juice, and *Portulaca Oleracea* Water. These ingredients are all reported to function as skin-conditioning agents in cosmetics.

Portulaca Oleracea Extract is the only ingredient included in this report that is reported to be used in cosmetic formulations. According to 2022 VCRP survey data, *Portulaca Oleracea* Extract is reported to be used in 541 formulations, of which 195 uses are in face and neck products and 139 are in moisturizing products. The results of the concentration of use survey conducted by the Council indicate *Portulaca Oleracea* Extract is used at a maximum concentration of 0.5% (in non – spray face and neck products).

The dermal LD₅₀ of a 10% ethanolic *Portulaca oleracea* extract was determined to be 1865 mg/kg in rabbits. The oral LD₅₀ of an ethanolic extract of whole *Portulaca oleracea* was determined to be ≤ 500 mg/kg bw in Swiss albino mice, while the oral LD₅₀ of a petroleum ether *Portulaca oleracea* leaf extract was determined to be > 2000 mg/kg bw in Sprague-Dawley rats. Maximum oral doses of 5000 mg/kg methanolic and chloroform *Portulaca oleracea* leaf extracts were well tolerated in rats.

No mortality occurred, and a significant increase in hypoglycemic activity was observed, in groups of 6 Swiss albino mice orally dosed with up to 400 mg/kg bw/d of a whole ethanolic *Portulaca oleracea* extract for 14 d. Albino rats dosed, orally, at up to 75 mg/kg bw/d of aqueous or methanolic extract of whole *Portulaca oleracea* for 30 d showed a significant decrease in white blood cell and neutrophil count in the 25 and 50 mg/kg bw/d aqueous extract groups, as well as a significant increase in mean corpuscular volume and mean corpuscular hemoglobin in the 25 and 75 mg/kg bw/d methanolic extract groups. No mortality occurred and a significant, dose-dependent increase in hematological parameters and cholesterol levels was observed in all Sprague-Dawley rats orally dosed with 500, 1000, or 2000 mg/kg bw/d petroleum ether extract of *Portulaca oleracea* leaves for 14 d. Groups of 16 male albino Wistar rats were orally administered 125, 250, or 500 mg/kg bw/d of methanolic or chloroform extract of *Portulaca oleracea* leaves, for 60 d; a significant decrease in the mean hematocrit on day 28 and a significant increase in white blood cell count on day 42 was observed in the 500 mg/kg group. Platelet count was increased in all treatment groups, and was significantly higher in the 125 and 500 mg/kg treated rats on day 60. Blood samples of male albino Wistar rats orally dosed with 1.5 ml/kg/d *Portulaca oleracea* juice extract for 12 d exhibited significant variability in serum levels of urea, creatine, glutathione, and bilirubin.

No pregnancies resulted from mating between male albino rats dosed, via gavage, with 75 mg/kg bw aqueous or methanolic *Portulaca oleracea* leaf extract for 50 d, and untreated female rats. Groups of 5 male Wistar rats were orally dosed with 0, 400, or 800 mg/kg bw methanolic *Portulaca oleracea* leaf and stem extract for 14 d. Significant increases in follicle-stimulating hormone and reduction in sperm counts were seen in the 800 mg/kg group, and sperm motility was significantly reduced in both 400 and 800 mg/kg groups, compared to controls; differences in luteinizing hormone and testosterone levels were not significant. Groups of 5 male albino rats orally dosed with 0, 25, 50, or 75 mg/kg/d aqueous or methanolic *Portulaca oleracea* leaf and stem extracts for 50 d exhibited significantly decreased testosterone levels at the maximum aqueous extract dose, and in all methanolic extract dose groups. Animals in all dose groups had significantly reduced sperm motility, sperm count, and increased sperm abnormalities. Groups of 16 male albino rats were orally dosed with 0, 125, 250, or 500 mg/kg/d chloroform or methanolic extract for 60 d; significant increases in sperm count were seen in the 250 mg/kg groups for both extracts on day 28, and testosterone levels were significantly decreased in the 125 and 500 mg/kg methanolic extracts groups on days 28 and 60. In two separate studies of groups of 5 - 6 female Wistar albino rats, ovary and uterine weights were significantly higher in mature rats, and significantly lower in immature bilaterally ovariectomized rats orally dosed with 0, 250, or 500 mg/kg bw/d *Portulaca oleracea* stem and leaf extract (indicated as “total flavonoid extract”). In a 14-d study examining the effect of orally administered 0, 400 mg/kg bw/d, or 800 mg/kg bw/d *Portulaca oleracea* extract upon ovariectomized rats, estrous cycle dysregulation and a statistically significant decrease in

estradiol and testosterone was observed in ovariectomized controls and both dose groups, and a significant increase in progesterone was only observed in the ovariectomized 400 mg/kg bw/d and 800 mg/kg bw d groups. In a 21-d study of female albino rats dosed, via gavage with either 75 mg/kg/d aqueous or methanolic *Portulaca oleracea* leaf and stem extracts, no significant changes in duration of estrous cycle phases were observed, however, upon withdrawal of both treatments in a 21-d follow-up period, a significant decrease in the proestrus phase was observed in both treated groups, as well as a significant increase in the estrous phase of the aqueous-treated rats, and increase in the metestrus phase of the methanolic extract group. Groups of 5 female albino rats orally dosed with 0, 125, 250, or 500 chloroform, or 80% aqueous methanolic extract for 21 d exhibited significant decreases in LH, from the 250 mg/kg chloroform extract group, and FSH from the 250 and 500 mg/kg chloroform extract groups. No other significant effects upon estrous cycle, ovarian and uterine histology, or LH, FSH, estrogen, or progesterone levels were observed. No significant pathological effects or changes in ovarian and uterine weights were observed in rats orally dosed with 75 mg/kg/d of aqueous or methanolic *Portulaca oleracea* leaf and stem extract for 25 d. In another study, dams orally dosed with up to 500 mg/kg bw/d of *Portulaca oleracea* leaf and stem extracts (indicated as “total flavonoid extracts”) had a statistically significant 30% abortion rate and 50% inhibition in implantation in the 250 mg/kg bw/d group, while animals in the 500 mg/kg bw/d group had a statistically significant 50% abortion rate and 70% inhibition in implantation, compared to controls. No significant differences in pregnancy stage, fetal development, or delivery were observed in albino rats dosed with 75 mg/kg/d aqueous or methanolic *Portulaca oleracea* leaf and stem extract, via gavage, during either day 1 to day 5 (implantation), day 6 to day 15 (mid-pregnancy), or day 16 to day 21 (late pregnancy).

Portulaca oleracea extracts were shown to significantly reduce LPS-induced synthesis of nitric oxide, the production of TNF- α , IL-6, and the expression levels of various transcription factors, in murine macrophage cells. An aqueous extract (80 μ g/ml) of *Portulaca oleracea* stems had the most protective effect against *E. coli* plasmid DNA damage in a DNA nicking assay, compared to leaf and flower extracts. A 70% ethanolic crude extract of whole *Portulaca oleracea*, tested at doses of up to 6.4 mg/ml on human peripheral lymphocytes, produced a non-significant increase in MI and BI values compared to the positive controls treated with phytohemagglutinin. In an MTT assay, the chloroform extract of whole *Portulaca oleracea* exhibited an IC₅₀ of 1132.02 μ g/ml in HCT-15 cells and 767.60 μ g/ml in Vero cells, compared to 460.13 μ g/ml and 2392.71 μ g/ml, for doxorubicin, respectively. The chloroform extract was not considered cytotoxic to HCT-15 cells, but was considered possibly toxic to Vero cells. Cell viability was recorded to be 67%, 31%, 21%, and 17% in HepG2 cells exposed to increasing doses of up to 500 μ g/ml *Portulaca oleracea* seed extracts. An aqueous *Portulaca oleracea* extract tested for cytotoxic potential against PANC-1 pancreatic cell lines exhibited an IC₅₀ of 500 μ g/ml in an MTT assay. The antiproliferative potential of aqueous and methanolic extracts of *Portulaca oleracea* leaves was examined in AMN3 cells, RD cells, and normal kidney epithelium cells of the African green monkey, at concentrations up to 10,000 μ g/ml, over 72 h. Both extracts exhibited time-dependent antiproliferative effects against both cancer cell lines, with more sensitivity in the AMN3 cells. Similarly, significantly reduced cell viability was seen in HeLa cervical cancer cells exposed to up to 1500 μ g/ml *Portulaca oleracea* stems and leaf extracts for up to 48 h.

No dermal irritation responses were seen in an SIOPT of a body lotion containing 0.1% *Portulaca Oleracea* Extract, in 22 subjects. The skin sensitization potential of a body moisturizer containing 0.1% *Portulaca Oleracea* Extract was tested in a maximization study involving 26 subjects; the test substance was deemed non-sensitizing. A face lotion containing 0.5% *Portulaca Oleracea* Extract and a face treatment product containing 0.5% *Portulaca Oleracea* Extract were not considered sensitizing when tested in a maximization study involving 27 and 26 subjects, respectively.

In a 3-wk use study, 46 subjects were instructed to apply a formulation containing 0.1% *Portulaca Oleracea* Extract at least two times a day to the entire face. Dermatological changes in skin texture and acne were determined to be within expected ranges; no irritation was observed.

DISCUSSION

This assessment reviews the safety of 4 *Portulaca oleracea*-derived ingredients as used in cosmetic formulations. 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 the safety assessment when formulated to be non-sensitizing.

Based on information from peer-reviewed literature, the Panel acknowledged that *Portulaca oleracea* leaves and stems, as well as the whole plant, are consumed as food, thereby mitigating potential concerns regarding systemic toxicity; food use also mitigated concerns about the lack of carcinogenicity and genotoxicity data. Adverse and contradictory effects in the reported developmental and reproductive toxicity studies were noted by the Panel, including: no changes in testosterone levels in one study, decreases in testosterone in 2 other studies, reduced sperm quality for male rats, and abortifacient activity for dams. However, the Panel noted that the effects seen in the developmental and reproductive toxicity studies occurred at high doses, and resulted from oral administration, thus, resulting in much higher blood concentrations of the extract components than would occur from topical cosmetic use.

Because final product formulations may contain multiple botanicals, each possibly containing similar constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. For *Portulaca oleracea*-derived ingredients, the Panel was concerned about the presence of terpenes as potential

sensitizers in cosmetics. Therefore, when formulating products, manufacturers should avoid reaching levels of plant constituents that may cause sensitization or other adverse health effects.

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.

The Panel discussed the issue of incidental inhalation exposure from use of these ingredients in products that could possibly be inhaled. For example, *Portulaca Oleracea* Extract has reported use in face powders (concentration of use is not available). Inhalation toxicity data were not available. However, the Panel noted that 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 physiological activity of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which the ingredients are used (or expected to be used) in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. 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>.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be assessed by the Panel. Therefore, the Panel has found the data insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the following 4 *Portulaca oleracea*-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.

Portulaca Oleracea Extract
Portulaca Oleracea Flower/Leaf/Stem Extract*

Portulaca Oleracea Juice*
Portulaca Oleracea Water*

**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 Portulaca Oleracea Extract.*

TABLES**Table 1. Definitions and functions of *Portulaca oleracea*-derived ingredients in this safety assesment¹**

Ingredient/CAS No.	Definition	Function
Portulaca Oleracea Extract 90083-07-1	Portulaca Oleracea Extract is the extract of the whole plant, <i>Portulaca oleracea</i> .	Skin- conditioning agent - humectant
Portulaca Oleracea Flower/Leaf/Stem Extract	Portulaca Oleracea Flower/Leaf/Stem Extract is the extract of the flowers, leaves and stems of <i>Portulaca oleracea</i> .	Antioxidants; skin-conditioning agent – misc.
Portulaca Oleracea Juice	Portulaca Oleracea Juice is the liquid expressed from the whole plant, <i>Portulaca oleracea</i> .	Skin-conditioning agent – misc.
Portulaca Oleracea Water	Portulaca Oleracea Water is the steam distillate obtained from the whole plant, <i>Portulaca oleracea</i> .	Skin-conditioning agent – misc.

Table 2. Constituents found in *Portulaca oleracea*, by plant part^{*30}

Classification	Whole plant	Flower, Leaf, and Stem**	Leaf and Stem***	Leaf	Stem
Flavonoids	genistein genistin luteolin myricetin quercetin	portulacanones a portulacanones b portulacanones c portulacanones d 2,2'-dihydroxy-4',6'-dimethoxychalcone	apigenin kaempferol		
Alkaloids	adenosine oleraceins A oleraceins B oleraceins C oleraceins D oleraceins E	aurantiamide aurantiamide acetate cyclo(L-tyrosinyl-L-tyrosinyl <i>N-trans</i> -feruloyltyramine (7 <i>R</i>)- <i>N</i> -feruloylnormetanephrene <i>N-cis</i> -feruloyltryramine <i>N-trans</i> -feruloyloctopamine <i>N-cis</i> -feruloyloctopamine Thymine trollisine uracil 1,5- dimethyl-6-phenyl-1,2-dihydro-1,2,4-triazin-3(2H)-one 1,5-dimethyl-6-phenyl-1,6,3,4-tetrahydro-1,2,4-2(1H)-triazin (3 <i>R</i>)-3,5-bis(3-methoxy-4-hydroxyphenyl)-2,3-dihydro-2(1H)-pyridinone	dopamine noradrenalin		oleraceins I oleraceins II
Terpenoids		friedelane lupeol portuloside A portuloside B portulene (2 α , 3 α)-3-((4- <i>O</i> -(β -D-glucopyranosyl)- β -D-xylopyranosyl)oxy)-2,23-dihydroxy-30-methoxy-30-oxoolean-12-en-28-oic acid (2 α , 3 α)-2,23,30-trihydroxy-3-((β -D-xylopyranosyl)oxy)olean-12-en-28-oic acid (3 <i>S</i>)-3- <i>O</i> -(β -D-glucopyranosyl)-3,7-dimethylocta-1,6-dien-3-ol (3 <i>S</i>)-3- <i>O</i> -(β -D-glucopyranosyl)-3,7-dimethylocta-1,5-dien-3,7-diol			
Organic Acids	<i>p</i> -Coumaric acid Ferulic acid	caffeic acid indole-3-carboxylic acid 3-quinolinecarboxylic acid		α - linoleic acid linoleic acid oleic acid oxalic acid palmitic acid stearic acid	docosapentaenoic acid
Vitamins				α -tocopherol folates hesperidin niacin pantothenic acid pyridoxine riboflavin thiamin vitamin A vitamin C	
Minerals			calcium copper iron phosphorus manganese	magnesium selenium zinc	

Table 2. Constituents found in *Portulaca oleracea*, by plant part^{*30}

Classification	Whole plant	Flower, Leaf, and Stem**	Leaf and Stem***	Leaf	Stem
Other compounds		β -sitosterol daucosterol portulacerebroside A		β -carotene chlorophyll glutathione melatonin proline tannin 1,4-di- <i>O</i> -acetyl-2,3,5-tri- <i>O</i> - methyl-L-arabinitol 1,4,5-tri- <i>O</i> -acetyl-2,3-di- <i>O</i> - methyl-L-arabinitol 1,5-di- <i>O</i> -acetyl-2,3,4,6-tetra- <i>O</i> -methyl-D-galactitol 1,4,5-tri- <i>O</i> -acetyl-2,3,6-tri- <i>O</i> -methyl-D-galactitol 1,3,4,5-tetra- <i>O</i> -acetyl-2,6-di- <i>O</i> -methyl-D-galactitol	

*the solvent used for extraction determines total constituent content

**defined as aerial part(s) in primary reference

***sometimes includes root or seed

Table 3. Frequency (2022)³⁷ and concentration of use (2019)^{38,39} data for Portulaca Oleracea Extract

	# of Uses	Max Conc of Use (%)
Totals*	541	0.001-0.5
Duration of Use		
Leave-On	461	0.001 – 0.5
Rinse-Off	80	0.002
Diluted for (Bath) Use	NR	NR
Exposure Type		
Eye Area	19	NR
Incidental Ingestion	1	NR
Incidental Inhalation-Spray	155 ^a ; 205 ^b	NR
Incidental Inhalation-Powder	2; 205 ^b ; 7 ^c	0.002 – 0.5 ^c
Dermal Contact	527	0.001 – 0.5
Deodorant (underarm)	NR	NR
Hair - Non-Coloring	13	NR
Hair-Coloring	NR	NR
Nail	NR	NR
Mucous Membrane	13	NR
Baby Products	10	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 that these products are sprays or powders, 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. Ingredients not reported to be in use³⁷⁻³⁹

Portulaca Oleracea Flower/Leaf/Stem Extract
Portulaca Oleracea Juice
Portulaca Oleracea Water

Table 5. Acute toxicity studies

Ingredient/ Extraction Method	Animals	No./Group	Vehicle/Control	Concentration/Dose/Protocol	LD ₅₀ /Results	Reference
DERMAL						
Portulaca oleracea extract Ethanol (10%)	Rabbits	strain and # not specified	90% ethanol	A 0.05 ml dose of the test article was administered via intradermal injection, after which the test article was applied to the shaved backs of the animals.	LD ₅₀ was determined to be 1865 mg/kg bw. No sensitivity was observed.	45
ORAL						
Portulaca oleracea extract (water:ethanol; 1:1)	Swiss albino mice (sex not specified)	2/group	2 % gum acacia	0, 500, 1000, 1500, or 2000 mg/kg bw, via gavage; Performed in accordance with OECD TG 423. The animals were observed 72 h for behavioral changes and mortality.	LD ₅₀ ≤ 500 mg/kg bw. After 48 h, half of the animals in the 500 mg/kg group, and all the animals in the 1000, 1500, and 2000 mg/kg bw groups showed sedation, respiratory arrest, convulsions, decreased motor activity, and mortality.	46,47
Portulaca oleracea leaf extract, Petroleum ether	Sprague-Dawley Rats (sex not specified)	6/group	10 ml/kg saline	0, 500, 1000, or 2000 mg/kg bw; The rats were observed up to 24 h for general changes in behavior, physiological function, and mortality.	LD > 2000 mg/kg bw. No mortality occurred, and no signs of toxicity were observed in the control and 500 mg /kg bw dose groups. The animals in the 1000 and 2000 mg /kg bw dose groups exhibited heightened asthenia, defecation, salivation, and urination compared to the control group.	48
Portulaca oleracea leaf extract, Chloroform/Methanolic extract	Rats (sex not specified)	strain and # not specified	80% aqueous methanol	NR	Well tolerated at the maximum dose of 5000 mg/kg. Not toxic.	49,50,56,60

NR- not reported

Table 6. Short-term oral toxicity studies

Ingredient Extraction method	Animals/Group	Study Duration	Vehicle/Control	Dose/Concentration	Results	Reference
ORAL						
<i>Portulaca oleracea</i> extract (water: ethanol; 1:1)	Swiss albino mice; 6/group	14 d	2% gum acacia	0, 200, or 400 mg/kg bw/d, via gavage	No mortality occurred during observation. Biochemical evaluations were performed on day 15. A statistically significant increase in hypoglycemic activity was observed in both treated groups, and to a greater extent in the 400 mg/kg bw group. The hepatotoxic potential of <i>Portulaca oleracea</i> extract was assessed by fixing and examining liver tissue. Histopathology results in treated mice showed no abnormalities and were comparable to control mice.	46,47
<i>Portulaca oleracea</i> extract Aqueous extract or 70% Methanolic extract	Albino rats; 5/group/sex	30 d	0.5 ml distilled water	25, 50, or 75 mg/kg bw; aqueous and methanolic extracts	Red blood cell production was not affected by oral administration of aqueous and methanolic extracts. Rats treated with 25 and 50 mg/kg bw of an aqueous extract for 15 d showed a statistically significant decrease in white blood cell and neutrophil counts, and significant increase in lymphocyte counts, relative to controls. Rats dosed with 25 mg/kg bw of a methanolic extract showed a significant increase in mean corpuscular volume and mean corpuscular hemoglobin relative to their respective controls. Thirty-day treatment with 25 mg/kg bw aqueous extract and 75 mg/kg bw methanolic extract produced a significant decrease in total plasma protein and albumin levels.	51
<i>Portulaca oleracea</i> leaf extract Petroleum ether extract	Sprague-Dawley rats; 6/group	14 d	10 ml/kg normal saline	500, 1000, or 2000 mg/kg/d, via gavage	Rats dosed with 2000 mg/kg <i>Portulaca oleracea</i> leaf extract exhibited decreased motor activity. Body weights were increased in the treatment groups, but the increase was not statistically significant. No mortality occurred during observation. Animals were sacrificed on the 15 th day, during which blood samples were collected for hematological assay, and liver, kidney, spleen, and stomach tissue were fixed and stained for examination. A significant, dose-dependent increase in hemoglobin, red blood cell count, packed cell volume, and mean corpuscular hemoglobin was observed, and total cholesterol levels were slightly increased, in all treated rats, compared to controls. Although renal weights had increased, and epithelial inflammation, oxalate stones, and hemorrhagic spots were observed in the 1000 and 2000 mg/kg groups, statistically relevant weight difference in the organ weights were not observed, compared to controls.	48
<i>Portulaca oleracea</i> leaf extract Chloroform/methanolic extract	Male albino Wistar rats; 16/group	60 d	0.5 ml/kg bw, 20% Tween 80	0, 125, 250, or 500 mg/kg bw/d, via gavage	Blood samples were collected on day 14, 28, 42, and 60 of treatment. The 500 mg/kg group showed a significant decrease in the mean hematocrit level on day 28, which was considered incidental, while a significant increase in white blood cell count was observed on day 42. Platelet count was increased in all treatment groups, and was significantly higher in the 125 and 500 mg/kg treated rats on day 60. No significant differences were observed in leukocyte (white blood cell) or erythrocyte (red blood cell) counts.	49,50
<i>Portulaca oleracea</i> juice Aqueous extract, 1.5 w/v	Male albino Wistar rats; 6/group	12 d	Distilled water	0.2 ml saline water (control) or 1.5 ml/kg/d extract/d, via gavage	Blood samples were obtained, prior to animal sacrifice, and analyzed to assess the effect of the extract upon liver and kidney function. Samples from rats treated with <i>Portulaca oleracea</i> juice showed a statistically significant increase in uric acid (28%), decrease in urea and creatine (33.2 and 28%), reduction in malondialdehyde of liver and kidney (30.9 and 8.7%), and an increase in glutathione, glutathione reductase, glutathione peroxidase, and glutathione-S-transferase in the liver, kidney, and testes (up to 94.1%). A significant reduction in AST, γ -GT, ALP, and bilirubin was observed (-7.4, -10.1, -31, and -13.3%), while the change in ALT was not significant.	52

Abbreviations: γ -GT- γ -glutamyl transpeptidase; AST – aspartate aminotransferase; ALP- alkaline phosphatase; ALT- alanine aminotransferase

Table 7. Oral reproductive and developmental toxicity studies

Test Article/ Extraction Solvent	Animals/Group	Vehicle	Dose/Concentration	Type of Study/Procedure	Results	Reference
ORAL						
<i>Portulaca oleracea</i> leaf and stem extract AEPO and MEPO	Male albino rats; 3/group	distilled water	0, 75 mg/kg bw AEPO or MEPO, via gavage	Fertility study in male albino rats (mating experiment). Three male albino rats were orally administered either 100 ml distilled water, 75 mg/kg bw AEPO, or 75 mg/kg bw MEPO for 50 d. Three untreated, fertile female rats were cohabitated with each of the treated male rats for 4 wk. Vaginal lavages were obtained from these females daily to identify the presence of sperm.	No pregnancy (or sterile mating) occurred between males from either extract group and the untreated female rats. Cohabitation of the control male rat with the untreated female rats resulted in pregnancy.	53
<i>Portulaca oleracea</i> leaf and stem extract MEPO	Male Wistar rats; 5/group	20 ml distilled water	0, 400, or 800 mg/kg bw MEPO, via gavage	Reproductive parameters in male Wistar rats. Animals were orally dosed for 14 d, fasted overnight after the last dosing, and then killed. Body weight was measured before and after administration of the test substance. After sacrifice, blood samples, sperm, testes, and epididymis were collected for serum hormones, sperm, and histological analyses.	Body weight significantly increased in both the control and 800 mg/kg MEPO group. No significant changes in serum LH and testosterone levels were observed in either MEPO group, compared to the controls. However, the 800 mg/kg bw group had a significant increase in FSH levels and reduction in sperm count, when compared to controls. Significant reduction in sperm motility was seen in both MEPO- treated groups compared to the controls. While the testis showed no abnormalities in its histology across groups, the epididymis showed some blood congestion in MEPO-treated groups.	54
<i>Portulaca oleracea</i> leaf and stem extract AEPO and MEPO	Male albino rats; 5/group	100 ml distilled water	0, 25, 50, 75 mg/kg AEPO or MEPO, via gavage	Reproductive parameters in male albino rats. Animals were orally dosed for 50 d. Body weight was monitored on a weekly basis. One day after the last dose (day 51), blood samples were collected to measure testosterone levels using ELISA and animals were sacrificed to collect semen and prepare testes for histological analysis.	Exposure to either <i>Portulaca oleracea</i> extract did not produce any significant changes in body weight, relative to controls. A statistically significant decrease in testosterone levels was observed in rats in the 75 mg/kg AEPO group, and in all MEPO groups. Testosterone decline may explain the concurrently observed acellular seminiferous tubules and Leydig cell hyperplasia in all-treated animals, which was most pronounced in the highest dosage group (75mg/kg). All animals dosed with the extracts had significantly reduced sperm motility, sperm count, and increased % of sperm abnormalities. These differences were mostly dose-dependent. A non-significant reduction in % of viable sperm was observed.	55
<i>Portulaca oleracea</i> leaf extract Chloroform and 80% aqueous methanol	Male albino rats; 16/group	0.5 ml 20% Tween 80	0, 125, 250, or 500 mg/kg chloroform or methanolic extract, via gavage	Reproductive parameters in male albino rats. Animals were orally dosed for 60 d. Blood samples, testes, and epididymis were harvested from 4 animals from each of the experimental groups on days 14, 28, 42, and 60.	Neither extract had a significant effect on the testicular weights, or sperm motility, viability, and morphology of treated rats, relative to the controls. A significant increase in sperm count was observed in the animals treated with the 250 mg/kg chloroform extract on days 14 and 28 and in the 250 mg/kg methanolic extract group on day 28, compared to controls. No effects on testosterone levels were observed in animals treated with the chloroform extract; a significant decrease in testosterone levels was observed in the animals treated with 125 and 500 mg/kg methanolic extract on days 28 and 60. No significant changes in testes histology were observed in animals from either treatment groups.	56

Table 7. Oral reproductive and developmental toxicity studies

Test Article/ Extraction Solvent	Animals/Group	Vehicle	Dose/Concentration	Type of Study/Procedure	Results	Reference
<i>Portulaca oleracea</i> leaf and stem extract “total flavonoid extract”*	Female Wistar albino rats; 5-6/group	1% Tween 80	0, 250, or 500 mg/kg bw/d, via gavage	Estrogenic/anti-estrogenic activity. Bilaterally ovariectomized, immature female rats received “total flavonoid extract” of <i>Portulaca oleracea</i> leaves and stems for 7 d. On day 8, all animals were sacrificed, uteri were fixed in Bouin’s fluid and dissected. Biochemical analysis of the adrenal glands and uteri of treated rats was also performed.	Administration of the “total flavonoids extract” at both doses caused a significant decrease in the uterine weight of the immature rats, and produced estrous cycles characterized by significantly longer diestrus phases. Protein and cholesterol (a precursor for steroidal hormone) content of the uterus was also significantly reduced in both doses, by 50% and 30%, respectively. Significant uterine changes included larger diameter and endometrial thickness.	57
<i>Portulaca oleracea</i> leaf and stem extract “total flavonoid extract”*	Female Wistar albino rats; 5/group	1% Tween 80	0, 250, or 500 mg/kg bw/d, via gavage	Flavonoid (estrogenic) effect on reproductive organ and body weight. All three groups were dosed for 10 d. On day 11, all animals were weighed and sacrificed. The ovaries and uteri were freed from surrounding tissue, weighed, and dissected.	The ovary and uterine weights were significantly higher in both extract-treated groups. The increase in the wet weight of the ovary was postulated to indicate inhibition of ovulation through suppression of follicular stimulating hormone.	57
<i>Portulaca oleracea</i> leaf and stem extract MEPO	Female Wistar rats; 5/group	Distilled water	0, 400, 800 mg/kg bw/d, MEPO, via gavage	Estrous cycle effects after bilateral ovariectomization. All four groups were dosed for 14 d. Negative controls were not ovariectomized, and received distilled water, while the positive controls were ovariectomized, but had no further treatment. Two test groups were ovariectomized and received either 400 or 800 mg/kg bw/d of the test article. Body weight changes, estrous cycle-phase, and blood samples were collected after dosing and analyzed for LH, FSH, E2, TT, and PG.	Significant body weight gain was observed in the positive control group (18.1%) and the 800 mg/kg bw/d group (44.5%), compared to the negative controls. A significant decrease in E2 and TT, and estrous cycle dysregulation, was observed in all 3 ovariectomized groups, while a statistically significant increase in PG levels was only observed in the 400 mg/kg bw/d and 800 mg/kg bw/d groups.	58
<i>Portulaca oleracea</i> leaf and stem extract AEPO and MEPO	Female albino rats; 5/group	100 ml distilled water	75 mg/kg/d AEPO or MEPO, via gavage	Estrous cycle effects. Animals were dosed for 21 d and vaginal smears were microscopically examined daily to classify rats into estrous cycle phase and determine cycle length. Vaginal smears were also evaluated for 21 d after cessation of dosing with the extracts; the experimental animals served as their own controls.	Rats were examined for changes in the estrous cycle, both during the 21 d of dosing with either extract, and for 21 d after termination of dosing. No significant changes in duration of estrous cycle phases were observed during dosing, relative to pre-treatment. However, during the 21-d withdrawal of treatment with both extracts, a statistically significant decrease occurred in the proestrus phase. A significant increase in the estrous phase was seen when the AEPO group ceased treatment, and a significant increase in the metestrus phase was seen when the MEPO group ceased treatment, relative to the pre-treatment period.	59
<i>Portulaca oleracea</i> leaf extract Chloroform and 80% aqueous methanol	Female albino rats; 5/group	0.5 ml 20% Tween 80	0, 125, 250, or 500 mg/kg chloroform or methanolic extract, via gavage	Estrous cycle and ovarian/uterine histology effects. Animals were dosed for 21 d, which began at the start of the estrous cycle. Vaginal smears were examined daily to assess the phase of the estrous cycle and blood samples were collected on day 21 for hormonal analysis of LH, FSH, progesterone, and estrogen serum levels. After the last dose, animals were killed and the uterine horns and ovaries were harvested for histological analyses (the estrous cycle phase at which the samples were collected was not stated).	No obvious or significant effects were observed on the estrous cycle and ovarian and uterine histology for animals treated with either extract, compared to controls. However, the ovarian sections from 125 mg/kg methanolic extract group showed hypertrophied ovarian follicles. Treatment with both extracts resulted in a decline in the mean serum levels of LH in the proestrus phase, which was not entirely significant. A significant decrease in LH was observed in the 250 mg/kg chloroform extract group; significant decreases in mean serum levels of FSH were also observed in the 250 and 500 mg/kg chloroform extract groups. No other significant effects were seen in LH, FSH, progesterone or estrogen serum levels in the estrus, metestrus, or diestrus phases.	60

Table 7. Oral reproductive and developmental toxicity studies

Test Article/ Extraction Solvent	Animals/Group	Vehicle	Dose/Concentration	Type of Study/Procedure	Results	Reference
<i>Portulaca oleracea</i> leaf and stem extract AEPO and MEPO	Female albino rats; 5/group	100 ml distilled water	0.5 ml distilled water, 75 mg/kg/d of AEPO or MEPO, via gavage	Ovarian and uterine histology. Rats showing at least 3 regular 4 - 5-d estrous cycles received either the control, AEPO, or MEPO extract for 25 d. On day 26, all rats were sacrificed and ovaries and uteri were weighed, fixed with Bouin's fluid, and dissected.	Changes in ovarian and uterine weights were not considered significant. No significant pathologic effects on the ovaries or uterus were observed. Both AEPO and MEPO were considered non-toxic to female rat reproductive function.	⁵⁹
<i>Portulaca oleracea</i> leaf and stem extract "total flavonoid extract"*	Female Wistar albino rats; 6/group	1% Tween 80	0, 250, or 500 mg/kg bw/d, via gavage	Abortifacient activity. Female rats of estrous phase were kept with male rats of proven fertility in a ratio of 2:1. Upon evidence of mating, they were separated from the male partner and divided into groups of 6. These rats received <i>Portulaca oleracea</i> extract, from day 7 to day 14 of gestation. On day 15, all animals were sacrificed and uterine horns were examined for aborted embryos.	Dams in the 250 mg/kg bw/d group had a 30% abortion rate, while animals in the 500 mg/kg bw/d group had a statistically significant 50% abortion rate.	⁵⁷
<i>Portulaca oleracea</i> leaf and stem extract "total flavonoid extract"*	Female Wistar albino rats; 6/group	1% Tween 80	0, 250, or 500 mg/kg bw/d, via gavage	Implantation study. Same mating strategy and female selection as above study. These rats were dosed from day 1 to day 7 of gestation. On day 10, all animals were sacrificed and uterine horns were examined for number of implants.	A 50% inhibition in implantation was seen at the 250 mg/kg dose, while a statistically significant, 70% inhibition in implantation was seen in the 500 mg/kg dose group (3.22 ± 0.02 vs. 8.12 ± 0.44 , in controls). The anti-implantation of the extract was observed after 24 h of the last administered dose.	⁵⁷
<i>Portulaca oleracea</i> leaf and stem extract AEPO and MEPO	Female albino rats; 5/group	100 ml distilled water	0.5 distilled water, or 75 mg/kg/d AEPO or MEPO, via gavage	Teratology study. Adult female rats exhibiting 4 - 5-d estrous cycles, found in the estrous phase, were caged, with virile males, in a 2:1 ratio. Pregnant rats were exposed to control or AEPO/MEPO from: - day 1 to day 5 (implantation/early pregnancy study); - day 6 to day 15 (mid-pregnancy/organogenesis study); or - day 16 to day 21 (late pregnancy study)	A non-significant increase in implantations occurred in rats treated from day 1 to day 5 of gestation with AEPO and MEPO. Treatment of rats from day 6 to 15 with AEPO and MEPO caused a decrease in fetal size for the pups of AEPO-treated dams, and an increase in fetal size for the pups of MEPO-treated dams, relative to controls. Changes in fetal size were not statistically significant. No premature births or abortions occurred, and pups were delivered normally. Treatment of rats from day 16 to 20 caused no significant increase in delivery litter size, and litter weights relative to controls. No resorption or gross malformations were observed in treated and control rats in mid or late pregnancy.	⁵³

Abbreviations: AEPO- aqueous extract *Portulaca oleracea*; ELISA- enzyme-linked immunosorbent assay; E2- estradiol; FSH-follicle-stimulating hormone; LH- luteinizing hormone; MEPO – methanolic extract *Portulaca oleracea*; NMRI- nuclear magnetic resonance imaging; PG – progesterone; TT - testosterone

*Methanol, ethanol, ethyl acetate, petroleum ether, diethyl ether, sulfuric acid, chloroform, HCL, potassium hydroxide, hexane, silica Gel 60-120 mesh, Tween 80 phosphate buffer saline, Folin- Ciocalteu reagent, are named as used chemicals, but are not specified as extract solvents.

Table 8. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Portulaca Oleracea Extract (body lotion containing 0.1%)	N/A	applied neat	22 subjects	24-h SIOPT; reference lotion used as control	Not irritating; PII = 0	⁶⁷
Portulaca Oleracea Extract (body moisturizer containing 0.1%)	N/A	0.5 ml; applied neat	26 subjects	Maximization study. Irritation was induced prior to each induction application (total of 5), with an occlusive, 24-h application of 0.05 ml 0.25% aqueous SLS. A 48-h (72 h over the weekend) occlusive application of the test article was applied to the pre-treated sites. After a 10-d non-treatment period, a 1-h occlusive application of 0.05 ml 5.0% aqueous SLS was made to a previously untreated site to induce irritation, after which a 48-h, occlusive challenge application of the test article was made. The challenge site was graded (on a 0-3 scoring scale) 15 - 30 min and 24 h after patch removal.	Not sensitizing; scores were 0 for both readings	⁶⁸
Portulaca Oleracea Extract (face lotion containing 0.5%)	N/A	applied neat	27 subjects	Maximization study. Identical to procedure described above	Not sensitizing	⁶⁹
Portulaca Oleracea Extract (face treatment product containing 0.5%)	N/A	applied neat	26 subjects	Maximization study. Identical to procedure described above	Not sensitizing	⁷⁰

Abbreviations: N/A – not applicable; PII – primary irritation index; SIOPT – single insult occlusive patch test; SLS – sodium lauryl sulfate

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2022 VCRP Frequency of Use Data – *Portulaca oleracea*- Derived Ingredients

Ingredient Name	Category	Code & Description	CPIS count
Portulaca Oleracea (Purslane) Extract			
Total Uses: 541			
Portulaca Oleracea (Purslane) Extract	01A	Baby Shampoos	1
Portulaca Oleracea (Purslane) Extract	01B	Baby Lotions, Oils, Powders, and Creams	7
Portulaca Oleracea (Purslane) Extract	01C	Other Baby Products	2
Portulaca Oleracea (Purslane) Extract	03D	Eye Lotion	9
Portulaca Oleracea (Purslane) Extract	03G	Other Eye Makeup Preparations	10
Portulaca Oleracea (Purslane) Extract	05A	Hair Conditioner	5
Portulaca Oleracea (Purslane) Extract	05F	Shampoos (non-coloring)	5
Portulaca Oleracea (Purslane) Extract	05I	Other Hair Preparations	2
Portulaca Oleracea (Purslane) Extract	07B	Face Powders	2
Portulaca Oleracea (Purslane) Extract	07C	Foundations	7
Portulaca Oleracea (Purslane) Extract	07E	Lipstick	1
Portulaca Oleracea (Purslane) Extract	07F	Makeup Bases	2
Portulaca Oleracea (Purslane) Extract	10A	Bath Soaps and Detergents	3
Portulaca Oleracea (Purslane) Extract	10D	Feminine Deodorants	3
Portulaca Oleracea (Purslane) Extract	10E	Other Personal Cleanliness Products	6
Portulaca Oleracea (Purslane) Extract	11A	Aftershave Lotion	1
Portulaca Oleracea (Purslane) Extract	12A	Cleansing	41
Portulaca Oleracea (Purslane) Extract	12C	Face and Neck (exc shave)	195
Portulaca Oleracea (Purslane) Extract	12D	Body and Hand (exc shave)	7
Portulaca Oleracea (Purslane) Extract	12F	Moisturizing	139
Portulaca Oleracea (Purslane) Extract	12G	Night	5
Portulaca Oleracea (Purslane) Extract	12H	Paste Masks (mud packs)	19
Portulaca Oleracea (Purslane) Extract	12I	Skin Fresheners	10
Portulaca Oleracea (Purslane) Extract	12J	Other Skin Care Preps	58
Portulaca Oleracea (Purslane) Extract	13A	Suntan Gels, Creams, and Liquids	1