
Safety Assessment of Palm Tree-Derived Ingredients as Used in Cosmetics

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

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



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Memorandum

To: CIR Expert Panel Members and Liaisons

From: Wilbur Johnson, Jr.
Senior Scientific Analyst

Date: March 15, 2019

Subject: Draft Report on Palm Tree-Derived Ingredients

Enclosed is a draft report on 8 palm tree-derived ingredients. This ingredient family comprises cosmetic ingredients that are derived from two palm tree species, *Euterpe edulis* and *Euterpe oleracea*. A Scientific Literature Review (SLR) was announced on January 22, 2019.

The attached report (*palmtr042019DR*) includes the following unpublished data that were received from the Council:

- 1) Use concentration data (*palmtr042019data1* and *palmtr042019data2*)
- 2) Compositional breakdown data on organic Euterpe Oleracea Juice (freeze dried) (*palmtr042019data3*)
- 3) Method of manufacturing data on Euterpe Oleracea Juice (freeze dried) (*palmtr042019data3*)
- 4) Compositional breakdown data on a Euterpe Oleracea Fruit Extract trade name material (*palmtr042019data3*)
- 5) Properties data (specifications) on a Euterpe Oleracea Fruit Extract trade name material (*palmtr042019data3*)
- 6) Method of manufacturing data on a Euterpe Oleracea Fruit Extract trade name material (*palmtr042019data3*)
- 7) In vitro dermal and ocular irritation data (in vitro models) on a Euterpe Oleracea Fruit Extract trade name material (*palmtr042019data3*)
- 8) In chemico skin sensitization data on a Euterpe Oleracea Fruit Extract trade name material (*palmtr042019data3*)
- 9) In vitro skin sensitization data on a Euterpe Oleracea Fruit Extract trade name material (*palmtr042019data3*)
- 10) In vitro genotoxicity data on a Euterpe Oleracea Fruit Extract trade name material (*palmtr042019data3*)

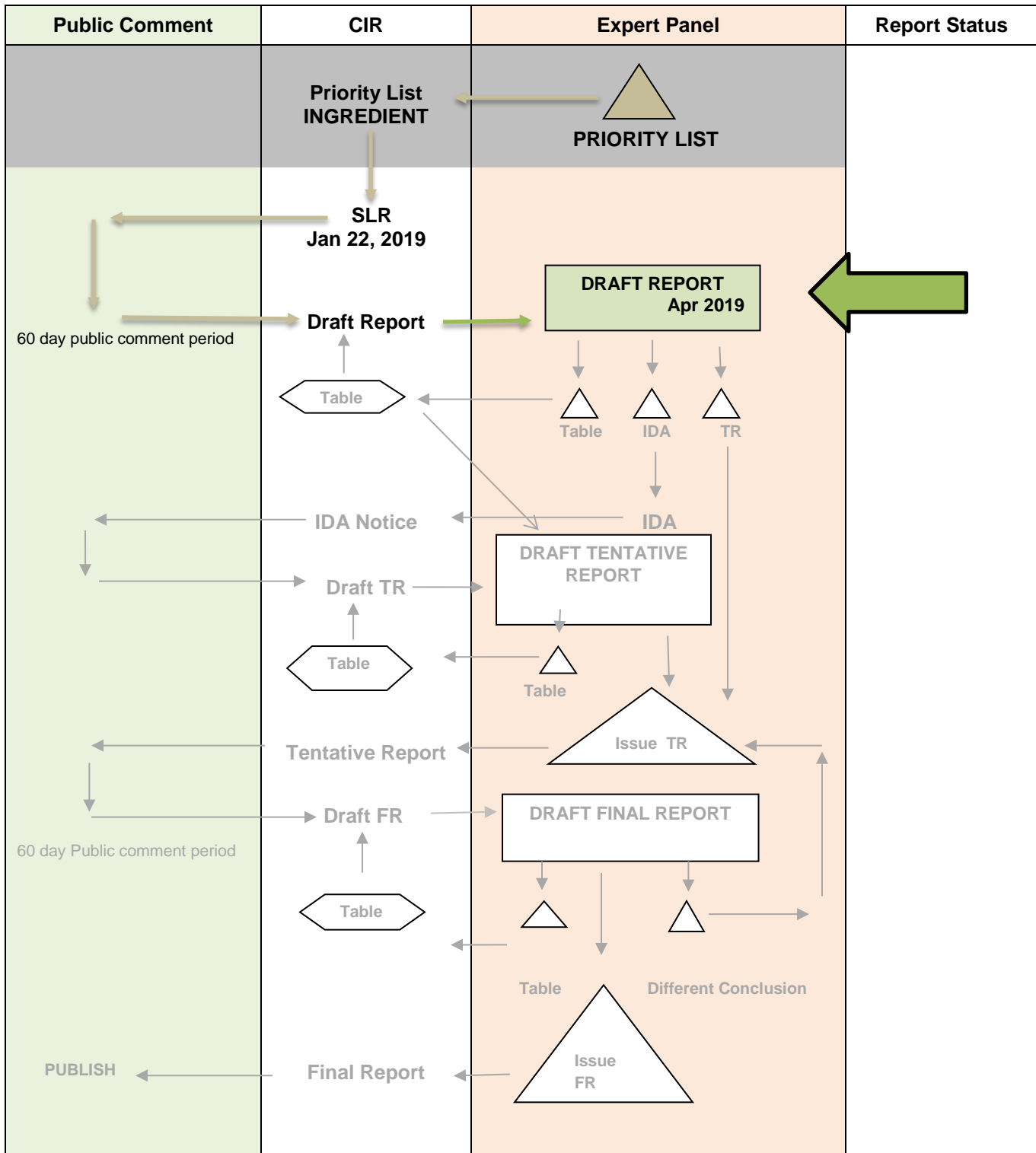
A cellular viability assay on a Euterpe Oleracea Fruit Extract trade name material (*palmtr042019data3*) was also submitted, but did not appear to be relevant to safety. Additionally, the attached comments on the SLR (*palmtr042019pcpc*) that were received from the Council have been addressed. Also included in this package for your review are the CIR report history (*palmtr042019hist*), flow chart (*palmtr042019flow*), literature search strategy (*palmtr042019strat*), ingredient data profile (*palmtr042019prof*), and 2019 FDA VCRP data (*palmtr042019fda*).

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

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Palm Tree-derived ingredients

MEETING April 2019



CIR History of:

Palm Tree-Derived Ingredients

A Scientific Literature Review (SLR) on Palm Tree-Derived Ingredients was issued on January 22, 2019. Comments and unpublished data were received from the Council before/after announcement of the SLR.

Draft Report, Teams/Panel: April 8-9, 201

The draft report has been revised to include the following unpublished data that were received from the Council:

- (1) Use concentration data
- (2) Compositional breakdown data on organic Euterpe Oleracea Juice (freeze dried)
- (3) Method of manufacturing data on Euterpe Oleracea Juice (freeze dried)
- (4) Compositional breakdown data on a Euterpe Oleracea Fruit Extract trade name material
- (5) Properties data (specifications) on a Euterpe Oleracea Fruit Extract trade name material
- (6) Method of manufacturing data on a Euterpe Oleracea Fruit Extract trade name material
- (7) In vitro dermal and ocular irritation data (in vitro models) on a Euterpe Oleracea Fruit Extract trade name material
- (8) In chemico skin sensitization data on a Euterpe Oleracea Fruit Extract trade name material
- (9) In vitro skin sensitization data on a Euterpe Oleracea Fruit Extract trade name material
- (10) In vitro genotoxicity data on a Euterpe Oleracea Fruit Extract trade name material
- (11) Cellular viability assay on a Euterpe Oleracea Fruit Extract trade name material

Comments on the safety assessment that were received from the Council have been addressed, and the report has also been updated to include current FDA VCRP data.

Palm Tree-Derived Ingredients Data Profile for April 8-9, 2019 Panel 1 Wilbur Johnson

[illegible]

[Palm Tree-Derived Ingredients--8/29/2018; updated on 2/24/2019]

| Ingredient | CAS # | InfoBase | SciFinder | PubMed | TOXNET | FDA | EU | ECHA | IUCLID | SIDS | HPVIS | NICNAS | NTIS | NTP | WHO | FAO | ECE-TOC | Web |
|---|-----------------------------|-----------------|------------------|---------------|---------------|---------------|-----------|-------------|---------------|-------------|--------------|---------------|-------------|------------|------------|------------|----------------|------------|
| Euterpe Oleracea Fruit Extract | 879496-95-4; 906351-38-0 | 1/1 | 50/12 | 0 | 2/0 | No | No | No | No | No | No | No | No | No | No | No | No | |
| Euterpe Edulis Fruit Extract | | 1/1 | 60/3 | 0 | 0 | No | No | No | No | No | No | No | No | No | No | No | No | |
| Euterpe Edulis Juice Extract | | 1/1 | 67/2 | 0 | 0 | No | No | No | No | No | No | No | No | No | No | No | No | |
| Euterpe Oleracea Juice | | 1/1 | 38/10 | 27/3 | 1/0 | No | No | No | No | No | No | No | No | No | No | No | No | |
| Euterpe Oleracea Palm Heart Extract | 879496-95-4; 906351-38-0 | 1/1 | 5/2 | 0 | 0 | No | No | No | No | No | No | No | No | No | No | No | No | |
| Euterpe Oleracea Pulp Powder | 879496-95-4; 906351-38-0 | 1/1 | 5/1 | 0 | 1/1 | No | No | No | No | No | No | No | No | No | No | No | No | |
| Euterpe Oleracea Seed Powder | 879496-95-4; 906351-38-0 | 1/1 | 100/5 | 0 | 0 | No | No | No | No | No | No | No | No | No | No | No | No | |
| Hydrolyzed Euterpe Oleracea Fruit | | | 276/6 | 0 | 0 | No | No | No | No | No | No | No | No | No | No | No | No | |
| Genus and Species Names (Not Cosmetic Ingredients) | | | | | | | | | | | | | | | | | | |
| Euterpe Oleracea | | | 40/3 | 155/4 | 2/0 | EAFUS on ext. | No | No | No | No | No | No | No | No | No | No | No | |
| Euterpe Edulis | | | 4/2 | 58/1 | 1/1 | No | No | No | No | No | No | No | No | No | No | No | No | |

Search Strategy

[document search strategy used for SciFinder, PubMed, and Toxnet]

[identify total # of hits /# hits that were useful or examined for usefulness]

LINKS

InfoBase (self-reminder that this info has been accessed; not a public website) - <http://www.personalcarecouncil.org/science-safety/line-infobase>
SciFinder (usually a combined search for all ingredients in report; list # of this/# useful) - <https://scifinder.cas.org/scifinder>
PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - <http://www.ncbi.nlm.nih.gov/pubmed>
Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) – <https://toxnet.nlm.nih.gov/> (includes Toxline; HSDB; ChemIDPlus; DAR; IRIS; CCRIS; CPDB; GENE-TOX)

FDA databases – <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm> (CFR); then, list of all databases: <http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm>; then, <http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true> (EAFUS); <http://www.fda.gov/food/ingredientpackaginglabeling/gras/default.htm> (GRAS); <http://www.fda.gov/food/ingredientpackaginglabeling/gras/scogs/ucm2006852.htm> (SCOGS database); <http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives> (indirect food additives list); <http://www.fda.gov/Drugs/InformationOnDrugs/default.htm> (drug approvals and database); <http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf> (OTC ingredient list); <http://www.accessdata.fda.gov/scripts/cder/iig/> (inactive ingredients approved for drugs)

EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - <http://ec.europa.eu/growth/tools-databases/cosing/>
ECHA (European Chemicals Agency – REACH dossiers) – <http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1>
IUCLID (International Uniform Chemical Information Database) - <https://iuclid6.echa.europa.eu/search>
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- <http://webnet.oecd.org/hpv/ui/Search.aspx>
HPVIS (EPA High-Production Volume Info Systems) - <https://ofmext.epa.gov/hpvis/HPVISlogin>
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- <https://www.nicnas.gov.au/>
NTIS (National Technical Information Service) - <http://www.ntis.gov/>
NTP (National Toxicology Program) - <http://ntp.niehs.nih.gov/>
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/> (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc
ECETOC (European Center for Ecotoxicology and Toxicology Database) - <http://www.ecetoc.org/>

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>

Fragrance Websites, if applicable

IFRA (International Fragrance Association) – <http://www.ifraorg.org/>

RIFM (the Research Institute for Fragrance Materials) should be contacted

Qualifiers

Absorption

Acute

Allergy

Allergic

Allergenic

Cancer

Carcinogen

Chronic

Development

Developmental

Excretion

Genotoxic

Irritation

Metabolism

Mutagen

Mutagenic

Penetration

Percutaneous

Pharmacokinetic

Repeated dose

Reproduction

Reproductive

Sensitization

Skin

Subchronic

Teratogen

Teratogenic

Toxic

Toxicity

Toxicokinetic

Toxicology

Tumor

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INTRODUCTION

The safety of the following 8 palm tree-derived ingredients, as used in cosmetics, is reviewed in this Cosmetic Ingredient Review (CIR) safety assessment.

Euterpe Edulis Fruit Extract
Euterpe Edulis Juice Extract
Euterpe Oleracea Fruit Extract
Euterpe Oleracea Juice
Euterpe Oleracea Palm Heart Extract
Euterpe Oleracea Pulp Powder
Euterpe Oleracea Seed Powder
Hydrolyzed Euterpe Oleracea Fruit

This group was formed based on the supposition that ingredients from a given genus and species (and closely related species (i.e., *edulis* and *oleracea*) source would have constituents in common. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), the palm tree-derived ingredients are reported to function mostly as skin conditioning agents in cosmetic products (See Table 1).¹ Euterpe Oleracea Pulp Powder and Euterpe Oleracea Seed Powder also are reported to function as abrasives and exfoliants in cosmetics.

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

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

Because the safety of *Euterpe oleracea*-derived ingredients is being reviewed in this safety assessment, it should be noted that the CIR Expert Panel (Panel) published a safety assessment on Euterpe Oleracea Fruit Oil and other plant-derived fatty acid oils in 2017.² Based on the available data, the Panel concluded that these ingredients are safe in the present practices of use and concentration described in the safety assessment. Though the safety of Euterpe Oleracea Fruit Oil is not being reviewed in this report on palm tree-derived ingredients, human repeated insult patch test (HRIPT) data on this ingredient from the published safety assessment are italicized within the report text for the Panel's consideration. Given some similarities in composition (based on the available data) between different parts of *Euterpe oleracea*, data on components that are not the names of cosmetic ingredients that are being reviewed in this safety assessment are included. Data on a component of *Euterpe edulis* (*Euterpe edulis* fruit oil) that is not among the names of cosmetic ingredients that are being reviewed are also included.

It is often not known how the substance being tested in a study compares to the ingredient that is being used in cosmetics. In the report text, if it is known that the material being tested is a cosmetic ingredient, the wINCI naming convention will be used (i.e., the names of cosmetic ingredients are capitalized, without italics). If it is not known that the test substance is that same as the cosmetic ingredient, then the taxonomic naming conventions will be used (i.e., with genus and species name, italicized).

CHEMISTRY

Definition and General Characterization

The palm species *Euterpe edulis* Martius, popularly known as juçara (or jussara) and açáidosol, is a native tree of the Atlantic Forest (South American forest).³ The juçara palm produces a spherical purple fruit. *Euterpe oleracea* Martius (açaí), is a native species of tree in the Amazon rainforest.⁴

The definitions and reported functions in cosmetics of these ingredients are presented in Table 1.¹

Method of Manufacture

Euterpe Oleracea Fruit Extract

The method of manufacture for a Euterpe Oleracea Fruit Extract trade name material (98% Euterpe Oleracea Fruit Extract and 2% lactobacillus ferment) provided by a supplier is as follows:⁵ *Euterpe oleracea* fruit is processed (mechanical grinding/milling). This process is followed by aqueous extraction (at specific pH and temperature) for a specified duration. The aqueous extract is then subjected to tangential flow filtration to isolate the desired components. Addition of lactobacillus ferment is the next step, and batch adjustments are made if needed (refiltration). A sample is then subjected to quality control, after which the material is packed and sampled for microbiological analysis prior to shipment.

Euterpe Oleracea Juice

According to one manufacturer of Euterpe Oleracea Juice, for use in foods, this juice is obtained by cold pressing the thin pulp of the ovoidal fruit (berry) of *Euterpe oleracea* Mart.⁶

The method of manufacture for organic Euterpe Oleracea Juice (undiluted, freeze dried), provided by a supplier, is as follows:⁷ Organic Euterpe Oleracea is cold-pressed for juice. This process is followed by filtration to remove unnecessary plant matter. The filtrate is then freeze dried, and batch adjustments are made, if necessary. A sample is then subjected to quality control, after which the material is packed. The packed material is then sampled for microbiological analysis prior to shipment. Reconstitution instructions for organic Euterpe Oleracea Juice (undiluted, freeze dried) are as follows: fill 25 g of powder up to 100 ml with water.

Euterpe Oleracea Pulp Powder

In this production method, the fruit pulp obtained from *Euterpe oleracea* fruit harvested in Brazil was frozen.⁸ Samples of spray-dried pulp were obtained using an industrial scale spray dryer system and manionic maltodextrin DE10 as a carrier agent.

Composition

Euterpe Edulis Fruit Extract

The composition of *Euterpe edulis* fruit extract has been determined using gas chromatography-mass spectrometry and solvents with different polarities (hexane, ethyl acetate, or chloroform) for extraction, and these data are presented in Table 2.⁹

According to research investigating the major anthocyanins (type of flavonoid) and non-anthocyanin phenolic compounds in *Euterpe edulis* fruit extract, high amounts of anthocyanins, approximately 26 mg/g dry weight basis (dwb), of a total of 31mg/g dwb of phenolic compounds, were detected.¹⁰ Cyanidin-3-*O*-rutinoside was the most abundant anthocyanin (73% of the total phenolic compounds content). It should be noted that an analysis of *Euterpe edulis* fruit for phenolics yielded a value of 4087 mg/100 g dwb for soluble phenolics in pulp from fruits collected in southeastern Brazil.¹¹ However, a lower value of 1695 mg/100 g dwb for soluble phenolics in this fruit (from Minas Gerais State, a state in the north of Southeastern Brazil) has also been reported.¹² Furthermore, *Euterpe edulis* fruit is rich in oleic and palmitic fatty acids.³

Additional data on the composition of Euterpe Edulis Fruit Extract, as well as data on the following other components of *Euterpe edulis*/component extracts are presented in Table 3: *Euterpe edulis* fruit, *Euterpe edulis* pulp extract, and *Euterpe edulis* pulp.^{10,11,12,13,14,15,16} Though not cosmetic ingredients, composition data on the 3 are included because they contain chemicals that may also be present in Euterpe Edulis Fruit Extract. Furthermore, data in Table 3 indicate that Euterpe Edulis Fruit Extract and one or more of the 3 fruit parts/extract have constituents in common.

Euterpe Oleracea Fruit Extract and Euterpe Oleracea Juice

Composition data on Euterpe Oleracea Fruit Extract (various extractants used) relating to phenolic compounds content (anthocyanins included) are presented in Table 4.^{17,18} As a food product, this material is reported to be a thin hygroscopic powder that is water soluble.⁴

It has been reported that total phenolic yields for *Euterpe oleracea* pulp (freeze-dried and mixed with ethyl acetate) ranged from 132.6 to 391.2 mg gallic acid equivalent (GAE)/100 g fresh weight (FW).¹⁹ Also, the total anthocyanin yield ranged from 4.2 to 90.0 mg/100 g FW. Data on the composition of *Euterpe oleracea* fruit, *Euterpe oleracea* fruit powder extract, *Euterpe oleracea* juice extract, Euterpe Oleracea Juice, and *Euterpe oleracea* pulp are presented in Table 5:^{20,21,22,23,24}

Taking into consideration the INCI names that represent the ingredients that are being reviewed in this safety assessment, except for *Euterpe Oleracea* Juice, these are not cosmetic ingredient names. Composition data on 4 *Euterpe oleracea*-derived botanicals are included because they contain chemicals that are also present in *Euterpe Oleracea* Fruit Extract (see Table 4 and Table 5). Particularly, data on *Euterpe oleracea* pulp are included because *Euterpe Oleracea* Pulp Powder is a cosmetic ingredient.

According to a supplier's specification for a *Euterpe Oleracea* Fruit Extract trade name material (98% *Euterpe Oleracea* Fruit Extract and 2% *Lactobacillus* ferment), the ferulic acid content ranges from 4% to 5%. This material is a clear to slightly hazy liquid.²⁵

The list of allergenic flavors or fragrances that *Euterpe Oleracea* Fruit Extract trade name material (98% *Euterpe Oleracea* Fruit Extract and 2% *Lactobacillus* ferment) does not contain, neither directly nor through cross contamination, are presented in Table 6.²⁶

A *Euterpe Oleracea* Fruit Extract trade name material consists of 98% *Euterpe Oleracea* Fruit Extract and 2% *Lactobacillus* ferment).⁴

***Euterpe oleracea* fruit (for *Euterpe Oleracea* Fruit Extract)**

The following trace elements have been detected in *Euterpe oleracea* fruit: potassium, magnesium, phosphorus, calcium, sodium, zinc, iron, and copper.²¹

***Euterpe Oleracea* Juice**

The list of allergenic flavors or fragrances that organic *Euterpe Oleracea* Juice (undiluted, freeze dried) does not contain, neither directly nor through cross contamination, is presented in Table 6.⁷

***Euterpe Oleracea* Seed Powder**

In the absence of data on *Euterpe Oleracea* Seed Powder constituents, composition data on *Euterpe oleracea* seed are presented in Table 7.¹⁸ It should also be noted that when *Euterpe oleracea* seeds were extracted with a solution of 95% ethanol/1.5 N HCL (85:15, v/v), the content of phenolic compounds was reported as a total only (3602 ± 88 mg GAE/100 g (dwb; chemical names not stated), and anthocyanins (content not stated) were among the types of phenolic compounds that were represented in the total.

Impurities

***Euterpe Edulis* Fruit Extract and *Euterpe Edulis* Juice Extract**

In the absence of impurities data on *Euterpe Edulis* Fruit Extract and *Euterpe Edulis* Juice Extract, data on heavy metal/mineral constituents of *Euterpe edulis* fruit and *Euterpe edulis* pulp are presented in Table 8.¹²

***Euterpe Oleracea* Fruit Extract**

The heavy metals content of *Euterpe Oleracea* Fruit Extract (powder) has been described as follows: arsenic (< 0.1 ppm), cadmium (< 0.01 ppm), mercury (< 0.005 ppm), lead (< 0.05 ppm), and copper (0.3 ppm).²⁷ Impurities data on related *Euterpe oleracea* components (*Euterpe oleracea* fruit and *Euterpe oleracea* pulp) are summarized below.

A supplier's impurities specifications for a *Euterpe Oleracea* Fruit Extract trade name material (98% *Euterpe Oleracea* Fruit Extract and 2% *Lactobacillus* ferment) include the following: heavy metals (< 20 ppm), lead (< 10 ppm), arsenic (< 2 ppm), microbial content (< 100 CFU/g; no pathogens), yeast and mold (< 100 CFU/g), and gram negative bacteria (0 CFU/g).²⁵ Data provided by the same supplier indicate that pesticides present in this trade name material do not exceed the Environmental Protection Agency's (EPA's) limits.²⁶ These data on pesticide levels are presented in Table 9.

***Euterpe oleracea* fruit (for *Euterpe Oleracea* Fruit Extract)**

Açaí (*Euterpe oleracea* Martius), as a native fruit of the Amazon rainforest, has been described as highly contaminated in microbiological terms.⁴ The fruit is said to be subject to natural microbiological contamination and one of the main sources of this contamination is water, considering that more than 50% of the municipalities located in the Brazilian Amazon do not use chlorinated water. *Euterpe oleracea* fruit from Brazil and the United States (US) was analyzed for 174 different pesticides, using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-tandem

mass spectrometry (GC-MS/MS).²⁸ *Euterpe oleracea* fruit that was harvested and lyophilized in Brazil had no detectable pesticides. There also were no detectable pesticides in 7 samples of *Euterpe oleracea* fruit in the US. However, the following pesticides were detected in 5 other samples (identified as samples 1, 4, 8, 9, and 10) of *Euterpe oleracea* fruit in the US: Sample 1 (methoxyfenozide [0.2 ng/g]), Sample 4 (metalaxyl [0.2 ng/g]), Sample 8 (boscalid [2.6 ng/g] and imidacloprid [0.9 ng/g]), Sample 9 (bifenazate [2.5 ng/g], carbendazim [0.9 ng/g]), and Sample 10 (bifenazate [1.6 ng/g], boscalid [3 ng/g], hexythiazox [0.6 ng/g], and pyraclostrobin [0.1 ng/g]).

The following heavy metals have been detected in *Euterpe oleracea* fruit: lead, cadmium, mercury, and arsenic.²¹ Ash has been detected in *Euterpe oleracea* fruit in an amount of 1.68 ± 0 g/100 g (dwb).¹⁸

***Euterpe oleracea* pulp (for Euterpe Oleracea Pulp Powder)**

Ash has been detected in *Euterpe oleracea* pulp in an amount of 3.78 ± 0.06 g/100 g (dwb).¹⁸

***Euterpe oleracea* seed (for Euterpe Oleracea Seed Powder)**

In the absence of impurities data on Euterpe Oleracea Seed Powder, there are data indicating that ash has been detected in *Euterpe oleracea* seed in an amount of 1.44 ± 0.01 g/100 g (dwb).¹⁸

USE

Cosmetic

The safety of palm tree-derived ingredients is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database.²⁹ Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.³⁰

According to 2019 VCRP data, Euterpe Oleracea Fruit Extract is reported to be used in 430 cosmetic products (297 leave-on products, 129 rinse-off products, 4 products that are diluted for (bath) use).²⁹ Of the palm tree-derived ingredients that are being reviewed in this safety assessment, this is the greatest reported use frequency. The results of a concentration of use survey conducted by the Council in 2017 indicate that Euterpe Oleracea Pulp Powder is being used at maximum use concentrations up to 3% in leave-on products (face and neck products [not spray]) and maximum use concentrations up to 0.6% in rinse-off products (moisturizing products [not spray] and paste masks [mud packs]).³⁰ These are the highest use concentrations in leave-on and rinse-off products that are being reported for the palm tree-derived ingredients that are being reviewed in this safety assessment. Further use data are presented in Table 10.

According to VCRP and Council survey data, the following 3 ingredients are not being used in cosmetic products: Euterpe Edulis Fruit Extract, Euterpe Edulis Juice Extract, and Euterpe Oleracea Seed Powder.

Cosmetic products containing palm tree-derived ingredients may be applied to the skin or, incidentally, may come in contact with the eyes (e.g., Euterpe Oleracea Fruit Extract). Euterpe Oleracea Fruit Extract, Euterpe Oleracea Juice, Euterpe Oleracea Palm Heart Extract, and Euterpe Oleracea Pulp Powder are ingredients that are used in products that come in contact with mucous membranes during product use (ingredient use concentrations: 0.0000083 - 0.3%). Additionally, Euterpe Oleracea Fruit Extract and Euterpe Oleracea Pulp Powder could be incidentally ingested (at maximum use concentrations up to 0.025% [lipstick] and 0.3% [lipstick], respectively). Products containing palm tree-derived ingredients may be applied as frequently as several times per day and may come in contact with the skin for variable periods following application. Daily or occasional use may extend over many years.

The following palm tree-derived ingredients are being used in products that are sprayed: Euterpe Oleracea Fruit Extract (0.001% in pump hair spray), Euterpe Oleracea Palm Heart Extract (0.001% in colognes and toilet waters), and Euterpe Oleracea Pulp Powder (0.015% in colognes and toilet waters). In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters $> 10 \mu\text{m}$, with propellant sprays yielding a greater fraction of droplets/particles below $10 \mu\text{m}$, compared with pump sprays.^{31,32,33,34} Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.^{31,32} The only use of palm tree-derived ingredients in powders is being reported for Euterpe Oleracea Juice, which is being used at concentrations up to 0.01% in face powders. 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.^{35,36,37}

The palm tree-derived ingredients reviewed in this safety assessment are not included on the European Union's list of substances that are restricted or list of substances that are prohibited in cosmetic products.³⁸

Non-Cosmetic

Euterpe Oleracea Fruit Extract

The Flavor and Extract Manufacturers Association (FEMA) has listed acai berry extract as a generally recognized as safe (GRAS) food flavoring ingredient.³⁹ According to the *Dictionary*, acai berry extract comprises Euterpe Oleracea Fruit Extract, propylene glycol, and water.¹ *Euterpe oleracea* is cultivated for both its fruit and edible hearts of palm; it should be noted that acai berry juice (GRAS ingredient) and hearts of palm are derived from the same species.⁴⁰

TOXICOKINETIC STUDIES

Dermal Penetration

Data on the dermal penetration of the palm tree-derived ingredients reviewed in this safety assessment were neither found in the published literature, nor were these data submitted. Dermal penetration data were not expected to be found because each botanical ingredient is a mixture of hundreds of constituents.

Absorption, Distribution, Metabolism, and Excretion

Human

Oral

Euterpe Oleracea Juice and *Euterpe oleracea* pulp

An acute 4-way crossover clinical trial that involved oral dosing with the following was performed using 12 subjects: Euterpe Oleracea Juice, *Euterpe oleracea* pulp, applesauce (control), and a non-antioxidant beverage (control).⁴¹ An oral dose of Euterpe Oleracea Juice or *Euterpe oleracea* pulp (7 mL/kg) was administered after a washout phase and overnight fast, and plasma was repeatedly sampled over 12 h. Urine was sampled over a 24-h period after dosing. Plasma anthocyanin (antioxidant) concentrations were determined over a period of 0 - 12 h. Noncompartmental pharmacokinetic analysis of total anthocyanins, quantified as cyanidin-3-*O*-glucoside, indicated maximum plasma concentration (C_{max}) values of 2321 and 1138 ng/L at maximum concentration times (t_{max}) of 2.2 and 2.0 h, and area under the concentration-time curve (AUC_{last} ; last refers to AUC up to the last measurable concentration) values of 8568 and 3314 ng h/L for *Euterpe oleracea* pulp and Euterpe Oleracea Juice, respectively. Nonlinear mixed effect modeling identified dose volume as a significant predictor of relative oral bioavailability in a negative nonlinear relationship for *Euterpe oleracea* pulp and Euterpe Oleracea Juice. Additionally, after consumption of *Euterpe oleracea* pulp, applesauce, and Euterpe Oleracea Juice, plasma antioxidant capacity was statistically significantly increased ($p < 0.01$) when compared to the non-antioxidant control beverage. Individual increases in plasma antioxidant capacity of up to 2.3- and 3-fold for Euterpe Oleracea Juice and *Euterpe oleracea* pulp, respectively, were observed. Both applesauce and *Euterpe oleracea* pulp induced statistically significantly higher plasma antioxidant activities than Euterpe Oleracea Juice ($p < 0.05$). The non-oxidant control beverage also caused an increase in the antioxidant capacity of the plasma when compared to the baseline, which may have resulted from its fructose content. The antioxidant capacity in the urine, generation of reactive oxygen species, and uric acid concentrations in plasma were not significantly altered by the treatments. The results of this study indicate that anthocyanins from *Euterpe oleracea* are bioavailable in human subjects after consumption of Euterpe Oleracea Juice and *Euterpe oleracea* pulp in moderate amounts.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

***Euterpe oleracea* pulp-enriched fruit and berry juice (for Euterpe Oleracea Juice)**

The acute toxicity of a *Euterpe oleracea* pulp-enriched fruit and berry juice (fortified with glucosamine) was evaluated in accordance with Organization for Economic Co-operation and Development (OECD) test guideline (TG) 423.⁴²

The concentration of *Euterpe oleracea* pulp in the juice was not stated. Two groups of Wistar rats (Crl:(WI) BR strain; 5 males and 5 females per group) received single oral doses by gavage of 5 g/kg and 20 g/kg, respectively. Dosing was followed by a 14-day observation period and gross necropsy was performed on day 15. None of the animals died and there were no treatment-related clinical or behavioral signs. For female rats, the mean body weight gain (on days 1 and 2 and during the last week) in the 20 g/kg dose group was statistically significantly lower when compared to the 5 g/kg group. However, the total body weight gain of females in the 20 g/kg dose group was not statistically significantly different when compared to the 5 g/kg dose group. At necropsy (both dose groups) on day 15, there was no evidence of gross lesions in any organ, and all organs were free of gross pathological changes. It was concluded that the acute oral LD₅₀ for the test substance was > 20 g/kg.

Short-Term Toxicity Studies

Oral

Euterpe oleracea fruit oil

The short-term oral toxicity of *Euterpe oleracea* fruit oil was evaluated using groups of 6 Wistar rats.⁴³ *Euterpe oleracea* fruit oil (doses of 30 mg/kg, 100 mg/kg, or 300 mg/kg) in 1% Tween 80 was administered by gavage daily (at 24-h intervals) for 14 consecutive days. At the dose of 300 mg/kg, but not at lower doses, some animals began to display signs of toxicity such as diarrhea and bristling of the hair.

Subchronic Toxicity Studies

Euterpe oleracea pulp-enriched fruit and berry juice

The subchronic oral toxicity of *Euterpe oleracea* pulp-enriched fruit and berry juice (fortified with glucosamine) was evaluated using groups of 40 Wistar rats (SPF Hsd.Brl.Han strain; 20 males and 20 females per group).⁴² The test substance was administered daily by gavage for 90 days to 3 groups at doses of 10, 20, and 40 g/kg, respectively. Necropsy was performed on day 91. The vehicle control group was dosed with saline, and there was also an untreated control group. When compared to the control groups, there were no treatment-related, statistically significant changes in the following in surviving animals of all 3 dose groups: body weight, food and water consumption, ophthalmology, organ weights, urinalysis, hematological and clinical chemistry, or gross pathology. Three animals died during the study (1 male at 20 g/kg; 1 male at 40 g/kg; and 1 female at 10 g/kg). The animals that died did not have clinical symptoms prior to death. With the exception of signs of suffocation/aspiration congestion (due to problems with the gavage administration of the test substance; not considered test substance-related), there was no evidence of histopathological lesions or injury to tissues or organs. The only statistically significant difference (not clinically meaningful) observed was in mean adrenal weight (values not stated) relative to the brain weight in the 20 mg/kg dose group when compared to untreated female controls. Whether or not the change in adrenal weight in treated animals was an increase or decrease when compared to controls was not stated. However, this statistically significant difference was not biologically significant. The no-observed-adverse-effect-level (NOAEL) was determined to be 40 g/kg/day for male and female rats.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Data on the developmental and reproductive toxicity of palm tree-derived ingredients reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

GENOTOXICITY STUDIES

The following genotoxicity studies on palm tree-derived ingredients are summarized below and in Table 11.

In Vitro

Euterpe edulis fruit pulp (9% in water) was genotoxic (at 25 to 250 µg/plate, but not at higher doses), without metabolic activation, in one *Salmonella typhimurium* strain in the Ames test, and in the micronucleus assay (RAW264.7 mouse macrophage-like cells; genotoxic at 0.27 to 10.8 mg/ml, range of concentrations tested).⁹ *Euterpe edulis* fruit oil was non-genotoxic in the cytokinesis-block micronucleus assay (human peripheral blood lymphocytes and HepG2 human hepatoma cells; concentrations up to 1000 µg/ml) and in the comet assay (human peripheral blood lymphocytes and HepG2 human hepatoma cells; concentrations up to 1000 µg/ml).⁴⁴

A *Euterpe Oleracea* Fruit Extract trade name material (98% *Euterpe Oleracea* Fruit Extract and 2% lactobacillus ferment) was non-genotoxic, with and without metabolic activation, in the Ames test (*S. typhimurium* strains and an

Escherichia coli strain; doses up to 5000 µg/plate).⁴⁵ *Euterpe oleracea* pulp-enriched fruit and berry juice (fortified with glucosamine) was non-genotoxic, with and without metabolic activation, in the Ames test (*S. typhimurium* strains; doses up to 5 µg/plate), and non-genotoxic, with and without metabolic activation, in the chromosomal aberration assay (Chinese hamster lung cells; concentrations up to 5000 µg/ml) and in the L5178Y/TK+/- mouse lymphoma assay (concentrations up to 500 µg/ml).⁴²

In Vivo

Euterpe edulis fruit pulp extract (9% in water) was genotoxic in the micronucleus assay (bone marrow erythrocytes from dosed rats) in which rats received doses up to 180 mg/kg by gavage.⁹ However, in a second study using the same protocol and doses, *Euterpe edulis* fruit pulp extract (9% in water) was non-genotoxic. Negative results were also obtained in the comet assay (single cell gel electrophoresis [SCGE] test) involving randomly selected cells in blood from rats receiving doses up to 180 mg/kg, and in another comet assay involving randomly selected cells in human blood that was drawn after a 300 ml dose.

Euterpe oleracea pulp-enriched fruit and berry juice (fortified with glucosamine) was non-genotoxic in the micronucleus assay (mouse bone marrow erythrocytes from mice receiving a dose of 100 µg/100 µl saline).⁴² *Euterpe oleracea* fruit pulp was non-genotoxic in the micronucleus assay (mouse bone marrow erythrocytes and peripheral blood erythrocytes from mice receiving doses up to 16.67 g/kg), and was non-genotoxic in the comet assay involving mouse peripheral blood erythrocytes, liver cells, and kidney cells from mice receiving doses up to 16.67 mg/kg.⁴⁶ In rats dosed with *Euterpe oleracea* fruit oil (doses up to 300 mg/kg), there was no significant induction of DNA strand breaks in the comet assay (peripheral blood, bone marrow, liver cells, and testicle cells), but there was minor DNA damage in a few nucleoids.⁴³ *Euterpe oleracea* fruit oil was non-genotoxic in the micronucleus assay (bone marrow erythrocytes from rats receiving doses up to 300 mg/kg).

CARCINOGENICITY STUDIES

Data on the carcinogenicity of palm tree-derived ingredients reviewed in this safety assessment were neither found in the published literature, nor were these data submitted.

ANTI-CARCINOGENICITY STUDIES

Euterpe Oleracea Fruit Extract

The anti-tumorigenicity of Euterpe Oleracea Fruit Extract (hydroalcoholic extract) was evaluated using 2 groups of 40 female Wistar rats.⁴⁷ Twenty rats were dosed orally (200 mg/kg, by gastric intubation) with a saline solution of the fruit extract for 16 consecutive weeks. The control group (20 rats) was dosed with saline according to the same procedure. One day after starting dosing with Euterpe Oleracea Fruit Extract, mammary carcinogenesis was induced in all animals by s.c. injection of 25 mg/kg of 7,12-dimethylbenz[a]anthracene (DMBA) in the mammary gland. The animals were palpated in the mammary gland once per week to detect the presence of breast tumors. At the end of the treatment period, the animals were killed and tumor tissues as well as heart, liver, and kidney samples were examined histologically. Survival analysis indicated that Euterpe Oleracea Fruit Extract increased survival ($P = 0.0002$, long-rank test) and reduced the number of deaths ($P = 0.0036$, Chi-square test). Cumulative survival periods of 15.15 weeks and 12.75 weeks were reported for test and control animals, respectively. The mortality rate in the control group was 65% (13 deaths), and the mortality rate was 15% (3 deaths) after dosing with Euterpe Oleracea Fruit Extract. There was no evidence of toxicity of the extract, based on food consumption, body weight, and activity levels, when compared to results for the 20 control rats. Histopathological results for the liver and kidneys indicated a protective effect of Euterpe Oleracea Fruit Extract, because, in the control group, there was an increase in fibrosis, atypical cells, and hemorrhagic microenvironment. There were no morphological differences in heart tissue between test and control rats.

In the control group, the tumor incidence rate was 100%. However, in the group dosed with Euterpe Oleracea Fruit Extract, the tumor incidence rate was markedly reduced to 50%. In both groups, mammary tumors displayed adhesions and a cystic pattern near the site of tumor induction. However, there was no significant difference in tumor volume (control: 4.151 ± 0.8 mL; Euterpe Oleracea Fruit Extract: 3.971 ± 1.3 mL) and tumor weight (control: 3.012 ± 0.5 g; Euterpe Oleracea Fruit Extract: 2.52 ± 0.7 g). It was concluded that Euterpe Oleracea Fruit Extract (hydroalcoholic extract) exhibited anti-tumorigenic activity in DMBA-induced breast cancer.⁴⁷

Euterpe Oleracea Pulp Powder

A study was performed to investigate the protective effect of Euterpe Oleracea Pulp Powder (spray-dried) intake on colon carcinogenesis induced by 1,2-dimethylhydrazine.⁴⁸ Four groups of 10 rats received 4 (s.c.) injections of 1,2-dimethylhydrazine (40 mg/kg) for 4 weeks (twice a week), for initiation of colon carcinogenesis. A fifth group (5 rats) received similar injections of ethylenediaminetetraacetic acid (EDTA; 1,2-dimethylhydrazine vehicle). The groups were then fed a standard diet containing 2.5% or 5.0% Euterpe Oleracea Pulp Powder, or a diet containing 0.2% *N*-acetylcysteine (antioxidant and anti-carcinogenic agent) for 10 weeks, using aberrant crypt foci (ACF) as the endpoint. Additionally, two groups were fed a standard diet or a diet containing 5.0% Euterpe Oleracea Pulp Powder for 20 weeks, using colon tumors as the endpoint. In the assay using ACF as the endpoint, a reduction in the number of aberrant crypts and ACF were observed in the groups fed 5.0% Euterpe Oleracea Pulp Powder (37% aberrant crypts and 47% ACF inhibition, $P = 0.036$) and 0.2% *N*-acetylcysteine (39% aberrant crypts and 41% ACF inhibition, $P = 0.042$). In the assay using colon tumors as the endpoint, a reduction in the number of invasive tumors ($p < 0.005$) and tumor multiplicity ($P = 0.001$) was observed in the group fed with 5.0% Euterpe Oleracea Pulp Powder. Also, a reduction in tumor Ki-67 (human protein strictly associated with cell proliferation) cell proliferation ($P = 0.003$) and net growth index ($P = 0.001$) was observed in the group fed 5.0% Euterpe Oleracea Pulp Powder. It was concluded that the results of this study indicate that Euterpe Oleracea Pulp Powder feeding may reduce the development of chemically-induced rat colon carcinogenesis.

Another study was performed to evaluate whether feeding with Euterpe Oleracea Pulp Powder attenuates the initiation step of chemically-induced mouse colon carcinogenesis.⁸ *Euterpe oleracea* fruit pulp was frozen and samples of spray-dried pulp (powder) were obtained. The production method for this powder is stated in the Method of Manufacture section of this report. This study involved male Swiss mice (3 groups of 15 (Groups 1 - 3); 1 group of 5 (Group 4)). Group 1 was fed a low fat diet and Groups 2 and 3 were fed a low fat diet containing 2.5% and 5% Euterpe Oleracea Pulp Powder, respectively, during weeks 1 to 4. The positive control group (Group 4) was fed a low fat diet containing 0.1% indole-3-carbinol during weeks 1 to 3. All groups received an intraperitoneal (i.p.) injection of the colon carcinogen azoxymethane (AOM) at week 3. Some mice from groups 1 to 3 and all mice from group 4 ($n = 5$ mice per group) were killed at week 3 ($n = 5$ mice/group) and liver samples were collected for immunohistochemical and glutathione analysis. The remaining mice (Groups 1-3; $n = 10$ mice/group) received a second i.p. injection of AOM at week 4 and were fed a high-fat diet to accelerate the development of preneoplastic ACF until week 14. At week 3, both dietary Euterpe Oleracea Pulp Powder doses (2.5% or 5.0%) reduced ($p < 0.001$) peripheral blood cell DNA damage induced by AOM. Also, 5.0% Euterpe Oleracea Pulp Powder increased ($p = 0.002$) hepatic total glutathione. At week 14, 5.0% Euterpe Oleracea Pulp Powder reduced ($p < 0.05$) ACF multiplicity. These findings indicate that feeding with Euterpe Oleracea Pulp Powder attenuates chemically-induced mouse colon carcinogenesis by increasing total GSH and attenuating DNA damage and preneoplastic lesion development.

OTHER RELEVANT STUDIES

Cytotoxicity

Euterpe Oleracea Fruit Extract

The anti-carcinogenicity of Euterpe Oleracea Fruit Extract (hydroalcoholic extract) was evaluated in a study using cell viability as the toxicity endpoint.⁴⁹ The malignant cell lines derived from human mammary adenocarcinoma (MCF-7 and MDA-MB-468 cells) and human colon adenocarcinomas (Caco-2 and HT-29) were treated with 10, 20, and 40 $\mu\text{g/ml}$ Euterpe Oleracea Fruit Extract for 24 h and 48 h. After treatment, cell viability was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays, and cell morphological features were observed by light and transmission electron microscopy. The data were analyzed statistically. Of all the cell lines tested, MCF-7 was the only line that responded to Euterpe Oleracea Fruit Extract treatment (cytotoxic effect). Significant reduction ($p < 0.01$) in cell viability and altered cell morphological features (by inducing the appearance of autophagic vacuoles) was noted at all concentrations. It was concluded that Euterpe Oleracea Fruit Extract possesses anti-tumorigenic potential in the MCF-7 cell line.

Euterpe oleracea pulp extract

The antiproliferative activity of *Euterpe oleracea* pulp extract (polyphenolic extract, concentrations ranging from 0.04 to 12 μg of gallic acid equivalents (GAE)/mL) was evaluated in a cell culture model using HT-29 colon carcinoma cell viability as the endpoint.⁵⁰ Cell numbers were determined after 48 h of incubation. Total cell numbers were indicative of the proliferative activity of HT-29 cells and the cytotoxic effect of *Euterpe oleracea* pulp extract. The extract caused significant ($p < 0.01$) decreases in total cell numbers in a concentration-dependent manner.

DERMAL IRRITATION AND SENSITIZATION STUDIES

In addition to the in vitro and in chemico sensitization data that are summarized in this section, human skin sensitization data on Euterpe Oleracea Fruit Oil that are summarized in the CIR Expert Panel's published safety assessment on Euterpe Oleracea Fruit Oil and other plant-derived fatty acid oils are included in the Sensitization section below for the Panel's consideration.²

Irritation

In Vitro

Euterpe Oleracea Fruit Extract

The skin irritation potential of a Euterpe Oleracea Fruit Extract trade name material (98% Euterpe Oleracea Fruit Extract and 2% lactobacillus ferment) was evaluated using the EpiDerm™ model (reconstructed human epidermis) assay.⁵¹ The test substance was applied to tissue inserts and incubated for 60 minutes. Cell viability was measured by dehydrogenase conversion of MTT, present in the cell mitochondria, into blue formazan salt. Skin irritation potential of the test substance is dictated by the reduction in tissue viability of exposed tissues when compared to the negative control (sterile Dulbecco's phosphate buffered saline). Sodium dodecyl sulfate (5%) served as the positive control. An irritant is predicted if the mean relative tissue viability of the 3 tissues exposed to the test substance is reduced by 50% of the mean viability of the negative controls, and a non-irritant's viability is > 50%. The trade name material was classified as a non-irritant in this assay.

Sensitization

In Vitro/In Chemico

Euterpe Oleracea Fruit Extract

The in vitro skin sensitization antioxidant/electrophile response element (ARE)-nuclear factor (erythroid-derived 2) (Nrf2) luciferase test method was used to evaluate the sensitization potential of a Euterpe Oleracea Fruit Extract trade name material (98% Euterpe Oleracea Fruit Extract and 2% lactobacillus ferment).⁵² This test method (validated by independent peer review by the European Union Reference Laboratory for Alternatives to Animal Testing (EURL)-European Center for the Validation of Alternative Methods (ECVAM) addresses the induction of genes that are regulated by AREs by skin sensitizers. The sensitization assay in this study utilizes the KeratinoSens™ method. Collectively, an immortalized adherent human keratinocyte cell line (HaCaT) was incubated for 48 h with 12 concentrations of the trade name material ranging from 0.98 µM to 2000 µM. Cinnamic aldehyde (4 µM to 64 µM) and 1% dimethyl sulfoxide (DMSO) served as positive and negative controls, respectively. There was no statistically significant increase in luciferase expression, and the Euterpe Oleracea Fruit Extract trade name material was not predicted to be a skin sensitizer.

The skin sensitization potential of a Euterpe Oleracea Fruit Extract trade name material (98% Euterpe Oleracea Fruit Extract and 2% lactobacillus ferment) was evaluated using the direct peptide reactivity assay (DPRA, an *in chemico* method).⁵³ This assay is designed to mimic the covalent binding of electrophilic chemicals to nucleophilic centers in skin proteins by quantifying the reactivity of chemicals towards the model synthetic peptides containing cysteine and lysine. The mean percent depletion of cysteine and lysine was 3.20%, interpreted as minimal reactivity in the assay and yielding a prediction of no sensitization.

Human

Euterpe Oleracea Fruit Oil

*The skin sensitization potential of 0.5% Euterpe Oleracea Fruit Oil in an eye treatment was evaluated using 104 subjects. The test substance (150 µl) was applied under semioclusive conditions in a human repeated insult patch test (HRIPT). It was concluded that the test substance was neither a dermal irritant nor a sensitizer in this study.*²

OCULAR IRRITATION STUDIES

In Vitro

The EpiOcular™ model (human corneal epithelial model) assay was used to evaluate the irritation potential of a Euterpe Oleracea Fruit Extract trade name material (98% Euterpe Oleracea Fruit Extract and 2% lactobacillus ferment).⁵¹ The test substance was applied to tissue inserts and incubated for 30 min. Cell viability was measured by dehydrogenase conversion of MTT, present in the cell mitochondria, into blue formazan salt. Ocular irritation potential of the test substance is dictated by the reduction in tissue viability of exposed tissues when compared to the negative control (sterile deionized

water). Methyl acetate served as the positive control. An irritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance is reduced by 60% of the mean viability of the negative controls, and a non-irritant's viability is > 40%. The trade name material was classified as a non-irritant in this assay.

SUMMARY

The safety of 8 palm tree-derived ingredients as used in cosmetics is reviewed in this CIR safety assessment. According to the *Dictionary*, these ingredients function mostly as skin conditioning agents in cosmetic products. Euterpe Oleracea Pulp Powder and Euterpe Oleracea Seed Powder also function as abrasives and exfoliants in cosmetics.

Information on the method of manufacture of a Euterpe Oleracea Fruit Extract trade name material (98% Euterpe Oleracea Fruit Extract and 2% lactobacillus ferment) from a supplier indicates that the process involves the aqueous extraction of Euterpe Oleracea Fruit. Additionally, this trade name material and Euterpe Oleracea Juice have not been found to contain many of the allergenic flavors or fragrances that have been identified in the published literature. The same supplier's impurities specifications for a Euterpe Oleracea Fruit Extract trade name material (98% Euterpe Oleracea Fruit Extract and 2% lactobacillus ferment) include the following: heavy metals (< 20 ppm), lead (< 10 ppm), arsenic (< 2 ppm), microbial content (< 100 CFU/g; no pathogens), yeast and mold (< 100 CFU/g), and gram negative bacteria (0 CFU/g). Data provided by the same supplier indicate that pesticides present in this trade name material do not exceed the EPA's limits.

According to 2019 VCRP data, Euterpe Oleracea Fruit Extract is reported to be used in 430 cosmetic products (297 leave-on products, 129 rinse-off products, and 4 products that are diluted for (bath) use). Of the palm tree-derived ingredients that are being reviewed in this safety assessment, this is the greatest reported use frequency. The results of a concentration of use survey conducted by the Council in 2017 indicate that Euterpe Oleracea Pulp Powder is being used at maximum use concentrations up to 3% in leave-on products (face and neck products [not spray]) and maximum use concentrations up to 0.6% in rinse-off products (moisturizing products [not spray] and paste masks [mud packs]). These are the highest use concentrations in leave-on and rinse-off products that are being reported for the palm tree-derived ingredients that are being reviewed in this safety assessment. According to VCRP and Council survey data, the following 3 ingredients that are being reviewed are not being used in cosmetic products: Euterpe Edulis Fruit Extract, Euterpe Edulis Juice Extract, and Euterpe Oleracea Seed Powder.

The results from a clinical trial involving 12 subjects who consumed an oral dose (7 ml/kg) of Euterpe Oleracea Juice or *Euterpe oleracea* pulp indicated that anthocyanins from *Euterpe oleracea* are bioavailable in human subjects after consumption of Euterpe Oleracea Juice and *Euterpe oleracea* pulp in moderate amounts.

The acute toxicity of a *Euterpe oleracea* pulp-enriched fruit and berry juice (fortified with glucosamine) was evaluated using 2 groups of 10 Wistar rats that received single oral doses of 5 g/kg and 20 g/kg, respectively. The acute oral LD₅₀ was reported as > 20 g/kg.

In groups of 6 Wistar rats, *Euterpe oleracea* fruit oil (doses of 30 mg/kg, 100 mg/kg, or 300 mg/kg) in 1% Tween 80 was administered by gavage daily for 14 consecutive days. At the dose of 300 mg/kg, but not at lower doses, some of the animals had signs of toxicity such as diarrhea and bristling of the hair. In a 16-week study involving 20 Wistar rats dosed orally with Euterpe Oleracea Fruit Extract and s.c. with DMBA, there was no evidence of toxicity of the extract, based on food consumption, body weight, and activity levels. There were no morphological differences in heart tissue between test and control rats.

The subchronic oral toxicity of *Euterpe oleracea* pulp-enriched fruit and berry juice (fortified with glucosamine) was evaluated using groups of 40 Wistar rats. The test substance was administered daily for 90 days to 3 groups at oral doses of 10, 20, and 40 g/kg, respectively. There were no treatment-related, statistically significant changes in the following in surviving animals of all 3 dose groups: body weight, food and water consumption, ophthalmology, organ weights, urinalysis, hematological and clinical chemistry, or gross pathology. The 3 animals that died during the study did not have clinical symptoms prior to death, and there was no evidence of histopathological lesions or injury to tissues or organs. An NOAEL of 40 g/kg/day was reported.

Components of *Euterpe edulis* and *Euterpe oleracea* were evaluated in in vitro genotoxicity tests. *Euterpe edulis* fruit pulp (9% in water) was genotoxic in one *S. typhimurium* strain in the Ames test, and in the micronucleus assay. *Euterpe edulis* fruit oil was non-genotoxic in the cytokinesis-block micronucleus assay and in the comet assay. A Euterpe Oleracea Fruit Extract trade name material (98% Euterpe Oleracea Fruit Extract and 2% lactobacillus ferment) was non-genotoxic, with and without metabolic activation, in the Ames test (*S. typhimurium* strains and an *E.coli* strain). *Euterpe oleracea* pulp enriched fruit and berry juice (fortified with glucosamine) was non-genotoxic in the Ames test, the chromosomal aberration assay, and in the L5178Y/TK+/- mouse lymphoma assay.

In vivo genotoxicity test results for components of *Euterpe edulis* and *Euterpe oleracea* have also been reported. *Euterpe edulis* fruit pulp (9% in water) was genotoxic in one micronucleus assay, but was non-genotoxic in another micronucleus assay or in comet assays. *Euterpe oleracea* pulp-enriched fruit and berry juice (fortified with glucosamine) was non-genotoxic in the micronucleus assay. *Euterpe oleracea* fruit pulp was non-genotoxic in the micronucleus assay and in the comet assay. Results for *Euterpe oleracea* fruit oil in the comet assay indicated no significant induction of DNA strand breaks, but there was minor DNA damage in a few nucleoids. *Euterpe oleracea* fruit oil was also non-genotoxic in the micronucleus assay.

The anti-tumorigenicity of Euterpe Oleracea Fruit Extract has been demonstrated both in vivo (rats, breast cancer study) and in vitro (human mammary adenocarcinoma cell line). In vivo anti-carcinogenic activity of Euterpe Oleracea Pulp Powder has been demonstrated in colon cancer studies involving rats. In another study, the antiproliferative activity of *Euterpe oleracea* pulp extract was evaluated in a cell culture model using colon carcinoma cells, and a significant decrease in total cell numbers was reported.

When compared to the control (details not provided), a Euterpe Oleracea Fruit Extract trade name material increased cellular metabolism and viability at all test concentrations (0.01%, 0.1%, and 1%) in human dermal fibroblasts in vitro. In an in vitro study in which IgE-sensitized mouse mast cells were treated with *Euterpe oleracea* pulp, the test material was found to be a potent inhibitor of IgE-mediated mast cell activation.

A Euterpe Oleracea Fruit Extract trade name material (98% Euterpe Oleracea Fruit Extract and 2% lactobacillus ferment) was classified as a non-irritant when skin irritation was evaluated using the EpiDerm™ model (reconstructed human epidermis) assay.

The in vitro skin sensitization ARE-Nrf2 luciferase test method was used to evaluate the sensitization potential of a Euterpe Oleracea Fruit Extract trade name material (98% Euterpe Oleracea Fruit Extract and 2% lactobacillus ferment). This test method involved incubation of the HaCaT cell line with concentrations ranging from 0.98 µM to 2000 µM, and the trade name material was not predicted to be a skin sensitizer. The same trade name material was evaluated for sensitization potential using the DPRA and was predicted to be a non-sensitizer.

The EpiOcular™ model (human corneal epithelial model) assay was used to evaluate the ocular irritation potential of a Euterpe Oleracea Fruit Extract trade name material (98% Euterpe Oleracea Fruit Extract and 2% lactobacillus ferment). The trade name material was classified as a non-irritant in this assay.

TABLES**Table 1.** Definitions, idealized structures, and functions of the ingredients in this safety assessment. ^(1: CIR Staff)

| Ingredient CAS No. | Definition & Structures | Function(s) |
|---|--|---|
| Euterpe Edulis Fruit Extract | Euterpe Edulis Fruit Extract is the extract of the fruit of <i>Euterpe edulis</i> . | Skin-Conditioning Agents - Miscellaneous |
| Euterpe Edulis Juice Extract | Euterpe Edulis Juice Extract is the extract of the sap of <i>Euterpe edulis</i> . | Skin-Conditioning Agents - Miscellaneous |
| Euterpe Oleracea Fruit Extract 879496-95-4 (generic) 906351-38-0 (generic) | Euterpe Oleracea Fruit Extract is the extract of the fruit of <i>Euterpe oleracea</i> . | Hair Conditioning Agents |
| Euterpe Oleracea Juice 879496-95-4 (generic) 906351-38-0 (generic) | Euterpe Oleracea Juice is the juice expressed from the fruit of <i>Euterpe oleracea</i> . | Skin-Conditioning Agents - Miscellaneous |
| Euterpe Oleracea Palm Heart Extract 879496-95-4 (generic) 906351-38-0 (generic) | Euterpe Oleracea Palm Heart Extract is the extract of the palm heart of <i>Euterpe oleracea</i> . | Skin-Conditioning Agents - Emollient |
| Euterpe Oleracea Pulp Powder 879496-95-4 (generic) 906351-38-0 (generic) | Euterpe Oleracea Pulp Powder is the powder obtained from the dried, ground pulp of <i>Euterpe oleracea</i> . | Abrasives; Antioxidants; Exfoliants; Skin-Conditioning Agents - Miscellaneous |
| Euterpe Oleracea Seed Powder 879496-95-4 906351-38-0 | Euterpe Oleracea Seed Powder is the powder obtained from the dried, ground seeds of <i>Euterpe oleracea</i> . | Abrasives; Exfoliants |
| Hydrolyzed Euterpe Oleracea Fruit | Hydrolyzed Euterpe Oleracea Fruit is the hydrolysate of the fruit of <i>Euterpe oleracea</i> derived by acid, enzyme, or other method of hydrolysis. | Skin-Conditioning Agents - Miscellaneous |

Table 2. Composition data on Euterpe Edulis Fruit Extract (various extractants).⁹

| Components | Principles Compound (Probability (%))* |
|--|---|
| <u>Hexane Extract</u> | |
| bis(2-methylpropyl)-1,2-benzenedicarboxylic acid ester | 20 |
| hexadecanamide | 54 |
| 9-(Z)-octadecenamide | 61 |
| phenethyl alcohol | 25 |
| squalene | 20 |
| <u>Ethyl Acetate Extract</u> | |
| 1,6-anhydro-β-D-glucopyranose, | 43 |
| hexadecanamide | 72 |
| 9-(Z)-octadecenamide | 54 |
| <u>Chloroform Extract</u> | |
| 2,4-(E,E)-decadienal | 23 |
| (Z)-2-hepten-1-al | 29 |
| naphthalene | 35 |
| phenethylalcohol | 55 |

*The chemical constituents of the extracts were identified by comparing their retention indices and making computer matches with the National Institute of Standards and Technology library provided by the computer controlling the gas chromatography-mass spectrometry system.

Table 3. Content of Ingredients/Fruit Parts derived from *Euterpe edulis*.^{10,13,12,14,15,11,16,54}

| Components | Euterpe Edulis Fruit Extract | <i>Euterpe edulis</i> fruit | <i>Euterpe edulis</i> pulp extract | <i>Euterpe edulis</i> pulp |
|--|---|-----------------------------|------------------------------------|----------------------------|
| <u>Carotenoids (µg/100 g fresh weight)</u> | | | | |
| apocarotenoid | | undetectable | | |
| all- <i>trans</i> -α-carotene | | 60.2 ± 6.0 | | |
| all- <i>trans</i> -β-carotene | | 266.5 ± 41.5 | | |
| all- <i>trans</i> -α-cryptoxanthin | | undetectable | | |
| all- <i>trans</i> -β-cryptoxanthin | | undetectable | | |
| all- <i>trans</i> -lutein | | 292.7 ± 3.3 | | |
| all- <i>trans</i> -neochrome | | undetectable | | |
| all- <i>trans</i> -zeaxanthin | | 5.4 ± 2.4 | | |
| all- <i>trans</i> -zeinoxanthin | | 7.7 ± 0.4 | | |
| <i>cis</i> -antheraxanthin | | undetectable | | |
| 9- <i>cis</i> -β-carotene | | 37.8 ± 3.5 | | |
| 13- <i>cis</i> -β-carotene | | 15.8 ± 1.9 | | |
| 15- <i>cis</i> -β-carotene | | 9.2 ± 0.3 | | |
| 9- <i>cis</i> -β-cryptoxanthin | | undetectable | | |
| 9'- <i>cis</i> -β-cryptoxanthin | | undetectable | | |
| 13- <i>cis</i> -β-cryptoxanthin | | undetectable | | |
| 13'- <i>cis</i> -β-cryptoxanthin | | undetectable | | |
| 15- <i>cis</i> -β-cryptoxanthin | | undetectable | | |
| <i>cis</i> -lutein | | 12.6 ± 1.3 | | |
| 9- <i>cis</i> -violaxanthin | | 5.5 ± 0.4 | | |
| 13- <i>cis</i> -violaxanthin | | 6.5 ± 4.3 | | |
| 9- <i>cis</i> -neoxanthin | | 13.2 ± 4.2 | | |
| 5,8-epoxy-β-carotene | | undetectable | | |
| 5,6-epoxy-β-cryptoxanthin | | undetectable | | |
| 5,8-epoxy-β-cryptoxanthin | | undetectable | | |
| phytoene | | undetectable | | |
| <u>Nutrients (%)</u> | | | | |
| Carbohydrate | | 85.7 ± 0.4 | | 42.5 ± 0.1 |
| Dietary fiber | | 71.8 ± 0.6 | | 27.1 |
| Lipid | | 6.9 ± 0.3 | | 46.6 |
| Moisture | | 51.9 ± 0.3 | | 83.8 ± 0.5 |
| Protein | | 5 ± 0.3 | | 7.5 ± 0.1 |
| <u>Anthocyanins (expressed as mg cyanidin 3-glucoside (C3G)/100 g fresh matter or as gallic acid equivalents (GAE)/100 g)</u> | | | | |
| cyanidin-3- <i>O</i> -glucoside | Amount not stated | | | |
| cyanidin 3-glucoside | Not assayed | 47.93 ± 1.52 | Amount not stated | |
| cyanidin 3-glucoside | Not assayed | 51.4 ± 3.1 (as GAE) | Amount not stated | |
| cyanidin 3,5-hexose pentose | Not assayed | 1.43 ± 0.05 | Not assayed | |
| cyanidin 3-rhamnoside | Not assayed | 0.30 ± 0.01 | Not assayed | |
| cyanidin-3- <i>O</i> -rutinoside | 73% of total phenolic compounds content | Not assayed | Not assayed | |
| cyanidin 3-rutinoside | Not assayed | 179.60 ± 5.77 | Amount not stated | |
| cyanidin 3-rutinoside | Not assayed | 141 ± 8.5 (as GAE) | Amount not stated | |
| cyanidin-3-sambubioside | Not assayed | Not assayed | Amount not stated | |
| delphinidin-3-glucoside | Not assayed | Not assayed | Amount not stated | |
| pelargonidin-3- <i>O</i> -glucoside | Amount not stated | Not assayed | Not assayed | |
| pelargonidin-3-glucoside | Not assayed | 1.66 ± 0.05 | Amount not stated | |
| pelargonidin 3-rutinoside | Not assayed | 2.87 ± 0.09 | Not assayed | |
| peonidin-3-rutinoside | Not assayed | 3.59 ± 0.11 | Amount not stated | |
| <u>Other Phenolic Compounds (expressed as gallic acid equivalents (GAE)/100 g)</u> | | | | |
| apigenin | Amount not stated | Not assayed | | |
| apigenin deoxyhexosidehexoside | Not assayed | 25.4 ± 1.5 | | |
| apigenin dihexoside | Not assayed | 11.06 ± 0.9 | | |
| apigenin hexoside | Not assayed | 13.2 ± 1 | | |
| caffeic acid | Not assayed | Amount not stated | | |

Table 3. Content of Ingredients/Fruit Parts derived from *Euterpe edulis*.^{10,13,12,14,15,11,16,54}

| Components | Euterpe Edulis Fruit Extract | <i>Euterpe edulis</i> fruit | <i>Euterpe edulis</i> pulp extract | <i>Euterpe edulis</i> pulp |
|------------------------------------|------------------------------|-----------------------------|------------------------------------|----------------------------|
| catechin | Amount not stated | Not assayed | | |
| chlorogenic acid | Not assayed | Amount not stated | | |
| chrysoeriol deoxyhexosylhexoside | Not assayed | 22.5 ± 0.7 | | |
| <i>m</i> -coumaric acid | Not assayed | Amount not stated | | |
| <i>p</i> -coumaric Acid | Not assayed | Amount not stated | | |
| dihydroluteolin | | | | |
| deoxyhexosylhexoside | Not assayed | 12.7 ± 0.5 | | |
| 4,5-dicaffeoylquinic acid | Amount not stated | Not assayed | | |
| dihydrokaempferol acetylhexoside | Not assayed | 2.8 ± 0.01 | | |
| dihydrokaempferol hexoside | Not assayed | 66.4 ± 2.6 | | |
| 3,4-dihydroxyphenylacetic acid | Not assayed | Amount not stated | | |
| ellagic acid | Amount not stated | Not assayed | | |
| ferulic acid | Not assayed | Amount not stated | | |
| gallic acid | Not assayed | Amount not stated | | |
| gallic acid hexoside | Not assayed | 1.7 ± 0.04 | | |
| <i>p</i> -hydroxybenzoic acid | Not assayed | Amount not stated | | |
| 4-hydroxyphenylacetic acid | Not assayed | Amount not stated | | |
| kaempferol | Amount not stated | Not assayed | | |
| kaempferol deoxyhexosylhexoside | Not assayed | 7.21 ± 0.9 | | |
| kaempferol-3- <i>O</i> -rutinoside | Amount not stated | Not assayed | | |
| luteolin | Amount not stated | Not assayed | | |
| luteolin deoxyhexosylhexoside | Not assayed | 37.6 ± 1.9 | | |
| myricetin | Amount not stated | Not assayed | | |
| protocatechuic acid | Not assayed | Amount not stated | | |
| quercetin | Amount not stated | Not assayed | | |
| rutin | Amount not stated | Not assayed | | |
| sinapinic acid | Not assayed | Amount not stated | | |
| syringic acid | Not assayed | Amount not stated | | |
| taxifolin hexoside | Not assayed | 13.3 ± 0.4 | | |
| <i>trans</i> -cinnamic acid | Not assayed | Amount not stated | | |
| vanillic acid | Not assayed | Not assayed | | |

Table 4. Composition data on Euterpe Oleracea Fruit Extract (various extractants).^{17,18}

| Components | Amount (mg GAE/100g [dwb])* |
|---|-----------------------------|
| <u>Sequential extraction with ethyl acetate, methanol, and methanol/water, yielding anthocyanins</u> | |
| cyanidin-di- <i>O</i> -glycoside | Not stated |
| cyanidin-3-glucoside | Not stated |
| cyanidin-3-rutinoside | Not stated |
| pelargonidin-3-glucoside | Not stated |
| peonidin-3-glucoside | Not stated |
| peonidin-3-rutinoside | Not stated |
| <u>Extraction with solution of ethanol and hydrochloric acid</u> | |
| Total phenolic compounds | 2370 ± 177 |
| Total anthocyanins | 81.62 ± 12.89 |

*dwb = dry weight basis

Table 5. Content of Ingredients/Components Derived From *Euterpe oleracea*.^{20,21,22,23,24,54}

| Components | <i>Euterpe oleracea</i> fruit | <i>Euterpe oleracea</i> fruit powder extract | <i>Euterpe oleracea</i> juice extract | <i>Euterpe Oleracea</i> Juice (data on the pulp [contains juice] identified as pulp below) |
|---|-------------------------------|--|---------------------------------------|--|
| <u>Anthocyanins</u> | | | | |
| cyanidin 3-acetyl hexose | Amount not stated | | | |
| cyanidin-3-arabinoside | Amount not stated | | | |
| cyanidin-3-glucoside | Not assayed | | Amount not stated | |
| cyanidin-3- <i>O</i> -glucoside | Amount not stated | | | |
| cyanidin-3-rutinoside | Not assayed | | Amount not stated | |
| cyanidin-3- <i>O</i> -rutinoside | Amount not stated | | | |
| cyanidin 3-sambubioside | Amount not stated | | | |
| peonidin 3-glucoside | Amount not stated | | | |
| peonidin 3-rutinoside | Amount not stated | | | |
| <u>Flavonoids (mg/100 g dry matter of juice extract; µg/g dry weight of juice)</u> | | | | |
| apigenin | Amount not stated | | | |
| apigenin 6,8-di- <i>C</i> -hexoside | Not assayed | | Amount not stated | |
| apigenin- <i>O</i> -hexoside- <i>C</i> -hexoside | Not assayed | | Amount not stated | |
| apigenin 6- <i>C</i> -hexoside-8- <i>C</i> -pentoside | Not assayed | | Amount not stated | |
| apigenin 6- <i>C</i> -pentoside-8- <i>C</i> -hexoside | Not assayed | | Amount not stated | |
| apigenin 8- <i>C</i> -(2"- <i>O</i> -pentosyl) hexoside | Not assayed | | Amount not stated | |
| astilbin | Amount not stated | | | |
| caffeic acid | Not assayed | | Amount not stated | Amount not stated |
| catechin | Amount not stated | | | 5.20 ± 1.08 |
| (+)-catechin | Not assayed | | 8.14 ± 0.80 | |
| chrysoeriol | Amount not stated | | 1.03 ± 0.03 | |
| crisoeirol | Amount not stated | | | |
| (+)-dihydrokaempferol | Not assayed | | 2.18 ± 0.02 | |
| (2R,3R)-dihydrokaempferol | Amount not stated | | | |
| 5,4'-dihydroxy-7, 3', 5'-trimethoxy flavone | Amount not stated | | | |
| epicatechin | Amount not stated | | | |
| (-)-epicatechin | Not assayed | | 4.43 ± 0.28 | |
| homoorientin | Not assayed | | 71.56 ± 5.81 | |
| isoorientin | Amount not stated | | | 89.74 ± 5.32 |
| isovitexin | Amount not stated | | Amount not stated | |
| kaempferol rhamnoside | Amount not stated | | | |
| kaempferol rutinoside | Amount not stated | | | |
| kaempferol-3-rutinoside | Not assayed | | Amount not stated | |
| luteolin | Not assayed | | Amount not stated | |
| luteoline diglicoside | Amount not stated | | | |
| orientin | Amount not stated | | 55.19 ± 0.76 | 189.49 ± 13.56 |
| procyanidin dimeric | Amount not stated | | | |
| protoanthocyanidin | Amount not stated | | | |
| quercetin | Amount not stated | | 1.77 ± 0.03 | |
| quercetin arabinopyranoside | Amount not stated | | | |
| quercetin-3-glucoside | Not assayed | | 1.57 ± 0.04 | |

Table 5. Content of Ingredients/Components Derived From *Euterpe oleracea*.^{20,21,22,23,24,54}

| Components | <i>Euterpe oleracea</i> fruit | <i>Euterpe oleracea</i> fruit powder extract | <i>Euterpe oleracea</i> juice extract | <i>Euterpe Oleracea</i> Juice (data on the pulp [contains juice] identified as pulp below) |
|---|-------------------------------|--|---------------------------------------|--|
| quercetin rhamnoside | Amount not stated | | | |
| quercetin rutinoside | Amount not stated | | | |
| rutin | Amount not stated | | 3.95 ± 0.07 | |
| scoparin | Amount not stated | | 4.71 ± 0.12 | |
| taxifolin | Not assayed | | Amount not stated | 1.57 ± 0.25 |
| taxifolin deoxyhexose | Amount not stated | | | |
| taxifolin deoxyhexose (or isomer) | Not assayed | | Amount not stated | |
| <u>Other Phenolic Compounds (µg/g dry weight of juice)</u> | | | | |
| benzoic acid | Amount not stated | | | |
| chlorogenic acid | Amount not stated | | | 4.23 ± 0.86 |
| <i>p</i> -coumaric acid | Not assayed | | | 4.67 ± 0.93 |
| <i>p</i> -coumarinic acid | Amount not stated | | | |
| dihydrokaempferol | Amount not stated | | | |
| (+)-dihydrokaempferol | Not assayed | | | |
| 4-hydroxybenzoic acid | Not assayed | | | 13.38 ± 1.50 |
| 3,4-dihydroxybenzoic acid | Not assayed | | | Amount not stated |
| ellagic acid | Amount not stated | | | |
| eriodictyol | Not assayed | | Amount not stated | |
| escoparine | Amount not stated | | | |
| ferulic acid | Amount not stated | | | 27.95 ± 2.48 |
| gallic acid | Amount not stated | | | |
| glycoside ellagic acid | Amount not stated | | | |
| <i>p</i> -hydroxybenzoic acid | Amount not stated | | | |
| 3-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanonadihydroconiferyl alcohol | Amount not stated | | | |
| isovitexin | Not assayed | | 7.07 ± 0.53 | |
| lariciresinol | Amount not stated | | | |
| pinoresinol | Amount not stated | | | |
| pirocatequic acid | Amount not stated | | | |
| protocatechuic acid | Not assayed | | Amount not stated | |
| syringaresinol | Amount not stated | | | |
| syringic acid | Not assayed | | | 0.69 ± 0.09 |
| vanillic acid | Amount not stated | | Amount not stated | 55.61 ± 5.26 |
| velutine | Amount not stated | | | |
| vitexin | Not assayed | | 6.26 ± 0.48 | |
| <u>Simple Benzenoids</u> | | | | |
| dihydroconiferyl alcohol | Amount not stated | | | |
| 3,4'-dihydroxy-3'-methoxypropiophenone | Amount not stated | | | |
| 3-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone | Amount not stated | | | |
| protocatechuic acid methyl ester | Amount not stated | | | |

Table 5. Content of Ingredients/Components Derived From *Euterpe oleracea*.^{20,21,22,23,24,54}

| Components | <i>Euterpe oleracea</i> fruit | <i>Euterpe oleracea</i> fruit powder extract | <i>Euterpe oleracea</i> juice extract | Euterpe Oleracea Juice (data on the pulp [contains juice] identified as pulp below) |
|--|-------------------------------|--|---------------------------------------|---|
| <u>Benzoquinone</u> | | | | |
| 2,6-dimethoxy-1,4-benzoquinone | Amount not stated | | | |
| <u>Monoterpenoids</u> | | | | |
| (E,Z)-2,6-dimethyl-2,6-octadiene-1,8-diol | Amount not stated | | | |
| (E,E)-2,6-dimethyl-2,6-octadiene-1,8-diol | Amount not stated | | | |
| (S)-menthiafolic acid | Amount not stated | | | |
| <u>Norisoprenoids</u> | | | | |
| (4R)-4-[(1E)-3-Hydroxy-1-butenyl]-3,5,5-trimethyl-2-cyclohexen-1-one | Amount not stated | | | |
| (-)-loliolide | Amount not stated | | | |
| <u>Saturated Fatty Acids (g/100g [dwb])</u> | | | | |
| behenic | Amount not stated | | | |
| butyric | Amount not stated | | | |
| caproic | Amount not stated | | | |
| caprylic | Amount not stated | | | |
| capric | Amount not stated | | | |
| eicosanoic | Amount not stated | | | |
| lauric | Amount not stated | | | |
| liognoceric | Amount not stated | | | |
| margaric | Amount not stated | | | |
| myristic | Amount not stated | | | |
| nonadecanoic | Amount not stated | | | |
| palmitic | Not assayed | | | 7.64 (pulp) |
| pentadecanoic | Amount not stated | | | |
| stearic | Amount not stated | | | 0.36 (pulp) |
| tricosanoic | Amount not stated | | | |
| tridecanoic | Amount not stated | | | |
| undecanoic | Amount not stated | | | |
| <u>Monounsaturated Fatty Acids (g/100g [dwb])</u> | | | | |
| elaidic | Amount not stated | | | |
| erucic | Amount not stated | | | |
| gadoleic | Amount not stated | | | |
| margaroleic | Amount not stated | | | |
| myristoleic | Amount not stated | | | |
| nervonic | Amount not stated | | | |
| oleic | Amount not stated | | | 18.20 (pulp) |
| palmitoleic | Amount not stated | | | 1.82 (pulp) |
| pentadecenoic | Amount not stated | | | |
| tridecenoic | Amount not stated | | | |

Table 5. Content of Ingredients/Components Derived From *Euterpe oleracea*.^{20,21,22,23,24,54}

| Components | <i>Euterpe oleracea</i> fruit | <i>Euterpe oleracea</i> fruit powder extract | <i>Euterpe oleracea</i> juice extract | <i>Euterpe Oleracea</i> Juice (data on the pulp [contains juice] identified as pulp below) |
|--|-------------------------------|--|---------------------------------------|--|
| <u>Polyunsaturated Fatty Acids (g/100g [dwb])</u> | | | | |
| arachidonic | Amount not stated | | | |
| docosadienoic | Amount not stated | | | |
| docosahexaenoic | Amount not stated | | | |
| eicosadienoic | Amount not stated | | | |
| eicosapentaenoic | Amount not stated | | | |
| eicosatrienoic | Amount not stated | | | |
| linoleic | Amount not stated | | | 3.64 (pulp) |
| linolenic | Amount not stated | | | |
| α -linolenic acid | Not assayed | | | 0.36 (pulp) |
| gamma linolenic | Amount not stated | | | |
| homogamma linolenic | Amount not stated | | | |
| <u>Sterols</u> | | | | |
| campesterol | Amount not stated | | | |
| beta-sitosterol | Amount not stated | | | |
| stigmasterol | Amount not stated | | | |
| <u>Amino Acids</u> | | | | |
| alanine | Amount not stated | | | |
| arginine | Amount not stated | | | |
| aspartic acid | Amount not stated | | | |
| cysteine | Amount not stated | | | |
| glutamic acid | Amount not stated | | | |
| glycine | Amount not stated | | | |
| histidine | Amount not stated | | | |
| hydroxyproline | Amount not stated | | | |
| isoleucine | Amount not stated | | | |
| leucine | Amount not stated | | | |
| lysine | Amount not stated | | | |
| methionine | Amount not stated | | | |
| phenylalanine | Amount not stated | | | |
| proline | Amount not stated | | | |
| serine | Amount not stated | | | |
| threonine | Amount not stated | | | |
| tryptophan | Amount not stated | | | |
| tyrosine | Amount not stated | | | |
| valine | Amount not stated | | | |
| <u>Sugars</u> | | | | |
| fructose | Amount not stated | | | |
| glucose | Amount not stated | | | |
| lactose | Amount not stated | | | |
| maltose | Amount not stated | | | |
| sucrose | Amount not stated | | | |

Table 5. Content of Ingredients/Components Derived From *Euterpe oleracea*.^{20,21,22,23,24,54}

| Components | <i>Euterpe oleracea</i> fruit | <i>Euterpe oleracea</i> fruit powder extract | <i>Euterpe oleracea</i> juice extract | Euterpe Oleracea Juice (data on the pulp [contains juice] identified as pulp below) |
|--|-------------------------------|--|---------------------------------------|---|
| <u>Lignans</u> | | | | |
| (-)-(7R,8S)-dihydrodehydroconiferyl alcohol | Amount not stated | | | |
| erythro-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-methoxy-phenoxy]-1,3-propanediol | Amount not stated | | | |
| (+)-isolariciresinol | Amount not stated | | | |
| (+)-(6R,7S,8S)-isolariciresinol | Amount not stated | | | |
| (+)-lariciresinol (8) | Amount not stated | | | |
| (+)-(7S,8R,8'R)-lariciresinol | Amount not stated | | | |
| (+)-(7R,8S)-5-methoxydihydrodehydroconiferyl alcohol | Amount not stated | | | |
| (+)-5-methoxy-isolariciresinol | Amount not stated | | | |
| (+)-(6R,7S,8S)-5-methoxyisolariciresinol | Amount not stated | | | |
| (+)-pinoresinol | Amount not stated | | | |
| (+)-syringaresinol | Amount not stated | | | |
| threo-1-(4-Hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol | Amount not stated | | | |
| <u>Neolignan glucosides</u> | | | | |
| (-)-(7R,8S)-7',8'-dihydroxy-dihydrodehydroconiferyl alcohol-9-O-β-D-glucopyranoside | | Amount not stated | | |
| (+)-(7S,8R)-7',8'-dihydroxy-dihydrodehydroconiferyl alcohol-9-O-β-D-glucopyranoside | | Amount not stated | | |
| 4-hydroxy-2-methoxyphenyl 1-O-[6-(hydrogen 3-hydroxy-3-methylpentanedioate)]-β-D-glucopyranoside | | Amount not stated | | |
| <u>Carotenoids</u> | | | | |
| α-carotene | Amount not stated | | | |
| β-carotene | Amount not stated | | | |
| chlorophyll | Amount not stated | | | |
| lutein | Amount not stated | | | |
| tocopherols A, B, C, and D | Amount not stated | | | |
| <u>Vitamins</u> | | | | |
| vitamin A | Amount not stated | | | |
| vitamin B1 | Amount not stated | | | |
| vitamin B2 | Amount not stated | | | |
| vitamin B3 | Amount not stated | | | |
| vitamin B5 | Amount not stated | | | |
| vitamin C | Amount not stated | | | |
| vitamin E | Amount not stated | | | |
| vitamin K | Amount not stated | | | |

Table 6. Allergens Not Present in Euterpe Oleracea Fruit Extract* or organic Euterpe Oleracea Juice (Freeze Dried).^{7,26}

| Allergen | CAS Number | European Union Limit (ppm) |
|--|-------------------|-----------------------------------|
| Alpha-IsoMethyl Ionone | 127-51-5 | < 0.02 |
| Amyl Cinnamal | 122-40-7 | < 0.10 |
| Anise Alcohol | 105-13-5 | < 0.00 |
| Benzyl Alcohol | 100-61-69 | < 0.01 |
| Benzyl Benzoate | 120-51-4 | < 0.09 |
| Benzyl Cinnamate | 103-41-3 | < 0.30 |
| Benzyl Salicylate | 118-58-1 | < 0.06 |
| Butylphenyl Methylpropional | 80-54-6 | < 0.50 |
| Cinnamal | 104-55-2 | < 0.01 |
| Cinnamyl Alcohol | 104-54-1 | < 0.30 |
| Citral | 5392-40-5 | <1.00 |
| Citronellol | 106-22-9 | < 1.00 |
| Coumarin | 91-64-5 | < 0.00 |
| Eugenol | 97-53-0 | < 0.70 |
| Farnesol | 4602-84-0 | < 0.04 |
| Geraniol | 106-24-1 | < 0.08 |
| Hexyl Cinnamal | 101-86-0 | < 0.40 |
| Hydroxycitronellal | 107-75-5 | < 1.00 |
| Hydroxymethylpentyl 3-Cyclohexene Carboxaldehyde | 31906-04-4 | < 0.00 |
| Isoeugenol | 97-54-1 | < 0.06 |
| Limonene | 5989-27-5 | < 0.05 |
| Linalool | 78-70-6 | < 0.00 |
| Methyl 2-Octynoate | 111-12-6 | < 0.20 |
| Evernia prunastri | 90028-68-5 | < 0.00 |
| Evernia furfuracea | 90028-67-4 | < 0.00 |
| Amylcinnamyl Alcohol | 101-85-9 | < 1.00 |

*Trade name material containing 98% Euterpe Oleracea Fruit Extract and 2% Lactobacillus Ferment

Table 7. Composition Data on *Euterpe oleracea* Seed.¹⁸

| Components | Amount (g/100 g [wwb])* |
|-------------------------------|--------------------------------|
| Moisture | 38.57 ± 0.07 |
| Protein | 3.95 ± 0.03 |
| Lipid | 1.04 ± 0.03 |
| Carbohydrates | 55.55 |
| Fatty Acid Composition | Amount (g/100 g [dwb]) |
| Saturated | 0.085 total |
| capric acid | 0.16 |
| myristic acid | 0.39 |
| palmitic acid | 0.28 |
| stearic acid | 0.02 |
| Monounsaturated | 0.46 total |
| oleic acid | 0.44 |
| palmitoleic acid | 0.02 |
| Polyunsaturated | 0.31 total |
| linoleic acid | 0.29 |
| α-linolenic | 0.02 |
| Other Fatty Acids | 0.08 |

*wwb = wet weight basis

Table 8. Heavy Metal/Mineral Constituents of *Euterpe edulis* Fruit and *Euterpe edulis* Pulp.¹²

| Constituents (mg/100 g, except ash [%]) | <i>Euterpe edulis</i> fruit | <i>Euterpe edulis</i> pulp |
|---|-----------------------------|----------------------------|
| Ash | 2.5% | 3.4% |
| Calcium | 63.8 ± 3.3 | 76.4 ± 2.9 |
| Copper | 0.3 ± 0 | 0.5 ± 0 |
| Iron | 1.67 ± 0.4 | 4.3 ± 0.6 |
| Magnesium | 32.1 ± 4.2 | 47.4 ± 4.2 |
| Manganese | 2.8 ± 0.9 | 3 ± 0 |
| Nickel | 0.5 ± 0 | 1 ± 0.1 |
| Phosphorus | 69.2 ± 12.2 | 41.2 ± 1.4 |
| Potassium | 361 ± 42 | 419.1 ± 26.9 |
| Sodium | 21.8 ± 2.5 | 17.3 ± 0.1 |
| Sulfur | 26.9 ± 2.9 | 35.4 ± 4.9 |
| Zinc | 0.6 ± 0.1 | 0.9 ± 0 |
| <u>Constituents (µg/100g)</u> | | |
| Cadmium | 1.1 ± 0.2 | 1.2 ± 0 |
| Cobalt | 13.6 ± 1.9 | 7.1 ± 0.2 |
| Selenium | 1 ± 0.1 | 0.5 ± 0.1 |

Table 9. List of Pesticides In Euterpe Oleracea Fruit Extract* That Do Not Exceed the EPA's Limits.²⁶

| Pesticide | EPA's Limit (mg/kg) |
|-----------------------------|---------------------|
| Alachlor | < 0.02 |
| Aldrin and Dieldrin | < 0.05 |
| Azinphos-methyl | < 1.00 |
| Bromopylate | < 3.00 |
| Chlordane (cis and trans) | < 0.05 |
| Chlorfenvinphos | < 0.50 |
| Chlorpyrifos | < 0.20 |
| Chlorpyrifos-methyl | < 0.10 |
| Cypermethrin | < 1.00 |
| DDT | < 1.00 |
| Deltamethrin | < 0.50 |
| Diazinon | < 0.50 |
| Dichlorvos | < 1.00 |
| Dithiocarbamates | < 2.00 |
| Endosulfan | < 3.00 |
| Endrin | < 0.05 |
| Enthion | < 2.00 |
| Fenitrothion | < 0.50 |
| Fenvalerate | < 1.50 |
| Fonofos | < 0.05 |
| Heptachlor | < 0.05 |
| Hexachlorobenzene | < 0.10 |
| Hexachlorocyclohexane | < 0.30 |
| Lindane | < 0.60 |
| Malathion | < 1.00 |
| Methidathion | < 0.20 |
| Parathion | < 0.50 |
| Parathion-methyl | < 0.20 |
| Permethrin | < 1.00 |
| Phosalone | < 0.10 |
| Piperonyl butoxide | < 3.00 |
| Pirimiphos-methyl | < 4.00 |
| Pyrethrins | < 3.00 |
| Quintozone (sum of 3 items) | < 1.00 |

*Trade name material containing 98% Euterpe Oleracea Fruit Extract and 2% Lactobacillus Ferment

Table 10. Frequency (2019) and Concentration of Use (2017) According to Duration and Type of Exposure.^{29,30}

| | Euterpe Oleracea Fruit Extract | | Euterpe Oleracea Juice | | Euterpe Oleracea Palm Heart Extract | |
|---------------------------------|---------------------------------------|-----------------------------|--|-------------|--|--------------------|
| | # of Uses | Conc. (%) | # of Uses | Conc. (%) | # of Uses | Conc. (%) |
| Totals***/Conc. Range | 430 | 0.0000001-0.38 | 1 | 0.04 | 3 | 0.001 |
| Duration of Use | | | | | | |
| <i>Leave-On</i> | 297 | 0.0000083-0.04 | 1 | 0.01-0.04 | 2 | 0.001 |
| <i>Rinse off</i> | 129 | 0.0000001-0.38 | NR | NR | 1 | 0.001 |
| <i>Diluted for (bath) Use</i> | 4 | 0.0005 | NR | NR | NR | 0.001 |
| Exposure Type | | | | | | |
| Eye Area | 3 | NR | NR | NR | NR | NR |
| Incidental Ingestion | 7 | 0.0000083-0.025 | 1 | NR | NR | NR |
| | | 0.001; | NR | NR | 1 | 0.001 |
| Incidental Inhalation - Sprays | 259 ^a | 0.00003- 0.001 ^a | | | | |
| Incidental Inhalation - Powders | NR | 0.0001-0.01 ^b | NR | 0.01 | NR | 0.001 ^b |
| Dermal Contact | 373 | 0.0000001-0.83 | NR | 0.01-0.04 | 3 | 0.001 |
| Deodorant (underarm) | NR | NR | NR | NR | NR | NR |
| Hair - Non-Coloring | 48 | 0.00000075-0.001 | NR | NR | NR | 0.001 |
| Hair-Coloring | 1 | 0.38 | NR | NR | NR | NR |
| Nail | NR | 0.04 | NR | NR | NR | NR |
| Mucous Membrane | 66 | 0.0000083-0.025 | 1 | NR | 1 | 0.001 |
| Baby Products | NR | NR | NR | NR | NR | NR |
| | Euterpe Oleracea Pulp Powder | | Hydrolyzed Euterpe Oleracea Fruit | | | |
| | # of Uses | Conc. (%) | # of Uses | Conc. (%) | | |
| Totals/Conc. Range | 11 | 0.003-3 | 1 | NR | | |
| Duration of Use | | | | | | |
| <i>Leave-On</i> | 9 | 0.033-3 | NR | NR | | |
| <i>Rinse off</i> | 2 | 0.003-0.6 | 1 | NR | | |
| <i>Diluted for (bath) Use</i> | NR | NR | NR | NR | | |
| Exposure Type | | | | | | |
| Eye Area | NR | NR | NR | NR | | |
| Incidental Ingestion | NR | 0.033-0.3 | NR | NR | | |
| Incidental Inhalation - Sprays | 5; 1 ^c | 0.015 | NR | NR | | |
| Incidental Inhalation - Powders | NR;1 ^c | 0.015-3 ^b | NR | NR | | |
| Dermal Contact | 9 | 0.015-3 | NR | NR | | |
| Deodorant (underarm) | NR | NR | NR | NR | | |
| Hair - Non-Coloring | 2 | 0.003-0.3 | NR | NR | | |
| Hair-Coloring | NR | NR | 1 | NR | | |
| Nail | NR | NR | NR | NR | | |
| Mucous Membrane | NR | 0.033-0.3 | NR | NR | | |
| Baby Products | NR | NR | NR | NR | | |

NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for Use Product Uses

^aIt is possible that these products may be sprays, but it is not specified whether the reported uses are sprays^bIt is possible that these products may be powders, but it is not specified whether the reported uses are powders^cNot specified that these products are sprays or powders, but it is possible the use can be as a spray or powder, therefore the information is captured in both categoriesNote: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum of total uses.

Table 11. Genotoxicity Studies on Palm Tree-derived ingredients and Related Components of *Euterpe edulis* and *Euterpe oleracea*.

| Ingredient | Strain/cell type | Assay | Dose/Concentration | Results |
|---|---|---|--|--|
| <i>In Vitro</i> | | | | |
| <i>Euterpe edulis</i> fruit pulp (9% in water) | <i>S. typhimurium</i> strains: TA97, TA98, TA100, and TA102 | Ames test, with and without metabolic activation. | Doses up to 500 µg/plate | Genotoxic in strain TA97 at doses ranging from 25 to 250 µg/plate without metabolic activation. Clear trend for geno-toxicity in strains TA98 and TA100 at doses ranging from 25 to 250 µg/plate without metabolic activation. Genotoxicity with metabolic activation was not reported for any strain tested. ⁹ |
| <i>Euterpe edulis</i> fruit pulp (9% in water) | RAW264.7 cells (mouse macrophage-like cells). | Micronucleus assay | Concentrations of 0.027, 0.108, 0.27, 0.54, and 1.08 mg per plate (0.27, 1.08, 2.7, 5.4, and 10.8 mg/ml, respectively) | Cytotoxic effect, suggested by a decrease in the mitotic index and survival rates, observed at all concentrations. When compared to negative control (sodium chloride), genotoxicity was significantly higher at all doses tested. ⁹ |
| <i>Euterpe edulis</i> fruit oil | Human peripheral blood lymphocytes and HepG2 (human hepatoma) cell line | Cytokinesis-block micronucleus assay | Concentrations up to 1000 µg/ml | Absence of significant DNA and chromosome damage in human lymphocytes and HepG2 cells. ⁴⁴ |
| <i>Euterpe edulis</i> fruit oil | Human peripheral blood lymphocytes and HepG2 (human hepatoma) cell line | Comet assay | Concentrations up to 1000 µg/ml in both assays | Absence of significant DNA and chromosome damage in human lymphocytes and HepG2 cells. ⁴⁴ |
| Euterpe Oleracea Fruit Extract trade name material (98% Euterpe Oleracea Fruit Extract and 2% lactobacillus ferment) in sterile distilled water | <i>S. typhimurium</i> strains TA98, TA100, TA1535, and TA1537 and <i>E. coli</i> strain WP2uvrA. | Ames test, with and without metabolic activation. | Doses up to 5000 µg/plate | Non-genotoxic, with and without metabolic activation in all bacterial strains tested. ⁴⁵ |
| <i>Euterpe oleracea</i> pulp-enriched fruit and berry juice (fortified with glucosamine) | <i>S. typhimurium</i> strains: TA98, TA100, TA1535, TA1537. <i>Eschericia coli</i> strain: WP2 (uvrA) | Ames test, with and without metabolic activation | Doses up to 5 µg/plate | Non-genotoxic, with and without meta-bolic activation. ⁴² |
| <i>Euterpe oleracea</i> pulp-enriched fruit and berry juice (fortified with glucosamine) | Chinese hamster lung cells | Chromosomal aberration assay, with and without metabolic activation (OECD TG 473) | Concentrations up to 5000 µg/ml | Structural chromosome aberrations not observed with or without metabolic activation. Non-clastogenic. ⁴² |
| <i>Euterpe oleracea</i> pulp-enriched fruit and berry juice (fortified with glucosamine) | L5178Y/TK+/- mouse lymphoma cells | L5178Y/TK+/- mouse lymphoma assay, with and without metabolic activation (OECD TG 476) | Concentrations up to 500 µg/ml | Non-genotoxic, with and without metabolic activation. ⁴² |
| <i>In Vivo</i> | | | | |
| <i>Euterpe edulis</i> fruit pulp extract (9% in water) | 4 groups of 5 male Wistar rats | Micronucleus assay (OECD TG 474). After dosing period, animals were killed and bone marrow smears prepared. Ratio of polychromatic to normochromatic erythrocytes (PCE/PCE + NCE x 100) calculated based on an evaluation of 2000 erythrocytes per slide (1000 per animal). | 4 groups received doses (by gavage) of 22.5, 45, 90, and 180 mg/kg, respectively, for 3 consecutive days. | Significant increase (P < 0.05) in frequency of micronucleated polychromatic erythrocytes in bone marrow, at daily doses of 45 to 180 mg/kg. ⁹ |

Table 11. Genotoxicity Studies on Palm Tree-derived ingredients and Related Components of *Euterpe edulis* and *Euterpe oleracea*.

| Ingredient | Strain/cell type | Assay | Dose/Concentration | Results |
|--|--|--|--|---|
| <i>Euterpe edulis</i> fruit pulp extract (9% in water) | 4 groups of 5 male Wistar rats | Micronucleus assay. Peripheral blood (500 µl) drawn from rats dosed according to preceding test procedure, and whole blood smears prepared. Frequency of lymphocytes with micronuclei per total lymphocytes determined using sample sized of 1000 lymphocytes per animal | Doses same as in preceding test | No statistically significant positive results for micronucleus frequency observed. Dose-related increase in mitotic index ($P > 0.05$) detected (at 90 to 180 mg/kg), suggesting induction of proliferation alongside acceptable survival rates of $>80\%$. ⁹ |
| <i>Euterpe edulis</i> fruit pulp extract (9% in water) | 4 groups of 5 male Wistar rats | Comet assay (Single cell gel electro-phoresis (SCGE) test). Blood drawn from rats dosed according to same test procedure. Slides prepared and extent and distribution of DNA damage evaluated by examining at least 200 randomly selected and non-overlapping cells. | Same doses | The SCGE score did not indicate significant DNA lesions, such as single or double breakages. ⁹ |
| <i>Euterpe edulis</i> fruit pulp (9%) | 5 human subjects | Comet assay. Subjects ingested single dose on 5 consecutive days. Peripheral blood drawn and slides prepared. Extent and distribution of DNA damage evaluated by examining at least 200 randomly selected and non-overlapping cells. | Single dose of 300 ml | SCGE score did not indicate significant DNA lesions, such as single or double breakages. No statistically significant positive genotoxicity response identified. ⁹ |
| <i>Euterpe oleracea</i> pulp-enriched fruit and berry juice (fortified with glucosamine) in saline | Groups of 16 BALB/c mice (8 males, 8 females) and 12 BALB/c mice (6 males, 6 females) | Micronucleus assay. Group divided into mice dosed orally or intraperitoneally daily for 7 days. Animals then killed, and bone marrow analyzed for micronuclei in polychromatic erythrocytes. Cytogenetic analysis performed by direct method of rinsing marrow of the femur and tibia. | Daily doses of 100µg/150µl | No increase in frequency of micronuclei in bone marrow polychromatic erythrocytes. ⁴² |
| <i>Euterpe oleracea</i> fruit pulp | Bone marrow cells and peripheral blood polychromatic erythrocytes (male Swiss albino mice) | Micronucleus assay. Assay performed using bone marrow cells and peripheral blood polychromatic erythrocytes. Number of micronucleated polychromatic erythrocytes in 2000 polychromatic erythrocytes per animal recorded. | Single (acute) oral doses (gavage) of 3.33 g/kg, 10 g/kg, and 16.67 g/kg were administered to groups of male Swiss albino mice (number per dose not stated). | No statistically significant differences ($p > 0.05$), between the negative control and groups treated with doses of the test substance, in the frequency of micronucleated polychromatic erythrocytes in bone marrow or blood. No genotoxic effects in this assay. ⁴⁶ |
| <i>Euterpe oleracea</i> fruit pulp | Bone marrow cells and peripheral blood polychromatic erythrocytes (male Swiss albino mice) | Micronucleus assay. Assay performed using bone marrow cells and peripheral blood polychromatic erythrocytes. Number of micronucleated polychromatic erythrocytes in 2000 polychromatic erythrocytes per animal recorded. | Oral doses (gavage) of 3.33 g/kg, 10 g/kg, and 16.67 g/kg administered to groups of male Swiss albino mice (number per dose not stated) daily for 14 consecutive days. | No statistically significant differences ($p > 0.05$), between the negative control and groups treated with doses of the test substance, in the frequency of micronucleated polychromatic erythrocytes in bone marrow or blood. No genotoxic effects in this assay. ⁴⁶ |

Table 11. Genotoxicity Studies on Palm Tree-derived ingredients and Related Components of *Euterpe edulis* and *Euterpe oleracea*.

| Ingredient | Strain/cell type | Assay | Dose/Concentration | Results |
|------------------------------------|--|---|--|--|
| <i>Euterpe oleracea</i> fruit pulp | Bone marrow cells and peripheral blood polychromatic erythrocytes (male Swiss albino mice) | Comet assay (DNA damage assay). Peripheral blood collected from mice and cellular suspensions prepared. Liver and kidney cells also collected (100 cells in each tissue visually scored) | Swiss albino mice dosed with test substance (same doses in acute and subacute dosing procedures in both micronucleus assays immediately above) | Absence of increased DNA damage (in peripheral blood, liver, and kidney cells) in mice dosed orally (all doses). Non-genotoxic. ⁴⁶ |
| <i>Euterpe oleracea</i> fruit oil | Groups of 6 Wistar rats | Comet assay. Doses administered by gavage (at 24-h intervals) for 14 consecutive days. At 24 h after last dose, peripheral blood from tail collected. Animals were killed and liver, bone marrow (from femur), and testicle cells also collected. DNA damage evaluated by examining at least 100 randomly selected and non-overlapping cells (50 cells per coded slide) per animal in blind analysis. | Doses of 30, 100, or 300 mg/kg in 1% Tween 80 | No significant induction of DNA strand breaks observed in tissues from any dose group. In the few nucleoids with DNA damage (also observed with vehicle control), damage was considered minor. ⁴³ |
| <i>Euterpe oleracea</i> fruit oil | Groups of 6 Wistar rats | Micronucleus assay. Doses and dosing procedure used in preceding test. Slides of bone marrow (femur) smears prepared and 2000 polychromatic Erythrocytes (PCE) per animal scored to determine clastogenic and/or aneugenic property of test substance. Clastogenic/aneugenic damage investigated by analyzing micronuclei formation in bone marrow PCE. | Doses of 30, 100, or 300 mg/kg in 1% Tween 80 | No significant increase in the micronucleus frequency in bone marrow cells, as well as no significant difference/increase in the PCE/NCE ratio ($P < 0.05$). ⁴³ |

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2019 FDA VCRP Data**Euterpe Edulis Fruit Extract - No Data****Euterpe Edulis Juice Extract - No Data****Euterpe Oleracea Fruit Extract**

| | |
|---|------------|
| 02A - Bath Oils, Tablets, and Salts | 1 |
| 02B - Bubble Baths | 2 |
| 02D - Other Bath Preparations | 1 |
| 03D - Eye Lotion | 1 |
| 03F - Mascara | 1 |
| 03G - Other Eye Makeup Preparations | 1 |
| 04E - Other Fragrance Preparation | 10 |
| 05A - Hair Conditioner | 18 |
| 05E - Rinses (non-coloring) | 1 |
| 05F - Shampoos (non-coloring) | 21 |
| 05G - Tonics, Dressings, and Other Hair Grooming Aids | 5 |
| 05I - Other Hair Preparations | 3 |
| 06H - Other Hair Coloring Preparation | 1 |
| 07C - Foundations | 2 |
| 07E - Lipstick | 7 |
| 07F - Makeup Bases | 1 |
| 07I - Other Makeup Preparations | 4 |
| 10A - Bath Soaps and Detergents | 44 |
| 10E - Other Personal Cleanliness Products | 11 |
| 12A - Cleansing | 27 |
| 12C - Face and Neck (exc shave) | 42 |
| 12D - Body and Hand (exc shave) | 19 |
| 12F - Moisturizing | 176 |
| 12G - Night | 2 |
| 12H - Paste Masks (mud packs) | 6 |
| 12I - Skin Fresheners | 2 |
| 12J - Other Skin Care Preps | 18 |
| 13A - Suntan Gels, Creams, and Liquids | 1 |
| 13B - Indoor Tanning Preparations | 1 |
| 13C - Other Suntan Preparations | 1 |
| Total | 430 |

Euterpe Oleracea Juice

| | |
|----------------|----------|
| 07E - Lipstick | 1 |
| Total | 1 |

Euterpe Oleracea Palm Heart Extract

| | |
|-----------------------------------|----------|
| 04E - Other Fragrance Preparation | 1 |
| 10A - Bath Soaps and Detergents | 1 |
| 12D - Body and Hand (exc shave) | 1 |
| Total | 3 |

| | |
|-------------------------------------|-----------|
| Euterpe Oleracea Pulp Powder | |
| 05A - Hair Conditioner | 1 |
| 05F - Shampoos (non-coloring) | 1 |
| 07I - Other Makeup Preparations | 1 |
| 12C - Face and Neck (exc shave) | 1 |
| 12D - Body and Hand (exc shave) | 4 |
| 12F - Moisturizing | 1 |
| 12J - Other Skin Care Preps | 2 |
| Total | 11 |

Euterpe Oleracea Seed Powder - No Data

| | |
|--|----------|
| Hydrolyzed Euterpe Oleracea Fruit | |
| 06F - Hair Lighteners with Color | 1 |
| Total | 1 |



Memorandum

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

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

DATE: December 13, 2017

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

Concentration of Use by FDA Product Categories – Palm-Derived Ingredients*

Euterpe Oleracea Fruit Extract
 Euterpe Edulis Fruit Extract
 Euterpe Edulis Juice Extract
 Euterpe Oleracea Juice

Euterpe Oleracea Palm Heart Extract
 Euterpe Oleracea Pulp Powder
 Euterpe Oleracea Seed Powder
 Hydrolyzed Euterpe Oleracea Fruit

| Ingredient | Product Category | Maximum Concentration of Use |
|-------------------------------------|---|-------------------------------------|
| Euterpe Oleracea Fruit Extract | Other bath preparations | 0.0005% |
| Euterpe Oleracea Fruit Extract | Hair conditioners | 0.00025% |
| Euterpe Oleracea Fruit Extract | Hair sprays Pump spray | 0.001% |
| Euterpe Oleracea Fruit Extract | Shampoos (noncoloring) | 0.00000075-0.00023% |
| Euterpe Oleracea Fruit Extract | Tonics, dressings and other hair grooming aids | 0.00003% |
| Euterpe Oleracea Fruit Extract | Hair shampoos (coloring) | 0.075% |
| Euterpe Oleracea Fruit Extract | Hair bleaches | 0.0001% |
| Euterpe Oleracea Fruit Extract | Other hair coloring preparations | 0.38% |
| Euterpe Oleracea Fruit Extract | Foundations | 0.04% |
| Euterpe Oleracea Fruit Extract | Lipstick | 0.0000083-0.025% |
| Euterpe Oleracea Fruit Extract | Cuticle softeners | 0.04% |
| Euterpe Oleracea Fruit Extract | Bath soaps and detergents | 0.0025% |
| Euterpe Oleracea Fruit Extract | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.0000001-0.083% |
| Euterpe Oleracea Fruit Extract | Face and neck products Not spray | 0.001% |
| Euterpe Oleracea Fruit Extract | Body and hand products Not spray | 0.0001-0.01% |
| Euterpe Oleracea Fruit Extract | Moisturizing products Not spray | 0.0001% |
| Euterpe Oleracea Fruit Extract | Skin fresheners | 0.001% |
| Euterpe Oleracea Fruit Extract | Other skin care preparations | 0.001% |
| Euterpe Oleracea Juice | Face powders | 0.01% |
| Euterpe Oleracea Juice | Foundations | 0.04% |
| Euterpe Oleracea Juice | Makeup bases | 0.01% |
| Euterpe Oleracea Palm Heart Extract | Other bath preparations | 0.001% |
| Euterpe Oleracea Palm Heart Extract | Colognes and toilet waters | 0.001% |
| Euterpe Oleracea Palm Heart Extract | Hair conditioners | 0.001% |
| Euterpe Oleracea Palm Heart Extract | Bath soaps and detergents | 0.001% |
| Euterpe Oleracea Palm Heart | Body and hand products | |

| | | |
|-------------------------------------|---|-------------|
| Extract | Not spray | 0.001% |
| Euterpe Oleracea Palm Heart Extract | Moisturizing products Not spray | 0.001% |
| Euterpe Oleracea Pulp Powder | Colognes and toilet waters | 0.015% |
| Euterpe Oleracea Pulp Powder | Hair conditioners | 0.3% |
| Euterpe Oleracea Pulp Powder | Hair straighteners | 0.003% |
| Euterpe Oleracea Pulp Powder | Shampoos (noncoloring) | 0.3% |
| Euterpe Oleracea Pulp Powder | Lipstick | 0.033-0.3%% |
| Euterpe Oleracea Pulp Powder | Bath soaps and detergents | 0.3% |
| Euterpe Oleracea Pulp Powder | Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.5% |
| Euterpe Oleracea Pulp Powder | Face and neck products Not spray | 3% |
| Euterpe Oleracea Pulp Powder | Body and hand products Not spray | 0.015% |
| Euterpe Oleracea Pulp Powder | Moisturizing products Not spray | 0.6% |
| Euterpe Oleracea Pulp Powder | Paste masks and mud packs | 0.6% |

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

Information collected in 2017
Table prepared December 13, 2017



Memorandum

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

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

DATE: February 5, 2019

SUBJECT: Euterpe Oleracea Juice and Euterpe Oleracea Fruit Extract

Arbor Organic Technologies. 2018. Compositional breakdown: Organic acai juice FD (Euterpe Oleracea Juice).

Arbor Organic Technologies. 2011. Manufacturing flow chart - organic acai juice FD (Euterpe Oleracea Juice).

Active Concepts. 2019. Compositional breakdown: Phyto-Biotics Acai® (Euterpe Oleracea Fruit Extract).

Active Concepts. 2017. Product specification: Phyto-Biotics Acai® (Euterpe Oleracea Fruit Extract).

Active Concepts. 2014. Manufacturing flow chart: Phyto-Biotics Acai® (Euterpe Oleracea Fruit Extract).

Active Concepts. 2017. Dermal and ocular irritation tests: Phyto-Biotics Acai (Euterpe Oleracea Fruit Extract).

Active Concepts. 2016. OECD TG 442C: *In chemico* skin sensitization (Phyto-Biotics Acai® - Euterpe Oleracea Fruit Extract).

Active Concepts. 2016. OECD TG 442D: *In vitro* skin sensitization (Phyto-Biotics Acai® -Euterpe Oleracea Fruit Extract).

Active Concepts. 2016. Bacterial reverse mutation test: Phyto-Biotics Acai® (Euterpe Oleracea Fruit Extract).

Active Concepts. 2014. Cellular viability assay analysis: Phyto-Biotics Acai® (Euterpe Oleracea Fruit Extract).



Arbor Organic Technologies



International
Certification
Services, Inc.

www.arbororganictechnologies.com

Organic Acai Juice FD Code: A60002

Compositional Breakdown:

| Ingredient | % |
|------------------------|--------|
| Euterpe Oleracea Juice | 100.00 |

Reconstitution Instructions: Fill 25 grams of powder up to 100 mL with water.

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Arbor Organic Technologies



www.arbororganictechnologies.com

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

| ALLERGENS listed in Annex III of EU Cosmetic Regulation(EC) No. 1223/2009 amending EU Directive 2003/15/EC | | |
|--|------------|-------------|
| INCI NAME | CAS NUMBER | Limit (ppm) |
| Alpha-IsoMethyl Ionone | 127-51-5 | < 0.02 |
| Amyl Cinnamal | 122-40-7 | < 0.10 |
| Anise Alcohol | 105-13-5 | < 0.00 |
| Benzyl Alcohol | 100-51-69 | < 0.01 |
| Benzyl Benzoate | 120-51-4 | < 0.09 |
| Benzyl Cinnamate | 103-41-3 | < 0.30 |
| Benzyl Salicylate | 118-58-1 | < 0.06 |
| Butylphenyl Methylpropional | 80-54-6 | < 0.50 |
| Cinnamal | 104-55-2 | < 0.01 |
| Cinnamyl Alcohol | 104-54-1 | < 0.30 |
| Citral | 5392-40-5 | < 1.00 |
| Citronellol | 106-22-9 | < 1.00 |
| Coumarin | 91-64-5 | < 0.00 |
| Eugenol | 97-53-0 | < 0.70 |
| Farnesol | 4602-84-0 | < 0.04 |
| Geraniol | 106-24-1 | < 0.08 |
| Hexyl Cinnamal | 101-86-0 | < 0.40 |
| Hydroxycitronellal | 107-75-5 | < 1.00 |
| Hydroxymethylpentyl 3-Cyclohexene carboxaldehyde | 31906-04-4 | < 0.00 |
| Isoeugenol | 97-54-1 | < 0.06 |
| Limonene | 5989-27-5 | < 0.05 |
| Linalool | 78-70-6 | < 0.00 |
| Methyl 2 Octynoate | 111-12-6 | < 0.20 |
| Evernia prunastri | 90028-68-5 | < 0.00 |
| Evernia furfuracea | 90028-67-4 | < 0.00 |
| Amylcinnamyl Alcohol | 101-85-9 | < 1.00 |

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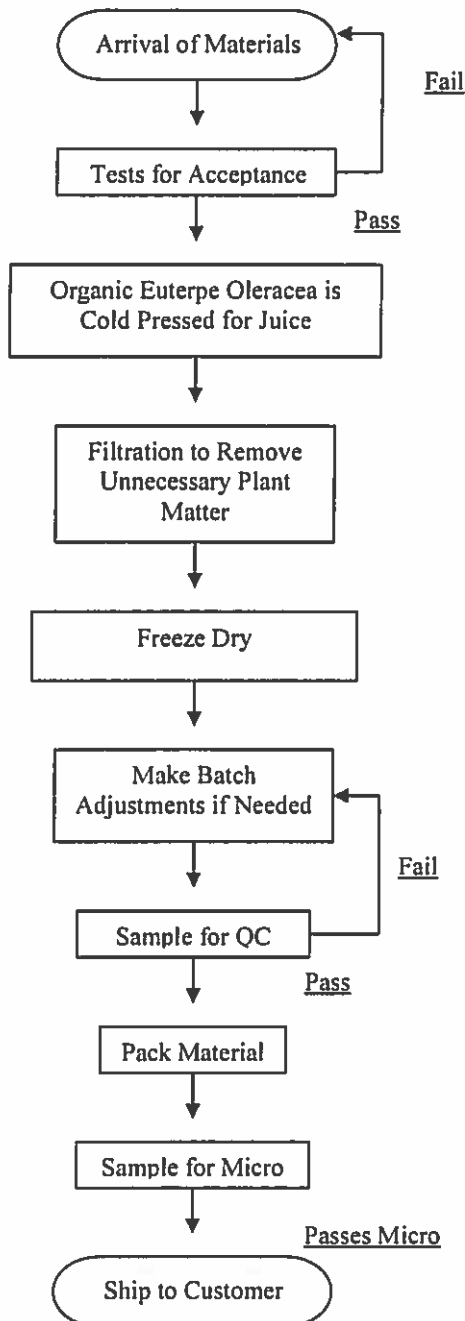
Arbor Organic Technologies



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MANUFACTURING FLOW CHART-ORGANIC ACAI JUICE FD-A60002



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Compositional Breakdown

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Phyto-Biotics Acai® Code: 16587

Compositional Breakdown:

| Ingredient | % |
|--------------------------------|-------|
| Euterpe Oleracea Fruit Extract | 98.00 |
| Lactobacillus Ferment | 2.00 |

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

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This is to certify that Phyto-Biotics Acai® does not contain pesticide levels exceeding the following (Reverse Phase High Performance Liquid Chromatography-Mass Spectrometer Coupled):

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

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

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Product Specification

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Product Name: Phyto-Biotics Acai®
Code Number: 16587
CAS #'s: 999999-99-4
EINECS #'s: 310-127-6
INCI Name: Euterpe Oleracea Fruit Extract
Status: Approved

| Specification | Parameter |
|------------------------|-------------------------------|
| Appearance | Clear to Slightly Hazy Liquid |
| Gardner Color | 9 Maximum |
| Odor | Characteristic |
| pH | 4.5 – 6.5 |
| Ferulic Acid Content | 4.0 – 5.0% |
| Heavy Metals | < 20 ppm |
| Lead | < 10 ppm |
| Arsenic | < 2 ppm |
| Cadmium | < 1 ppm |
| Microbial Content | < 100 CFU/g; No pathogens |
| Yeast & Mold | < 100 CFU/g |
| Gram Negative Bacteria | 0 CFU/g |

May Sediment upon Standing; Mix Well Prior to Use

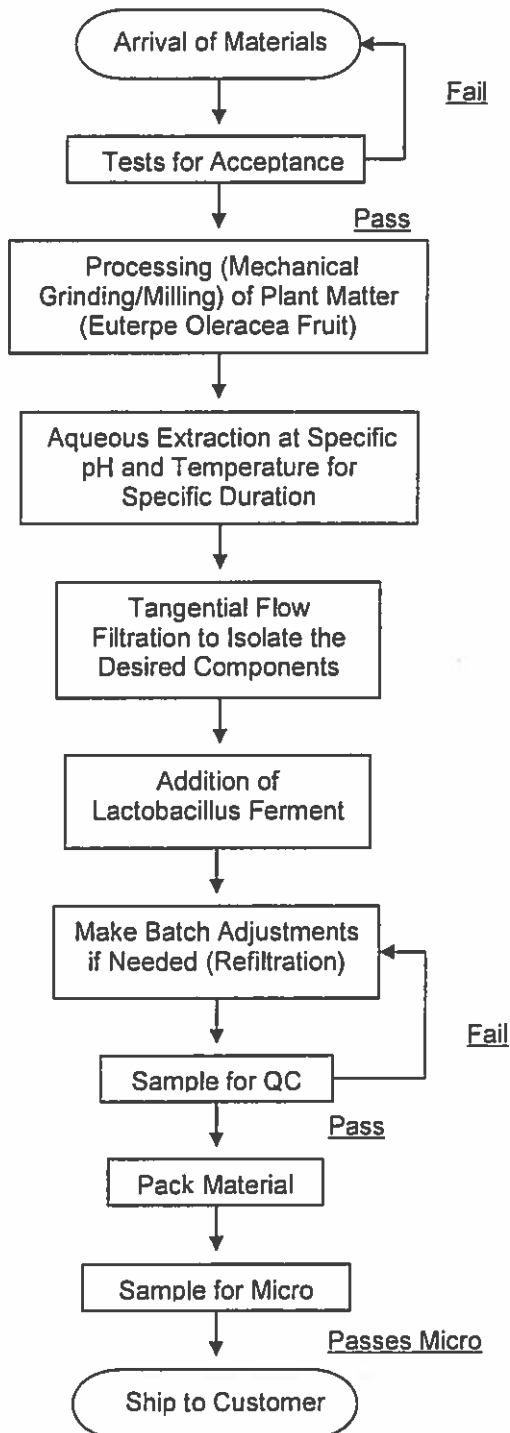
Product should be stored at room temperature. Excess heat may cause Instability.

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16587-Phyto-Biotics Acai®- Manufacturing Flow Chart

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Dermal and Ocular Irritation Tests

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Sample: Phyto-Biotics Acai

Code: 16587

CAS #: 999999-99-4

Test Request Form/Submission #: 443

Lot #: NC121205-A

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

Study Director: Maureen Danaher

Principle Investigator: Jennifer Goodman

Test Performed:

In Vitro EpiDerm™ Dermal Irritation Test (EPI-200-SIT)

EpiOcular™ Eye Irritation Test (OCL-200-EIT)

SUMMARY

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

The product was tested according to the manufacture's protocol. The test article solution was found to be **non-irritating**. Reconstructed human epidermis and cornea epithelial model were incubated in growth media overnight to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substances were applied to the tissue inserts and incubated for 60 minutes for liquid and solid substances in the EpiDerm™ assay and 30 minutes for liquid substances and 90 minutes for solid substances in the EpiOcular™ assay at 37°C, 5% CO₂, and 95% relative humidity (RH). Tissue inserts were thoroughly washed and transferred to fresh plates with growth media. After post substance dosing incubation is complete, the cell viability test begins. Cell viability is measured by dehydrogenase conversion of MTT [(3-4,5-dimethyl thiazole 2-yl)], present in the cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The irritation potential of the test chemical is dictated by the reduction in tissue viability of exposed tissues compared to the negative control.

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

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Dermal and Ocular Irritation Tests

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I. Introduction

A. Purpose

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

II. Materials

- A. Incubation Conditions:** 37°C at 5% CO₂ and 95% relative humidity
- B. Equipment:** Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes
- C. Media/Buffers:** DMEM based medium; DPBS; sterile deionized H₂O
- D. Preparation:** Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
- E. Tissue Culture Plates:** Falcon flat bottom 96-well, 24-well, 12-well, and 6-well tissue culture plates
- F. Reagents:** MTT (1.0mg/mL); Extraction Solution (Isopropanol); SDS (5%); Methyl Acetate
- G. Other:** Nylon Mesh Circles (EPI-MESH); Cotton tip swabs; 1mL tuberculin syringes; Ted Pella micro-spatula; 220mL specimen containers; sterile disposable pipette tips; Parafilm

III. Test Assay

A. Test System

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

B. Negative Control

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

C. Positive Control

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

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Dermal and Ocular Irritation Tests

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D. Data Interpretation Procedure

a. EpiDerm™

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

b. EpiOcular™

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

IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Concepts, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO₂ and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO₂ and 95% relative humidity for an additional 18 to 21 hours.

B. Test Substance Exposure

a. EpiDerm™

30µL (liquid) or 25mg (solid) of the undiluted test substance is applied to 3 tissue inserts and allowed to incubate for 60 minutes in a humidified incubator (37°C, 5% CO₂, 95% RH).

b. EpiOcular™

Each tissue is dosed with 20µL DPBS prior to test substance dosing. 50µL (liquid) or 50mg (solid) of the undiluted test substance is applied to 2 tissue inserts and allowed to incubate for 90 minutes in a humidified incubator (37°C, 5% CO₂, 95% RH).

C. Tissue Washing and Post Incubation

a. EpiDerm™

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

b. EpiOcular™

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

D. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO₂, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD₅₇₀) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

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B. Positive Control

a. EpiDerm™

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

b. EpiOcular™

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

C. Standard Deviation

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

VI. Results

A. Tissue Characteristics

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

B. Tissue Viability Assay

The results are summarized in Figure 1. In no case was the tissue viability $\leq 50\%$ for EpiDerm™ or $\leq 60\%$ for EpiOcular™ in the presence of the test substance. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited substantial loss of tissue viability and cell death.

C. Test Validity

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

VII. Conclusion

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

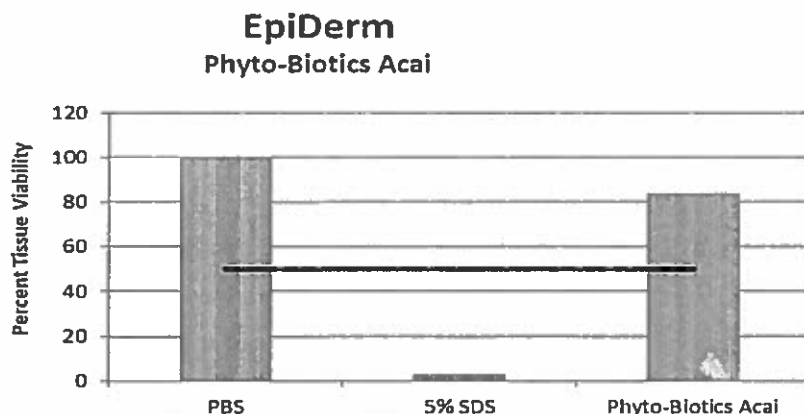


Figure 1: EpiDerm tissue viability

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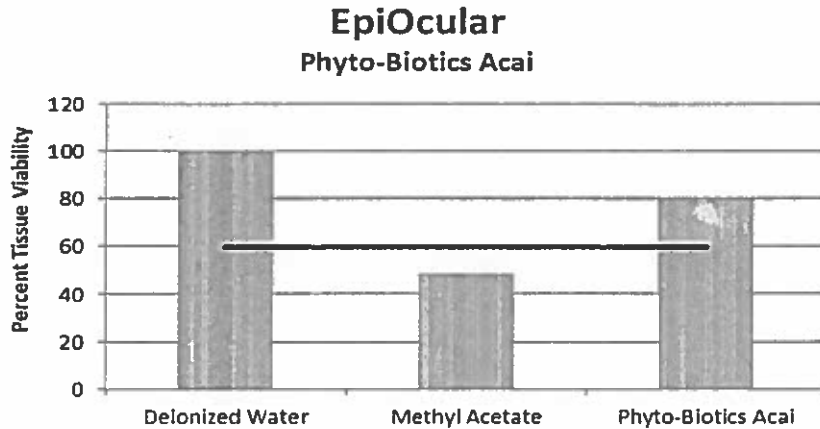


Figure 2: EpiOcular tissue viability

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OECD TG 442C: *In Chemico* Skin Sensitization

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Tradename: Phyto-Biotics Acai®

Code: 16587

CAS #: 999999-99-4

Test Request Form #: 2257

Lot #: NC160523-D

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

Study Director: Maureen Danaher

Principle Investigator: Jennifer Goodman

Test Performed:

OECD TG 442C: *In Chemico* Skin Sensitization

Direct Peptide Reactivity Assay (DPRA)

Introduction

A skin sensitizer is a substance that will lead to an allergic response following skin contact¹. Haptenation is the covalent binding of a hapten, or low-molecular weight substance or chemical, to proteins in the skin. This is considered the prominent mechanism which defines a chemical as a sensitizer. Haptenation is described as a "molecular initiating event" in the OECD Adverse Outcome Pathway (AOP) for skin sensitization which summarizes the key events known to be involved in chemically-induced allergic contact dermatitis². The direct peptide reactivity assay (DPRA) is designed to mimic the covalent binding of electrophilic chemicals to nucleophilic centers in skin proteins by quantifying the reactivity of chemicals towards the model synthetic peptides containing cysteine and lysine. The DPRA is able to distinguish sensitizers from non-sensitizer with 82% accuracy (sensitivity of 76%; specificity of 92%)³.

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

Assay Principle

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

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

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OECD TG 442C: In Chemico Skin Sensitization

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Materials

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

Methods

Solution Preparation:

- 0.667mM Cysteine Peptide in 100mM Phosphate Buffer (pH 7.5)
- 0.667mM Lysine Peptide in 100mM Ammonium Acetate Buffer (pH 10.2)
- 100mM Cinnamic Aldehyde in Acetonitrile
- 100mM* Phyto-Biotics Acai® in Acetonitrile

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

Reference Controls:

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

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

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

| 1:10 Ratio, Cysteine Peptide 0.5mM Peptide, 5mM Test Chemical | 1:50 Ratio, Lysine Peptide 0.5mM Peptide, 25mM Test Chemical |
|--|---|
| <ul style="list-style-type: none"> • 750µL Cysteine Peptide Solution (or 100mM Phosphate Buffer, pH 7.5, for Co-Elution Controls) • 200µL Acetonitrile • 50µL Test Chemical Solution (or Acetonitrile for Reference Controls) | <ul style="list-style-type: none"> • 750µL Lysine Peptide Solution (or 100mM Ammonium Acetate Buffer, pH 10.2, for Co-Elution Controls) • 250µL Test Chemical Solution (or Acetonitrile for Reference Controls) |

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Calibration Curve:

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

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

HPLC Analysis:

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

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

Data and Reporting

Acceptance Criteria:

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

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OECD TG 442C: In Chemico Skin Sensitization

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Prediction Model:

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

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

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

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

| Cysteine 1:10/Lysine 1:50 Prediction Model | | |
|--|--------------------|----------------|
| Mean of Cysteine and Lysine % Depletion | Reactivity Class | Prediction |
| 3.29 | Minimal Reactivity | Non-sensitizer |
| 3.23 | Minimal Reactivity | Non-sensitizer |
| 3.25 | Minimal Reactivity | Non-sensitizer |

| Cysteine 1:10 Prediction Model | | |
|---|--------------------|----------------|
| Mean of Cysteine and Lysine % Depletion | Reactivity Class | Prediction |
| 3.16 | Minimal Reactivity | Non-sensitizer |
| 3.10 | Minimal Reactivity | Non-sensitizer |
| 3.18 | Minimal Reactivity | Non-sensitizer |

Results and Discussion

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

Percent peptide depletion is determined by the following equation:

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

Based on HPLC-UV analysis of Phyto-Biotics Acai® (16587) we can determine this product is not classified as a sensitizer and is not predicted to cause allergic contact dermatitis. The Mean Percent Depletion of Cysteine and Lysine was 3.20% causing minimal reactivity in the assay giving us the prediction of a non-sensitizer.

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OECD TG 442D: *In Vitro* Skin Sensitization

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Tradename: Phyto-Biotics Acai®

Code: 16587

CAS #: 999999-99-4

Test Request Form #: 2112

Lot #: NC160406-F

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

Study Director: Maureen Danaher

Principle Investigator: Jennifer Goodman

Test Performed:

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

Introduction

Skin sensitization refers to an allergic response following skin contact with the tested chemical, as defined by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals¹. Substances are classified as skin sensitizers if there is evidence in humans that the substance can lead to sensitization by skin contact or positive results from appropriate tests, both *in vivo* and *in vitro*. Utilization of the KeratinoSens™ cell line allows for valid *in vitro* testing for skin sensitization.

This assay was conducted to determine skin sensitization potential of Phyto-Biotics Acai® in accordance with the UN GHS.

Assay Principle

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

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

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

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OECD TG 442D: *In Vitro* Skin Sensitization

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Materials

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

Methods

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

24 hour post KeratinoSens™ seeding, the culture media was removed and replaced with fresh media containing 10% FBS without G418 geneticin. 50 µL of the above described test concentrations was added to the appropriate wells. The treated plates were then incubated for 48 hours at 37°C in the presence of 5% CO₂ and 95% relative humidity. After treatment incubation was complete the media was removed and the wells were washed with PBS 3 times.

One of the four plates was used for a cytotoxicity endpoint, where MTT was added to the wells and incubated for 4 hours at 37°C in the presence of 5% CO₂. SLS was then added to the wells and incubated overnight at room temperature. A spectrometer measured the absorbance at 570 nm. The absorbance values (optical density) were then used to determine the viability of each well by comparing the optical density of each test material treated well to that of the solvent control wells to determine the IC₅₀ and IC₃₀ values.

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

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Data and Reporting

Acceptance Criteria:

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

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

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

Results

| Compound | Classification | EC _{1.5} (μ M) | IC ₅₀ | I _{max} |
|---------------------|----------------|------------------------------|------------------|------------------|
| Cinnamic aldehyde | Sensitizer | 19 | 289.19 μ M | 31.43 |
| DMSO | Non-Sensitizer | No Induction | 243.24 μ M | 0.17 |
| Phyto-Biotics Acai® | Non-Sensitizer | No Induction | > 1000 μ M | 0.36 |

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



OECD TG 442D: *In Vitro* Skin Sensitization

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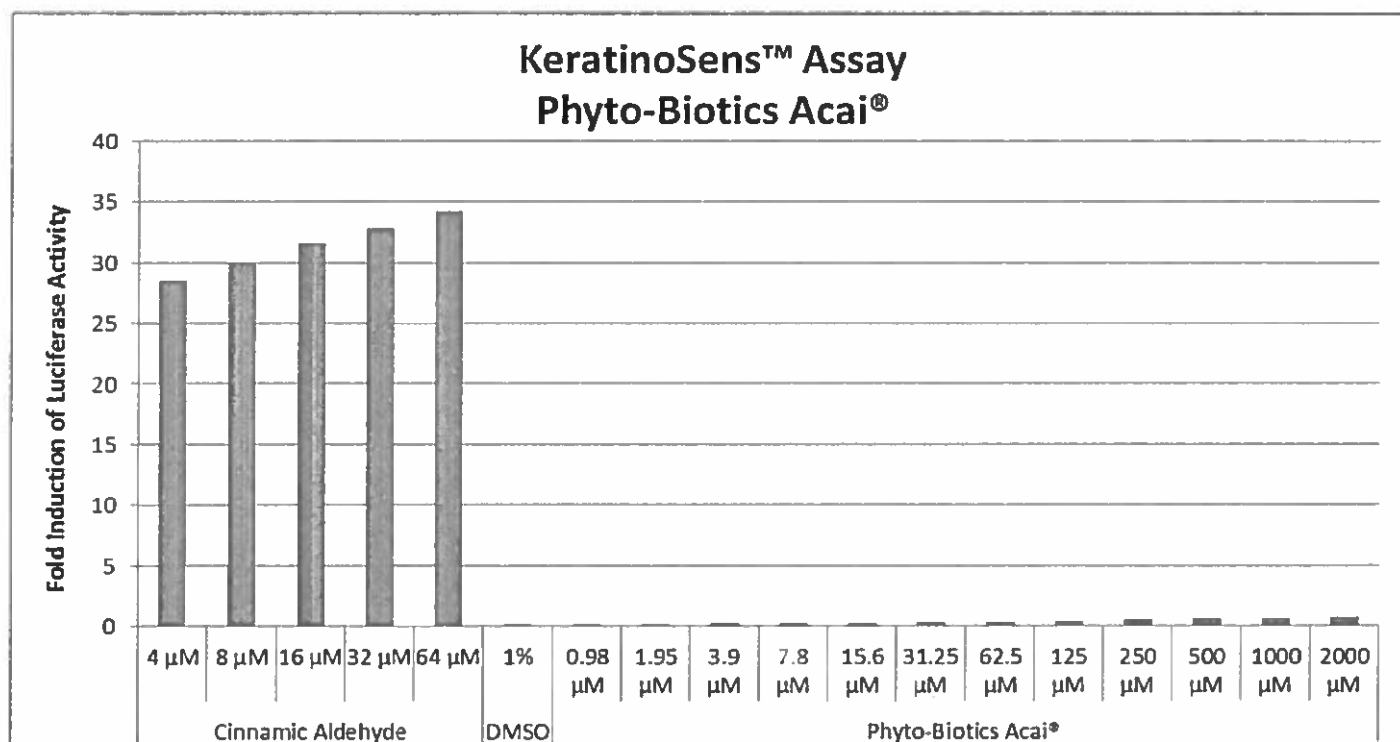


Figure 1: Fold Induction of Luciferase

Discussion

As shown in the results, **Phyto-Biotics Acai® (16587)** was not predicted to be a skin sensitizer based on the KeratinoSens™ ARE-Nrf2 Luciferase Test Method as there was not a significant increase in luciferase expression. It can be concluded that **Phyto-Biotics Acai®** can be safely used in cosmetics and personal care products at typical use levels.

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Bacterial Reverse Mutation Test

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Test Article: Phyto-Biotics Acai®
Code Number: 16587
CAS #: 999999-99-4

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

Study Director: Maureen Danaher
Principle Investigator: Monica Beltran

Test Performed:
Genotoxicity: Bacterial Reverse Mutation Test

Reference:
OECD471/ISO10993.Part 3

Test Request Number: 2041

SUMMARY

A *Salmonella typhimurium*/*Escherichia coli* reverse mutation standard plate incorporation study described by Ames *et al.* (1975) was conducted to evaluate whether a test article solution Phyto-Biotics Acai® would cause mutagenic changes in the average number of revertants for histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and tryptophan-dependent *Escherichia coli* strain WP2uvrA in the presence and absence of Aroclor-induced rat liver S9. This study was conducted to satisfy, in part, the Genotoxicity requirement of the International Organization for Standardization: Biological Evaluation of Medical Devices, Part 3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity.

The stock test article was tested at eight doses levels along with appropriate vehicle control and positive controls with overnight cultures of tester strains. The test article solution was found to be noninhibitory to growth of tester strain TA98, TA100, TA1537, TA1535 and WP2uvrA after Sport Inhibition Screen.

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

Under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* tester strain WP2uvrA. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

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Bacterial Reverse Mutation Test

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I. Introduction

A. Purpose

A *Salmonella typhimurium*/*Escherichia coli* reverse mutation standard plate incorporation study was conducted to evaluate whether a test article solution would cause mutagenic changes in the average number of revertants for *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2uvrA in the presence and absences of the S9 metabolic activation. Bacterial reverse mutation tests have been widely used as rapid screening procedures for the determination of mutagenic and potential carcinogenic hazards.

II. Materials

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

III. Test System

A. Test System

Each *Salmonella typhimurium* and *Escherichia coli* tester strain contains a specific deep rough mutation (*rfa*), the deletion of *uvrB* gene and the deletion in the *uvrA* gene that increase their ability to detect mutagens, respectively. These genetically altered *Salmonella typhimurium* strains (TA98, TA100, TA1537 and TA1535) and *Escherichia coli* strain (WP2uvrA) cannot grow in the absence of histidine and tryptophan, respectively. When placed in a histidine-tryptophan free medium, only those cells which mutate spontaneously back to their wild type states are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

| <u>Tester strain</u> | <u>Mutations/Genotypic Relevance</u> |
|----------------------|--|
| TA98 | hisD3052, Dgal chlD bio <i>uvrB rfa</i> pKM101 |
| TA100 | hisG46, Dgal chlD BIO <i>uvrB rfa</i> pKM101 |
| TA1537 | hisC3076, <i>rfa</i> , Dgal chlD bio <i>uvrB</i> |
| TA 1535 | hisG46, Dgal chlD bio <i>uvrB rfa</i> |
| WP2uvrA | trpE, <i>uvrA</i> |

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

B. Metabolic Activation

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

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C. Preparation of Tester strains

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

D. Negative Control

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

E. Positive Control

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

F. Titer of the Strain Cultures:

Fresh cultures of bacteria were grown up to the late exponential or early stationary phase of growth; to confirm this, serial dilutions from each strain were conducted, indicating that the initial population was in the range of 1 to 2×10^9 /ml.

IV. Method

A. Standard Plate Incorporation Assay:

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

V. Evaluation

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

VI. Results and Discussion

A. Solubility:

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

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B. Dose levels tested:

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

C. Titer (Organisms/ml):

5×10^8 UFC/ml plate count indicates that the initial population was in the range of 1 to 2×10^9 UFC/ml.

C. Standard Plate Incorporation Assay

In no case was there a 2-fold or greater increase in the mean number of revertant testing strains TA98, TA100, TA1537, TA1535 and WP2uvrA in the presence of the test solution compared with the mean of vehicle control value. The positive controls mean exhibited at least a 3-fold increase over the respective mean of the *Salmonella typhimurium* and *Escherichia coli* tester strains used. The results are summarized in Appendix 2.

VII. Conclusion

All criteria for a valid study were met as described in the protocol. The results of the Bacterial Reverse Mutation Assay indicate that under the conditions of this assay, the test article solution was considered to be Non-Mutagenic to *Salmonella typhimurium* tester strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2uvrA. The negative and positive controls performed as anticipated. The results of this study should be evaluated in conjunction with other required tests as listed in ISO 100993, Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicology.

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Appendix 2:

Bacterial Mutation Assay Plate Incorporation Assay Results

| | Concentration µg per Plate | TA98 | | |
|----------------------------------|-------------------------------|-------------------------------|-----|------|
| | | Revertants per plate (CFU) | | Mean |
| Test Solution w/ S9 | 5000 | 35 | 38 | 37 |
| | 1500 | 25 | 32 | 29 |
| | 500 | 25 | 21 | 23 |
| | 150 | 32 | 32 | 32 |
| | 50 | 29 | 35 | 28 |
| | 15 | 27 | 42 | 35 |
| | 5.0 | 22 | 44 | 33 |
| | 1.5 | 18 | 37 | 28 |
| Test Solution w/o S9 | 5000 | 25 | 41 | 33 |
| | 1500 | 16 | 21 | 19 |
| | 500 | 33 | 22 | 28 |
| | 150 | 25 | 25 | 25 |
| | 50 | 47 | 41 | 44 |
| | 15 | 35 | 21 | 28 |
| | 5.0 | 25 | 17 | 21 |
| | 1.5 | 45 | 15 | 30 |
| DI Water w/S9 | | 52 | 48 | 50 |
| DI Water w/o S9 | | 55 | 47 | 51 |
| 2-aminoanthracen w/ S9 | | 221 | 232 | 227 |
| 2-nitrofluorene w/o S9 | | 217 | 205 | 211 |
| Historical Count Positive w/S9 | | 43-1893 | | |
| Historical Count Positive w/o S9 | | 39-1871 | | |
| Historical Count Negative w/S9 | | 4-69 | | |
| Historical Count Negative w/o S9 | | 3-59 | | |

*CFU = Colony Forming Units

*Mean = Average of duplicate plates

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| | Concentration µg per Plate | TA100 | | |
|----------------------------------|-------------------------------|-------------------------------|-----|------|
| | | Revertants per plate (CFU) | | Mean |
| Test Solution w/ S9 | 5000 | 215 | 232 | 224 |
| | 1500 | 210 | 187 | 199 |
| | 500 | 132 | 125 | 129 |
| | 150 | 117 | 127 | 122 |
| | 50 | 148 | 121 | 135 |
| | 15 | 115 | 118 | 117 |
| | 5.0 | 126 | 147 | 137 |
| | 1.5 | 132 | 123 | 128 |
| Test Solution w/o S9 | 5000 | 117 | 131 | 124 |
| | 1500 | 98 | 95 | 97 |
| | 500 | 101 | 135 | 118 |
| | 150 | 114 | 123 | 119 |
| | 50 | 178 | 163 | 171 |
| | 15 | 140 | 115 | 128 |
| | 5.0 | 115 | 138 | 127 |
| | 1.5 | 110 | 102 | 106 |
| DI Water w/S9 | | 208 | 211 | 210 |
| DI Water w/o S9 | | 192 | 166 | 179 |
| 2-aminoanthracen w/ S9 | | 600 | 598 | 599 |
| Sodium azide w/o S9 | | 615 | 633 | 624 |
| Historical Count Positive w/S9 | | 224-3206 | | |
| Historical Count Positive w/o S9 | | 226-1837 | | |
| Historical Count Negative w/S9 | | 55-268 | | |
| Historical Count Negative w/o S9 | | 47-250 | | |

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*Mean = Average of duplicate plates

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| | Concentration µg per Plate | TA1537 | | |
|----------------------------------|-------------------------------|-------------------------------|-----|------|
| | | Revertants per plate (CFU) | | Mean |
| Test Solution w/ S9 | 5000 | 35 | 25 | 30 |
| | 1500 | 21 | 18 | 20 |
| | 500 | 20 | 21 | 21 |
| | 150 | 35 | 33 | 34 |
| | 50 | 17 | 20 | 19 |
| | 15 | 21 | 17 | 19 |
| | 5.0 | 25 | 20 | 23 |
| | 1.5 | 25 | 22 | 24 |
| Test Solution w/o S9 | 5000 | 19 | 32 | 26 |
| | 1500 | 16 | 28 | 22 |
| | 500 | 17 | 22 | 20 |
| | 150 | 22 | 21 | 22 |
| | 50 | 23 | 24 | 24 |
| | 15 | 21 | 36 | 29 |
| | 5.0 | 18 | 21 | 20 |
| | 1.5 | 21 | 23 | 22 |
| DI Water w/S9 | | 46 | 56 | 51 |
| DI Water w/o S9 | | 60 | 66 | 63 |
| 2-aminoanthracen w/ S9 | | 456 | 475 | 466 |
| 2-aminoacridine w/o S9 | | 301 | 308 | 305 |
| Historical Count Positive w/S9 | | 13-1934 | | |
| Historical Count Positive w/o S9 | | 17-4814 | | |
| Historical Count Negative w/S9 | | 0-41 | | |
| Historical Count Negative w/o S9 | | 0-29 | | |

*CFU = Colony Forming Units

*Mean = Average of duplicate plates

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| | Concentration µg per Plate | TA1535 | | |
|----------------------------------|-------------------------------|-------------------------------|-----|------|
| | | Revertants per plate (CFU) | | Mean |
| Test Solution w/ S9 | 5000 | 45 | 22 | 34 |
| | 1500 | 29 | 31 | 30 |
| | 500 | 26 | 27 | 27 |
| | 150 | 23 | 35 | 29 |
| | 50 | 30 | 31 | 31 |
| | 15 | 25 | 24 | 25 |
| | 5.0 | 16 | 26 | 21 |
| | 1.5 | 20 | 26 | 23 |
| Test Solution w/o S9 | 5000 | 33 | 30 | 32 |
| | 1500 | 20 | 20 | 20 |
| | 500 | 24 | 30 | 27 |
| | 150 | 32 | 45 | 39 |
| | 50 | 20 | 25 | 23 |
| | 15 | 15 | 22 | 19 |
| | 5.0 | 19 | 19 | 19 |
| | 1.5 | 17 | 13 | 15 |
| DI Water w/S9 | | 66 | 51 | 59 |
| DI Water w/o S9 | | 47 | 42 | 45 |
| 2-aminoanthracen w/ S9 | | 285 | 264 | 275 |
| Sodium azide w/o S9 | | 615 | 627 | 621 |
| Historical Count Positive w/S9 | | 22-1216 | | |
| Historical Count Positive w/o S9 | | 47-1409 | | |
| Historical Count Negative w/S9 | | 1-50 | | |
| Historical Count Negative w/o S9 | | 1-45 | | |

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*Mean = Average of duplicate plates

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| | Concentration µg per Plate | WP2uvrA | | |
|----------------------------------|-------------------------------|-------------------------------|-----|------|
| | | Revertants per plate (CFU) | | Mean |
| Test Solution w/ S9 | 5000 | 31 | 28 | 30 |
| | 1500 | 18 | 38 | 28 |
| | 500 | 21 | 25 | 23 |
| | 150 | 23 | 18 | 21 |
| | 50 | 20 | 23 | 22 |
| | 15 | 21 | 35 | 28 |
| | 5.0 | 20 | 15 | 18 |
| | 1.5 | 23 | 22 | 23 |
| Test Solution w/o S9 | 5000 | 28 | 33 | 31 |
| | 1500 | 13 | 16 | 15 |
| | 500 | 22 | 16 | 19 |
| | 150 | 25 | 20 | 23 |
| | 50 | 31 | 31 | 31 |
| | 15 | 23 | 20 | 22 |
| | 5.0 | 20 | 20 | 20 |
| | 1.5 | 28 | 27 | 28 |
| DI Water w/S9 | | 57 | 61 | 59 |
| DI Water w/o S9 | | 58 | 66 | 62 |
| 2-aminoanthracen w/ S9 | | 235 | 263 | 249 |
| Methylmethanesulfonate w/o S9 | | 267 | 246 | 257 |
| Historical Count Positive w/S9 | | 44-1118 | | |
| Historical Count Positive w/o S9 | | 42-1796 | | |
| Historical Count Negative w/S9 | | 8-80 | | |
| Historical Count Negative w/o S9 | | 8-84 | | |

*CFU = Colony Forming Units

*Mean = Average of duplicate plates

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Cellular Viability Assay Analysis

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Tradename: Phyto-Biotics Acai®

Code: 16587

CAS #: 999999-99-4

Test Request Form #: 361

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

Study Director: Erica Segura

Principle Investigator: Meghan Darley

Test Performed:

Cellular Viability Assay

Introduction

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

The cellular viability assay was conducted to assess the ability of **Phyto-Biotics Acai®** to increase cellular metabolic activity in cultured dermal fibroblasts.

Assay Principle

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

| |
|---|
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|---|



Cellular Viability Assay Analysis

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Materials

- | | |
|----------------------------------|---|
| A. Kit: | PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261) |
| B. Incubation Conditions: | 37°C at 5% CO ₂ and 95% relative humidity (RH) |
| C. Equipment: | Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes |
| D. Cell Line: | Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511) |
| E. Media/Buffers: | Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS) |
| F. Culture Plate: | Falcon flat bottom 96-well tissue culture treated plates |
| G. Reagents: | PrestoBlue™ reagent (10X) |
| H. Other: | Sterile disposable pipette tips |

Methods

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

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



Cellular Viability Assay Analysis

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Results

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

Phyto-Biotics Acai® at all concentrations is able to increase cellular metabolism compared to the control.

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

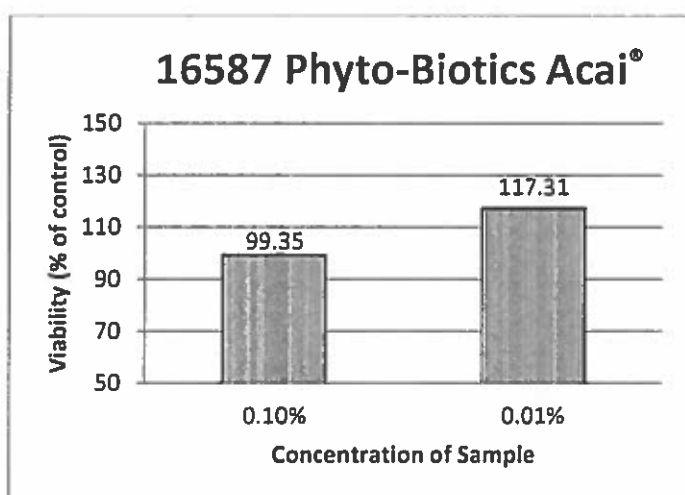
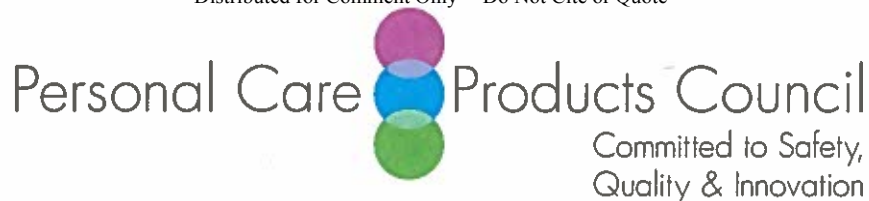


Figure 1: Cellular Metabolism of **Phyto-Biotics Acai®**-treated fibroblasts expressed in terms of percent of control.

Discussion

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

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Memorandum

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

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

DATE: February 15, 2019

SUBJECT: Scientific Literature Review: Safety Assessment of Palm Tree-Derived Ingredients as Used in Cosmetics (release date January 23, 2019)

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

The Council has no suppliers listed for the following ingredients included in this report:

Euterpe Edulis Juice Extract
Euterpe Oleracea Palm Heart Extract
Hydrolyzed Euterpe Oleracea Fruit

Key Issues

The Introduction should state that Euterpe Oleracea Fruit Oil was included in the CIR report on plant oils (published 2017) and found safe for use. If there are studies regarding Euterpe Oleracea Fruit Oil in the 2017 report, it would be helpful if they were summarized in a table in this report.

The Introduction should indicate why the ingredients in this report are being reviewed together. Do they have common constituents?

Additional Considerations

Introduction - As there is no organization that sets standards for ingredients used in cosmetics, it is not appropriate to use the term "cosmetic-grade".

Composition, Euterpe Oleracea Fruit Extract - Please include the units for the 0.53 polyphenol content of Euterpe Oleracea Fruit Extract (cited to reference 16).

Impurities - Potassium, magnesium, phosphorus, calcium, zinc, iron and copper are not considered heavy metals. As many of these metals are essential, they should be considered constituents rather than impurities. If the concentrations of the metals in the acai berries were stated, they should be included in the CIR report.

ADME - Since applesauce was used as a control, how did the increase in antioxidant activity after injection of Euterpe Oleracea Juice and pulp compare to applesauce?

Subchronic, *Euterpe Oleracea* Fruit Extract - It is not clear how the control group was treated (or was there more than one control group)?

Subchronic, *Euterpe oleracea* pulp-enriched fruit and berry juice - Were the relative adrenal weights increased or decreased (reference 37)?

Genotoxicity, In Vitro, Summary - Please indicate whether or not metabolic activation was used in the *in vitro* genotoxicity assays.

Genotoxicity, In Vivo - Please include the doses that were used in these studies.

Table 3 - Is percent the correct units for this table? The amount in each extract is well over 100%. Therefore, if percent is correct, what does it represent e.g., is each a percentage of a different fraction of the extract?

Table 4 - Please define FW

Table 5 - Please define dwb

Table 7 - Please define ww

Table 8 - It is not correct to call an element such as phosphorus, e.g., 69.2 mg/100 g in the fruit, an impurity. It is a constituent of the fruit. The title of the table needs to be revised.

Table 16, In Vitro - The Assay column indicates that the Ames assay on *Euterpe edulis* fruit pulp was completed with and without metabolic activation, but the results column only describes the results without metabolic activation. What were the results with metabolic activation? Whether or not metabolic activation was included should be stated for each *in vitro* study.