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Safety Assessment of *Vitis vinifera* (Grape)-Derived Ingredients as Used in Cosmetics

Monice M. Fiume[1], Wilma F. Bergfeld[2], Donald V. Belsito[2], Ronald A. Hill[2], Curtis D. Klaassen[2], Daniel C. Liebler[2], James G. Marks Jr[2], Ronald C. Shank[2], Thomas J. Slaga[2], Paul W. Snyder[2], and F. Alan Andersen[3]

Abstract

The Cosmetic Ingredient Review Expert Panel (Panel) assessed the safety of 24 *Vitis vinifera* (grape)-derived ingredients and found them safe in the present practices of use and concentration in cosmetics. These ingredients function in cosmetics mostly as skin-conditioning agents, but some function as antioxidants, flavoring agents, and/or colorants. The Panel reviewed the available animal and clinical data to determine the safety of these ingredients. Additionally, some constituents of grapes have been assessed previously for safety as cosmetic ingredients by the Panel, and others are compounds that have been discussed in previous Panel safety assessments.

Keywords

Vitis vinifera, grape, safety, cosmetics

Introduction

As given in the Code for Federal Regulations (21CFR101, subpart C), grapes are among the 20 most frequently consumed raw fruits and are subject to regulation by the Food and Drug Administration (FDA) as foods.

This report assesses the safety of the following 24 *Vitis vinifera* (grape)-derived ingredients for use in cosmetic formulations:

vitis vinifera (grape);
vitis vinifera (grape) bud extract;
vitis vinifera (grape) flower extract;
vitis vinifera (grape) fruit extract;
vitis vinifera (grape) fruit powder;
vitis vinifera (grape) fruit water;
vitis vinifera (grape) juice;
vitis vinifera (grape) juice extract;
vitis vinifera (grape) leaf extract;
vitis vinifera (grape) leaf oil;
vitis vinifera (grape) leaf/seed/skin extract;
vitis vinifera (grape) leaf water;
vitis vinifera (grape) leaf wax;
vitis vinifera (grape) root extract;
vitis vinifera (grape) seed;
vitis vinifera (grape) seed extract;
vitis vinifera (grape) seed powder;
vitis vinifera (grape) shoot extract;
vitis vinifera (grape) skin extract;
vitis vinifera (grape) skin powder;
vitis vinifera (grape) vine extract;
vitis vinifera (grape) vine sap;
Hydrolyzed grape fruit;
Hydrolyzed grape skin.

These ingredients are reported to have many functions in cosmetics, most frequently as skin-conditioning agents.[1,2] Some of these ingredients are reported to function as antioxidants, flavoring agents, and/or colorants (Table 1).

The safety of *Vitis vinifera* (grape) seed oil and hydrogenated grapeseed oil was reviewed previously by the Cosmetic Ingredient Review (CIR) Expert Panel (Panel) in the Safety Assessment of Plant-Derived Fatty Acid Oils as Used in Cosmetics, at which time the Panel concluded that these ingredients are safe as used in cosmetics.[3] These 2 ingredients are not included in this safety assessment.

The detailed chemical composition of *vitis vinifera* (grape) is given later in this assessment. As shown in Table 2, some of

[1] Cosmetic Ingredient Review Senior Scientific Analyst/Writer, Washington, DC, USA
[2] Cosmetic Ingredient Review Expert Panel Member, Washington, DC, USA
[3] Former Director, Cosmetic Ingredient Review, Washington, DC, USA

Corresponding Author:
Lillian J. Gill, Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, DC 20036, USA.
Email: cirinfo@cir-safety.org

Table 1. Definitions, Functions, and Chemical Class.[1,2]

Ingredient (CAS no.)	Definition	Reported functions	Chemical class
Vitis vinifera (Grape; 85594-37-2)	A plant material derived from the whole plant, *Vitis vinifera*	Not reported	Botanical products and botanical derivatives
Vitis vinifera (grape) bud extract (85594-37-2)	The extract of the buds of *Vitis vinifera*	Skin-conditioning agent—misc	Botanical products and botanical derivatives
Vitis vinifera (grape) flower extract (85594-37-2)	The extract of the flowers of *Vitis vinifera*	Skin-conditioning agent—emollient; fragrance ingredient	Botanical products and botanical derivatives
Vitis vinifera (grape) fruit extract (84929-27-1; 85594-37-2)	The extract of the fruit of *Vitis vinifera*	Skin-conditioning agent—misc; antioxidant	Botanical products and botanical derivatives
Vitis vinifera (grape) fruit powder (85594-37-2)	The powder obtained from the dried, ground fruit of *Vitis vinifera*	Skin-conditioning agent—misc; antioxidant; colorant; flavoring agent	Botanical products and botanical derivatives
Vitis vinifera (grape) fruit water (85594-37-2)	An aq solution of the steam distillate obtained from the fruit of *Vitis vinifera*	Skin-conditioning agent—misc	Essential oils and waters
Vitis vinifera (grape) juice (85594-37-2)	The liquid expressed from the fresh pulp of the grape	Skin-conditioning agent—misc, antioxidant, colorant, flavoring agent	Botanical products and botanical derivatives
Vitis vinifera (grape) juice extract (85594-37-2)	The extract of the juice of *Vitis vinifera*	Antioxidant, colorant, flavoring agent	Botanical products and botanical derivatives
Vitis vinifera (grape) leaf extract (84929-27-1; 85594-37-2)	The extract of the leaves of *Vitis vinifera*	Skin-conditioning agent—misc	Botanical products and botanical derivatives
Vitis vinifera (grape) leaf oil 8016-21-5	The essential oil derived from the leaves of the grape, *Vitis vinifera*	Fragrance ingredient	Essential oils and waters
Vitis vinifera (grape) leaf/seed/skin extract (85594-37-2)	The extract of the leaves, skin, and seeds of *Vitis vinifera*	Antioxidant	Botanical products and botanical derivatives
Vitis vinifera (grape) leaf water (85594-37-2)	An aq solution of the steam distillate obtained from the leaves of *Vitis vinifera*	Skin-conditioning agent—misc	Essential oils and waters
Vitis vinifera (grape) leaf wax (85594-37-2)	A wax obtained from the vine leaf of *Vitis vinifera*	Not reported	Waxes (natural and synthetic)
Vitis vinifera (grape) root extract (84929-27-1; 85594-37-2)	The extract of the roots of *Vitis vinifera*	Skin-conditioning agent—misc	Botanical products and botanical derivatives
Vitis vinifera (grape) seed (85594-37-2)	The seed of *Vitis vinifera*	Skin-conditioning agent—misc	Botanical products and botanical derivatives
Vitis vinifera (grape) seed extract (84929-27-1; 85594-37-2)	The extract of the seeds of *Vitis vinifera*	Anticaries agent, antidandruff agent, antifungal agent, antimicrobial agent, antioxidant, flavoring agent, light stabilizer, oral care agent, oral health care drug, sunscreen agent	Botanical products and botanical derivatives
Vitis vinifera (grape) seed powder (85594-37-2)	The powder obtained from the dried, ground seeds of *Vitis vinifera*	Abrasive, exfoliant	Botanical products and botanical derivatives
Vitis vinifera (grape) shoot extract	The extract of the shoots of the vines of *Vitis vinifera*	Antioxidant, skin protectant	Botanical products and botanical derivatives
Vitis vinifera (grape) skin extract (85594-37-2)	Extract of the skin of the grape, *Vitis vinifera*	Antioxidant, colorant, flavoring agent	Botanical products and botanical derivatives
Vitis vinifera (grape) skin powder (85594-37-2)	The powder obtained from the dried, ground skin of *Vitis vinifera*	Skin-conditioning agent—misc; antioxidant; binder; colorant	Botanical products and botanical derivatives
Vitis vinifera (grape) vine extract (85594-37-2)	The extract of the vine of *Vitis vinifera*	Skin-conditioning agent—misc	Botanical products and botanical derivatives
Vitis vinifera (grape) vine sap	The sap obtained from the vines of *Vitis vinifera*	Skin-conditioning agent—misc	Botanical products and botanical derivatives
Hydrolyzed grape fruit	The hydrolysate of the fruit of *Vitis vinifera* derived by acid, enzyme or other method of hydrolysis	Cosmetic astringent, skin protectant, skin-conditioning agent—misc	Botanical products and botanical derivatives
Hydrolyzed grape skin	The hydrolysate of the skin of *Vitis vinifera* derived by acid, enzyme or other method of hydrolysis	Antioxidant, light stabilizer, skin protectant, skin-conditioning agent-emollient	Botanical products and botanical derivatives

Abbreviations: aq, aqueous; misc, miscellaneous.

Table 2. Conclusions of CIR Safety Assessments on Ingredients That Are Constituents of *Vitis vinifera* (Grape).

Component reviewed	Conclusion	Reference
Acetic acid	Safe as used (≤0.0004% in leave-ons; ≤0.3% in rinse-offs)	4
Ascorbic acid	Safe as used (≤10% in leave-ons; ≤5% in rinse-offs)	5
Benzoic acid	Safe as used (≤5% in leave-ons; ≤5% in rinse-offs; 0.08% in diluted for [bath] use formulations)	6
Benzyl alcohol	Safe as used (≤3% in leave-ons; ≤10% in rinse-offs; ≤0.9% in diluted for [bath] use formulations)	6
Biotin	Safe as used (≤0.6% in leave-ons; ≤0.01% in rinse-offs)	7
Cholesterol	Safe as used (≤5% in leave-ons; ≤1% in rinse-offs)	8
Citric acid	Safe as used (≤4% in leave-ons; ≤10% in rinse-offs; ≤39% in diluted for [bath] use formulations)	9
Fumaric acid	Safe as used (≤0.2% in leave-ons; ≤0.2% in rinse-offs; ≤5% in diluted for [bath] use formulations)	10
Lactic acid	Safe for use at ≤10%, final formulation pH ≥3.5, when formulated to avoid increasing sun sensitivity or when directions for use include the daily use of sun protection; safe for use in salon products at ≤30%, final formulation pH ≥3.0, in products designed for brief, discontinuous use followed by thorough rinsing from the skin, when applied by trained professionals, and when application is accompanied by directions for the daily use of sun protection	11
Malic acid	Safe for use as a pH adjuster; insufficient for other uses in cosmetic ingredients	12
Myristic acid	Safe as used (≤10% in leave-ons; ≤19% in rinse-offs)	13
Niacin	Safe as used (≤0.1% in leave-ons)	14
Oleic acid	Safe as used (≤20% in leave-ons; ≤19% in rinse-offs)	15,16
Palmitic acid	Safe as used (≤16% in leave-ons; ≤20% in rinse-offs)	15,16
Pantothenic acid	Safe as used (≤0.01% in leave-ons: 0.00001% in rinse-offs)	16,17
Salicylic acid	Safe as used when formulated to avoid skin irritation and when formulated to avoid increasing the skin's sensitivity to sun, or, when increased sun sensitivity would be expected, directions for use include the daily use of sun protection (≤3% in leave-ons; ≤3% in rinse-offs)	106
Stearic acid	Safe as used (≤22% in leave-ons; ≤43% in rinse-offs)	15,16
Succinic acid	Safe as used (≤0.2% in leave-ons; ≤26% in rinse-offs)	18
Tocopherol	Safe as used (≤2% in leave-ons; ≤0.4% in rinse-offs; ≤0.8% in products diluted for use)	19

Abbreviation: CIR, Cosmetic Ingredient Review.

the constituents of grape, such as ascorbic acid, biotin, malic acid, and so on, are cosmetic ingredients for which a Panel safety assessment is available; others are compounds that have been discussed in previous Panel safety assessments.[4-19]

Although many studies conducted using *vitis vinifera* (grape)-derived ingredients address health claims, antioxidant activity, and so on, this safety assessment only includes studies that relate directly to the safety of the cosmetic use of these ingredients.

In many of the published studies, it is not known how the substance being tested is compared to the cosmetic-grade ingredient. Therefore, if it is not known whether the ingredient being discussed is a cosmetic ingredient, the test substance will be identified as "grape . . . " (eg, grape seed extract); if it is known that the substance is a cosmetic ingredient, the terminology "*Vitis vinifera* (grape) . . . " (eg, *vitis vinifera* [grape] seed extract) will be used.

Chemistry

Definition

The definitions of the *vitis vinifera* (grape)-derived ingredients are provided in Table 1. *Vitis vinifera* is also known as wine grape, European grape,[20] and grapevine.[21]

Chemical and Physical Properties

Chemical and physical property data are provided in Table 3.[22-29]

Composition

Grapes contain fruit acids, and the unripe fruit contains 34 ppm oxalic acid.[20,26] Grape seeds contain 6% to 20% oil. Phenols are the third most abundant constituent in grapes; carbohydrates and fruit acids are the most and second most abundant, respectively.[30] The total extractable phenolics in grapes are present at ≤10% in the pulp, 60% to 70% in the seeds, and 28% to 35% in the skin.

The amount of a constituent present in the plant varies with the region in which it is grown.[26] For example, fruit of grapes from Africa and Asia contained 50.0 μg β-carotene equivalents per 100 g of fruit while elsewhere trace β-carotene equivalent were present in the fruit. The cultivar, climate condition, and degree of maturation also affect the composition, as does whether the grapes are red or white.[30]

It has also been shown that the amount of a constituent present in an extract is dependent on the medium used during extraction and the variety of *vitis vinifera* (grape) used.[31] For example, a red grape methanolic extract, red grape water extract, white grape methanolic extract, and white grape water extract each contained 0.22, 0.04, 0.01, and 0.02 mg/g trans-resveratrol, respectively; 0.9, 0.35, 2.25, and 4.09 mg/g (+)-catechin, respectively; 1.1, 0.32, 1.08, and 2.10 mg/g (-)-epicatechin, respectively; and 0, 0.13, 0.04, and 0.03 mg/g quercetin, respectively.

Melatonin (*N*-acetyl-5-methoxytryptamine) is present in grapes.[21] Depending on variety and location, levels of

Table 3. Chemical and Physical Properties.

Property	Description	Reference
Vitis vinifera (grape) fruit extract		
Mixture containing 75%-100% glycerin (solvent), 50%-75% *Vitis vinifera* (grape) fruit extract, and 10%-25% water		
Appearance	Clear yellow liquid with a faint fruity odor	22
Density	1.225-1.245	22
Refractive index	1.445-1.465	22
pH	4.0-5.0	22
Solubility	In water clear soluble	22
Vitis vinifera (grape) leaf extract		
Mixture containing 75%-100% glycerin (solvent), 5%-10% *Vitis vinifera* (grape) leaf extract, and 10%-25% water		
Appearance	Dark brownish-red colored liquid with a faint herbal odor	23
Density	1.215-1.235	23
Refractive index	1.445-1.465	23
pH	4.0-5.0	23
Solubility	Soluble in water	23
Vitis vinifera (grape) seed extract		
Appearance	Red to brown powder	24
Water content	8% (upper limit)	24
Vitis vinifera (grape) skin extract		
Appearance	Red to purple powder or liquid	25
	Purplish-red liquid	26
	Purplish-red liquid, lump, powder, or paste with a characteristic odor	27
Appearance in solution	Red in acid solution; violet or blue in neutral to alkaline solution	25
Solubility	Soluble in water	27
Hydrolyzed grape skin		
Appearance	Ruby red aq solution	28
Odor	Characteristic, fruity	28,29
Boiling point	98°C-102°C (760 mm Hg)	29
Density	$\approx$1 g/cm^3	29
pH	2.6-3.5	28
	2.8-4	29
Solubility	Completely soluble in water; soluble in alcohol and acetone	29
Dry residue	$\geq$1.5%, w/w	28
Water content	$\geq$90%	29
Phenol content	700-1500 mg/kg	28

Abbreviation: aq, aqueous.

melatonin in grape skin have ranged from 0.005 to 1.2 ng/g. The stage of growth also affects the amount present. Studies have indicated that melatonin may also be present in the flesh and seeds of grapes.

A detailed list of chemical constituents by plant part is presented in Table 4,[20] and a more focused listing of constituents of *vitis vinifera* is provided in Table 5.[30,33,34] As stated earlier, Table 2 provides the conclusions from CIR safety assessments that exist for some of the constituents of grape, and Table 6 includes information on the toxicity of some constituents.[32,35-47]

Vitis vinifera (grape) fruit extract. Fruit acids, sugars, minerals, pectin, tannins, proteins, anthocyanins, waxes, flavonoids, xanthophylls, carotene, vitamins, polysaccharides, aromatic substances, and procyanidins are part of the composition of *vitis vinifera* (grape) fruit extract.[22]

Vitis vinifera (grape) juice. A commercial brand grape juice contained 4.4 mg/L quercetin and 6.2 mg/L myricetin.[48]

Vitis vinifera (grape) leaf extract. Potassium and calcium bitartrate, calcium malate, fruit acids, sugar, flavonoids, and tannins are part of the composition of *vitis vinifera* (grape) leaf extract.[23]

Vitis vinifera (grape) seed extract. The main constituents of grape seeds are reported to be phenolic compounds. Those phenolic compounds from standardized grape seed extracts are reported to be 92% to 95% oligomeric proanthocyanidins.[49] Proanthocyanidin structures vary depending upon the source of the flavanol(s) building blocks (monomer units), the degree of oligomerization (how many flavanol repeat units), and the presence of modifications (such as esterification) of the 3-hydroxyl group.[50] The most prominent grape seed extract proanthocyanidin is depicted in Figure 1.[49] Catechin, epicatechin, and taxifolin are the primary flavanols present in grape seeds and comprise the majority of the remaining phenols in grape seed extracts (Figure 2). Heating of oligomeric proanthocyanidins, under acidic conditions, leads to the release of anthocyanins, and in turn, flavanols. Accordingly, the length of oligomeric proanthocyanidins and the concentration of flavanols in grape seed

Table 4. Chemical Constituents by Plant Part.[20]

Chemical	Amount, ppm	Chemical	Amount, ppm
Plant			
2,6-Dimethyl-trans-octa-2,7-dien-1,6-diol-β-D-glucopyranoside	NS	Oleic acid	230-1183
Delphinidin	NS	Petunidin-3-caffeoylglucoside	NS
Leucocyanidin	NS	Riboflavin	0.5-0.2
Limonene	NS	Stigmasterol	NS
Malic acid	NS	Vitispirane	NS
Fruit			
2,2,6-Trimethyl-8-(1-hydroxy-ethyl)-7-oxa-bicyclo-(4,3,0)-nona-4,9-diene	NS	Lutein	0.7-7
2,6-Dimethyl-trans,trans-octa-2,6-dien-1,8-diol	NS	Lutein-5,6-epoxide	NS
2,6-Dimethyl-trans-octa-2,7-dien-1,6-diol-6-o-α-D-arabinofuranosyl-β-D-β-D-glucopyranoside	NS	Lutein-5-8-epoxide	NS
3,7-Dimethyl-oct-1-ene-3,6,7-triol	NS	Luteoxanthin	NS
3,7-Dimethyl-oct-1-ene-3,7-diol	NS	Lycopene	NS
3,7-Dimethyl-octa-1,5,7-trien-3-ol	NS	Lysine	150-772
3,7-Dimethyl-octa-1,5-dien-3,7-diol	NS	Magnesium	58-2310
3,7-Dimethyl-octa-1,6-dien-3,5-diol	NS	Malic acid	1500-2000
3,7-Dimethyl-octa-1,7-dien-3,6-diol	NS	Malvidin	NS
a-Hemicellulose	NS	Malvidin-3-(6-p-coumaroylglucoside)-5-glucoside	NS
Abscissic acid	NS	Malvidin-3-(p-coumaroylglucoside)	NS
Acetic acid	1500-2000	Malvidin-3-caffeoylglucoside	NS
Alanine	280-1440	Malvidin-3-chlorogenic-acid-glucoside	NS
α-Carotene	NS	Malvidin-3-glucoside	NS
α-Hydroxycarotene	NS	Malvidin-3-o-β-D-glucoside	NS
α-Linolenic acid	390-2006	Manganese	0.5-54
α-Tocopherol	6-31	Melibiose	NS
Aluminum	1-154	Mercury	0.011
Antheraxanthin	NS	Methionine	220-1132
Anthocyanins	NS	Molybdenum	0-0.539
Arginine	490-2520	Mono-p-coumaryl-acid	NS
Arsenic	0.001-0.889	Monocaffeic acid	NS
Ascorbic acid	99-600	Monounsaturated fatty acids	230-1183
Ascorbic acid oxidase		Mutatoxanthin	NS
Ash	4290-77 000	Myricetin	NS
Aspartic acid	810-4167	Myricetin-3-monoglucoside	NS
b-Hemicellulose		Myristic acid	50-257
Barium	0.66-15.4	Neo-chlorogenic acid	NS
Benzoic acid		Neoxanthin	NS
β-Carotene	0.25-2.1	Neoxanthin	NS
β-Ionone	NS	Nerol-6-0-α-L-arabinofuranosyl-β-D-glucopyranoside	NS
β-Sitosterol	NS	nerol-6-0-α-L-rhamnopyranosyl-β-D-glucopyranoside	NS
Biotin	NS	Niacin	3-15.4
Boron	1-50	Nickel	0.01-0.77
Bromine	NS	Nitrogen	1100-7220
Cadmium fruit 0.001-0.231 ppm	0.001-0.231	Nonacosane	NS
Caffeic acid	NS	Oxalic acid	34
caffeoyl-tartrate	NS	p-Coumaric-acid	NS
Caffeyltartaric acid	NS	p-Coumaroyl-cis-tartrate	NS
Calcium	92-4774	p-Coumaroyl-trans-tartrate	NS
Carbohydrates	177 700-914 095	Paeonidin	NS
Catalase	NS	Paeonidin-3-(6-p-coumaroylglucoside)	NS
Catechol oxidase	NS	Paeonidin-3-5,-diglucoside	NS
Chlorogenic acid	NS	Paeonidin-3-caffeoylglucoside	NS
Cholesterol	NS	Paeonidin-3-o-β-D-glucoside	NS
Chromium	0.005-0.385	Palmitic acid	1620-8333

(continued)

Table 4. (continued)

Chemical	Amount, ppm	Chemical	Amount, ppm
Cinnamic acid	NS	Pantothenic acid	0.2-1.3
Cis-caffeic acid	NS	Pectin	300-3900
Citric acid	NS	Pectin-methyl-esterase	NS
Cobalt	0.005-0.22	Pelargonidin	NS
Copper	0.7-11.6	Peroxidase	NS
Coumarin	NS	Petunidin-3,5-diglucoside	NS
Cryptochlorogenic acid	NS	Petunidin-3-(6-p-coumaroylglucoside)	NS
Cryptoxanthin	NS	Petunidin-3-glucoside	NS
Cyanidin	NS	Petunidin-3-o-β-D-glucoside	NS
Cyanidin-3-galactoside	NS	Phenylalanine	140-720
Cyanidin-3-glucoside	NS	Phosphorus	117-1848
Cystine	110-566	Phytoene	NS
D-Catechin	NS	Phytofluene	NS
Delphinidin-3,5-diglucoside	NS	Phytosterols	40-206
Delphinidin-3-(6-p-coumaroylglucoside)	NS	Polyphenol oxidase	NS
Delphinidin-3-(p-coumaroylglucoside)-5-glucoside	NS	Potassium	1784-24 640
Delphinidin-3-0-β-D-glucoside	NS	Procyanidin-b-2-3′-o-gallate	NS
Delphinidin-3-caffeoylglucoside	NS	Procyanidins	NS
Dihydrophaseic-acid-4′-β-D-glucoside	NS	Praline	220-1132
Ellagic acid	NS	Protein	6350-35 236
Enomelanin	NS	Protopectinase	NS
Epicatechin	NS	Polyunsaturated fatty acids	1690-8693
Epicatechin-3-gallate	NS	Quercetin	NS
Ergosterol	NS	Quercetin-glucuronoside	NS
Fat	5010-33 898	Quinic acid	NS
Ferulic acid	NS	Protein	70 000-10 000
Fiber	4210-24 640	Raffinose	NS
Fluorine	0.1-0.6	Roseoside	NS
Folacin	0.03-0.23	Rubidium	0.4-5.5
Formic acid	NS	Selenium	0.012
Fructose	NS	Serine	320-1646
Gaba	NS	Saturated fatty acids	1890-9722
Galactose	NS	Silicon	1-28
Galacturonic acid	NS	Silver	0.022-0.077
Gallic acid	NS	Sodium	2-454
Gamma-carotene	NS	Stachyose	
Geraniol	NS	Strontium	1.54-38.5
Geraniol-6-o-α-L-arabinofuranosyl-β-D-glucopyranoside	NS	Succindehydrogenase	NS
Geraniol-6-o-α-L-rhamnopyranosyl-β-D-glucopyranoside	NS	Succinic acid	NS
Glucose	NS	Sugar	30 000-189 000
Glucose-6-phosphate-dehydrogenase:	NS	Sulfur	7-888
Glutamic acid	1380-7099	Tartaric acid	15-20
Glycine	200-1029	Tartaric acid-caffeoyl-ester	15-20
Hentriacontane		Thiamin	0.8-4.9
Hexokinase		Threonine	180-926
Histidine	240-1235	Titanium	0.11-7.7
Iron	1.5-154	Trans-caffeic acid	
Isochlorogenic acid		Tryptophan	30-154
Isoleucine	50-257	Tyrosine	120-617
Kaempferol-3-monoglucoside	NS	Valine	180-926
Lactic acid	NS	Violaxanthin	NS
Lead	0.02-9	Vitamin B6	1-6
Leucine	140-720	Vomifoliol	NS
Leucoanthocyanidole	NS	Water	761 000-897 000
Linalol	NS	Xylose	NS

(continued)

Table 4. (continued)

Chemical	Amount, ppm	Chemical	Amount, ppm
Linalol-6-0-α-L-arabinofuranosyl-β-D-glucopyranoside	NS	Zeaxanthin	NS
Linalol-6-0-α-L-rhamnopyranosyl-β-D-glucopyranoside	NS	Zinc	0.4-27
Linoleic acid	1300-6687	Zirconium	0.44-1.54
Lithium	0.088-0.308		
Fruit juice			
2-Phenylethylamine	NS	Diethylamine	NS
3-Hydroxy-β-damascone	NS	Dihydrofuran	NS
9-Hydroxy-megastigm-4,6,7-trien-3-one	NS	Dimethylamine	NS
Acuminoside	NS	Ethylamine	NS
α-3-Oxo-damascone	NS	Geraniol-β-D-glucoside	NS
α-3-Oxo-ionone	NS	Isoamylamine	NS
α-Amylamine	NS	Isobutylamine	NS
Benzyl-6-o-β-D-apiofuranosyl-β-D-glucoside	NS	Linalol-6-0-β-D-apiofuranosyl-β-D-glucoside	NS
β-3-Oxo-damascone	NS	Linalol-β-D-glucoside	NS
β-Phenylethanol-6-β-D-arabinofuranosyl-β-D-glucopyranoside	NS	Megastigm-5-en-7-yne-3,9-diol	NS
β-Phenylethanol-β-D-glucoside	NS	n-Propylamine	NS
β-Phenylethanol-β-D-rutinoside	NS	Nerol-6-0-β-D-apiofuranosyl-β-D-glucoside	NS
Betaine	NS	Nerol-β-D-glucoside	NS
Damascenone	0.013-0.085	Pyrrolidine	NS
Leaf			
(DL)-Gallocatechin	NS	Hirsutrin	NS
2-Phenylethan-1-ol	NS	Inositol	NS
Acetic acid	NS	Isoquercitrin	NS
α-Viniferin	23 400	Isovitilagin	163
Ascorbic acid	3490-3870	Kaempferol	NS
Benzyl-alcohol	NS	Lupeol	NS
Benzyl-alcohol-6-o-L-arabinofuranosyl-β-D-glucopyranoside	NS	Luteolin	NS
Benzyl-alcohol-β-D-glucoside	NS	Mono-p-coumaryl acid	NS
Benzyl-alcohol-β-D-rutinoside:	NS	Monocaffeic acid	NS
Brevilagin	533	Monoferulylsuccinic acid	NS
Calcium-pectate	69 000	Nerol	NS
Citric acid	NS	Oleanolic acid-methyl-ester	NS
Citronellol	NS	Pterostilbene	NS
D-Catechin	NS	Quercitrin	NS
Epsilon-viniferin	30.900	Quinic acid	NS
Flavonoids	40 000-50 000	Resveratrol 90 400 ppm	NS
Fumaric acid	NS	Selenium	NS
Gallocatechin	NS	Vitilagin	89
Glyceric acid	NS		
Leaf wax			
Oleanolic acid			
Leaf—essential oil			
α-Terpineol	108 000	Geraniol	145 200
Elemol-acetate	130.2	Linalol	273 000
Essential oil			
Hydroxy-citronellol	NS		
Flower			
Asragalin			
Stem			
2-Methoxy-3-isobutyl-pyrazine	NS	Magnesium	4360
24-Methyl-cycloartenol	NS	Niacin	NS
α-Amyrin	1030	Obtusifoliol	NS
Ascorbic acid	310	Octan-1-ol	NS
Ash	88 000	Oleanolic aldehyde	NS

(continued)

Table 4. (continued)

Chemical	Amount, ppm	Chemical	Amount, ppm
β-Amyrin	NS	Phosphorus	1710
β-Carotene	43	Potassium	20 100
Calcium	17 700	Riboflavin	6.9
Chromium	9	Selenium	NS
Citrostadienol	NS	Silicon	365
Cobalt	33	Sodium	156
Cycloartenol	NS	Thiamin	11
Germanicol	NS	Tin	12
Iron	900	Water	792 000
Manganese	986	Zinc	75
Root			
30-Nor-lupan-3-β-ol-20-one	NS	Pyrophosphatase nucleotide	NS
Betulinic acid	NS	Salicylic acid	NS
Heptacosan-1-ol	NS	Sinapic acid	NS
Phosphodiesterase	NS	Triacontan-1-ol-tridecanoate	NS
Seed			
Enotannin	NS	Oleic acid	22 200-74 000
Epicatechin-3-gallate	NS	Palmitic acid	3300-11 000
Fat	60 000-200 000	Protein	89 000
Linoleic acid	33 000-110 000	Stearic acid	1440-4800
Hull husk			
Gentisic acid	NS	Syringic acid	NS
o-Hydroxybenzoic acid	NS	Vanillic acid	NS
p-Hydroxybenzoic acid	NS		
Petiole			
Oenin	NS		

Abbreviation: NS, not specified.

extracts are highly dependent on the extraction techniques used.

Grape seed oligomeric proanthocyanidins (United States Pharmacopeia [USP] grade for dietary supplements) contain no more than 10 ppm heavy metals, no more than 19.0% catechin and epicatechin on the anhydrous basis, no more than 8.0% water, and no more than 2% water-insoluble fraction.[51]

Vitis vinifera (grape) seed extract, as the trade name ActiVin, contains 54% dimeric, 13% trimeric, and 7% tetrameric oligomeric proanthocyanidins and a small amount of catechin derivatives, flavonoids, and other oligomeric proanthocyanidins.[52]

Vitis vinifera (grape) skin extract. Grape skin extract (enocianina) is an approved food color additive exempt from batch certification. The FDA describes the color additive as containing the common components of grape juice: anthocyanins, tartaric acid, tannins, sugars, and minerals (21CFR73.170). A small amount of residual sulfur dioxide may be present following aqueous (aq) extraction in the presence of sulfur dioxide. The grape anthocyanins are usually either monoglycerides or diglycosides.[46] The Food Chemicals Codex states the primary color components of grape skin extract are anthocyanins, such as the glucosides of malvidin, peonidin, petunidin, delphinidin, or cyanidin. Food-grade grape skin extract is to contain no more than 1 mg/kg arsenic and no more than 5 mg/kg lead.

Preparation/Extraction

Vitis vinifera (grape) fruit extract. A product information sheet submitted by industry on a mixture that contains *vitis vinifera* (grape) fruit extract states that the solvent of extraction is glycerin.[22] The resulting composition of the mixture is 75% to 100% glycerin, 50% to 75% *vitis vinifera* (grape) fruit extract, and 10% to 25% water, and the ratio of extract to botanical is 2:1. Potassium sorbate and sodium benzoate, 0.3% each, are used as preservatives. The extract is filtered clear after preparation.

Vitis vinifera (grape) leaf extract. A product information sheet submitted by industry on a mixture that contains *vitis vinifera* (grape) leaf extract states that the solvent of extraction for this product is also glycerin.[23] The resulting composition of the mixture is 75% to 100% glycerin, 10% to 25% water, and 5% to 10% *vitis vinifera* (grape) leaf extract. As mentioned earlier, potassium sorbate and sodium benzoate, 0.3% each, are used as preservatives, and the extract is filtered clear after preparation.

Another source reported the extraction of grape leaves with a propylene glycol solution.[34] The composition of this extract was not provided.

Vitis vinifera (grape) seed extract. One manufacturer reported that *vitis vinifera* (grape) seed extract is prepared as a concentrated extract by separating the seeds from the fruit, cleaning and comminuting the seeds, extracting with alcohol, and then filtering the

Table 5. Additional Constituent Data.

Plant part not specified
- Polyphenols
  - Cinnamic acids: coumaric, caffeic, ferulic, chlorogenic, and neochlorogenic acid[30]
  - Benzoic acids: p-hydroxybenzoic acid, protocatechuic, vanillic, and gallic acid[30]
- Trans-resveratrol (trans-3,5,40-trihydroxystilbene)[32]

Fruit
- Polyphenols
  - Flavones: quercetin (traces) and quercitrin; quercetin-, kaempferol-, and myricetin-3-monoglucoside; quercetin-glucuronoside; astilbin; and engeletin.[33]
  - Catechins: catechin; epicatechin, gallocatechin, and epicatechingallage.[33]
  - Anthocyanins: delphinidin-, petunidin-, malvidin- (41.2%), cyanidin-, and peonidin-3-monoglucosides;[33] 3-glucosides; 3-acetylglucosides; 3-coumaroylglucosides; 3-caffeoylglucosides; 3,5-diglucosides; 3-acetyl-5-diglucosides; 3-coumaroyl-5-diglucosides; and 3-caffeoyl-5-diglucosides of cyanidin, delphinidin, peonidin, petunidin, and malvidin.[32]
  - Procyanidins: procyanidin B_1, B_2, B_3, B_4, B_5, B_7, B_8;[33] acylated procyanidins that are esters of gallic acid; 14 dimeric, 11 trimeric, and 1 tetrameric procyanidin.[32]
- α-Hydroxy acids: tartaric, citric, and malic acids[33]
- Esters: containing cinnamic and tartaric acids[33]
- Aldehydes: vanillin; protocatechuic; cinnamic; and coniferyl aldehydes[33]
- Vitamins: C, B group, PP[33]
- Carotene[33]
- Sugars: fructose, glucose[33]
- Polysaccharides: containing galactose, mannose, arabinose, rhamnose, and galacturonic acid[33]
- Proteins[33]
- Volatile constituents[33]
- Waxes[33]
- Pectin[33]

Seeds
- Polyphenols (5-8 by wt%;[30] 60%-70% of grape polyphenols are found in grape seeds;[32] they are flavan-3-ol derivatives)
  - Catechins: (+)-catchins; (−)-epicatechin; (−)-epicatechin-3-O-gallate.[32]
  - Procyanidins: procyanidin B_1, B_2, B_3, B_4, B_5, B_7, B_8[33]; procyanidins C1; procyanidins B5-3′-gallate.[32]
  - Proanthocyanidins (mostly hexamers).[32]
  - Flavonoids (4%-5%): kaemperferol-3-O-glucosides; quercetin-3-O-glucosides; quercetin; myricetin.[32]
- Proteins (7%-10%): containing arginine, cystine, leucine (11.4%), valine, phenylalanine[33]
- Triglycerides (6%-20%): containing palmitic, stearic, oleic (37%), and linoleic (55%) acids[33]
- Unsaponifiables (0.5%-1%): phytosterols: b-sitosterol[33]
- Phospholipids: phosphatidylserine, phosphatidylinositol, lecithin, cephalin, cerebrosides, and phosphatidic acid[33]
- Vitamin E[33]

Leaves
- Polyphenols
  - Anthocyanins,[33]
  - catechins: catechin; epicatechin; gallocatechin; epicatechin-3-O-gallate,[33]
  - ellagitannins: brevilagin-1; vitilagin; and isovitilagin,[33]
  - flavones: traces of quercitrin, quercetin, kaempferol, rutin, iso-quercitrin, and luteolin.[33]
- Organic acids: tartaric, malic, oxalic, fumaric, succinic, citric, and glyceric acids[33]
- Phenol acids: o- and p-hydroxybenzoic acid; protocatechuic, gallic, vanillic, syringic, and ellargic acids[33]
- Esters: containing cinnamic acids and tartaric acid[33]
- Vitamins: C, PP, B group, folic acid[33]
- Carotenoids[33]
- Volatile constituents[33]
- Waxes[33]
- Proteins[33]
- Mineral salts (5%-7%)[33]

extract.[24] The filtrate is concentrated by distillation and then spray-dried. The ratio of fresh plant material to extract is 133:1.

The USP-grade grape seed oligomeric proanthocyanidins (dietary supplement) is a fraction of an extract of ripe *vitis vinifera* seeds.[51] The extract is prepared using alcohol, methanol, acetone, ethyl acetate, water, or mixtures of these solvents. The extract is then further enriched in oligomeric proanthocyanidins by fractionation with ethyl acetate or by other means.

Vitis vinifera (grape) skin extract. Grape skin extract (enocianina), the FDA-approved color additive, is prepared by the aq extraction (steeping) of the fresh deseeded marc remaining after

Table 6. Toxicity Information on Some Components of *Vitis vinifera* (Grape).

Component	Toxicity information	Reference
Polyphenol		
Resveratrol	In rats given daily oral administration of resveratrol (300, 1000, and 3000 mg/kg for 28 days), nephrotoxicity, and other signs of toxicity were observed at the high-dose level, dehydration and loss of body wt were observed at the mid-dose level, and the NOAEL was 300 mg/kg/d; in several mammary cancer cell lines, resveratrol showed mixed estrogen agonist/antagonist activities, whereas in the presence of 17β-estradiol, it was an antiestrogen; progesterone receptor (PR) protein expression was induced with the compound alone, but when combined with estradiol, the expression was suppressed; exhibited estradiol antagonist activity for estrogen receptor (ER)-α with select estrogen response elements and no such activity with ER-β; in vivo, resveratrol was not an agonist at the ER; when resveratrol and 17β-estradiol were administered in combination, a synergistic effect was observed; oral or subcutaneous (sc) administration of trans-resveratrol produced no estrogenic response in the uterine tissue of the animals; trans-resveratrol was not mutagenic in an Ames test, induced dose-dependent chromosome aberrations in the Chinese hamster lung, and induced micronuclei, polynuclei, and karyorrhectic cells in an SCE assay	[34]
	Not an ocular or dermal irritant in rabbits; not a sensitizer in a local lymph node assay (≤25%, w/v in dimethylformamide); not mutagenic in an Ames test, was clastogenic in a chromosomal aberrations assay in human lymphocytes, nongenotoxic in an in vivo bone marrow micronucleus test in rats, not adverse effect in rats in repeated dose studies (up to 90 days with up to 700 mg/kg bw/d); 750 mg/kg bw/d was not embryotoxic in rats; readily absorbed, metabolized, and excreted in rats	[36]
	Concentrations of 1 nmol/L to 100 μmol/L trans-resveratrol in DMSO, evaluated in a yeast estrogen screen, did not have estrogenic activity at any of the concentrations tested; when the same concentrations were measured for estrogenic activity in CHO-K1 cells, concentration-dependent ERα and ERβ agonist activity was observed and ERβ showed greater activation; compared to estradiol, resveratrol had weaker activity, and the agonist activity was inhibited by 4-hydroxytamoxifen	[37]
Anthocyanins	Do not appear to be readily absorbed or metabolized; low acute oral toxicity; weight-of-evidence analysis indicates anthocyanins are not genotoxic	[38]
Carotenoids	No evidence of adverse biological activity	[39]
Lutein/esters	Single-dose, 4-week, and 13-week oral studies found no evidence of toxicity	[39]
Chlorogenic acid	An antioxidant that inhibited tumor promotion by phorbol esters in mice; some controversy exists over allergic reactions in green coffee beans, but it was accepted that chlorogenic acid was not the allergen	[39]
	In mice, 2% (20 000 ppm) chlorogenic acid in the diet for 96 weeks induced papillomas and carcinomas of the forestomach, alveolar type II-cell tumors of the lung, and renal cell adenomas; few toxic effects resulted from acute exposure; subchronic dietary exposures did not induce clinical symptoms of toxicity, however, reduced kidney and adrenal wts and hyperplasia of the forestomach were observed; some genotoxic effects seen in vitro but not in vivo	[40]
Coumarin	*Limited evidence* in experimental animals for carcinogenicity; *not classifiable as to its carcinogenicity in humans* (IARC)	[41]
Flavonoids	Epidemiological studies implicated high dietary intake levels of flavonoids in heart disease, but a study of cancer risk failed to find a link; some evidence of genotoxicity in bacterial assays, but a European Organization of Cosmetic Ingredients Industries and Services (UNITIS) report stated that flavonoids do not appear to be genotoxic to mammals in vivo; flavonoids are not considered allergens	[39]
Quercetin	Genotoxic in vitro but not in vivo; some evidence for carcinogenicity (renal tumors) was found in one of the several studies, in 1 species (rat), in 1 gender (male); antioxidant properties noted; estrogenic properties, similar to other flavonoids, were noted; overall conclusion by the Council of Europe Committee of Experts on Cosmetic Products was that quercetin did not present potential risks for human health, but that skin effects and dermal penetration data were needed to complete a toxicological profile; a weight of evidence approach supported a finding that at estimated dietary levels of as a dietary supplement (200-1200 mg/d), adverse health effects would not be produced; reduced histamine release from antigen-induced human basophil cells	[39]
	Quercetin alone, 100 μmol/L, increased the spontaneous number of SCEs in human lymphocytes; however, 50 and 100 μmol/L inhibited mitomycin C (MMC)-induced SCEs in a dose-dependent manner	[31]
(+)-Catechin; (-)-epicatechin	No effect on SCEs in human lymphocytes in the presence or absence of MCC	[31]
Kaempferol	Increased the frequency of SCEs in cultured hamster cells; shown to mutate and transform human and mouse cells in culture	[42]
Monoterpenes	These chemicals may be skin irritants	[39]
Phenolic acids		
Caffeic acid	In an MMC-induced SCE assay in human lymphocytes, 100 μmol/L caffeic acid enhanced MMC-induced SCEs by 55%; 100 μmol/L caffeic acid alone enhanced MMC-induced SCEs by 26%	[31]

(continued)

Table 6. (continued)

Component	Toxicity information	Reference
	Caffeic acid is reported to penetrate skin and have UV photoprotective activity; an IARC report stated that there was sufficient evidence for carcinogenicity in animals, but no data on carcinogenicity in humans—caffeic acid was possibly carcinogenic to humans	39,43
	The carcinogenic potency of caffeic acid, estimated based on an average human intake of 1 mg/kg bw/d, was less than 1000 cancer cases per 1 000 000 individuals; in rats 1% or 2% (10 000 or 20 000 ppm) caffeic acid in the diet for 51 weeks to 2 years induced papillomas of the forestomach and renal adenomas; 1 study in which rats were exposed to 2% (20 000 ppm) caffeic acid in the diet for 2 years showed treatment-induced carcinomas of the forestomach, whereas 2 studies with shorter exposure durations showed no such effect; caffeic acid was shown to exert strong promotion activity for forestomach carcinogenesis; chronic exposure to caffeic acid in the diet induced hyperplasia of the forestomach (mice, rats, and hamsters), hyperplasia of the kidney (mice and rats), and increased liver and kidney wts (rats); few toxic effects resulted from acute exposure; subchronic dietary exposures did not induce clinical symptoms of toxicity; however, hyperplasia of the forestomach was observed; some genotoxic effects seen in vitro but not in vivo	41
Ferulic acid	In an SCE assay, ferulic acid did not affect SCEs in the presence or absence of MMC	31
	This acid is reported to penetrate skin and have UV photoprotective activity	39
Phytosterols	Oral studies demonstrate that phytosterols and phytosterol esters are not significantly absorbed and do not result in systemic exposure; small amounts did appear in the ovaries; well-defined phytosterols and phytosterol esters are not estrogenic and do not pose a hazard to reproduction; phytosterols were not mutagenic in bacterial and mammalian systems	44
Tannins	IARC has concluded that tannins are not classifiable to their carcinogenicity	45
Leucocyanidin	Without stating any details, a review source stated this substance has been reported to be toxic to some laboratory animals; symptoms included cardiac failure and hepatic lesions	46
Terpene alcohols		
Noncyclic		
Citronellol	Percutaneous absorption, 954 $\mu g/cm^2/h$ through human cadaver skin; ocular irritant in rabbit eyes (undiluted)	47
D,L-Citronellol	Dermal LD_{50} in rabbits, 2650 mg/kg; oral LD_{50} in rats, 3450 mg/kg; dietary NOAEL in rats in a 12-week study, 50 mg/kg bw/d; inhalation NOAEC in rats in a 100 day inhalation study, 0.3 mg/m^3; not mutagenic in an Ames assay with activation, a rec-assay, or a host-mediated assay; undiluted, dermal irritant in guinea pigs and rabbits in most tests; mostly not an irritant in clinical testing at up to 40%, irritation was reported in a study at 32% in acetone; not a sensitizer in a Buehler (2.5%-25%) or maximization (max) test (10%) in guinea pigs, positive reaction at 50% (but not $\leq$25% in mice; not a sensitizer in an HRIPT at 25%	47
Geraniol	Dermal LD_{50} in rabbits, >5000 mg/kg; oral LD_{50} in rats, 3600 mg/kg; no adverse effects in rats in dietary studies with $\leq$1000 mg/kg bw/d for up to 16 weeks and with 100 mg/kg bw/d for 27 weeks; not mutagenic in an Ames test or rec-assay, equivocal results with regard to polyploidy in 1 chromosome aberration test at up to 0.125 mg/mL in DMSO and inconclusive results in another at up to 156.3 $\mu g/mL$, and not genotoxic in a bone marrow micronucleus assay; undiluted was a dermal irritant in rabbits in most single application tests and a primary irritation study and 30% and 100% in ethanol caused irritation in a primary irritation study in guinea pigs; mixed irritation results in clinical studies, but generally <10% was not irritating; ocular irritant in rabbit eyes (12.5% and undiluted); mixed results in LLNA assays, but mostly sensitizing at 30 and 50, and mixed results in guinea pig sensitization studies, with both positive and negative results at 10%; not a sensitizer in multiple HRIPTs at 2%-12.5%, 20 positive reactions in a max study at 5% in petrolatum in 25 subjects, 2 positive reactions in a modified Draize test at 10% in alcohol in 73% volunteers, not a sensitizer in other clinical max studies with 5%-6% in petrolatum not phototoxic at 5% in petrolatum in clinical testing	47
Nerol	Dermal LD_{50} in rabbits, >5000 mg/kg; oral LD_{50} in rats, 4500 mg/kg; some erythema ($+$rxn in 2 and $\pm$rxn in 8/314 subjects) with up to 0.5%; ocular irritant in rabbit eyes (undiluted); not a sensitizer in guinea pigs at up to 4%; not a sensitizer at 4% in petrolatum in a clinical max study	47
Cyclic		
α-Terpineol	Oral LD_{50} in mice, 2830 mg/kg; 1000 mg/kg bw/d for 2 weeks caused reduced body wt gains and an increase in serum cholesterol; not mutagenic in an Ames test or mouse lymphoma assay; did not induce pulmonary tumors in mice given ip injections; a derma irritant in animals studies, but not a dermal irritant in a 4-hour clinical study; not a sensitizer in guinea pigs; in clinical patch tests, 5% in petrolatum had 1/1606 positive and 11/1606 questionable reactions in 1 study and 2/1200 positive reactions in another	47
Triterpene alcohols	Hepatoprotective and anticarcinogenic activities have been suggested for lupeol; no toxicity data were available; triterpene alcohols were considered to have intermediate risk	39

Abbreviations: DMSO, dimethyl sulfoxide; IARC, International Agency for Research on Cancer; ip, intraperitoneal; LD_{50}, median lethal dose; NOAEL, no-observed adverse effect level; SCE, sister chromatid exchange; UV, ultraviolet; wt, weight.

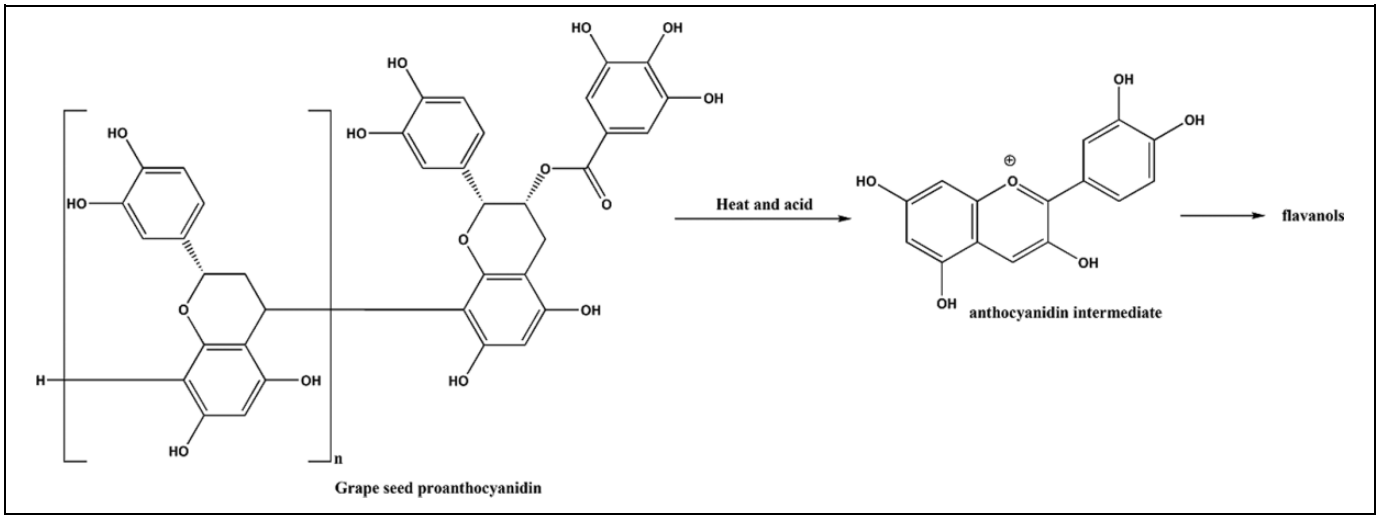

Figure 1. Grape seed acid proanthocyanidin.

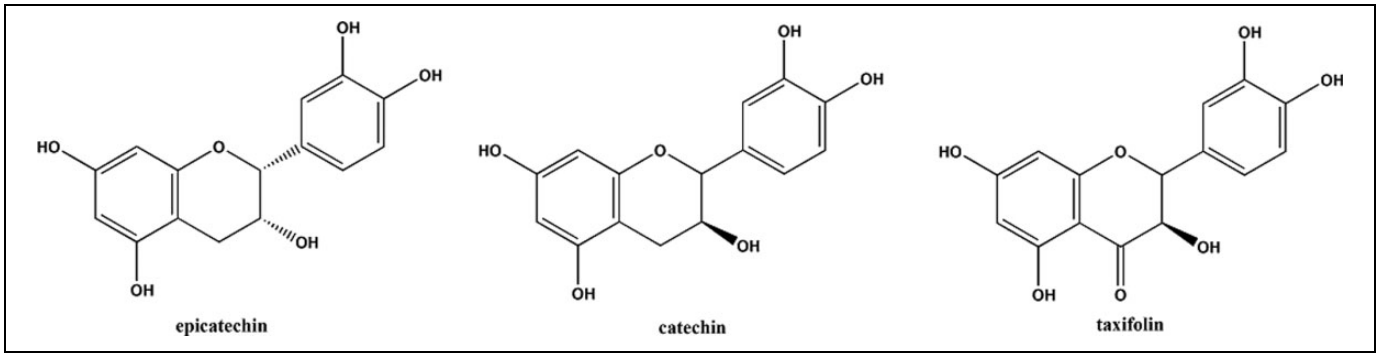

Figure 2. Primary flavanols in grape seeds.

grapes have been pressed to produce grape juice or wine (21CFR73.170). During the steeping process, sulfur dioxide is added and most of the extracted sugars are fermented to alcohol. The extract is concentrated by vacuum evaporation, during which practically all of the alcohol is removed.

Use

Cosmetic

The *vitis vinifera* (grape)-derived ingredients included in this safety assessment are reported to have many possible functions in cosmetic formulations. *Vitis vinifera* (grape) seed extract is reported to function as an anticaries agent, antidandruff agent, antifungal agent, antimicrobial agent, antioxidant, flavoring agent, light stabilizer, oral care agent, oral health care drug, and sunscreen agent.[53] Many of the other *vitis vinifera* (grape) ingredients are reported to function as skin-conditioning agents, and a few are reported to function as antioxidants. Five ingredients—the seed extract, the fruit powder, the juice, the juice extract, and the skin extract—are reported to function as flavoring agents and 4 of those 5 (all except the seed extract), as well as the skin powder, are reported to function as colorants. The *International Cosmetic Ingredient Dictionary and Handbook* does not list the functions for *vitis vinifera* (Grape) and *vitis vinifera* (grape) leaf wax. A list of all the reported functions for each ingredient is provided (Table 1).

The FDA collects information from manufacturers on the use of individual ingredients in cosmetics as a function of cosmetic product category in its Voluntary Cosmetic Registration Program (VCRP). The VCRP data obtained from the FDA in 2012 indicate that *vitis vinifera* (grape) seed extract is used in 495 cosmetic formulations, *vitis vinifera* (grape) fruit extract is used in 238 cosmetic formulations, and *vitis vinifera* (grape) leaf extract is used in 80 cosmetic formulations.[54] The other in-use *vitis vinifera* (grape)-derived ingredients are used in less than 15 formulations, and no uses were reported for 11 other *vitis vinifera* (grape)-derived ingredients.

The *vitis vinifera* (grape)-derived ingredients are used at relatively low concentrations in cosmetic formulations. *Vitis vinifera* (grape) leaf extract is included at up to 3% in leave-on formulations (perfumes); *vitis vinifera* (grape) fruit extract and *vitis vinifera* (grape) juice are included at up to 2% in rinse-off skin cleansing products and paste masks and mud packs,

Table 7. Frequency and Concentration of Use According to Duration and Type of Exposure.

	Vitis vinifera (grape)		*Vitis vinifera* (grape) bud extract		*Vitis vinifera* (grape) fruit extract	
	# of uses[54]	Max conc of use, %[55]	# of uses[54]	Max conc of use, %[55]	# of uses[54]	Max conc of use, %[55]
Totals[a]	4	0.1	NR	0.08	238	0.000001-2
Duration of use						
Leave-On	3	NR	NR	NR	195	0.00001-0.7
Rinse-off	1	0.1	NR	0.08	41	0.000001-2
Diluted for (bath) use	NR	NR	NR	NR	2	0.05
Exposure type						
Eye area	NR	NR	NR	NR	21	0.002-0.6
Incidental ingestion	NR	NR	NR	NR	13	0.0005-0.6
Incidental inhalation—spray	NR	NR	NR	NR	1	0.0001[b]-0.05
Incidental inhalation—powder	NR	NR	NR	NR	1	0.00005-0.002
Dermal contact	3	0.1	NR	NR	209	0.000001-2
Deodorant (underarm)	NR	NR	NR	NR	1[c]	NR
Hair—noncoloring	1	NR	NR	0.08	12	0.0005-0.3
Hair—coloring	NR	NR	NR	NR	4	0.002-0.3
Nail	NR	NR	NR	NR	NR	0.00001-0.00007
Mucous membrane	NR	0.1	NR	NR	20	0.000002-0.6
Baby products	NR	NR	NR	NR	NR	0.00001
	Vitis vinifera (grape) fruit powder		*Vitis vinifera* (grape) fruit water		Vitis Vinifera (grape) juice	
	# of uses[54]	Max conc of use, %[55]	# of uses[54]	Max conc of use, %[55]	# of uses[54]	Max conc of use, %[55]
Totals[a]	2	NR	10	0.7-0.8	9	0.01-2
Duration of use						
Leave-On	NR	NR	9	0.7-0.8	7	0.01-0.2
Rinse-off	NR	NR	1	NR	2	2
Diluted for (bath) use	2	NR	NR	NR	NR	NR
Exposure type						
Eye area	NR	NR	NR	NR	1	NR
Incidental ingestion	NR	NR	NR	0.8	NR	NR
Incidental inhalation—spray	NR	NR	1	NR	NR	NR
Incidental inhalation—powder	NR	NR	NR	0.7	NR	0.01
Dermal contact	2	NR	10	0.7	9	0.01-2
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair—noncoloring	NR	NR	NR	NR	NR	NR
Hair—coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous membrane	2	NR	NR	0.8	NR	NR
Baby products	NR	NR	NR	NR	NR	NR
	Vitis vinifera (grape) juice extract		*Vitis vinifera* (grape) leaf extract		*Vitis Vinifera* (grape) seed	
	# of uses[54]	Max conc of use, %[55]	# of uses[54]	Max conc of use, %[55]	# of uses[54]	Max conc of use, %[55]
Totals[a]	7	NR	80	0.01-3	3	0.05-0.08
Duration of use						
Leave-on	1	NR	60	0.01-3	1	0.05-0.08
Rinse-off	6	NR	17	NR	1	NR
Diluted for (bath) use	NR	NR	3	NR	1	NR
Exposure type						
Eye area	NR	NR	3	NR	NR	NR
Incidental ingestion	NR	NR	NR	0.02	NR	NR
Incidental inhalation—spray	NR	NR	5[b]	3	NR	NR
Incidental inhalation—powder	NR	NR	NR	NR	NR	NR
Dermal contact	1	NR	74	0.01-3	3	0.05-0.08
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair—noncoloring	5	NR	6	NR	NR	NR
Hair—coloring	1	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR

(continued)

Table 7. (continued)

	Vitis vinifera (grape)		*Vitis vinifera* (grape) bud extract		*Vitis vinifera* (grape) fruit extract	
	# of uses[54]	Max conc of use, %[55]	# of uses[54]	Max conc of use, %[55]	# of uses[54]	Max conc of use, %[55]
Mucous membrane	NR	NR	10	0.02	1	NR
Baby products	NR	NR	NR	NR	NR	NR
	Vitis vinifera (grape) seed extract		*Vitis vinifera* (grape) seed powder		*Vitis vinifera* (grape) shoot extract	
	# of uses[54]	Max conc of use, %[55]	# of uses[54]	Max conc of use, %[55]	# of uses[54]	Max conc of use, %[2]
Totals[a]	495	0.00002-0.2	1	NR	NR	0.00005-0.003
Duration of use						
Leave-on	369	0.00002-0.2	1	NR	NR	0.00005
Rinse-off	118	0.00008-0.1	NR	NR	NR	0.003
Diluted for (bath) use	8	0.002-0.003	NR	NR	NR	NR
Exposure type						
Eye area	19	0.0002-0.09	NR	NR	NR	NR
Incidental ingestion	18	0.0002	NR	NR	NR	NR
Incidental inhalation—spray	28[b]	Pump spray: 0.00002 0.0002-0.02	NR	NR	NR	NR
Incidental inhalation—powder	4	0.0002	NR	NR	NR	0.00005
Dermal contact	411	0.0002-0.2	1	NR	NR	0.003
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair—noncoloring	62	0.00002-0.1	NR	NR	NR	NR
Hair—coloring	1	NR	NR	NR	NR	NR
Nail	1	0.001	NR	NR	NR	NR
Mucous membrane	60	0.0002-0.02	NR	NR	NR	0.003
Baby products	NR	NR	NR	NR	NR	NR
	Vitis vinifera (grape) vine extract					
	# of uses[54]	Max conc of use, %[55]				
Totals[a]	11	0.004				
Duration of use						
Leave-on	10	0.004				
Rinse-off	1	NR				
Diluted for (bath) use	NR	NR				
Exposure type						
Eye area	2	NR				
Incidental ingestion	NR	NR				
Incidental inhalation—spray	NR	NR				
Incidental inhalation—powder	NR	NR				
Dermal contact	10	0.004				
Deodorant (underarm)	NR	NR				
Hair—noncoloring	1	NR				
Hair—coloring	NR	NR				
Nail	NR	NR				
Mucous membrane	NR	NR				
Baby products	NR	NR				

Abbreviations: max conc, maximum concentration; NR, not reported.
[a]Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal to the sum of total uses.
[b]Includes suntan preparations, and it is not known whether or not those product are sprays.
[c]It is not known whether or not this product is a pump or a spray.

respectively.[55] All others are used at <1% in formulation. Although no reported uses were received in the VCRP for *vitis vinifera* (grape) shoot extract, use concentration data were provided in the industry survey. Thus, it should be presumed that *vitis vinifera* (grape) shoot extract is used in at least 2 cosmetic formulations.

Frequency and concentration of use data categorized by exposure and duration of use are provided in Table 7, and the ingredients for which no uses are reported are listed in Table 8.

Various products containing *vitis vinifera* (grape)-derived ingredients may be applied to the eye area or mucous membranes or could be incidentally ingested. Additionally, *vitis vinifera* (grape) fruit extract, *vitis vinifera* (grape) fruit water, *vitis vinifera* (grape) juice, *vitis vinifera* (grape) leaf extract,

Table 8. Ingredient Not Reported to be Used.

Vitis vinifera (grape) flower extract
Vitis vinifera (grape) leaf oil
Vitis vinifera (grape) leaf/seed/skin extract
Vitis vinifera (grape) leaf water
Vitis vinifera (grape) leaf wax
Vitis vinifera (grape) root extract
Vitis vinifera (grape) skin extract
Vitis vinifera (grape) skin powder
Vitis vinifera (grape) vine sap
Hydrolyzed grape fruit
Hydrolyzed grape skin

and *vitis vinifera* (grape) seed extract are used in cosmetic products that could possibly be inhaled; concentrations of use for ingredients used in products that could be inhaled range from 0.00002% *vitis vinifera* (grape) seed extract in pump hairsprays to 3% *vitis vinifera* (grape) leaf extract in perfumes. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μm.[56-59] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (ie, they would not enter the lungs) in any appreciable amount.[56,59]

All *vitis vinifera* (grape)-derived ingredients named in this safety assessment, with the exception of hydrolyzed grape skin, are listed in the European Union inventory of cosmetic ingredients.[60]

Noncosmetic

As given in the Code for Federal Regulations (21CFR101, subpart C), grapes are among the 20 most frequently consumed raw fruit and are subject to regulation by the Food and Drug Administration (FDA) as foods.

Vitis vinifera (grape) seed extract. Grape seed extracts are used as nutritional supplements.[49]

Vitis vinifera (grape) skin extract. Grape skin extract (enocianina) is a food color additive exempt from batch certification that can be used for coloring only still and carbonated drinks and ades, beverage bases, and, with restrictions, alcoholic bases (21CFR73.170). According to the evaluation of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the acceptable daily intake (ADI) of grape skin extract is 0 to 2.5 mg/kg bw.[61]

Toxicokinetics

It has been reported that most phenolic compounds in grapes are readily metabolized by the gut flora, producing metabolites that potentially can be absorbed into the bloodstream by passive diffusion or active transport systems.[62] A number of factors may play a role in the bioavailability of polyphenols, but maximum plasma values are generally reached between 5 minutes and 2 hours after administration. Oligomeric procyanidins and other higher molecular-weight phenols are not appreciably absorbed, but they can release monomer and dimer units and epicatechin that can be absorbed.

Toxicological Studies

Single Dose (Acute) Toxicity

Dermal

Vitis vinifera (grape) seed extract. The acute dermal toxicity of *vitis vinifera* (grape) seed extract (trade name ActiVin; a water-ethanol extract) was evaluated in 5 male and 5 female albino rats.[52] A single dose of 2 g/kg moistened with 0.3 mL deionized water was applied to the clipped intact dorsal skin of each animal for 24 hours, and the dose covered approximately 5% to 6% of the total body surface. The test site was covered with a gauze bandage that was secured with tape, and collars were placed on the animals to avoid ingestion. The animals were observed for 14 days. None of the animals died during the study, and there were no test material-related clinical findings, body weight changes, or findings at necropsy. Very slight to slight erythema and desquamation was observed in all animals; these dermal responses subsided in all but 3 animals by day 12. One male rat had edema from days 6 to 9. The dermal median lethal dose (LD_{50}) of *vitis vinifera* (grape) seed extract in albino rats was >2 g/kg; this dose was also the no-observed effect level (NOEL) for systemic toxicity in this dermal study.

Oral

Vitis vinifera (grape) seed extract. Five male and five female albino rats were given a single dose of 5 g/kg *vitis vinifera* (grape) seed extract (trade name ActiVin) by gavage.[52] The animals were observed for 14 days. One female died on day 1 of the study. Matting and test material around the mouth, hypoactivity, and ocular discharge were noted for some animals; all animals appeared normal by day 3. The oral LD_{50} of *vitis vinifera* (grape) seed extract in albino rats was >5 g/kg.

The acute oral toxicity of a grape seed extract (extracted in water and ethanol) containing 89.3% proanthocyanidins was determined using groups of 5 male and 5 female F344/DuCrj rats.[63] The extract was dissolved in purified water, and the animals were dosed by gavage with 0, 2, or 4 g/kg of the extract at a rate of 10 mL/kg bw. None of the animals died, and the LD_{50} of the grape seed extract was >4 g/kg.

Vitis vinifera (grape) seed/(grape) skin extract. The acute oral toxicity of a mixed grape seed and grape skin extract (extracted in ethanol) containing 76% total polyphenols was determined in a litmus test using female Wistar rats.[62] Three rats were given a single oral dose by gavage of 5 g/kg in saline at a rate of 10 mL/kg. Three negative control rats were dosed with saline only. There were no signs of toxicity for up to 14 days after dosing, and no gross lesions were observed at necropsy. The LD_{50} of the mixed grape seed/skin extract was >5 g/kg.

Table 9. Repeated Dose Toxicity Studies.

Ingredient	Extraction solvent	Animals/group	Study duration	Dose/concentration	Results	Reference
Dietary						
Vitis vinifera (grape) seed extract						
Grape seed extract containing 89.3% proanthocyanidin	Not specified	SKH-1 hairless mice, 20F	3 weeks	0%, 0.2%, or 0.5%	- No significant difference in body weights or other signs of toxicity; -no gross differences observed in the organs of treated and untreated mice.	64
As above	Water and ethanol	F344/DuCrj rats, 10M/10F	90 days	0%, 0.02%, 0.2%, or 2%	- No mortality in any of the grps; - no clinical signs of toxicity; - the few slight but statistically significant changes in organ weights noted, primarily in the 0.2% group, were not dose dependent; - no treatment-related microscopic changes were observed.	63
Grape seed extract composed of ~90.5% total phenols	Not stated	Sprague-Dawley rats, 20M/20F	90 days	0%, 0.62%, 1.25%, or 2.50%; mean test article intake was 434, 860, and 1788 mg/kg bw/d for males; 540, 1052, and 2167 mg/kg bw/d for females)	- No mortality; - a mild head tilt in 6 of the 20 female rats in the 2.5% grp; the researchers remarked that it was doubtful this observation was treatment related; - a small but statistically significant increase in feed consumption by males of the 2.5% grp from day 7 until study termination; similar increases were observed for males of the 1.25% grp, but the occurrence was at irregular intervals; - body wts and body wt gains were similar for treated and control grps; - a decrease in heart–body wt ratio in females of the 1.25% grp was not considered treatment related; - no gross or microscopic lesions were reported at necropsy; - the NOAEL was ~2150 mg/kg bw/d for female rats and 1780 mg/kg bw/d for male rats.	65
Grape seed extract that contained <5.5% catechin monomers	Water	Sprague-Dawley rats, 20M/20F	90 days	0%, 0.5%, 1.0%, or 2.0%; extract intake was 348, 642, and 1586 mg/kg bw/d for males; 469, 883, and 1928 mg/kg bw/d for females	- No mortality and no clinical signs of toxicity; - feed consumption was increased in test grps compared to controls; increases by males of the 2.0% grp reached statistical significance, with no corresponding increase in body wts or body wt gains; - no differences in organ wts between the test and control groups;	66

(continued)

Table 9. (continued)

Ingredient	Extraction solvent	Animals/group	Study duration	Dose/concentration	Results	Reference
					- differences in clinical chemistry and hematology parameters between the test and control grps were not considered to be toxicologically significant; - no test article-related gross or microscopic lesions were observed.	
Vitis vinifera (grape) seed extract (as ActiVin)	Water–ethanol	Female B6C3F$_1$; no/grp not specified	6 months	0, 100, 250, or 500 mg/kg bw/d	- No treatment-related mortality; - no significant changes in body wt or physical appearance; - no significant differences in BUN levels or ALT and CK activity between treated and control animals; - no gross or microscopic lesions were observed.	52
Vitis vinifera (grape) seed extract (as ActiVin)	Water–ethanol	male B6C3F$_1$; no/grp not specified	12 months; animals were killed at 90-day intervals	100 mg/kg bw/d	- No treatment-related mortality; - no significant changes in body weight or physical; - no significant differences in BUN levels or ALT and CK activity; - no gross or microscopic lesions; - hepatic genomic DNA fragmentation was monitored as an index of oxidative DNA damage; no significant changes were observed.	52
Vitis vinifera (grape) skin extract						
Grape skin extract containing 87.3% total phenols expressed as gallic acid equivalents	Not specified	Sprague-Dawley rats, 20M/20F	90 days	2.5%; mean test article intake was 1788 mg/kg bw/d for males and 2167 mg/kg bw/d for females	- No mortality; no clinical signs of toxicity; - small but statistically significant increase in feed consumption by treated male however, body wts and body wt gains were similar for treated and control grps; - statistically significant changes in some hematology measurements were noted at study termination, but none were considered clinically relevant; - statistically significant decrease in absolute and relative heart wt of female test animals was not considered treatment related; - no gross lesions were reported; - a common renal cortical inflammation of minimal severity, comprised predominantly of lymphocytic interstitial filtrates, was observed in 11 of the male test animals; this was stated to be a common lesion seen in	65

(continued)

Table 9. (continued)

Ingredient	Extraction solvent	Animals/group	Study duration	Dose/concentration	Results	Reference
					male rats and not considered treatment related; - the NOAEL was approximately 2150 mg/kg bw/d for female rats and 1780 mg/kg bw/d for male rats.	
Grape color extract "Grape color powder" consisting of 40% of the naturally occurring grape-color extract in a malto-dextrin carrier	–	Beagle dogs, 4M/4F	90 days	0, 7.5, or 15% (w/w) - a control grp was fed a diet containing 9% maltodextrin (w/w)	- Physical appearance and behavior were normal for all dogs; - body wt gains in the high-dose grp were statistically significantly decreased compared to the controls, while feed consumption was comparable for test and control animals; - no significant differences in absolute or relative organ wts between treated and control animals; - no significant differences in ophthalmic, clinical chemistry, hematology, or urinary parameters between the group; - no gross or microscopic lesions were noted.	[67]

Abbreviations: ALT, serum alanine aminotransferase; BUN, blood urea nitrogen; CK, serum creatinine kinase; F, female; grp, groups; M, male; NOAEL, no-observed adverse effect level; wt, weight.

Repeated Dose Toxicity

Dietary repeated dose toxicity studies are presented in Table 9.[52,63-67]

In a 3-week study in which female SKH-1 hairless mice were fed a diet containing 0%, 0.2%, or 0.5% grape seed extract containing 89.3% proanthocyanidins for 3 weeks, no treatment-related signs of toxicity were reported.[64] In 90-day dietary repeated dose studies in rats, the no-observed adverse effect levels (NOAELs) of grape seed extract and grape skin extract were approximately 2150 and 1780 mg/kg bw/d for male and female rats, respectively.[65] No toxic effects were observed in female B6C3F_1 mice after 6 months of dietary administration of up to 500 mg/kg bw/d *vitis vinifera* (grape) seed extract or in male rats fed 100 mg/kg bw/d *vitis vinifera* (grape) seed extract for 12 months.[52] Dietary administration of 7.5% or 15% of a grape color extract to Beagle dogs for 90 days resulted in a statistically significant decrease in body weight gains in the high-dose group; however, feed consumption was comparable, leading the researchers to suggest that the decrease in body weight gain was due to the lower calorific value per gram of feed supplemented with grape color extract. No other significant changes were observed.[67]

Effect on Ultraviolet-induced skin pigmentation

Vitis vinifera (grape) seed extract. The lightening effect of the oral administration of a grape seed extract (extracted in water and ethanol) containing 89.3% proanthocyanidins on ultraviolet (UV)-induced pigmentation of guinea pig skin was examined.[68] The extract did not contain resveratrol or other phenolic compounds, such as anthocyanidins and flavonols. Using a PEN-RAY lamp (UV containing UVA and UVB, peak at 366 nm), 2 areas on the backs of male and female brownish guinea pigs were irradiated 2×/week for 3 weeks with 0.9 J/cm^2 UV. One week after the final UV exposure, groups of 5 irradiated animals were fed a diet containing 1% of the grape seed extract or a standard diet for 8 weeks. The lightening effect was determined every 2 weeks by measuring the L*-value (lightness) and the melanin index at the 2 irradiated sites and an unexposed site. The L*-value was measured with a reflectance spectrophotometer, and the melanin index was calculated using these data. After 8 weeks of dosing, blood samples were taken from each animal, and the animals were then killed. Skin samples were taken from UV-irradiated and a nontreated sites and evaluated for 3,4-dihydroxyphenylalanine (DOPA)-positive melanocytes and markers of oxidative DNA damage.

There were no differences in body weights between the groups. The UV-induced skin pigmentation was reduced in the group fed grape seed extract, as indicated by the increase in L*-value and the decrease in melanin index in UV-induced pigmented skin throughout the study as compared to control values; these differences were not statistically significant. These parameters were similar for both groups in unirradiated skin. The number of DOPA-positive melanocytes in the grape seed extract group was decreased compared to the control group. The number of melanin 8-hydroxy-2′-deoxyguanosine-positive cells, melanin-Ki-67-positive cells, and melanin proliferating cell nuclear antigen-positive cells in irradiated skin also decreased in the grape skin extract group compared to controls; the decrease observed with melanin-Ki-67-positive cells was statistically significant.

Reproductive and Developmental Toxicity

Published reproductive and developmental toxicity data were not found for *vitis vinifera* (grape)-derived ingredients. A reproduction study on grape color extract is described subsequently. Information on estrogenic activity of some of the constituents of *vitis vinifera* is provided in Table 6.

Grape Color Extract

A 2-generation reproductive study on grape color extract was performed using Sprague-Dawley rats.[69] The Code of Federal Regulations (21CFR73.169) states that the color additive grape color extract is an aq solution of anthocyanin grape pigments made from Concord grapes (*Vitis labrusca*) or a dehydrated water soluble powder prepared from the aq solution. The aq solution is prepared by extracting the pigments from precipitated lees produced during the storage of Concord grape juice. It contains the common components of grape juice, namely anthocyanins, tartrates, malates, sugars, and minerals, and so on, but not in the same proportion as found in grape juice. The dehydrated water soluble powder is prepared by spray drying the aq solution containing added maltodextrin. Groups of 25 male and 25 female rats (F_0 generation) were fed diets containing 0%, 7.5%, or 15% (w/w) grape color powder or a diet containing 9% by wt maltodextrin for 3 weeks; after 3 weeks, the rats were mated within their respective groups. Female F_0 rats, which were allowed to deliver, were fed the test diets throughout mating, gestation, and lactation. Each litter (the F_1 generation) was culled to 10 pups (5 males and 5 females if possible) on day 4. On day 21 of lactation, 2 F_1 males and 2 F_1 females were selected for a subsequent 13-week study followed by a reproduction study. The F_0 parents and the remaining offspring were killed.

The selected F_1 animals were fed the same dietary levels of grape color extract as their parents. After 13 weeks of dosing, the rats were mated within their respective groups. The F_1 rats were also allowed to deliver and were fed the test diets throughout mating, gestation, and lactation. The F_2 generation litters were culled as described previously. On day 21 of lactation, all F_1 parents and F_2 pups were killed.

All animals, except 1 F_1 male of the maltodextrin group, survived until scheduled termination. Dietary administration of up to 15% grape color powder had no effect on reproductive parameters or fertility. In the F_1 animals fed the test diets for 13 weeks prior to dosing, the group mean body weight gain was statistically significantly decreased in the high-dose females. Body weights of the F_1 and F_2 pups of both test groups were statistically significantly decreased compared to controls at day

21 of lactation, and, compared to controls, the body weights of F_0 pups of the high-dose group were statistically significantly decreased on day 4, while the body weights of F_1 pups of both test groups were statistically significantly decreased at birth. The researchers suggested because there were no significant differences in food-conversion data between groups, the decrease in body-weight gain was due to the lower calorific value per gram of food of the diet supplemented with grape color powder compared with the control diet.

Statistically significant differences in several clinical chemistry parameters were observed between groups after 6 weeks of dosing; the values, which were within the normal physiological range for Sprague-Dawley rats, were comparable at the end of 13 weeks of dosing. At necropsy, absolute and relative liver weights were decreased in males and females of both test groups; absolute adrenal gland weights were decreased in males of both test groups and high-dose females; and relative thyroid gland weights were decreased in males of both test groups; the researchers stated that it was unlikely these changes were related to feeding of the test article because there were no corresponding effects on clinical chemistry values or microscopic observations. No microscopic lesions were reported in any of the neonate groups.

Genotoxicity

Genotoxicity testing on grape-derived extracts is summarized in Table 10.[31,62,63,70-76] In vitro, mixed results were reported in the genotoxicity of *vitis vinifera* (grape)-derived ingredients, but in vivo, mostly negative results were obtained (Table 10). Fractions of raw grapes demonstrated potent mutagenic activity in an Ames test,[70] and water and ethanol extracts of red and white grapes enhanced mitomycin-C (MMC)-induced sister chromatid exchanges (SCEs) in an SCE assay in human lymphocytes, but there was no effect on SCEs without MMC.[31] Grape juice was also mutagenic in vitro, as demonstrated in the Ames test.[71,72] However, grape seed extract was not mutagenic in vitro in an Ames test or chromosomal aberration assay[63] nor in vivo in the mouse micronucleus test.[63,76] A mouse micronucleus test with grape skin extract was negative.[76] In vitro, grape seed/grape skin extract was weakly mutagenic in an Ames test but not genotoxic in a chromosomal aberration assay, and the mixed extract demonstrated a statistically significant increase in micronuclei after 48 hours, but not after 72 hours.[62] (Table 6 includes information on the genotoxic potential of some of the constituents of *vitis vinifera*.)

Carcinogenicity

Oral

Vitis vinifera (grape) seed extract. In a photocarcinogenicity study (described later in this report in Table 10), a group of 20 SKH-1 hairless mice were fed a diet containing 1% grape seed extract that contained 89.3% proanthocyanidins for 30 weeks to determine whether dietary grape seed extract alone had any effect on skin tumor formation.[64] No skin tumors formed.

Tumor Promotion

The effect on tumor promotion by *vitis vinifera* has been assessed in many studies; some of these studies are summarized in Table 11.[64,74,77-83] Seed polyphenols and extracts in particular were shown to inhibit 7,12-dimethylbenz[a]anthracene (DMBA)-initiated and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-promoted tumors in mouse skin; both dermal application and dietary administration had significant inhibitory activity.[74,79,80,82,84] Dietary grape seed extract also inhibited UV-initiated, UV-promoted, or UV-initiated and promoted skin tumors in hairless mice,[64] and it inhibited the formation of azoxymethane (AOM)-induced aberrant crypt foci (ACF) in the intestines of rats.[83] Some of the studies summarized in Table 11 examined the effect of applying DMBA to mice and then later either treating the animals topically or in the diet with grape seed extract without TPA.[79,81,84] Mice did not develop tumors when dosed dermally or orally with grape seed extract after initiation with DMBA.

Irritation and Sensitization

Skin Irritation/Sensitization

Dermal irritation and sensitization data are presented in Table 12.[52,85-99] In in vitro predictive model testing, a product containing 3% *vitis vinifera* (grape) fruit extract was a nonirritant in a dermal irritection test in human skin,[85] a product containing 10% *vitis vinifera* (grape) fruit extract was non-/minimally irritating in an Epiderm MTT viability assay,[86] and hydrolyzed grape skin was nonirritating in an MTT assay.[87] In a single-dose study in NZW rabbits, *vitis vinifera* (grape) seed extract applied neat was classified as moderately irritating[52]; in a human 2-week use study, a formulation containing 0.15% *vitis vinifera* (grape) seed extract was not an irritant.[88] In an in vitro assay of prosensitizing potential, hydrolyzed grape skin did not increase the expression of the investigated markers and did not show any stimulating potential of the immune cellular response mediated by monocytes/macrophages.[89] In clinical testing, products containing up to 10% *vitis vinifera* (grape) fruit extract,[90-93] a formulation containing 0.1% *vitis vinifera* (grape) juice,[94] cosmetic formulations containing 0.5% *vitis vinifera* (grape) juice extract, and *vitis vinifera* (grape) seed extract tested at a maximum concentration of 1% in a raw material[95-99] were not irritants or sensitizers in human repeated insult patch testing (HRIPTs).

Occupational exposure. A skin prick-to-prick test was performed on vineyard workers to assess the prevalence of sensitization to grapes with occupational exposure.[100] Three groups of vineyard workers, 120/group, were tested: harvesters (group A), workers in grape selection (group B), and workers operating destemming/crushing/pressing machines (group C); a group of 120 office employees (group D) was used as a negative control group. For the test, the needle was inserted into a cleaned grape and then inserted into the skin. Normal saline was used as a negative control. Eight harvesters in group A (6.7%) and 5 grape selection workers in group B (4.2%) had positive prick-to-prick tests to grapes; an additional 15 workers in group A and 9 workers in

Table 10. Genotoxicity Studies.

Concentration/vehicle	Procedure	Test system	Results	Reference
In vitro				
Grape fruit				
Fractions of raw grapes (concentration not specified)	Ames test	*Salmonella typhimurium* TA98 and TA100, with and without metabolic activation; grapes were washed, peeled, trimmed, and seeded; 250 g sample was blended with 500 mL water and fractionated; fractions were obtained with chloroform and n-butanol (fraction 5), water (fraction 7), methanol (fraction 3), or hexane (fraction 4)	Was mutagenic in TA98 and TA100 without metabolic activation for all fractions except fraction 7	[70]
75-350 μg/mL methanolic extracts of red grapes	SCE assay; MMC induced	Human lymphocytes	Enhanced MMC-induced SCEs in a dose-dependent manner; no effect on SCEs without MMC	[41]
75-350 μg/mL water extracts of red grapes	SCE assay; MMC induced	Human lymphocytes	Statistically significant increase in MMC-induced SCEs at 300 μg/mL; no effect on SCEs without MMC	[41]
75-350 μg/mL methanolic extract of white grapes	SCE assay; MMC induced	Human lymphocytes	Enhanced MMC-induced SCEs in a dose-dependent manner; no effect on SCEs without MMC	[41]
75-350 μg/mL water extract of white grapes	SCE assay; MMC induced	Human lymphocytes	Enhanced MMC-induced SCEs in a dose-dependent manner; no effect on SCEs without MMC	[41]
Grape juice				
Grape juice fractions (genus and species not stated) from canned or bottled juice in DMSO	Ames test	*S typhimurium* TA98 and TA100, with and without metabolic activation	Marked mutagenic activity	[71]
0.25-1.0 mL commercially available white grape juice (genus and species not stated)	Ames test	*S typhimurium* TA97, TA98, TA100, TA102, TA104, and TA1530 with and without metabolic activation	Without metabolic activation, a positive mutagenic response was observed in all strains except TA102; toxicity was observed with TA102; TA104 was the most sensitive; metabolic activation did not affect response; response was not due to histidine	[72]
0.25-1.0 mL of 3 commercial brands of white grape juice (genus and species not stated)	Ames test	*S typhimurium* TA104 without metabolic activation	Positive response with all 3 brands, but there was considerable difference in the potency of the response that was not attributable to the amount of solids	[72]
0.25-1.0 mL fresh grape juice (genus and species not stated)	Ames test	*S typhimurium* TA104 without metabolic activation	Concentration-dependent mutagenic response	[72]
White grape juice (genus and species not stated)	Examined the role of phenols, quinones, and reactive oxygen species in the mutagenicity of white grape juice in the Ames test		Mutagenicity was markedly suppressed by reduced glutathione, but was not influenced by superoxide dismutase or catalase; polyphenol oxidase-mediated oxidation of grape juice	[73]

(continued)

Table 10. (continued)

Concentration/vehicle	Procedure	Test system	Results	Reference
			phenolics generates species that can induce mutations	
Grape seed extract				
19-1250 μg/plate; extracted with water and ethanol; extract contained 89.3% proanthocyanidins	Ames test	*S typhimurium* TA98 and TA100, with and without metabolic activation	Negative	63
156-5000 μg/plate; extracted with water and ethanol; extract contained 89.3% proanthocyanidins	Ames test	*S typhimurium* TA1535 and TA1537, with and without metabolic activation	Negative	63
9.4-37.5 μg/mL; extracted with water and ethanol; extract contained 89.3% proanthocyanidins	Chromosomal aberration assay	CHL cells exposed for 24-48 hours without metabolic activation	Negative	63
18.8-75 μg/mL; extracted with water and ethanol; extract contained 89.3% proanthocyanidins	Chromosomal aberration assay	CHL cells exposed for 18 hours without metabolic activation	Negative	63
18.8-300 μg/mL; extracted with water and ethanol; extract contained 89.3% proanthocyanidins	Chromosomal aberration assay	CHL cells exposed for 6 hours with metabolic activation	Negative	63
1, 4, or 20 μmol/L; extract contained 95% proanthocyanidins	Comet assay	3 murine keratinocytes cell line were pretreated with the extract	Protective effect; comet length decreased in a dose-dependent manner	74
Grape seed/grape skin extract				
50-5000 μg/plate; extracted with ethanol; extract contained 76% of total phenols	Ames test	*S typhimurium* TA1535, TA1537, TA98, and TA100, with and without metabolic activation	Weakly mutagenic	62
9.7 and 19.5 μg/mL; extracted with ethanol; extract contained 76% of total phenols	Chromosomal aberration assay	Human lymphocytes	Negative	62
Photomutagenicity—in vitro				
Grape skin				
0.001-10 mg/mL grape skin color (*Vitis vinifera* or *Vitis labrusca*) in PBS	Ames test of irradiated color: the color was irradiated with 4 black light bulbs (FL15BL-B) that emit light between 300 and 400 nm; most of the UVB was filtered; the bacterial suspension was irradiated for 30 minutes with 1.25 J/cm^2 UVA	*S typhimurium* TA98, TA100, and TA102 with and without metabolic activation	No significant increase in mutations compared to irradiated suspension with grape skin color; 10 mg/mL nonirradiated grape-skin color was not mutagenic	75
0.01-1 mg/mL grape skin color (*Vitis vinifera* or *Vitis labrusca*) in PBS	Photocytotoxicity; cell survival was measured before UVA, 1 hour after UVA, and after 1-hour UVA irradiation and 24-hour incubation	WTK-1 cells	Delayed cytotoxicity was observed with 1 mg/mL following 24-hour incubation after UVA exposure	75

(continued)

Table 10. (continued)

Concentration/vehicle	Procedure	Test system	Results	Reference
In vivo				
Grape seed extract				
0, 0.5, 1, or 2 g/kg in distilled water; extracted with water and ethanol; extract contained 89.3% proanthocyanidins	Micronucleus test	5 or 6 mice were dosed orally; dose was repeated after 24 hours	Negative	[63]
0, 0.5, 1, or 2 g/kg in 0.5% aq CMC); extract contained 90.5% total phenols by wt (genus and species not stated)	Micronucleus test	6 male mice/group were dosed by gavage at a volume of 20 mL/kg; 24-hour harvest for all doses; 48-hour harvest for 0 and 2 g/kg groups	1 high-dose animal found dead 1 hour after dosing; cytotoxic (statistically significant decrease in the PCE:NCE ratio) at the 2 g/kg—48-hour harvest; no other cytotoxic effects were observed; not clastogenic	[76]
Grape seed/grape skin extract				
2 g/kg in saline; extracted with ethanol; extract contained 76% of total phenols	Micronucleus test	6 female Wistar rats; blood samples were taken after 48 and 72 hours	Statistically significant increase in micronuclei after 48 hours, but not after 72 hours	[62]
Grape skin extract				
0, 0.5, 1, or 2 g/kg in 0.5% aq CMC; extract contained 87.3% total phenols by wt (genus and species not stated)	Micronucleus test	6 male mice/group were dosed by gavage at a volume of 20 mL/kg; 24-hour harvest for all doses; 48-hour harvest for 0 and 2 g/kg groups	No clinical signs of toxicity; not cytotoxic or clastogenic	[76]

Abbreviations: aq, aqueous; CMC, carboxymethylcellulose; DMSO, dimethyl sulfoxide; MMC, mitomycin C; PBS, phosphate-buffered saline; PCE:NCE, polychromatic erythrocyte:normochromatic erythrocyte; SCE, sister chromatid exchange; UV, ultraviolet; wt, weight.

group B had weak positive reactions that were considered negative in this study. None of the workers in the other 2 groups had positive reactions. (Workers in groups A and B had greater exposure to grapes than did workers in groups C or D.) The reported sensitization to grapes was asymptomatic; none of the employees tested had any reported history or symptoms upon exposure.

Case report. A female grape farmer presented with an eczematous dermatitis of the hand.[101] The genus and species of grape were not stated. Patch testing with a crushed bud that had not been exposed to gibberellin (a vegetable hormone she applied to the grapes), an ethanol extract of a bud, a crushed leaf, an ethanol extract of a leaf, and with gibberellin was performed using Finn chambers, as was patch testing with standard allergens and several photoallergens. The only positive reactions were to the crushed and ethanol-extracted bud preparations. Irradiation with 0.7 J/cm^2 UVA and 15 mJ/cm^2 UVB light increased the erythema and edema. The minimal response dose of UVA was >1.4 J/cm^2, and the minimal erythema dose of UVB was 45 mJ/cm^2. In similar testing of 22 farmers, a weak positive reaction to the bud and/or leaf was observed in 6 subjects. The reactions did not increase with UV irradiation and subsided within 96 hours.

Ocular Irritation

In vitro

Vitis vinifera (grape) fruit extract. In an EpiOcular assay, a product containing 3% *vitis vinifera* (grape) fruit extract was predicted to be a minimal ocular irritant.[102] The ocular irritation potential of a single sample of a blend containing 3% *vitis vinifera* (grape) fruit extract, extracted in water, was evaluated in a standard volume-dependent dose–response study using the ocular irritection test method. The irritection Draize equivalent scores ranged from 4.5 to 6.4/80 for neat samples of the product tested at volumes ranging from 25 to 125 µL.

The irritancy classification for a product containing 10% *vitis vinifera* (grape) fruit extract was nonirritating/minimal.[103] An EpiOcular MTT viability assay was performed to determine the ocular irritation potential of a product containing 10% *vitis vinifera* (grape) fruit extract that was extracted with water. The tissue samples were treated with neat test article for 16, 64, and 256 minutes. The effective time to immobilize 50% of the exposed individuals was >256 minutes.

Vitis vinifera (grape) seed extract. A product containing 0.15% *vitis vinifera* (grape) seed extract was classified as a mild ocular irritant during in vitro testing.[104] A bovine corneal opacity and permeability assay (BCOP) was performed with undiluted

Table 11. Effect on Tumor Promotion.

Test article	Dose/ Vehicle	Animals/ Group	Procedure	Results	Reference
Dermal application					
Grape					
Total extract of *Vitis vinifera* (all active ingredients of the plant); ethanolic fraction was used	5 and 10 mg/ kg	20 Swiss albino female mice	- DMBA initiation (40 μg/0.2 mL acetone); - after 2 weeks, TPA promotion (5 μg/0.2 mL acetone); - extract topically applied 1 hour prior to TPA; -applications made 2×/wk for 20 weeks.	Time of appearance of first tumor was delayed by 3 weeks (week 9 vs week 6); dose-dependent inhibition of skin tumorigenesis; the number of mice with tumors was inhibited 40%-50% and the number of tumors per mouse (tumor multiplicity) was inhibited 16%-27%	[77]
Grape seed					
Grape seed polyphenols as a lyophilized powder containing 95% (w/w) polyphenols; extracted with ethyl acetate	0, 0.5, and 1.5 mg/ mouse applied in 0.1 mL acetone	20 female SENCAR mice	- DMBA initiation (10 μg/0.1 mL acetone); 1 week after initiation: Group 1-0.1 mL acetone applied. Group 2-0.5 mg grape seed powder in acetone. Group 3-1.5 mg grape seed powder in acetone. - 30 minutes after application, TPA promotion (2 μg/0.1 mL acetone) in groups 1-3; applications were made 2×/wk for 19 weeks; Group 4-0.1 mL acetone applied; no DMBA initiation. Group 5-1.5 mg grape seed powder in acetone applied, starting 1 week after DMBA initiation, 2×/wk for 19 weeks. -no TPA promotion in groups 4 or 5.	Groups 1-3: time of appearance of the tumor in groups 2 and 3 was delayed by 1 and 2 weeks, respectively, compared to group 1; grape seed powder significantly inhibited TPA tumor promotion in a dose-dependent manner as evidenced by a reduction in tumor incidence (35% and 60% inhibition), total number of tumors (61%-83% inhibition), and tumor volume per mouse (48% and 63% decrease); tumor growth was not significantly inhibited Group 4: no skin tumors were observed when grape seed powder was evaluated as a promoter - there were no differences in wt gain between animals exposed to grape seed powder and those that were not	[84]
Grape seed polyphenolic fraction	0, 5, 10, or 20 mg in 0.4 mL acetone	20 female CD-1 mice	- DMBA initiation (50 μg/0.2 mL acetone); - 2 weeks later, grape seed was topically applied; - 20 minutes after application, TPA promotion (5.2 μg/0.2 mL acetone); - applications were made 2×/wk for 15 weeks.	Tumor incidence was inhibited by 30%, 40%, and 60% with 5, 10, or 20 mg grape pretreatment, respectively; tumor multiplicity was significantly reduced 63%, 51%, and 94%, respectively; the percentage of tumors classified as papillomas was 94%, 88%, 97%, and 100% in the 0, 5, 10, and 20 mg groups, and the remaining tumors were carcinomas	[79]
Grape seed polyphenolic fraction	0 or 20 mg in 0.4 mL acetone	10 female CD-1 mice	- DMBA initiation, as above; - 2 weeks later, acetone or grape seed extract was applied dermally 2×/wk for 15 weeks; - no TPA promotion.	No tumors were observed in animals of either group	[79]
Grape seed extract					
Grape seed extract containing 95% proanthocyanidins	0, 1, 2.5, or 5 μmol in 0.2 mL acetone	Female SENCAR mice, no. per group	- DMBA (0.1 μmol in 0.2 mL acetone) applied topically 2×/wk for 4 weeks;	DMBA alone induced dermal hyperplasia, increasing epidermal thickness by 4.6 times the normal average; grape seed	[74]

(continued)

Table 11. (continued)

Test article	Dose/ Vehicle	Animals/ Group	Procedure	Results	Reference
		not specified	- extract applied 20 minutes prior to DMBA.	extract inhibited DMBA-induced hyperplasia in a dose-dependent manner; DMBA induced mutations in the Ha-ras oncogene; the extract had a dose-dependent inhibitory effect on the number of animals with Ha-ras mutations	
Grape seed extract containing 95% proanthocyanidins	0, 1, and 2.5 μmol	Female SENCAR mice, no. per group not specified	- DMBA (0.1 μmol in 0.2 mL acetone) applied topically 2×/wk for 4 weeks; - extract applied 20 minutes prior to DMBA.	DMBA alone increased epidermal thickness 5× as well as the PCNA level; application of the extract statistically significantly inhibited both increases in a dose-dependent manner	80
Grape fruit powder/grape seed extract					
Freeze-dried grape powder (from fresh red, green, and blue-black Cal. grapes; genus/species not stated); powdered grape seed extract containing 95% proanthocyanidins	1, 2, or 4 mg each	15 female SENCAR mice	- DMBA (0.1 μmol; vol. 0.2 mL), 2×/wk for 4 weeks; - 30 minutes after DMBA application, grape test article was applied; - 5 mice/group were killed 2 days, 4 weeks, or 8 weeks after dosing; - some animals were dosed for 24 weeks.	DMBA treatment produced epidermal hyperplasia, and both grape test substances inhibited the hyperplasia; % PCNA-positive cells decreased in a dose-dependent manner, and the change was statistically significant with 4 mg topical powder for the animals killed after 24 weeks, there was clear reduction in the number of papillomas in animals dosed with 2 mg grape powder	81
Dietary administration					
Grape fruit powder					
Freeze-dried grape powder (from fresh red, green, and blue-black Cal. grapes genus/species not stated)	1%, 2%, or 5%	15 female SENCAR mice	Mice were given treated feed 2 weeks prior to DMBA for up to 12 weeks; - DMBA (0.1 μmol; vol 0.2 mL), 2×/wk for 4 weeks; - some animals were given treated feed for 24 weeks.	DMBA treatment produced epidermal hyperplasia, dietary grape powder inhibited the hyperplasia; percentage PCNA-positive cells decreased in a dose-dependent manner with treated feed, and the change was statistically significant with 2% and 5% powder in feed for 12 weeks for the animals dosed for 24 weeks, there was clear reduction in the number of papillomas in animals fed the grape powder	81
Grape seed extract					
Grape seed extract containing 95% proanthocyanidins	2% and 4% in feed	Female SENCAR mice, no. per group not specified	- Rats were fed the extract in the diet; - after 2 weeks of treated diet, DMBA (0.1 μmol in 0.2 mL acetone) applied topically 2×/wk for 4 weeks.	DMBA alone increased epidermal thickness 5× and increased the PCNA level; dietary exposure to the extract statistically significantly inhibited both increases in a dose-dependent manner	80
Grape seed extract containing 89% proanthocyanidins	0%, 0.2%, and 0.5% in feed	20 female C3H/ HeN mice	DMBA-initiation (0.4 μmol/0.2 mL acetone)	Time of appearance of first tumor was delayed by 4 weeks (0.2% group) and 10 weeks (0.5% group); tumor incidence	82

(continued)

Table 11. (continued)

Test article	Dose/ Vehicle	Animals/ Group	Procedure	Results	Reference
			- after 1 week, TPA promotion (0.01 μg/0.1 mL acetone); 2×/ wk for 27 weeks; - treated diet was started with TPA application.	decreased 20% in the 0.2% group (not statistically significant) and 35% in the 0.5% group (statistically significant; 12, 8, and 5 mice of the 0%, 0.2%, and 0.5% groups had tumors); number of tumors per group decreased by 43% (0.2% group) and 70% (0.5% group); tumor size was significantly decreased in both test groups; 20% of the mice given untreated feed developed carcinoma, while only 5% of the mice of the 0.2% group and none in the 0.5% group developed carcinoma	
As above	0.5% in feed	10 female C3H/ HeN mice	DMBA initiation as above - after 1 week, fed treated diet for 27 weeks; no TPA promotion; - a control group for spontaneous tumors was treated with 0.2 mL acetone 2×/wk.	No tumors were observed in animals of either group	[82]
As above	0.5% in feed	5 female C3H/ HeN mice	- Mice were fed treated feed; - either 1 week later, a single application of 5 μg TPA was made and the mice were killed after 6, 12, or 24 hours or TPA was applied 3× on alternate days and the mice were killed 6 hours after the last application; - skin edema was measured using skin punches and bi-fold skin thickness measurements.	- TPA caused an increase in mean epidermal thickness and vertical thickness of epidermal cell layers - grape seed extract significantly reduced the epidermal thickness after multiple TPA applications and in mice killed 12 and 24 hours after a single application of TPA - dietary extract without TPA treatment did not induce an epidermal hyperplastic response - TPA-induced increases in skin punch wt were reduced by feeding the extract; bi-fold skin thickness was also reduced	[82]
Grape seed extract containing 89.3% proanthocyanidins	0%, 0.25%, and 0.5% in feed	7 male F344 rats	Group 1: control feed for 10 weeks Group 2: control feed for 10 weeks; after 1 week, sc AOM 1×/wk for 2 weeks Group 3: 0.25% in feed for 10 weeks; after 1 week of treated feed, sc AOM 1×/wk for 2 weeks Group 4: 0.5% in feed for 10 weeks; after 1 week of treated feed, sc AOM 1×/wk for 2 weeks Group 5: sc AOM 1×/wk for 2 weeks; 4 weeks later, 0.25% in feed for 4 weeks Group 6: sc AOM 1×/wk for 2 weeks; 4 weeks later, 0.5% in feed for 4 weeks Group 7: 0.5% in feed for 10 weeks	Intestinal AOM-induced ACF were statistically significantly decreased in groups 3-6 compared to group 2—the inhibition was stronger in groups 3 and 4 (50%-60% inhibition) than in groups 5 and 6 (34%-37% inhibition); the number of ACF consisting of 1-4 crypts or >4 crypts was decreased in groups 3-6 compared to group 2; PCNA-positive cells were decreased in groups 3-6 compared to group 2, and the AOM-induced PCNA labeling index in the colonic mucosa was decreased; induction of apoptosis in groups 3-6 as evidence by a	[83]

(continued)

Table 11. (continued)

Test article	Dose/ Vehicle	Animals/ Group	Procedure	Results	Reference
				significant increase in the number of TUNEL-positive cells	
Antiphotocarcinogenesis with dietary administration					
Grape seed extract					
Grape seed extract containing 89.3% proanthocyanidins	0%, 0.2%, and 0.5% in feed	20 female SKH-1 hairless mice	- Mice were fed treated feed for 14 days; - starting on day 15, the mice were irradiated with 180 mJ/cm^2 every day for 10 days; - 1 week after the last UV exposure, mice were again irradiated with 180 mJ/cm^2 3×/wk for 29 weeks.	Latency period of tumors was increased by 2 weeks by feeding the extract; inhibition of tumor incidence was statistically significant in the 0.5% group (35% inhibition; tumor multiplicity (46 and 65% with 0.2% and 0.5%, respectively), tumor size expressed in terms of total tumor volume per group or total tumor volume per tumor bearing mouse, and avg tumor volume per tumor was significantly inhibited at both doses	64
Grape seed extract containing 89.3% proanthocyanidins	0% and 0.5% in feed	20 female SKH-1 hairless mice	Same protocol as above performed to examine effect on malignant conversion of papillomas into carcinomas	45% prevention by extract in terms of carcinoma incidence; prevention of UVB-induced transformation of benign papillomas to carcinomas was 65%, but when analyzed in terms of number carcinomas per carcinoma bearing mouse, there was no inhibition by the extract	64
Grape seed extract containing 89.3% proanthocyanidins	0% and 0.5% in feed	20 female SKH-1 hairless mice	- Mice were fed treated feed for 14 days; - starting on day 15, the mice were irradiated with 180 mJ/cm^2 every day for 10 days; - 1 week after the last UV exposure, both groups were treated topically with TPA (0.01 μmol/0.1 mL acetone); 3×/wk for 23 weeks.	Latency period of tumors was increased by 2 weeks by feeding the extract; a highly significant reduction in tumor incidence was observed (95%); between weeks 13-15 of promotion, 10%-20% of extract-fed mice developed tumors that regressed later; since these tumors were not present at the termination of the study, they were not included in tumor multiplicity and tumor multiplicity decreased by 95%; total tumor volume per group and per tumor bearing mouse was reduced	64
Grape seed extract containing 89.3% proanthocyanidins	0% and 0.5% in feed	20 female SKH-1 hairless mice	- DMBA initiation (51.2 μg/0.01 mL acetone); - after 1 week, UVB irradiation (promotion; 180 mJ/cm^2); 3×/wk for 24 weeks; - treated diet was started with UVB exposure.	Latency period of tumors was increased by 3 weeks by feeding the extract; feeding the extract resulted in a 60% reduction in the total number of tumors per group, a 74% reduction in total tumor volume per group, a 63% reduction in terms of tumor volume per tumor bearing mouse, and a 29% reduction in average tumor volume per tumor	64

Abbreviations: ACF-aberrant crypt foci; AOM, azoxymethane; avg, average; DMBA, dimethylbenz[a]anthracene; PCNA, proliferating cell nuclear antigen; sc, subcutaneous; TPA, 12-O-tetradecanoylphorbol-13-acetate; UV, ultraviolet.

Table 12. Dermal Irritation and Sensitization.

Test article	Concentration	Test pop	Procedure	Results	Reference
In vitro—irritation					
Vitis vinifera (grape) fruit extract					
3% in a sample product blend (extracted in water)	Neat; test vol, 25-125 μL	–	Dermal irritection test method, standard volume-dependent dose-response study	Predicted to be a nonirritant in human skin; human irritancy equivalent scores ranged from 0.46 to 0.61	85
Product containing 10% (extracted in water)	Neat	–	Epiderm MTT viability assay; tissue samples treated for 1, 4, and 24 hours	Nonirritating/minimal ET_{50} was >24 hours; irritancy classification	86
Hydrolyzed grape skin					
Hydrolyzed grape skin	Neat	Cultured human	keratinocytes (HaCaT cells)	MTT cytotoxicity test; 0.15-5 mg/mL were tested; SLS was used as a positive control	Predicted to be
nonirritating; the IC_{50} was >5 mg/mL	87				
IC_{50} of SLS was 0.083 mg/mL (irritating)					
Non-human—irritation					
Vitis vinifera (grape) seed extract					
As trade name ActiVin	Neat	New Zealand White rabbits; 3 M/3 F	4-hour semiocclusive application; 0.5 g of the extract moistened with 0.3 mL deionized water; applied to an intact 1 in × 1 in area of clipped skin; collars were used	Classified as moderately irritating all rabbits had slight to severe erythema, very slight to slight edema, and desquamation; erythema completely subsided by day 6, edema by day 8; exfoliation in 1 animal, eschar in 2 animals; all dermal irritation subsided by day 12	52
Human—irritation					
Vitis vinifera (grape) seed extract					
0.15% in an after shave balm (extraction solvents were butylene glycol and water)	Neat	31 male subjects	2-week in-use study; product was applied at least once daily to shave skin of the face and neck	Not an irritant; no evidence of erythema, edema, or drying	88
In vitro—sensitization					
Hydrolyzed grape skin					
Hydrolyzed grape skin in ethanol	4 and 20 μg/mL	Monocyte-like human cell line, THP-1 cells	Cells were exposed for 48 hours; CD80 and CD86 were used as costimulatory molecules; MFI was measured using a FACS; MFI of nontreated THP-1 cells was used as an internal control; nickel sulfate was used as a positive control	Did not increase the expression of the investigated markers and did not show any stimulating potential of the immune cellular response mediated by monocyte/macrophage	89
Human—irritation and sensitization					
Vitis vinifera (grape) fruit extract					
0.0239% in a foundation	Neat	103 subjects	Modified HRIPT—semiocclusive;; 0.15 mL on a 20 × 20 mm pad; nine 24-hour induction applications; 24-hour challenge application at treated and untreated sites followed a 17 or 24-day nontreatment period	Not an irritant or sensitizer	90
Blend containing 3%	Tested at 1% aq	108 subjects	HRIPT—semiocclusive; 0.02-0.05 mL on a 7.5 mm paper disc; nine 24-hour induction applications; challenge application at a	Not an irritant or sensitizer	91

(continued)

Table 12. (continued)

Test article	Concentration	Test pop	Procedure	Results	Reference
			previously untreated site after a 10-14 day nontreatment period		
Product containing 6%	10% in deionized water	97 subjects	Modified HRIPT—semiocclusive;; 150 mg on a 20 × 20 mm pad; nine 24-hour induction applications; 24-hour challenge at treated site and 48-hour challenge at untreated site followed a 10-day nontreatment period	Not an irritant or sensitizer	[92]
Product containing 10% (extracted in water)	Neat	54 subjects	HRIPT—occlusive; 0.2 mL on a 20 × 20 mm^2 Webril pad; nine 24-hour induction applications;; 24 hours challenge at a previously untreated site after a 10-14 day nontreatment period	Not an irritant or sensitizer	[93]
Vitis vinifera (grape) juice					
Make-up primer containing 0.1%	Neat	208 subjects	HRIPT—semiocclusive; same induction protocol; 24-hour challenge application applied to a previously untreated site after a 2-week nontreatment period	Not an irritant or sensitizer with the exception of an occasional ± score (barely perceptible erythema), no visible reactions were noted	[94]
Vitis vinifera (grape) juice extract					
Hair styling product containing 0.5%	Neat	100 subjects	Modified HRIPT—occlusive; 21-day induction period, 10-24 day nontreatment period, 4-day challenge	Not an irritant or sensitizer	[95]
Vitis vinifera (grape) seed extract					
Body lotion formulation containing 0.0002%	Neat	101 subjects	Modified HRIPT—occlusive; 21-day induction period, 10-24 day nontreatment period, 4-day challenge	Not an irritant or sensitizer	[96]
Hair conditioner containing 0.1%	10% aq dilution	105 subjects	Modified HRIPT—semiocclusive;; 0.2 mL on a 20 × 20 mm^2 pad; nine 24-hour induction applications, 24-hour challenge application at treated and untreated sites followed a 10-day nontreatment period	Not an irritant or sensitizer	[97]
After shave balm containing 0.15% (extraction solvents were butylene glycol and water)	Not stated; presumed neat	105 subjects	HRIPT—occlusive; 0.2 mL; air-dried at 20+ minutes prior to application; nine 24-hour induction applications; 24-hour challenge followed a 10-day nontreatment period	Not a sensitizer; no reactions at challenge during induction, 1 subject had a minimal/doubtful response at readings 2-4 and erythema (+) was observed at readings 5-8; 1 subject had a response at readings 1-2 and 1 subject had a response at reading 2	[98]
Raw material containing 1%	Neat	107 subjects	Modified HRIPT—semiocclusive;; 0.15 mL on a 20 × 20 mm^2 pad; nine 24-hour induction applications, 24-hour challenge application at treated and untreated sites followed a 10-day nontreatment period	Not an irritant or sensitizer 5 grade 1 and 1 grade 2 response noted during induction; grade 1 response were noted for 3 subjects during challenge	[99]

Abbreviations: ET50, effective time to immobilize 50% of the exposed individuals; FACS, fluorescence activated cell sorter; HRIPT, human repeated insult patch test; IC50, half maximal inhibitory concentration; MFI, mean fluorescence intensity; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; pop, population; SLS, sodium lauryl sulfate.

samples of an after shave lotion containing 0.15% *vitis vinifera* (grape) seed extract; the extract was prepared with the extraction solvents butylene glycol and water. Sterile deionized water served as the negative control and ethanol as the positive control. The in vitro score for the test article was 1.0. (Test materials with in vitro scores of 0 to 25 are classified as mild irritants.) The

positive control had an in vitro score of 43.2; test materials with in vitro scores of 25.1 to 55 are classified as moderate irritants.

Hydrolyzed grape skin. Hydrolyzed grape skin was predicted to be nonirritating to eyes in a cytotoxicity assay evaluating ocular irritation potential.[105] A neutral red uptake (NRU) assay using fibroblast cultures was performed with 0.15 to 5 mg/mL hydrolyzed grape skin. Sodium lauryl sulfate (SLS) was used as a positive control. The half maximal inhibitory concentration (IC_{50}) value (ie, the concentration of test compound that induces a 50% decrease in cell growth/survival) for hydrolyzed grape skin was >5 mg/mL. The IC_{50} value for the positive control was 0.063 mg/mL.

Nonhuman

Vitis vinifera (grape) seed extract. The ocular irritation potential of *vitis vinifera* (grape) seed extract (trade name ActiVin) was evaluated in 6 female NZW rabbits.[52] The test article, 85 mg, was instilled into the conjunctival sac of the right eye, the eyelid was held closed for 1 second, and the eye was not rinsed. The contralateral eye served as an untreated control. The eyes were scored for irritation using the Draize method at 1, 24, 48, and 72 hours and 4, 7, and 14 days after instillation of the test article. Conjunctival irritation was observed in all animals, 4 animals had iridal reactions, and 3 had corneal reactions. The irritation was reversible and completely subsided by day 14. The maximum average score (MAS) at 24 hours for *vitis vinifera* (grape) seed extract was 16.7/110.

Summary

This report addresses the safety of 24 *vitis vinifera* (grape)-derived ingredients as used in cosmetics. These ingredients are reported to have many functions in cosmetics, but the most frequently reported function is as a skin-conditioning agent. According to VCRP data obtained from the FDA, *vitis vinifera* (grape) seed extract is used in 495 cosmetic formulations, *vitis vinifera* (grape) fruit extract is used in 238 cosmetic formulations, and *vitis vinifera* (grape) leaf extract is reported to be used in 80 cosmetic formulations; 9 other *vitis vinifera*-derived ingredients are reported to be in use, and they are used in less than 15 formulations. These ingredients are used at relatively low concentrations in cosmetic formulations. For example, *vitis vinifera* (grape) leaf extract is included at up to 3% in leave-on formulations (perfumes) and *vitis vinifera* (grape) fruit extract and *vitis vinifera* (grape) juice are included at up to 2% in rinse-off skin cleansing products. All others are used at <1% in formulation.

Fruit acids and trans-resveratrol are constituents of *vitis vinifera*, and polyphenols are found in all parts of the plant. The main constituents of grape seeds are reported to be phenolic compounds, and standardized grape seed extracts are reported to contain 92% to 95% oligomeric proanthocyanidins. Grape skin extract contains anthocyanins, tartaric acid, tannins, sugars, and minerals. The oral LD_{50} values of grape seed extract and grape skin extract in rats were >4 to 5 and >5 g/kg, respectively, and the dermal LD_{50} (and NOEL for systemic toxicity) in albino rats was >2 g/kg.

In a 3-week dietary study in which female SKH-1 hairless mice were fed a diet containing 0%, 0.2%, or 0.5% grape seed extract containing 89.3% proanthocyanidins for 3 weeks, no signs of toxicity were reported. In 90-day dietary repeated dose studies in rats, the NOAELs of grape seed extract and grape skin extract were approximately 2150 and 1780 mg/kg bw/d for male and female rats, respectively. No toxic effects were observed in female $B6C3F_1$ mice after 6 months of dietary administration of up to 500 mg/kg bw/d *vitis vinifera* (grape) seed extract or in male rats fed 100 mg/kg bw/d *vitis vinifera* (grape) seed extract for 12 months. Dietary administration of 7.5% or 15% of a grape color extract to Beagle dogs for 90 days resulted in a statistically significant decrease in body weight gains in the high-dose group; however, feed consumption was comparable, leading the researchers to suggest that the decrease in body weight gain was due to the lower calorific value per gram of feed supplemented with grape color extract. No other significant changes were observed. Grape seed extract reduced UV-induced skin pigmentation in guinea pigs, but the difference was not statistically significant when compared to controls that did not receive grape skin extract.

A 2-generation reproductive study in which 7.5% or 15% grape color extract was fed in the diet was performed using Sprague-Dawley rats. The only statistically significant effects observed were decreases in the body weights of F_1 and F_2 pups of both test groups and in body weights of F_1 animals fed the test article for 30 days prior to mating; because there were no significant differences in food-conversion data between groups, the researchers suggested the decrease in body weight gain was due to the lower calorific value per gram of food of the diet supplemented with grape color powder compared with the control diet. Liver, adrenal gland, and thyroid gland weights in F_1 animals fed the test article for 30 days prior to mating were statistically significantly decreased; these changes were not attributed to the test article because there were no corresponding effects on clinical chemistry values or microscopic observations.

In vitro, mixed results were reported in the genotoxicity of *vitis vinifera* (grape)-derived ingredients but in vivo, mostly negative results were obtained. Fractions of raw grapes demonstrated potent mutagenic activity in an Ames test, and water and ethanol extracts of red and white grapes enhanced MMC-induced SCEs in an SCE assay in human lymphocytes, but there was no effect on SCEs without MMC. Grape juice was also mutagenic in vitro, as demonstrated in the Ames test. However, grape seed extract was not mutagenic in vitro in an Ames test or chromosomal aberration assay nor in vivo in the mouse micronucleus test.[63,76] A mouse micronucleus test with grape skin extract was negative. In vitro, grape seed/grape skin extract was weakly mutagenic in an Ames test but not genotoxic in chromosomal aberration assay, and the mixed extract demonstrated a statistically significant increase in micronuclei after 48 hours but not after 72 hours.

Vitis vinifera, the seed extract in particular, was shown to inhibit DMBA-initiated and TPA-promoted tumors in mouse skin; both dermal application and dietary administration had significant inhibitory activity. Dietary grape seed extract also inhibited UV-initiated, UV-promoted, or UV-initiated and promoted skin tumors in hairless mice. The formation of AOM-induced ACF in the intestines of rats was also inhibited by dietary grape seed extract. Dietary administration of 1% grape seed extract for 30 weeks did not produce skin tumors in mice, and grape seed extract and grape seed powder were not tumor promoters when applied dermally to mice following initiation with DMBA.

In in vitro predictive model testing, a product containing 3% *vitis vinifera* (grape) fruit extract was a nonirritant in a dermal irritection test in human skin, a product containing 10% *vitis vinifera* (grape) fruit extract was non-/minimally irritating in an Epiderm MTT viability assay, and hydrolyzed grape skin was nonirritating in an MTT assay. In a single-dose study in NZW rabbits, *vitis vinifera* (grape) seed extract applied neat was classified as moderately irritating; in a human 2-week use study, a formulation containing 0.15% *vitis vinifera* (grape) seed extract was not an irritant. In an in vitro assay of prosensitizing potential, hydrolyzed grape skin did not increase the expression of the investigated markers and did not show any stimulating potential of the immune cellular response mediated by monocytes/macrophages. In clinical testing, products containing up to 10% *vitis vinifera* (grape) fruit extract, a formulation containing 0.1% *vitis vinifera* (grape) juice, cosmetic formulations containing 0.5% *vitis vinifera* (grape) juice extract, and *vitis vinifera* (grape) seed extract tested at a maximum concentration of 1% in a raw material were not irritant or sensitizers in HRIPTs. Some asymptomatic sensitization reactions were seen in an occupational setting in vineyard workers who had substantial exposure to grapes. One case study was found that reported positive reactions to grape bud preparations.

Products containing 3% and 10% *vitis vinifera* (grape) fruit extract were predicted to be minimal ocular irritants in in vitro testing. In a study using rabbits, the MAS at 24 hours for *vitis vinifera* (grape) seed extract was 16.7/110. A product containing 0.15% *vitis vinifera* (grape) seed extract was classified as a mild ocular irritant during a BCOP assay, and hydrolyzed grape skin was predicting to be nonirritating to eyes in an NRU study.

Discussion

The Panel recognizes that there are data gaps for some of these *vitis vinifera*-derived ingredients. However, the overall information available on the types of products in which these ingredients are used and at what concentration indicate a pattern of use, which was considered by the Panel in assessing safety. Additionally, the Panel noted that *vitis vinifera* (grape) seed oil has previously been found safe.

Most of the irritation and sensitization testing performed on the *vitis vinifera*-derived ingredients included in this report demonstrated that these ingredients are not dermal irritants or sensitizers, with the exception of one 4-hours semiocclusive study of *vitis vinifera* (grape) seed extract that reported moderate irritation using rabbits when the test substance was applied neat. Additionally, in clinical testing with *vitis vinifera* (grape) seed extract at a maximum concentration of 1% in a raw material was not an irritant or sensitizer; the grape seed extract is reported to be used at a maximum leave-on concentration of 0.2%. Also, because all the other irritation and sensitization tests were negative, including a human study using up to 10% *vitis vinifera* (grape) fruit extract in a product, the Panel was of the opinion that the 1 study was an outlier and that the weight of evidence supports the view that these ingredients are not irritants or sensitizers.

The Panel discussed the findings of mutagenic activity of grape and grape juice in some of the bacterial mutagenicity tests. The Panel is aware that there is a history of positive Ames tests with some foods, including grape. Although positive results for mutagenicity occur in bacterial assays, based on the expertise of the Panel and information provided by the European Organization of Cosmetic Ingredients Industries and Services (UNITIS), the constituents of foods in grapes, for example, flavonoids, do not appear to be genotoxic to mammals in vivo. Additionally, *vitis vinifera*-derived extracts have demonstrated an inhibition of tumor promotion. Therefore, the mutagenic effects in bacterial systems were not considered relevant to the safety of these cosmetic ingredients.

The *vitis vinifera* plant parts contain some constituents, such as ascorbic acid, biotin, and malic acid, which are cosmetic ingredients for which separate Panel safety assessments are available. Others constituents are compounds that have been discussed in previous CIR assessments. For example, *vitis vinifera*, and therefore derived extracts, contains a variety of phytochemicals. The Panel has discussed in previous safety assessments that although some of the phytochemicals present in grapes could exert significant biological effects, the low levels in conjunction with the currently reported exposure routes and low use concentrations preclude significant effects. Also, although no dermal absorption data were available for constituents of *vitis vinifera*, extensive data are available showing that these phytosterol constituents at the potential exposure levels are not estrogenic, are not reproductive toxicants, are not genotoxic, and are not carcinogenic. The Panel also noted that 1 particular constituent that could be of concern, that is, quercetin, can be present at low levels in some components of *vitis vinifera*. However, again, because the *vitis vinifera*-derived ingredients are used at very low concentrations in cosmetics, and because the concentrations of quercetin in the plant parts are low, the presence of quercetin was below the level of toxicological concern.

The leaf extract, which is used at up to 3% in perfumes, is a highly colored component, and the Panel discussed the possibility that the leaf extract could be photoactive. The dermatologists on the Panel remarked that phototoxicity issues have not been reported in vineyard workers, and the Panel relied on this clinical experience to alleviate the concern of possible phototoxic effects of *vitis vinifera* (grape) leaf extract.

The Panel discussed the issue of incidental inhalation exposure to *vitis vinifera* (grape)-derived ingredients from products that may be aerosolized. There were no inhalation toxicity data available. *Vitis vinifera* (grape) seed extract is reportedly used at a concentration of 0.00002% in pump hairsprays and *vitis vinifera* (grape) leaf extract is reportedly used at a concentration of 3% in perfumes. The Panel noted that 95% to 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. The Panel considered the data available to characterize the potential for *vitis vinifera* (grape)-derived ingredients to cause systemic toxicity, irritation, sensitization, or other effects. They noted that *vitis vinifera* (grape)-derived ingredients did not produce systemic toxicity in oral single-dose or long-term (up to 12 months) repeated dose studies; grape color extract was not a reproductive or developmental toxicant; *vitis vinifera* (the seed extract in particular) inhibits the promotion of tumors; and the *vitis vinifera* (grape)-derived ingredients do not appear to be irritants or sensitizers. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products are available at http://www.cir-safety.org/cir-findings.

Finally, the Panel expressed concern regarding pesticide residues and heavy metals that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use the necessary procedures to limit these impurities in the ingredient before blending into cosmetic formulation.

Conclusion

The CIR Expert Panel concluded the *vitis vinifera* (grape)-derived ingredients listed subsequently are safe in the present practices of use and concentration in cosmetics.

vitis vinifera (grape);
vitis vinifera (grape) bud extract;
vitis vinifera (grape) flower extract;*
vitis vinifera (grape) fruit extract;
vitis vinifera (grape) fruit powder;
vitis vinifera (grape) fruit water;
vitis vinifera (grape) juice;
vitis vinifera (grape) juice extract;
vitis vinifera (grape) leaf extract;
vitis vinifera (grape) leaf oil;*
vitis vinifera (grape) leaf/seed/skin extract;*
vitis vinifera (grape) leaf water;*
vitis vinifera (grape) leaf wax;*
vitis vinifera (grape) root extract;*
vitis vinifera (grape) seed;
vitis vinifera (grape) seed extract;
vitis vinifera (grape) seed powder;
vitis vinifera (grape) shoot extract;
vitis vinifera (grape) skin extract;*
vitis vinifera (grape) skin powder;*
vitis vinifera (grape) vine extract;
vitis vinifera (grape) vine sap;*
Hydrolyzed grape fruit;*
Hydrolyzed grape skin.*

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

Authors' Contribution

Fiume contributed to conception, design acquisition, analysis, and interpretation, and drafted the article. L. Gill contributed to conception, design, analysis, and interpretation, critically revised the article, and gave final approval. W. Bergfeld, D. Belsito, R. Hill, C. Klaasen, D. Liebler, J. Marks, R. Shank, T. Slaga, and P. Snyder contributed to conception, design acquisition, analysis, and interpretation, critically revised the article, and gave final approval.

Authors' Note

Unpublished sources cited in this report are available from the Lillian J. Gill, Director, Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, DC 20036, USA.

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