Tentative Report

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

June 14, 2012

All interested persons are provided 60 days from the above date to comment on this Tentative Report and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. F. Alan Andersen.

The 2012 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Monice M. Fiume, Senior Scientific Analyst/Writer.

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ABSTRACT

The Expert Panel assessed the safety of 24 Vitis vinifera (grape)-derived ingredients and found them safe in the present practices of use and concentration. These ingredients are most frequently reported to function in cosmetics as skin conditioning agents. Some of these ingredients are reported to function as antioxidants, flavoring agents, and/or colorants. The Panel reviewed the available animal and clinical data to determine the safety of these ingredients. Various constituents of grapes have been assessed previously for safety as cosmetic ingredients; others are compounds that have been discussed in previous CIR safety assessments.

INTRODUCTION

This report is a safety assessment 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; they are most frequently reported to function as skin conditioning agents. Some of these ingredients are reported to function as antioxidants, flavoring agents, and/or colorants. In the Food and Drug Administration (FDA) Food Labeling regulations (21CFR101) subpart C, which addresses Specific Nutrition Labeling Requirements and Guidelines, grapes are listed as one of the 20 most frequently consumed raw fruits.

The safety of Vitis Vinifera (Grape) Seed Oil and Hydrogenated Grapeseed Oil was reviewed previously in 2011 by the Cosmetic Ingredient Review (CIR) Expert 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.[1] Consequently, these two ingredients are not included in this safety assessment.

The detailed chemical composition of *Vitis vinifera* is given later in this assessment. Some of the constituents of grape, such as ascorbic acid, biotin, malic acid, etc., are cosmetic ingredients for which a CIR safety assessment is available; others are compounds that have been discussed in previous CIR safety assessments.

Many studies have been conducted with *Vitis vinifera* (grape)-derived ingredients with regard to health claims, anti-oxidant activity, and so forth. This safety assessment only includes studies and study-types that relate directly to the safety of the cosmetic use of these ingredients.

Note: In many of the published studies, it is not known how the substance being tested compares 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…" (e.g. grape seed extract); if it is known that the substance is a cosmetic ingredient, the terminology "Vitis Vinifera (Grape)…" (e.g. 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,[2] and grapevine.[3]

Chemical and Physical Properties

Chemical and physical property data are provided in Table 2.

Composition

A detailed list of chemical constituents by plant part is presented in Table 3, and a more focused listing of constituents of *Vitis vinifera* is provided in Table 4. Table 5 provides the conclusions from CIR safety assessments that exist for some of the constituents of grape. Table 6 includes information on the toxicity of some constituents.

Grapes contain fruit acids, and the unripe fruit contains 34 ppm oxalic acid.[2,4] Grape seeds contain 6-20% oil. Phenols are the third most-abundant constituent in grapes; carbohydrates and fruit acids are the most- and second most-abundant, respectively.[5] The total extractable phenolics in grapes are present at ≤10% in the pulp, 60-70% in the seeds, and 28-35% in the skin.

The amount of a constituent present in the plant present varies with the region in which it is grown.[4] 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.[5]

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* used.[6] 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.[3] Depending on variety and location, levels of melatonin in grape skin have ranged from 0.005-1.2 ng/g. The stage of growth also affects the amount present. Recent studies have indicated that melatonin may also be present in the flesh and seeds of grapes.

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.[7]

Vitis Vinifera (Grape) Juice

A commercial brand grape juice contained 4.4 mg/L quercetin and 6.2 mg/L myricetin.[8]

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.[9]

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 92-95% oligomeric proanthocyanidins.[10] 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.[11] The most prominent grape seed extract proanthocyanidin is depicted in Figure 1.[10] 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 extracts are highly dependent on the extraction techniques used.

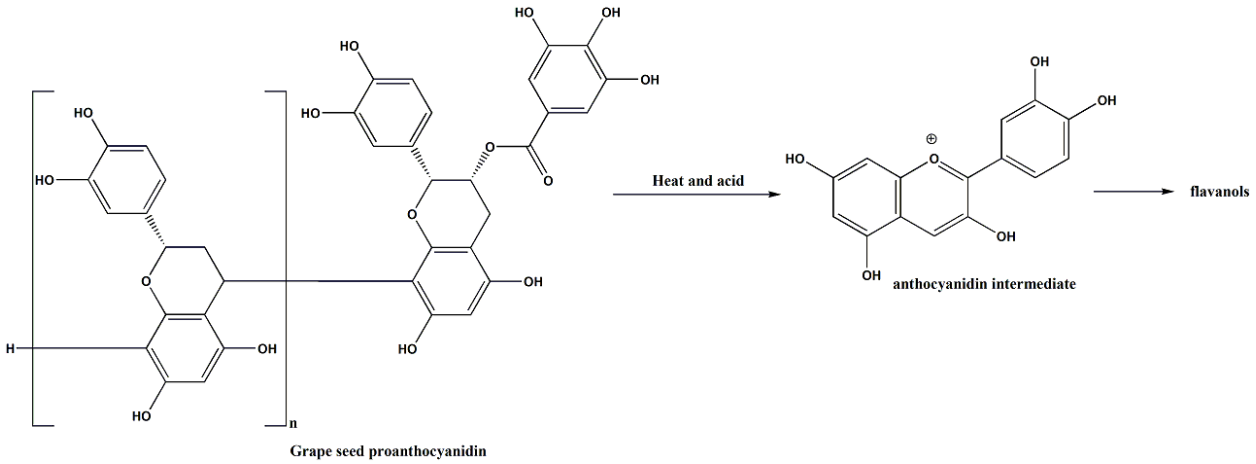

Figure 1. Grape seed acid proanthocyanidin

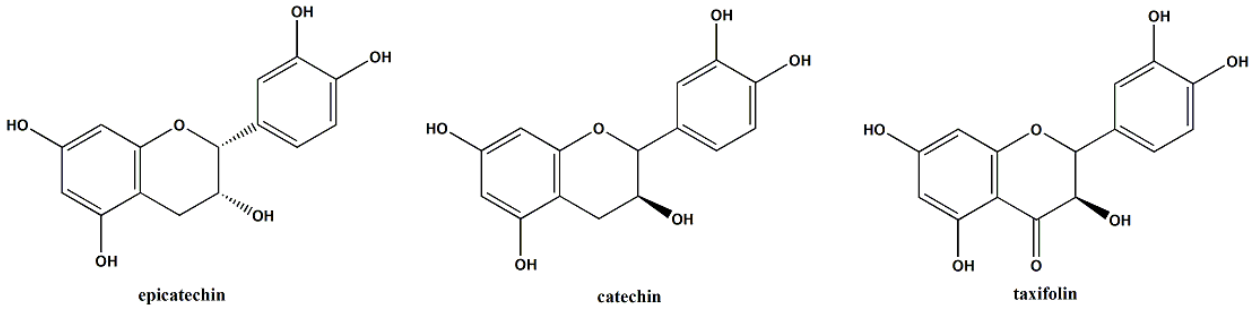

Figure 2. Primary flavanols in grape seeds

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.[12]

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.[13]

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.[14] The Food Chemicals Codes 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 on a mixture that contains Vitis Vinifera (Grape) Fruit Extract states that the solvent of extraction is glycerin.[7] The resulting composition of the mixture is 75-100% glycerin, 50-75% Vitis Vinifera (Grape) Fruit Extract, and 10-25% 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 on a mixture that contains Vitis Vinifera (Grape) Leaf Extract states that the solvent of extraction for this product is also glycerin.[9] The resulting composition of the mixture is 75-100% glycerin, 10-25% water, and 5-10% Vitis Vinifera (Grape) Leaf Extract. As above, 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.[15] 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 extract.[16] The filtrate is concentrated by distillation, and then spray-dried. The ratio of fresh plant material to extract is 133:1.

USP-grade grape seed oligomeric proanthocyanidins (dietary supplement) is a fraction of an extract of ripe *Vitis vinifera* seeds.[12] 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 deseed-ed marc remaining after 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 anti-caries agent, anti-dandruff agent, anti-fungal agent, anti-microbial agent, antioxidant, flavoring agent, light stabilizer, oral care agent, oral health care drug, and sunscreen agent.[17] 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 of the ingredients - the seed extract, the fruit powder, the juice, the juice extract, and the skin extract – are reported to function as flavoring agents and four of those five (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 listing of all the reported functions for each ingredient is provided in 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). 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.[18] The nine other in-use *Vitis vinifera* (grape)-derived ingredients are used in less than 15 formulations, and 12 *Vitis vinifera* (grape)-derived ingredients are not reported to be used.

The *Vitis vinifera* (grape)-derived ingredients are used at 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, respectively.[19] All others are used at <1% in formulation. 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.

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, and Vitis Vinifera (Grape) Seed Extract are used in cosmetic products that could possibly be inhaled; concentrations of use for ingredients that used in product 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.[20-23] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) in any appreciable amount.[20,23]

All of the *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.[24]

Non-Cosmetic

Vitis Vinifera (Grape) Seed Extract

Grape seed extracts are used as nutritional supplements.[10]

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 0-2.5 mg/kg bw.[25]

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.[26] A number of factors may play a role in the bioavailability of polyphenols, but maximum plasma values are generally reached between 5 min and 2 h 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 five male and five female albino rats.[13] 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 h, and the dose covered approximately 5-6% 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 three animals by day 12. One male rat had edema from day 6 to day 9. The dermal 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.[13] 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.[27] 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.[26] 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.

Repeated Dose Toxicity

Oral

Vitis Vinifera (Grape) Seed Extract

Groups of 20 female SKH-1 hairless mice were fed a diet containing 0, 0.2, or 0.5% grape seed extract (solvent of extraction not given) containing 89.3% proanthocyanidins for 3 wks.[28] No significant difference in body weights or other signs of toxicity were observed. No gross differences were observed in the organs of treated and untreated mice.

Groups of 10 male and 10 female F344/DuCrj rats were fed a diet that contained 0, 0.02, 0.2, or 2% grape seed extract (extracted in water and ethanol) containing 89.3% proanthocyanidins for 90 days.[27] There was no mortality in any of the groups, and there were no clinical signs of toxicity. A few statistically significant changes in organ weights were noted, primarily in the 0.2% group; these slight changes were not dose-dependent. No treatment-related microscopic changes were observed.

A 90-day dietary study was performed in which group of 20 male and 20 female Sprague-Dawley rats were fed a diet containing 0, 0.62, 1.25, or 2.50% of a grape seed extract (genus and species and solvent of extraction not stated) that was composed of approximately 90.5% total phenols.[29] (The mean test article intake was 434, 860, and 1788 mg/kg bw/day for males and 540, 1052, and 2167 mg/kg bw/day for females.) All animals survived until study termination. The only notable observation was a mild head-tilt in 6 of 20 female rats in the 2.5% group; the researchers remarked that it was doubtful this observation was treatment-related. There was a small but statistically significant increase in feed consumption by males of the 2.5% group from day 7 until study termination; similar increases were observed for males of the 1.25% group, but the occurrence was at irregular intervals. However, body weights and body weight gains were similar for treated and control groups. No ophthalmic changes were found, and there were no significant changes in hematology values that were considered clinically relevant. A decrease in heart/body weight ratio in females of the 1.25% group was not considered treatment-related. No gross or microscopic lesions were reported at necropsy. The no-observed adverse effect level (NOAEL) was approximately 2150 mg/kg bw/day for female rats and 1780 mg/kg bw/day for male rats.

In another 90-day dietary study, groups of 20 male and 20 female Sprague-Dawley rats were fed diets containing 0, 0.5, 1.0, or 2.0% water-extracted grape seed extract that contained less than 5.5% catechin monomers.[30] (The intake of the extract was 348, 642, and 1586 mg/kg bw/day for males and 469, 883, and 1928 mg/kg bw/day for females). All animals survived until study termination, and no clinical signs of toxicity were noted. Again, feed consumption was increased in test groups compared to controls, with increases by males of the 2.0% group reaching statistical significance, with no corresponding increase in body weights or body weight gains. There were no differences in organ weights between the test and control groups. Differences in clinical chemistry and hematology parameters between the test and control groups were not considered to be toxicologically significant. No test-article related gross or microscopic lesions were observed at necropsy.

Groups of female $B6C3F_1$ mice were fed a diet containing 0, 100, 250, or 500 mg/kg bw/day Vitis Vinifera (Grape) Seed Extract (trade name ActiVin) for 6 mos.[13] (The number of animals used per group was not specified). No treatment-related mortality was

reported, and no significant changes in body weight or physical appearance were observed during the study. There were no significant differences in blood urea nitrogen (BUN) levels or serum alanine aminotransferase (ALT) and serum creatinine kinase (CK) activity between treated and control animals. No gross or microscopic lesions were observed in the organs examined at necropsy.

Groups of male B6C3F$_1$mice were fed a diet containing 100 mg/kg bw/day Vitis Vinifera (Grape) Seed Extract (trade name ActiVin) for 12 mos, with sub-groups killed at 90-day intervals.[13] (The number of animals used per group was not specified). As in the 6 mos study in female mice, no treatment-related mortality and no significant changes in body weight or physical appearance were observed during the study, there were no significant differences in BUN levels or ALT and CK activity, and there were no gross or microscopic lesions observed in the organs examined at necropsy. Hepatic genomic DNA fragmentation was monitored as an index of oxidative DNA damage; no significant changes were observed with feeding of Vitis Vinifera (Grape) Seed Extract to male mice for up to 12 mos.

Vitis Vinifera (Grape) Skin Extract

A diet containing 2.5% of a grape skin extract (genus and species and solvent of extraction not stated) that contained 87.3% total phenols expressed as gallic acid equivalents was fed to a group of 20 male and 20 female Sprague-Dawley rats for 90 days.[29] (The mean test article intake was 1788 and 2167 mg/kg bw/day for males and females, respectively). The negative control group was given untreated feed. All animals survived until study termination, and there were no clinical signs of toxicity. No ophthalmic changes were found. There was a small but statistically significant increase in feed consumption by treated males, however, body weights and body weight gains were similar for treated and control groups. Statistically significant changes in some hematology measurements were noted at study termination, but none were considered clinically relevant. A statistically significant decrease in absolute and relative heart weight of female test animals was not considered treatment-related by the researchers. No gross lesions were reported at necropsy. Microscopically, the occurrence of 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 male rats and not considered treatment-related. The NOAEL was approximately 2150 mg/kg bw/day for female rats and 1780 mg/kg bw/day for male rats.

Grape Color Extract

Groups of four male and four female Beagle dogs were fed diets containing 0, 7.5, or 15% (w/w) "grape color powder" for 90 days, and another group of four males and four females was fed a diet containing 9% malto-dextrin (w/w).[31] The researchers defined grape colour powder as consisting of 40% of the naturally occurring grape-color extract in a malto-dextrin carrier. It is stated in the Code of Federal Regulations (21CFR73.169) 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 aqueous solution. The aqueous 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, etc., but not in the same proportion as found in grape juice. The dehydrated water soluble powder is prepared by spray drying the aqueous solution containing added malto-dextrin.

All animals were killed at the termination of dosing. Physical appearance and behavior were normal for all dogs during the study. Body weight gains for dogs of the high dose group were statistically significantly decreased compared to the controls, while feed consumption was comparable for test animals and controls. There were no significant differences in ophthalmic, clinical chemistry, hematology, or urinary parameters between the groups. No gross or microscopic lesions were noted, and there were no significant differences in absolute or relative organ weights between the treated and control animals.

Skin Lightening Effect

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% proantho-cyanidins on UV-induced pigmentation of guinea pig skin was examined.[32] 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), two areas on the backs of male and female brownish guinea pigs were irradiated 2x/wk for 3 wks with 0.9 J/cm^2 UV. One wk 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 wks. The lightening effect was determined every 2 wks by measuring the L*-value (lightness) and the melanin index at the two 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 wks of dosing, blood samples were taken from each animal and the animals were then killed. Skin samples were taken from UV-irradiated and a non-treated sites and evaluated for 3,4-dihydroxyphenylalanine (DOPA)-positive melanocytes and markers of oxidative DNA damage.

There were no differences in body wts 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 un-irradiated 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 (8-OHdG)-positive cells, melanin-Ki-67-positive cells, and melanin proliferating cell nuclear antigen (PCNA)-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 below. Information on estrogenic activity of some of the constituents of *Vitis vinifera* is provided in Table 6.

Grape Color Extract

A two-generation reproductive study on grape color extract was performed using Sprague-Dawley rats.[33] 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% malto-dextrin by wt for 3 wks; after 3 wks, 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, two F_1 males and two F_1 females were selected for a subsequent 13-wk 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 wks 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 above. On day 21 of lactation, all F_1 parents and F_2 pups were killed.

All animals, except one F_1 male of the malto-dextrin group, survived until scheduled termination. Dietary administration of up to 15% grape color powder had no effect on reproductive parameters or fertility. 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. Also, 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. No microscopic lesions were reported in any of the groups.

In the F_1 animals fed the test diets for 13 wks prior to dosing, the group mean body weight gain was statistically significantly decreased in the high dose females. Statistically significant differences in several clinical chemistry parameters were observed between groups after 6 wks of dosing; the values were comparable at the end of 13 wks of dosing. The following statistically significant differences were recorded at necropsy regarding body and organ weights of the F_1 animals: body weights of the high dose animals were decreased; 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.

GENOTOXICITY

The results of genotoxicity testing on grape-derived extracts are summarized in Table 9. (Table 6 includes information on the genotoxic potential of some of the constituents of *Vitis vinifera*).

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,[34] and water and ethanol extracts of red and white grapes enhanced mitomycin-C (MMC)-induced sister chromatid exchanges (SCEs) in a SCE assay in human lymphocytes, but there was no effect on SCEs without MMC.[6] Grape juice was also mutagenic *in vitro*, as demonstrated in the Ames test.[35,36] However, grape seed extract was not mutagenic *in vitro* in an Ames test or chromosomal aberration assay,[27] nor *in vivo* in the mouse micronucleus test.[27,37] A mouse micronucleus test with grape skin extract was negative.[37] *In vitro*, grape seed/grape skin extract was weakly mutagenic in an Ames test but not genotoxic in a chromosomal aberration, and the mixed extract demonstrated a statistically significant increase in micronuclei after 48 h, but not after 72 h.[26]

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 wks to determine whether dietary of grape seed extract alone had any effect on skin tumor formation.[28] No skin tumors formed.

Inhibition of Tumor Promotion

The inhibition of tumor promotion by *Vitis vinifera* has been assessed in many studies, and these studies are summarized in Table 10.

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; dermal application and dietary administration both had significant inhibitory activity.[38-42] Dietary grape seed extract also inhibited UV-initiated, UV-promoted, or UV-initiated and promoted skin tumors in hairless mice,[28] and it inhibited the formation of azoxymethane (AOM)-induced aberrant crypt foci (ACF) in the intestines of rats.[43] Some of the studies summarized in Table 10 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.[38,39,44] 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 11.

In *in vitro* testing, a product containing 3% Vitis Vinifera (Grape) Fruit Extract was predicted to be a non-irritant in a dermal irritection test in human skin, a product containing 10% Vitis Vinifera (Grape) Fruit Extract was predicted to be non-irritating/minimal in an Epiderm MTT viability assay, and hydrolyzed grape skin was predicted to be non-irritating 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-wk use study, a formulation containing 0.15% Vitis Vinifera (Grape) Seed Extract was not an irritant. 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 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.[45] Three groups of vineyard workers, 120/group, were tested: harvesters (Group A), workers in grape selection (Group B), and workers operating de-stemming/crushing/pressing machines (Group C); a group of 120 office employees (Group D) was used 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 five 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 in Group B had weak positive reactions that were considered negative in this study. None of the workers in the other two 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.[46] 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 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 ultraviolet A (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 h.

Ocular Irritation

In Vitro

Vitis Vinifera (Grape) Fruit Extract

A product containing 3% Vitis Vinifera (Grape) Fruit Extract was predicted to be a minimal ocular irritant.[47] 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 for neat samples of the product tested at volumes ranging from 25 -125 µl.

The irritancy classification for a product containing 10% Vitis Vinifera (Grape) Fruit Extract was non-irritating/minimal.[48] 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 min. The ET_{50} was >256 min.

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.[49] A bovine corneal opacity and permeability assay (BCOP) was performed with undiluted 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 0-25 are classified as mild irritants). The positive control had an in vitro score of 43.2; test materials with in vitro scores of 25.1-55 are classified as moderate irritants.

Hydrolyzed Grape Skin

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

Non-Human

Vitis Vinifera (Grape) Seed Extract

The ocular irritation potential of Vitis Vinifera (Grape) Seed Extract (trade name ActiVin) was evaluated in six female NZW rabbits.[13] The test article, 85 mg, was instilled into the conjunctival sac of the right eye, the eyelid was held closed for 1 sec, 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 h and 4, 7, and 14 days after instillation of the test article. Conjunctival irritation was observed in all animals, four animals had iridal reactions, and three had corneal reactions. The irritation was reversible and completely subsided by day 14. The Maximum Average Score (MAS) at 24 h 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 of the Vitis Vinifera (Grape) ingredients 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; nine other Vitis vinifera-derived ingredients are reported to be in use, and they are used in less than 15 formulations.

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 92-95% oligomeric proanthocyanidins. Grape skin extract contains anthocyanins, tartaric acid, tannins, sugars, and minerals.

Grapes are one of the 20 most frequently consumed raw fruits. The oral LD_{50} values of grape seed extract and grape skin extract in rats were > 4-5 and >5 g/kg, respectively, and the dermal LD_{50} (and NOEL for systemic toxicity) in albino rats was >2 g/kg. In an *in vitro* study using a reconstructed three-dimensional skin equivalent model, grape seed extract stimulated keratinocyte proliferation, fibrillin-1, elastin, and collagen type-1. Grape seed extract inhibited mushroom tyrosinase activity, and it inhibited melanogenesis in cultured B16 mouse melanoma cells.

In dietary repeated dose studies in rats, the NOAELs of grape seed extract and grape skin extract were approximately 2150 and 1780 mg/kg bw/day for male and female rats, respectively. No toxic effects were observed in female $B6C3F_1$ mice after 6 mos of dietary administration of up to 500 mg/kg bw/day Vitis Vinifera (Grape) Seed Extract or in male rats fed 100 mg/kg bw/day Vitis Vinifera (Grape) Seed Extract for 12 mos. Dietary administration of 7.5 or 15% of a grape colour extract to Beagle dogs for 90 days resulted in a statistically significant decrease in body weight gains in the high dose group; 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 two-generation reproductive study in which 7.5 or 15% grape colour 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 and liver, adrenal gland, and thyroid gland weights in F_1 animals fed the test article for 30 days prior to mating.

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 mitomycin-C (MMC)-induced sister chromatid exchanges (SCEs) in a 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.[27,37] 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, and the mixed extract demonstrated a statistically significant increase in micronuclei after 48 h, but not after 72 h.

Vitis vinifera, the seed extract in particular, was shown to inhibit DMBA-initiated and TPA-promoted tumors in mouse skin; dermal application and dietary administration both 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 wks

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* testing, a product containing 3% Vitis Vinifera (Grape) Fruit Extract was predicted to be a non-irritant in a dermal irritection test in human skin, a product containing 10% Vitis Vinifera (Grape) Fruit Extract was predicted to be non-irritating/minimal in an Epiderm MTT viability assay, and hydrolyzed grape skin was predicted to be non-irritating 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-wk use study, a formulation containing 0.15% Vitis Vinifera (Grape) Seed Extract was not an irritant. In an in vitro assay of pro-sensitizing 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 (Cucumber) 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 human repeated insult patch testing (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 non-human study using rabbits, the MAS at 24 h 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 non-irritating to eyes in a NRU study.

DISCUSSION

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-h semi-occlusive study of Vitis Vinifera (Grape) Seed Extract that reported moderate irritation using rabbits. Because all the other irritation and sensitization test were negative, including a human study using up to 10% Vitis Vinifera (Grape) Fruit Extract in a product, the CIR Expert Panel was of the opinion that the one 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, it is known that constituents of foods such as grapes, e.g. 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 ingredients.

The *Vitis vinifera* plant parts contain a number of constituents and some of the constituents, such as ascorbic acid, biotin, and malic acid, are cosmetic ingredients for which a CIR safety assessment is available. Others are compounds that have been discussed in previous CIR assessments. For example, *Vitis vinifera*, and therefore derived extracts, contains a variety of phytochemicals, all present at relatively low concentrations. The Panel has discussed in previous CIR safety assessments that although some of these phytochemicals could exert significant biological effects (e.g., isoflavones), the low levels preclude significant effects. Also, although no dermal absorption data were available, in the Panel's experience, phytosterols and phytosterol esters are not significantly absorbed and do not result in systemic exposure and extensive data are available showing that these phytosterol constituents are not estrogenic, are not reproductive toxicants, are not genotoxic, and are not carcinogenic.

Because some of the *Vitis vinifera* (grape)-derived ingredients are reported to be used in preparations which may be aerosolized, with concentrations ranging from 0.00002% Vitis Vinifera (Grape) Seed Extract in pump hairsprays to 3% Vitis Vinifera (Grape) Leaf Extract in perfumes, the Panel discussed the issue of incidental inhalation exposure. In the absence of inhalation data, the Panel noted that the *Vitis vinifera* (grape)-derived ingredients did not produce systemic toxicity in oral single-dose or long-term (up to 12 mos) 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. Further, these ingredients are reportedly used at low concentrations, most at <1%, in cosmetic products that may be aerosolized. The Panel noted that 95% – 99% of droplets/particles produced in cosmetic aerosols 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 concern based on the chemical and biological properties of these ingredients.

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 below are safe for use 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.

TABLES

Table 1. Definitions, Functions, and Chemical Class[17]

Ingredient (CAS No.)	Definition	Reported Function(s)	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* (grape)	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	anti-caries agent; anti-dandruff agent; anti-fungal agent; anti-microbial 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	***monograph development in progress***		
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

Table 2. 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	7
density	1.225-1.245	7
refractive index	1.445-1.465	7
pH	4.0-5.0	7
solubility	in water clear soluble	7
	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	9
density	1.215-1.235	9
refractive index	1.445-1.465	9
pH	4.0-5.0	9
solubility	in water clear soluble+	9
	Vitis Vinifera (Grape) Seed Extract	
appearance	red to brown powder	16
water content	8% (upper limit)	16
	Vitis Vinifera (Grape) Skin Extract	
appearance	red to purple powder or liquid	51
	purplish-red liquid	4
	purplish-red liquid, lump, powder, or paste with a characteristic odor	52
appearance in solution	red in acid solution; violet or blue in neutral to alkaline solution	51
solubility	soluble in water	52
	Hydrolyzed Grape Skin	
appearance	ruby red aq. solution	53
odor	characteristic, fruity	53,54
boiling point	98-102°C (760 mm Hg)	54
density	≈1 g/cm^3	54
pH	2.6 – 3.5	53
	2.8-4	54
solubility	completely soluble in water; soluble in alcohol and acetone	54
dry residue	≥1.5% w/w	53
water content	≥90%	54
phenol content	700 – 1500 mg/kg	53

Table 3. Chemical constituents by plant part [2]

Chemical	Amount (ppm)	Chemical	Amount (ppm)
	Plant		
2,6-dimethyl-trans-octa-2,7-dien-1,6-diol-beta-d-glucopyranoside	NS	oleic-acid	230-1183
delphinidin	NS	petunidin-3-caffeoylglucoside	NS
leucocyanidin	NS	riboflavin	0.5- .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-alpha-d-arabinofuranosyl-beta-d-beta-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
alpha-carotene	NS	malvidin-3-glucoside	NS
alpha-hydroxycarotene	NS	malvidin-3-o-beta-d-glucoside	NS
alpha-linolenic acid	390-2006	manganese	0.5-54
alpha-tocopherol	6-31	melibiose	NS
aluminum	1-154	mercury	0.011
antheraxanthin	NS	methionine	220-1132

Table 3. Chemical constituents by plant part [2]

Chemical	Amount (ppm)	Chemical	Amount (ppm)
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 acds	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
beta-carotene	0.25-2.1	neoxanthin	NS
beta-ionone	NS	nerol-6-0-alpha-l-arabinofuranosyl-beta-d-glucopyranoside	NS
beta-sitosterol	NS	nerol-6-0-alpha-l-rhamnopyranosyl-beta-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-beta-d-glucoside	NS
chromium	0.005-0.385	palmitic-acid	1620-8333
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-beta-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	1784-24640
delphinidin-3-0-beta-d-glucoside	NS	procyanidin-b-2-3'-o-gallate	NS
delphinidin-3-caffeoylglucoside	NS	procyanidins	NS
dihydrophaseic-acid-4'-beta-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-alpha-l-arabinofuranosyl-beta-d-glucopyranoside	NS	succindehydrogenase	NS
geraniol-6-o-alpha-l-rhamnopyranosyl-beta-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

Table 3. Chemical constituents by plant part [2]

Chemical	Amount (ppm)	Chemical	Amount (ppm)
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
linalol-6-0-alpha-l-arabinofuranosyl-beta-d-glucopyranoside	NS	zeaxanthin	NS
linalol-6-0-alpha-l-rhamnopyranosyl-beta-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-beta-damascone	NS	dihydrofuran	NS
9-hydroxy-megastigm-4,6,7-trien-3-one	NS	dimethylamine	NS
acuminoside	NS	ethylamine	NS
alpha-3-oxo-damascone	NS	geraniol-beta-d-glucoside	NS
alpha-3-oxo-ionone	NS	isoamylamine	NS
alpha-amylamine	NS	isobutylamine	NS
benzyl-6-o-beta-d-apiofuranosyl-beta-d-glucoside	NS	linalol-6-0-beta-d-apiofuranosyl-beta-d-glucoside	NS
beta-3-oxo-damascone	NS	linalol-beta-d-glucoside	NS
beta-phenylethanol-6-beta-d-arabinofuranosyl-beta-d-glucopyranoside	NS	megastigm-5-en-7-yne-3,9-diol	NS
beta-phenylethanol-beta-d-glucoside	NS	n-propylamine	NS
beta-phenylethanol-beta-d-rutinoside	NS	nerol-6-0-beta-d-apiofuranosyl-beta-d-glucoside	NS
betaine	NS	nerol-beta-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
alpha-viniferin	23,400	isovitilagin	163
ascorbic-acid	3490-3870	kaempferol	NS
benzyl-alcohol	NS	lupeol	NS
benzyl-alcohol-6-o-l-arabinofuranosyl-beta-d-glucopyranoside	NS	luteolin	NS
benzyl-alcohol-beta-d-glucoside	NS	mono-p-coumaryl-acid	NS
benzyl-alcohol-beta-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
alpha-amyrin	1030	obtusifoliol	NS
ascorbic-acid	310	octan-1-ol	NS
ash	88,000	oleanolic-aldehyde	NS
beta-amyrin	NS	phosphorus	1710
beta-carotene	43	potassium	20,100
calcium	17,700	riboflavin	6.9
chromium	9	selenium	NS

Table 3. Chemical constituents by plant part [2]

Chemical	Amount (ppm)	Chemical	Amount (ppm)
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-beta-ol-20-one	NS	pyrophosphatase-nucloetide	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	3300-11000
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		

NS – not specified

Table 4. Additional constituent data

Polyphenols
- Cinnamic acids: coumaric, caffeic, ferulic, chlorogenic, and neochlorogenic acid[5]
- Benzoic acids: p-hydroxybenzoic acid; protocatechuic, vanillic, and gallic acid[5]

trans-Resveratrol (trans-3,5,40-trihydroxystilbene)[55]

Fruit

Polyphenols
- Flavones: quercetin (traces) and quercitrin; quercetin-, kaempferol-, and myricetin-3-monoglucoside; quercetin-glucuronoside; astilbin; engeletin[15]
- Catechins: catechin; epicatechin, gallocatechin, epicatechingallage[15]
- Anthocyanins: delphinidin-, petunidin-, malvidin- (41.2%), cyanidin-, and peonidin-3-monoglucosides;[15] 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[55]
- Procyanidins: procyanidin B_1, B_2, B_3, B_4, B_5, B_7, B_8;[15] acylated procyanidins that are esters of gallic acid; 14 dimeric, 11 trimeric, and one tetrameric procyanidin[55]

α-Hydroxy acids: tartaric, citric, and malic acids[15]

Esters: containing cinnamic and tartaric acids[15]

Aldehydes: vanillin; protocatechuic; cinnamic and coniferyl aldehydes[15]

Vitamins: C, B group, PP[15]

Carotene[15]

Sugars: Fructose, Glucose[15]

Polysaccharides: containing galactose, mannose, arabinose, rhamnose, galacturonic acid[15]

Proteins[15]

Volatile constituents[15]

Waxes[15]

Pectin[15]

Table 4. Additional constituent data

Seeds

Polyphenols (5-8 by wt%;[5] 60-70% of grape polyphenols are found in grape seeds;[55] they are flavan-3-ol derivatives)

- Catechins: (+)-catchins; (-)-epicatechin; (-)-epicatechin-3-O-gallate[55]
- Procyanidins: procyanidin B_1, B_2, B_3, B_4, B_5, B_7, B_8[15]; procyanidins C1; procyanidins B5-3'-gallate[55]
- Proanthocyanidins (mostly hexamers)[55]
- Flavonoids (4-5%): kaemperferol-3-O-glucosides; quercetin-3-O-glucosides; quercetin; myricetin[55]

Proteins (7-10%): containing arginine, cystine, leucine (11.4%), valine, phenylalanine[15]

Triglycerides (6-20%): containing palmitic, stearic, oleic (37%), and linoleic (55%) acids[15]

Unsaponifiables (0.5-1%): phytosterols: b-sitosterol[15]

Phospholipids: phosphatidylserine, phosphatidylinositol, lecithin, cephalin, cerebrosides, phosphatidic acid[15]

Vitamin E[15]

Leaves

Polyphenols

- Anthocyanins[15]
- Catechins: catechin; epicatechin; gallocatechin; epicatechin-3-O-gallate[15]
- Ellagitannins: brevilagin-1; vitilagin; isovitilagin[15]
- Flavones: traces of quercitrin, quercetin, kaempferol, rutin, iso-quercitrin, luteolin[15]

Organic Acids: tartaric, malic, oxalic, fumaric, succinic, citric, and glyceric acids[15]

Phenol acids: o- and p-hydroxybenzoic acid; protocatechuic, gallic, vanillic, syringic, and ellargic acids[15]

Esters: containing cinnamic acids and tartaric acid[15]

Vitamins: C, PP, B group, folic acid[15]

Carotenoids[15]

Volatile constituents[15]

Waxes[15]

Proteins[15]

Mineral salts (5-7%)[15]

Table 5. 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)	56
Ascorbic Acid	safe as used (≤10% in leave-ons; ≤5% in rinse-offs)	57
Benzoic Acid	safe as used (≤5% in leave-ons; ≤5% in rinse-offs; 0.08% in diluted for (bath) use formulations)	58
Benzyl Alcohol	safe as used (≤3% in leave-ons; ≤10% in rinse-offs; ≤0.9% in diluted for (bath) use formulations)	58
Biotin	safe as used (≤0.6% in leave-ons; ≤0.01% in rinse-offs)	59
Cholesterol	safe as used safe as used (≤5% in leave-ons; ≤1% in rinse-offs)	60
Citric Acid	safe as used (≤4% in leave-ons; ≤10% in rinse-offs; ≤39% in diluted for (bath) use formulations)	61
Fumaric Acid	safe as used (≤0.2% in leave-ons; ≤0.2% in rinse-offs; ≤5% in diluted for (bath) use formulations)	62
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	63
Malic Acid	safe for use as a pH adjuster; **insufficient for other uses**	64
Myristic Acid	safe as used (≤10% in leave-ons; ≤19% in rinse-offs)	65
Niacin	safe as used (≤0.1% in leave-ons)	66
Oleic Acid	safe as used (≤20% in leave-ons; ≤19% in rinse-offs)	67,68
Palmitic Acid	safe as used (≤16% in leave-ons; ≤20% in rinse-offs)	67,68
Pantothenic Acid	safe as used (≤0.01% in leave-ons: 0.00001% in rinse-offs)	68,69
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)	70
Stearic Acid	safe as used (≤22% in leave-ons; ≤43% in rinse-offs)	67,68
Succinic Acid	safe as used (≤0.2% in leave-ons; ≤26% in rinse-offs)	71
Tocopherol	safe as used (≤2% in leave-ons; ≤0.4% in rinse-offs; ≤0.8% in products diluted for use)	72

Table 6. Toxicity information on some components of Vitis Vinifera (grape)

Component	Toxicity information	
Polyphenol		
Resveratrol	-in rats given daily oral administration of resveratrol (300, 1000, 3000 mg/kg for 28 days), nephrotoxicity and other signs of toxicity was 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/day; in several mammary cancer cell lines, resveratrol showed mixed estrogen agonist/antagonist activities, whereas in the presence of 17β-estradiol, it was an anti-estrogen; 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 (s.c.) 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 a sister chromatid exchange assay	[73]
	- not genotoxic in a mouse or rat micronucleus test or in an Ames test	[74]
	- 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, non-genotoxic 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/day); 750 mg/kg bw/day was not embryotoxic in rats; readily absorbed, metabolized, and excreted in rats	[75]
	-concentrations of 1 nM - 100 μM 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	[76]
Anthocyanins	do not appear to be readily absorbed or metabolized; low acute oral toxicity; weight-of-evidence analysis indicates anthocyanins are not genotoxic	[77]
Carotenoids	no evidence of adverse biological activity	[78]
Lutein/Esters	single-dose, 4-wk,and 13-wk oral studies found no evidence of toxicity	[78]
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	[78]
	-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	[79]
Coumarin	*limited evidence* in experimental animals for carcinogenicity; *not classifiable as to its carcinogenicity in humans* (IARC)	[80]
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	[78]
Quercetin	- genotoxic in vitro but not in vivo; some evidence for carcinogenicity (renal tumors) was found in one of several studies, in one species (rat), in one 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	[78]
	- quercetin alone, 100 μM, increased the spontaneous number of sister chromatid exchanges (SCEs) in human lymphocytes; however, 50 and 100 μM inhibited mitomycin C (MMC)-induced SCEs in a dose-dependent manner	[6]
(+)-Catechin; (-)-Epicatechin	no effect on SCEs in human lymphocytes in the presence or absence of MCC	[6]
Kaempferol	increased the frequency of sister chromatid exchanges in cultured hamster cells; shown to mutate and transform human and mouse cells in culture	[81]
Monoterpenes	these chemicals may be skin irritants	[78]
Phenolic Acids		
Caffeic Acid	- in a MMC-induced SCE assay in human lymphocytes, 100 μM caffeic acid enhanced MMC-induced SCEs by 55%; 100 μM caffeic acid alone enhanced MMC-induced SCEs by 26%	[6]
	- 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	[78,82]

Table 6. Toxicity information on some components of Vitis Vinifera (grape)

Component	Toxicity information	
	- the carcinogenic potency of caffeic acid, estimated based on an average human intake of 1 mg/kg bw/day, 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; one study in which rats were exposed to 2% (20,000 ppm) caffeic acid in the diet for 2 yrs showed treatment-induced carcinomas of the forestomach, whereas two 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	[79]
Ferulic Acid	- in an SCE assay, ferulic acid did not affect SCEs in the presence of absence of MMC	[6]
	- this acid is reported to penetrate skin and have UV photoprotective activity	[78]
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	[83]
Tannins	IARC has concluded that tannins are not classifiable to their carcinogenicity	[84]
Leucocyanidin	this substance has been reported to be toxic to some laboratory animals; symptoms included cardiac failure and hepatic lesions	[14]
Terpene Alcohols Non-Cyclic		
citronellol	- percutaneous absorption, 954 µg/cm^2/h through human cadaver skin; ocular irritant in rabbit eyes (undiluted)	[85]
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 wk study, 50 mg/kg bw/day; inhalation NOAEL 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 ≤25%0 in mice; not a sensitizer in an HRIPT at 25%	[85]
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 ≤1000 mg/kg bw/day for up to 16 wks and with 100 mg/kg bw/day for 27 wks; not mutagenic in an Ames test or rec-assay, equivocal results with regard to polyploidy in one chromosome aberration test at up to 0.125 mg/ml in DMSO and inconclusive results in another at up to 156.3 µg/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 pet. 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 pet; not phototoxic at 5% in pet. in clinical testing	[85]
nerol	- dermal LD_{50} in rabbits, >5000 mg/kg; oral LD_{50} in rats, 4500 mg/kg; some erythema (+ rxn in 2 and ± 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 pet. in a clinical max. study	[85]
Cyclic α-terpineol	- oral LD_{50} in mice, 2830 mg/kg; 1000 mg/kg bw/day for 2 wks 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 i.p. injections; a derma irritant in animals studies, but not a dermal irritant in a 4-h clinical study; not a sensitizer in guinea pigs; in clinical patch tests, 5% in pet. had 1/1606 positive and 11/1606 questionable reactions in one study and 2/1200 positive reactions in another	[85]
Triterpene Alcohols	hepatoprotective and anti-carcinogenic activity has been suggested for lupeol; no toxicity data were available; triterpene alcohols were considered to have intermediate risk	[78]

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[18]	*Max. Conc. of Use (%)*[19]	*# of Uses*[18]	*Max. Conc. of Use (%)*[19]	*# of Uses*[18]	*Max. Conc. of Use (%)*[19]
Totals*	**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[a]-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[b]	NR
Hair - Non-Coloring	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[18]	*Max. Conc. of Use (%)*[19]	*# of Uses*[18]	*Max. Conc. of Use (%)*[19]	*# of Uses*[18]	*Max. Conc. of Use (%)*[19]
Totals*	**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 - Non-Coloring	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[18]	*Max. Conc. of Use (%)*[19]	*# of Uses*[18]	*Max. Conc. of Use (%)*[19]	*# of Uses*[18]	*Max. Conc. of Use (%)*[19]
Totals*	**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[a]	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 - Non-Coloring	5	NR	6	NR	NR	NR
Hair-Coloring	1	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	10	0.02	1	NR
Baby Products	NR	NR	NR	NR	NR	NR

Table 7. Frequency and concentration of use according to duration and type of exposure

	Vitis Vinifera (Grape) Seed Extract		Vitis Vinifera (Grape) Seed Powder		Vitis Vinifera (Grape) Vine Extract	
	# of Uses[18]	*Max. Conc. of Use (%)*[19]	*# of Uses*[18]	*Max. Conc. of Use (%)*[19]	*# of Uses*[18]	*Max. Conc. of Use (%)*[19]
Totals*	**495**	**0.00002 -0.2**	**1**	**NR**	**11**	**0.004**
Duration of Use						
Leave-On	*369*	*0.00002- 0.2*	*1*	*NR*	*10*	*0.004*
Rinse Off	*118*	*0.00008–0.1*	*NR*	*NR*	*1*	*NR*
Diluted for (Bath) Use	*8*	*0.002-0.003*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	19	0.0002-0.09	NR	NR	2	NR
Incidental Ingestion	18	0.0002	NR	NR	NR	NR
Incidental Inhalation-Spray	28[a]	pump spray: 0.00002 0.0002–0.02	NR	NR	NR	NR
Incidental Inhalation-Powder	4	0.0002	NR	NR	NR	NR
Dermal Contact	411	0.0002-0.2	1	NR	10	0.004
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	62	0.00002-0.1	NR	NR	1	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	NR
Baby Products	NR	NR	NR	NR	NR	NR

* Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types my not equal the sum of total uses
NR – not reported
[a] Includes suntan preparations, and it t is not known whether or not those product are sprays
[b] It is not known whether or not this product is a pump or a spray

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) Shoot Extract
Vitis Vinifera (Grape) Skin Extract
Vitis Vinifera (Grape) Skin Powder
Vitis Vinifera (Grape) Vine Sap
Hydrolyzed Grape Fruit
Hydrolyzed Grape Skin

Table 9. Genotoxicity studies

Concentration/Vehicle	Procedure	Test System	Results	Reference
		IN VITRO		
Grape Fruit				
fractions of raw grapes (concentration not specified);	Ames test	*Salmonella typhimurium* TA 98 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	34
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	6
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	6
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	6
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	6
Grape Juice				
grape juice fractions (genus and species not stated) from canned or bottled juice in DMSO	Ames test	*S. typhimurium* TA 98 and TA100, with and without metabolic activation	marked mutagenic activity	35
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	36
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	36
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	36
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 phenolics generates species that can induce mutations	86
Grape Seed Extract				
19-1250 µg/plate; extracted with water and ethanol; extract contained 89.3% proanthocyanidins	Ames test	*S. typhimurium* TA 98 and TA100, with and without metabolic activation	negative	27
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	27

Table 9. Genotoxicity studies

Concentration/Vehicle	Procedure	Test System	Results	Reference
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 h without metabolic activation	negative	27
18.8-75 µg/ml; extracted with water and ethanol; extract contained 89.3% proanthocyanidins	chromosomal aberration assay	CHL cells exposed for 18 h without metabolic activation	negative	27
18.8-300 µg/ml; extracted with water and ethanol; extract contained 89.3% proanthocyanidins	chromosomal aberration assay	CHL cells exposed for 6 h with metabolic activation	negative	27
1, 4, or 20 µM; 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	41
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	26
9.7 and 19.5 µg/ml; extracted with ethanol; extract contained 76% of total phenols	chromosomal aberration assay	human lymphocytes	negative	26
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 300-400 nm; most of the UVB was filtered; the bacterial suspension was irradiated for 30 min 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 non-irradiated grape-skin color was not mutagenic	87
0.01-1 mg/ml grape skin color (*Vitis vinifera* or *Vitis labrusca*) in PBS	photocytotoxicity; cell survival was measured before UVA, 1 h after UVA, and after 1 h UVA irradiation and 24h incubation	WTK-1 cells	delayed cytotoxicity was observed with 1 mg/ml following 24 h incubation after UVA exposure	87
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 h	negative	27
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 h harvest for all doses; 48 h harvest for 0 and 2 g/kg groups	1 high-dose animal found dead 1h after dosing; cytotoxic (statistically significant decrease in the PCE:NCE ratio) at the 2 g/kg - 48-h harvest; no other cytotoxic effects were observed; not clastogenic	37
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 h	statistically significant increase in micronuclei after 48 h, but not after 72 h	26
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 h harvest for all doses; 48 h harvest for 0 and 2 g/kg groups	no clinical signs of toxicity; not cytotoxic or clastogenic	37

Abbreviations: CMC = carboxymethylcellulose; DMSO – dimethyl sulfoxide; MMC = mitomycin C; PBS = phosphate-buffered saline; PCE:NCE = polychromatic erythrocyte: normochromatic erythrocyte; SCE = sister chromatid exchange;

Table 10. Inhibition of 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 wks, TPA-promotion (5 µg/0.2 ml acetone) - extract topically applied 1 h prior to TPA -applications made 2x/wk for 20 wks	time of appearance of first tumor was delayed by 3 wks (wk 9 vs. wk 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%	88
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 wk 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 min after application, TPA promotion (2 µg/0.1 ml acetone) in groups 1-3; applications were made 2x/wk for 19 wks Group 4 – 0.1 ml acetone applied; no DMBA initiation Group 5 – 1.5 mg grape seed powder in acetone applied, starting 1 wk after DMBA initiation, 2x/wk for 19 wks -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 wks, 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	38
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 wks later, grape seed was topically applied - 20 min after application, TPA promotion (5.2 µg/0.2 ml acetone) - applications were made 2x/wk for 15 wks	tumor incidence was inhibited by 30, 40,and 60% with 5, 10, or 20 mg grape pre-treatment, respectively; tumor multiplicity was significantly reduced 63, 51, and 94%, respectively; the % 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	39
grape seed polyphenolic fraction	0 or 20 mg in 0.4 ml acetone	10 female CD-1 mice	- DMBA initiation, as above - 2 wks later, acetone or grape seed extract was applied dermally 2x/wk for 15 wks - no TPA promotion	no tumors were observed in animals of either group	39
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 not specified	- DMBA (0.1 µmol in 0.2 ml acetone) applied topically 2x/wk for 4 wks - extract applied 20 min prior to DMBA	DMBA alone induced dermal hyperplasia, increasing epidermal thickness by 4.6 times the normal average; grape seed 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	41
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 2x/wk for 4 wks - extract applied 20 min prior to DMBA	DMBA alone increased epidermal thickness 5x as well as the PCNA level; application of the extract statistically significantly inhibited both increases in a dose-dependent manner	42
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), 2x/wk for 4 wks - 30 min after DMBA application, grape test article was applied - 5 mice/group were killed 2 days, 4 wks, or 8 wks after dosing - some animals were dosed for 24 wks	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 after24 wks, there was clear reduction in the number of papillomas in animals dosed with 2 mg grape powder	44

Table 10. Inhibition of Tumor Promotion

Test Article	Dose/Vehicle	Animals/Group	Procedure	Results	Reference
			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 wk prior to DMBA for up to 12 wks - DMBA (0.1 µmol; vol. 0.2 ml), 2x/wk for 4 wks - some animals were given treated feed for 24 wks	DMBA treatment produced epidermal hyperplasia, dietary grape powder inhibited the hyperplasia; % 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 wks for the animals dosed for 24 wks, there was clear reduction in the number of papillomas in animals fed the grape powder	44
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 2ks of treated diet, DMBA (0.1 µmol in 0.2 ml acetone) applied topically 2x/wk for 4 wks	DMBA alone increased epidermal thickness 5x and increased the PCNA level; dietary exposure to the extract statistically significantly inhibited both increases in a dose-dependent manner	42
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) - after 1 wk, TPA promotion (0.01 µg/0.1 ml acetone); 2x/wk for 27 wks - treated diet was started with TPA application	time of appearance of first tumor was delayed by 4 wks (0.2% group) and 10 wks (0.5% group); tumor incidence 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	40
as above	0.5% in feed	10 female C3H/HeN mice	DMBA initiation as above - after 1 wk, fed treated diet for 27 wks; no TPA promotion - a control group for spontaneous tumors was treated with 0.2 ml acetone 2x/wk	no tumors were observed in animals of either group	40
as above	0.5% in feed	5 female C3H/HeN mice	- mice were fed treated feed - either 1 wk later, a single application of 5 µg TPA was made and the mice were killed after 6, 12, or 24 h or TPA was applied 3x on alternate days and the mice were killed 6 h 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 h 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	40
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 wks Group 2: control feed for 10 wks; after 1 wk, s.c. AOM 1x/wk for 2 wks Group 3: 0.25% in feed for 10 wks; after 1 wk of treated feed, s.c. AOM 1x/wk for 2 wks Group 4: 0.5% in feed for 10 wks; after 1 wk of treated feed, s.c. AOM 1x/wk for 2 wks Group 5: s.c. AOM 1x/wk for 2 wks; 4 wks later, 0.25% in feed for 4 wks Group 6: s.c. AOM 1x/wk for 2 wks; 4 wks later, 0.5% in feed for 4 wks Group 7: 0.5% in feed for 10 wks	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 significant increase in the number of TUNEL-positive cells	43

Table 10. Inhibition of Tumor Promotion

Test Article	Dose/Vehicle	Animals/Group	Procedure	Results	Reference
ANTI-PHOTOCARCINOGENESIS 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 wk after the last UV exposure, mice were again irradiated with 180 mJ/cm^2 3x/wk for 29 wks	latency period of tumors was increased by 2 wks 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	[28]
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	[28]
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 wk after the last UV exposure, both groups were treated topically with TPA (0.01 µmol/0.1 ml acetone); 3x/wk for 23 wks	latency period of tumors was increased by 2 wks by feeding the extract; a highly significant reduction in tumor incidence was observed (95%); between wks 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	[28]
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 wk, UVB irradiation (promotion; 180 mJ/cm^2); 3x/wk for 24 wks - treated diet was started with UVB exposure	latency period of tumors was increased by 3 wks 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	[28]

Abbreviations: ACF – aberrant crypt foci; AOM = azoxymethane; DMBA = dimethylbenz[a]anthracene; PCNA = proliferating cell nuclear antigen; TPA = 12-O-tetradecanoylphorbol-13-acetate

Table 11. 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 non-irritant in human skin; human irritancy equivalent scores ranged from 0.46 to 0.61	89
product containing 10% (extracted in water)	neat	-----	Epiderm MTT viability assay; tissue samples treated for 1, 4, and 24 h	non-irritating/minimal ET_{50} was >24 h; irritancy classification	90
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 non-irritating; the IC_{50} was >5 mg/ml IC_{50} of SLS was 0.083 mg/ml (irritating)	91
NON-HUMAN - IRRITATION					
Vitis Vinifera (Grape) Seed Extract					
as trade name ActiVin	neat	New Zealand White rabbits; 3M/3F	4-h semi-occlusive application; 0.5 g of the extract moistened with 0.3 ml deionized water; applied to an intact 1 in x 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 one animal, eschar in 2 animals; all dermal irritation subsided by day 12	13
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-wk 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	92
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 h; CD80 and CD86 were used as co-stimulatory molecules; MFI was measured using a FACS; MFI of non-treated 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	93
HUMAN – IRRITATION AND SENSITIZATION					
Vitis Vinifera (Grape) Fruit Extract					
0.0239% in a foundation	neat	103 subjects	modified HRIPT – semi-occlusive; ; 0.15 ml on a 20 x 20 mm pad; 9 24-h induction applications; 24-h challenge application at treated and untreated sites followed a 17 or 24-day non-treatment period	not an irritant or sensitizer	94
blend containing 3%	tested at 1% aq.	108 subjects	HRIPT - semi-occlusive; 0.02-0.05 ml on a 7.5 mm paper disc; 9 24-h induction applications; challenge application at a previously untreated site after a 10-14 day non-treatment period	not an irritant or sensitizer	95
product containing 6%	10% in deionized water	97 subjects	modified HRIPT - semi-occlusive; ; 150 mg on a 20 x 20 mm pad; 9 24-h induction applications;24-h challenge at treated site and 48-h challenge at untreated site followed a 10-day non-treatment period	not an irritant or sensitizer	96
product containing 10% (extracted in water)	neat	54 subjects	HRIPT – occlusive; 0.2 ml on a 20 x 20 mm Webril pad; 9 24-h induction applications; ; 24 h challenge at a previously untreated site after a 10-14 day non-treatment period	not an irritant or sensitizer	97
Vitis Vinifera (Grape) Juice					
make-up primer containing 0.1%	neat	208 subjects	HRIPT – semi-occlusive; same induction protocol;24-h challenge application applied to a previously untreated site after a 2-wk non-treatment period	not an irritant or sensitizer with the exception of an occasional ± score (barely perceptible erythema), no visible reactions were noted	98

Table 11. Dermal irritation and sensitization

Test Article	Concentration	Test Pop.	Procedure	Results	Reference
Vitis Vinifera (Grape) Juice Extract					
hair styling product containing 0.5%	neat	100 subjects	modified HRIPT – occlusive; 21-day induction period, 10-24 day non-treatment period, 4-day challenge	not an irritant or sensitizer	[99]
Vitis Vinifera (Grape) Seed Extract					
body lotion formulation containing 0.0002%	neat	101 subjects	modified HRIPT – occlusive; 21-day induction period, 10-24 day non-treatment period, 4-day challenge	not an irritant or sensitizer	[100]
hair conditioner containing 0.1%	10% aq. dilution	105 subjects	modified HRIPT – semi-occlusive; ; 0.2 ml on a 20 x 20 mm pad; 9 24-h induction applications, 24-h challenge application at treated and untreated sites followed a 10-day non-treatment period	not an irritant or sensitizer	[101]
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+ min prior to application; 9 24-h induction applications; 24-h challenge followed a 10-day non-treatment 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 one subject had a ? response at reading 2	[102]
raw material containing 1%	neat	107 subjects	modified HRIPT – semi-occlusive; ; 0.15 ml on a 20 x 20 mm pad; 9 24-h induction applications, 24-h challenge application at treated and untreated sites followed a 10-day non-treatment period	not an irritant or sensitizer five grade 1 and 1 grade 2 response noted during induction; grade 1 response were noted for 3 subjects during challenge	[103]

Abbreviations: FACS = fluorescence activated cell sorter; HRIPT = human repeated insult patch test; MFI = mean fluorescence intensity; MTT = 3-(4,5-di¬methyl¬thiazol-2-yl)-2,5-diphenyl tetrazolium bromide; SLS = sodium lauryl sulfate

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