Safety Assessment of Tocopherols and Tocotrienols as Used in Cosmetics

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The 2014 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This re-review was prepared by Monice M. Fiume, Assistant Director/Senior Scientific Analyst, and Bart A. Heldreth, Ph.D., Chemist.

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

ABSTRACT

The Expert Panel assessed the safety of 14 tocopherols and tocotrienols, and concluded these ingredients are safe as used in cosmetics. The tocopherols are reported to function in cosmetics as antioxidants or skin conditioning agents; in contrast, tocotrienols is not reported to function as an anti-oxidant in cosmetics, but as a light stabilizer, oral care agent, or skin conditioning agent. The Panel reviewed the new and existing animal and clinical data to determine the safety of these ingredients, and found it appropriate to extrapolate the existing information to conclude on the safety of all the tocopherols and tocotrienols.

INTRODUCTION

In 2002, the Panel published a review on tocopherol, the component most commonly associated with vitamin E. Although it has not been 15 years since the report on the tocopherols was published, the Panel opened a re-review of the tocopherols to include tocotrienols (a 2013 priority), as well as a few additional tocopherols that were not a part of the original report. In the original safety assessment of tocopherols, the Panel concluded these ingredients are safe as used in cosmetics:[1]

tocopherol
tocopheryl acetate
tocopheryl linoleate
tocopheryl linoleate/oleate
tocopheryl nicotinate
tocopheryl succinate
dioleyl tocopheryl methylsilanol
potassium ascorbyl tocopheryl phosphate
tocophersolan

The Panel stated the following cosmetic ingredients should be included in this safety assessment:

tocotrienols
ascorbyl tocopheryl acetate
ascorbyl tocopheryl maleate
tocopheryl phosphate
sodium tocopheryl phosphate

All relevant published literature that has become available since the CIR safety assessment was issued on tocopherols, and all relevant literature on tocotrienols and the tocopherols that are being proposed for addition to this report, are presented in this review. For the purpose of the review, relevant literature refers to that which applies to the safety of tocopherols and tocotrienols as used in cosmetics. Health claims have been made for vitamin E; that information is not relevant to the safety of vitamin E in cosmetics, and therefore, the studies investigating those claims are not included in this review.

Tocopherol and tocopheryl acetate are generally recognized as safe (GRAS) food ingredients. Because systemic exposure via cosmetic use is not expected to exceed the levels found safe for food use, oral toxicity data on tocopherol and tocopheryl acetate are not included in this review.

Some of the ingredients included in this report are ascorbyl-containing compounds. In 2005, the Panel concluded that L-ascorbic acid, ascorbyl phosphates, and ascorbates are safe as used in cosmetics.[2]

Excerpts from the 2002 report summary on tocopherols are included in each appropriate report section, and are indicated by *italicized text*.

CHEMISTRY

Definition and Structure

Tocopherols and tocotrienols are amphiphilic lipids that share in common a substituted chromanol core.[3] Together, the tocopherols and tocotrienols comprise vitamin E, differing structurally by substitution on the polar, chromanol core by either a lipophilic saturated, phytyl side chain, or by an unsaturated, isoprenoid (geranylgeranyl) side chain, respectively. Tocopherols have three stereocenters (*) which are all of the *R*-configuration (D-form) when obtained from natural sources. Synthetically produced tocopherols, however, are commonly racemic mixtures.[4] Tocotrienols have one stereocenter and three double bonds, which are in the *R*-configuration and are all *trans*-geometries, respectively, when obtained from natural sources. Synthetically produced tocotrienols, however, can potentially have any combination of stereochemistry and double bond geometries, or mixtures thereof.

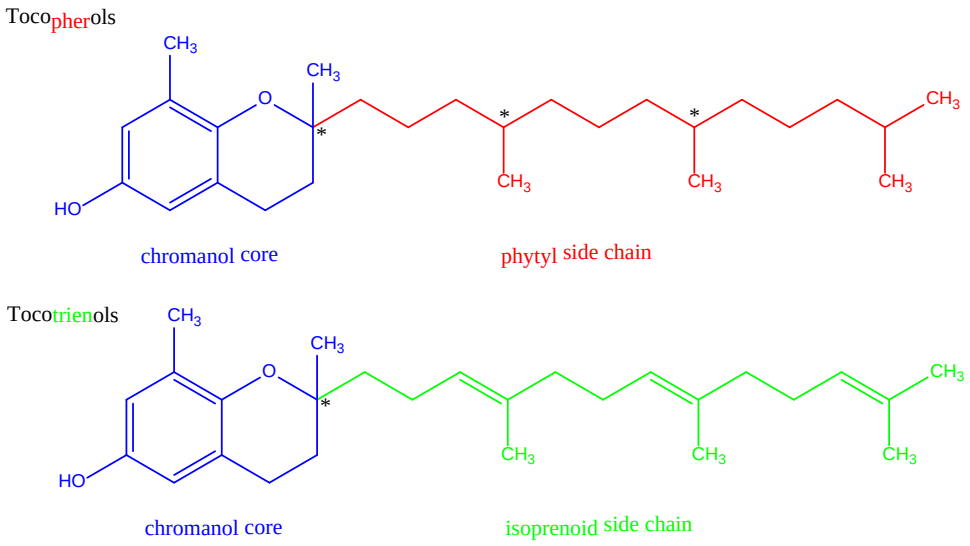


Figure 1. Tocopherols and Tocotrienols

In addition to possible stereochemistries and double bond geometries, vitamin E is comprised of eight naturally occurring structural analogs: four tocopherols (α-, β-, γ-, and δ-analogs) and four tocotrienols (α-, β-, γ-, and δ-analogs).[4] These analogs differ structurally by the presence and placement of additional methyl groups around the aromatic ring of the chromanol core. All four of the tocopherol analogs are listed in the definition for Tocopherol. According to the definition for the cosmetic ingredient, however, tocotrienols only contains the α-, γ-, and δ-analogs (i.e., no β-tocotrienol).

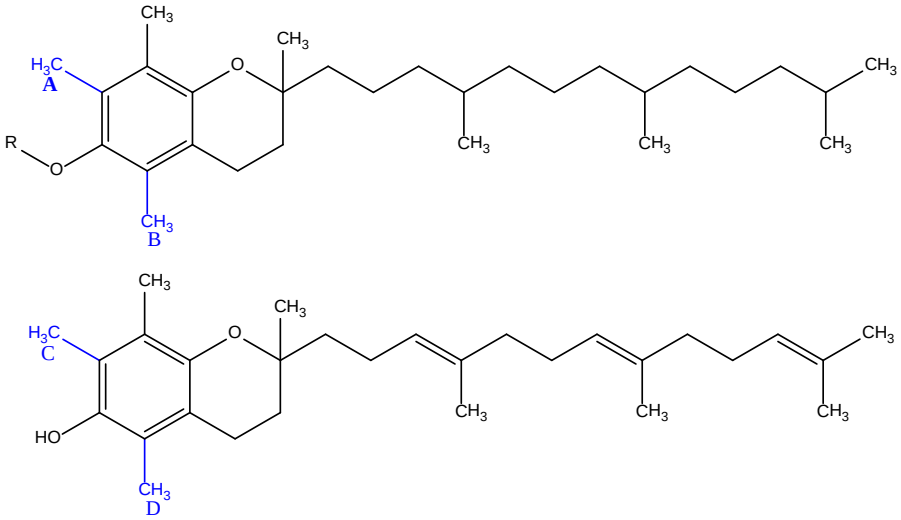


Figure 2. α-, β-, γ-, and δ-analogs
-wherein methyl groups A and B are present in the case of α-tocopherol, only B for β-tocopherol, only A for γ-tocopherol, and neither for δ-tocopherol
-wherein methyl groups C and D are present in the case of α-tocotrienol, only D for β-tocotrienol, only C for γ- tocotrienol, and neither for δ- tocotrienol
-wherein R is hydrogen or, in the case of the tocopheryl ingredients, constitutes, together with the attached oxygen, an ester

Further substituted tocopheryl ingredients, including tocopheryl acetate, tocopheryl linoleate, tocopheryl linoleate/oleate, tocopheryl nicotinate, tocopheryl succinate, dioleyl tocopheryl methylsilanol, potassium ascorbyl tocopheryl phosphate, tocophersolan, ascorbyl tocopheryl acetate, ascorbyl tocopheryl maleate, tocopheryl phosphate, and sodium tocopheryl phosphate, involve ester linkages of the corresponding acid to the free alcohol of tocopherol (e.g., tocopheryl nicotinate is the tocopheryl ester of nicotinic acid).

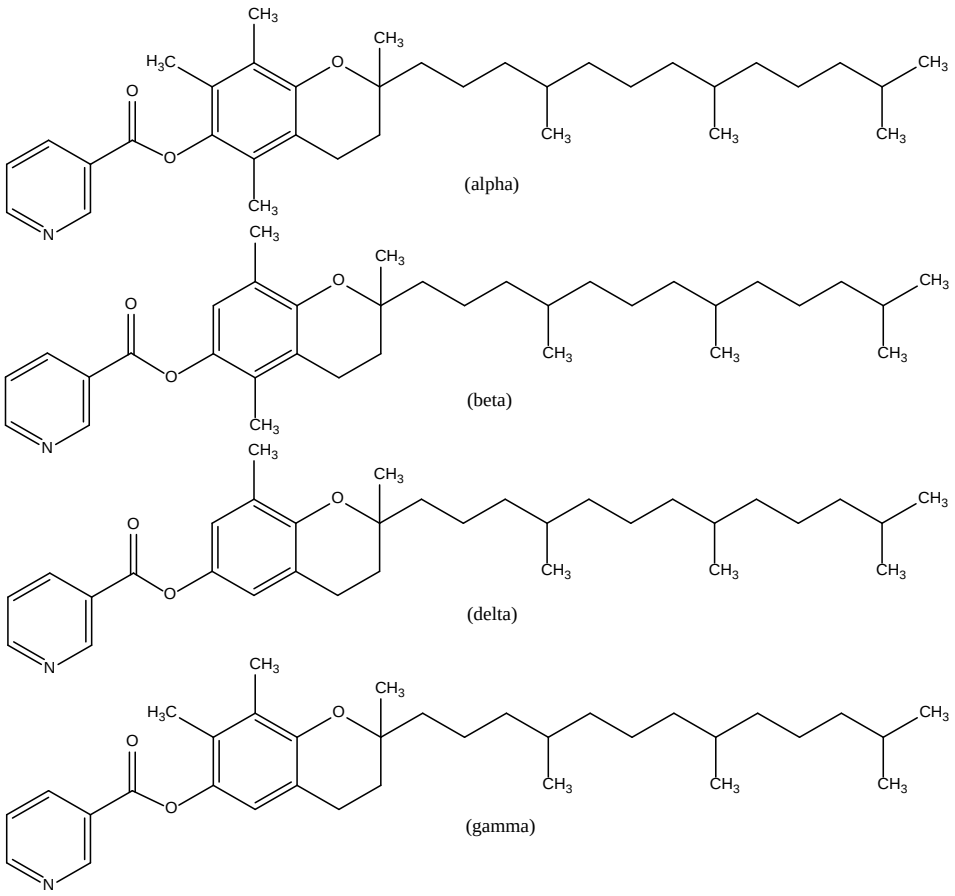


Figure 3. Tocopheryl Nicotinate.

The definitions and structures of each ingredient are found in Table 1.

Physical and Chemical Properties

α-, β-, γ- and δ-Tocopherols and tocotrienols are lipophilic molecules.[5] Tocopherol is readily oxidized upon exposure to atmospheric conditions or light, but the oxidation propensity varies among α-, β-, γ- and δ- analogs, because of differences in oxidation potential and reactivity with molecular oxygen. Limited physical and chemical properties data are provided in Table 2.

Method of Manufacture

From the original report on Tocopherols

Tocopherol is isolated on a commercial scale from vegetable oils. It is produced synthetically by condensing isophytol with tri-, di-, or monomethylhydroquinone; when produced synthetically, racemic mixtures of eight stereoisomers are formed. Tocopheryl acetate, linoleate/oleate, and nicotinate are prepared by the esterification of tocopheryl with the respective acid. Tocopheryl succinate is obtained by the vacuum steam distillation and succinylation of edible vegetable oil products; it can also be prepared by treating α-tocopherol with succinic acid anhydride in pyridine. Potassium ascorbyl tocopheryl phosphate is manufactured using a phosphate diester linkage of vitamin E and vitamin C, formulated as a potassium salt. Tocophersolan is prepared from crystalline d-α-tocopheryl succinate by esterification of the acid group with polyethylene glycol.[1]

Tocotrienols can be isolated from a number of plant sources, including *Vitis vinifera* and *Avena sativa*.[3] The extraction method with the highest efficiency is Soxhlet extraction using hexane. Tocotrienols can also be synthesized via a multitude of synthetic pathways, most of which involve multi-step processes to alkenylate the chromanol core.[6]

Natural Occurrence

From the original report on Tocopherols

Tocopherol occurs naturally in four forms, i.e., d-α-, d-β-, d-γ-, and d-δ-. α-, β-, γ-, and δ-tocotrienols are also found in nature; these compounds differ from tocopherol only in that the side chain is saturated in tocopherol and unsaturated in tocotrienol. Tocopherol is found largely in plant materials; it is also found in vegetable fats and oils, dairy products, meat, eggs, cereals, and nuts.[1]

Tocopherols and tocotrienols are synthesized by photosynthetic organisms, and because this synthesis is accomplished stereo-specifically via enzymes, the resulting tocopherols always possess the same stereochemical configurations, with the R configuration at C-2 (ring-sidechain junction) and the sidechain methyl substituents.[7-10] Tocotrienols are present in seeds, fruits, and latex. Tocopherols are the exclusive form of vitamin E in leaves of plants and the seeds of most dicots; tocotrienols are found in the seed endosperm of a limited number of dicots. Tocotrienols are the primary form of vitamin E found in the seed endosperm of most monocots, including wheat, rice, and barley, and tocotrienols are also found in coconut oil, cocoa butter, and soybeans.

Natural sources of tocopherols include sunflower, peanut, walnut, sesame, and olive oils; tocotrienols are not found in these oils.[9] γ-Tocopherol is often the most prevalent form of vitamin E in plant seeds and the products derived from them; corn, soybean and sesame oil and walnuts, pecans, and peanuts are good sources of γ-tocopherol.[11] Tocopheryl phosphate has been identified in various seeds and nuts, dairy products, green vegetables, fruits, and cereals.[12]

Palm oil is one of the richest sources of tocotrienols; tocotrienols compose up to 70% of the vitamin E in palm oil.[9] Crude palm oil extracted from the fruits of *Elaeis guineensis* contains up to 800 mg/kg tocotrienols.[8] Different analogs are more abundant in some plants than others; for example, rice bran oil is a major source of γ-, but not α-, tocotrienol.[7] Some other examples of plants containing significant amounts of tocotrienols are provided in Table 3.

Ultraviolet Absorbance

From the original report on Tocopherols

Tocopherol, tocopheryl acetate, and tocopheryl succinate absorb in the ultraviolet B (UVB) range. Reported absorption maxima are 292-295 nm (in ethanol) for tocopherol, 284 nm (in ethanol) and 285.5 nm (in cyclohexane) for tocopheryl acetate, and 286 nm (in ethanol) for tocopheryl succinate.[1]

USE

Cosmetic

Most of the tocopherols are reported to function in cosmetics as antioxidants or skin conditioning agents.[13] In contrast, tocotrienols is not reported to function in cosmetics as an antioxidant, but instead as a light stabilizer, oral care agent, or skin conditioning agent (Table 1.) The Food and Drug Administration (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 2014 report that the frequency of use increased considerably for both tocopherol and tocopheryl acetate. The reported use of tocopherol increased from 1072 (1998 data) to 6635 uses (2014 data), and the reported use of tocopheryl acetate increased from 1322 (1998 data) to 9677 uses (2014 data).[14] For both of these ingredients, the 2014 VCRP data includes uses under more than one name. Tocopherol has 6470 reported uses as tocopherol, 119 reported uses as tocopherol *d*-alpha, and 46 reported uses tocopherol *dl*-alpha. Tocopheryl acetate has 9098 reported uses as tocopheryl acetate and 579 reported uses as tocopheryl acetate *dl*-alpha. Both of these ingredients are used in almost every product category. The use of the other tocopherols that were included in the original safety assessment did not change much.

The use concentration of tocopherol, but not of tocopheryl acetate, has increased since the original assessment. According to the survey conducted by the Personal Care Products Council (Council), the concentration of use of tocopherol in leave-on products increased from 2% in 1999 to 5.4% in 2013.[15] Tocopheryl acetate continued to have the highest reported use concentration; it is used at up to 36% in leave-on formulations, and that use is in cuticle softeners. The original safety assessment reported that tocopheryl acetate was used at 100% in “vitamin E oil”.

Ascorbyl tocopherol acetate and tocopheryl phosphate are not reported to be used according to VCRP data and Council survey data.[14]

The current and historical frequency and concentration of use data for the previously-reviewed tocopherols are provided in Table 4. Table 5 provides the frequency[14] and concentration[15,16] of use data for the ingredients that have been included in this re-review.. The tocopherols are used in baby product formulations (0.1% tocopheryl acetate in baby lotions, oils, and creams) and formulations that can be incidentally ingested (3% tocopheryl acetate in lipsticks).

Several of the tocopherols are used in products that can possibly be inhaled. For example, tocopheryl acetate is used at up to 5% in foot powders and sprays and up to 0.2% in aerosol hair spray formulations, and tocopherol is used at up to 1% in pump hair spray formulations. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μm, with propellant sprays yielding a greater fraction of droplets/particles <10 μm compared with pump sprays.[17,18] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[19,20] Tocopheryl acetate is used at up to 0.1% in aerosol deodorants, and there is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[19] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays.

All tocopherols named in this report are listed in the European Union inventory of cosmetic ingredients.[21] α-Tocopherol acetate is the most common form of vitamin E used in commercial sunscreen and skin care products.[22]

Non-Cosmetic

α-Tocopherol and α-tocopheryl acetate are GRAS food ingredients when used as a nutrient, and α-tocopherol is GRAS as a chemical preservative in food when used in accordance with good manufacturing practices. (21CFR182.8890; 21CFR182.8892; 21CFR182.8390) The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a group allowable daily intake (ADI) of 0.15-2 mg/kg for *dl*-α-tocopherol and *d*-α-tocopherol, concentrate, singly or in combination.[23]

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

From the original report on Tocopherols

In a dermal absorption study using human subjects, tocopheryl acetate was substantially absorbed in the skin, but systemic availability was not observed. Also, conversion to tocopherol was not seen. In a study using rats, approximately 6% of the applied dose penetrated into the epidermis after 5 days. Most studies found that some tocopheryl acetate was converted to tocopherol. Irradiation of the animals dosed with tocopheryl acetate resulted in a significant increase in the amount of tocopheryl acetate found in the skin as compared to non-irradiated animals, and irradiation significantly increased the amount of tocopherol found in the skin of test animals. Tocopherol was "more efficiently" absorbed from w/o than o/w emulsions. The liposomal form increased absorption from o/w emulsions but did not have an effect on w/o emulsions.[1]

In oral ingestion studies with human subjects, administration of tocopherol and tocopheryl acetate resulted in increased tocopherol concentration; the mean serum concentration peaked at approximately 7-8 h following dosing. In one study, d-*α-tocopherol had a greater bioavailability than dl-α-tocopheryl. In an oral study using rats, tocopheryl acetate generally had a greater uptake than tocopheryl nicotinate. The liver was the principal storage site, but the adrenal glands had the greatest uptake. Most of the radioactivity was recovered as tocopheryl quinone, but in a few tissues, such as the adrenal glands, it was recovered mostly as tocopherol. In another study using rats that were fed tocopheryl acetate for up to 14 wks, a linear relationship was found between time and tissue concentration of tocopherol. In mice that were fed tocopheryl acetate and irradiated, a dose-dependent increase was seen for tocopherol in the ventral skin compared to controls, whereas irradiation decreased dorsal skin tocopherol concentrations.*[1]

Oral

The structural differences between tocopherol and tocotrienols result in a difference in the penetration of these compounds into tissues. The presence of three unsaturated bonds in the carbon sidechain allows tocotrienols to penetrate tissues with saturated fatty layers, such as the brain and the liver, more readily than tocopherol, which has a fully saturated side chain.[8,9]

Many studies have been conducted on the distribution of tocopherols and tocotrienols with oral administration, and the studies demonstrate that tocopherols distribute to almost all tissues in the body, and the distribution and metabolism varies among the tissues. α-Tocopherol is the predominant form of vitamin E in human and animal tissues, and it has the highest oral bioavailability, and *RRR*-α-tocopherol (natural vitamin E) has approximately twice the systemic availability of synthetic tocopherol (*all-rac*-tocopherol). Humans also have significantly greater plasma and tissue concentrations of α- than γ-tocopherol, and supplementation with α-tocopherol actually decreases plasma and tissue γ-tocopherol. The tocotrienols are not as prevalent in the body as the tocopherols, and oral absorption of the tocotrienols has been reported to be incomplete. One study reported that α-tocopherol out-competes α-tocotrienols for transport to systems in the body when these ingredients are co-supplemented. Orally administered tocopherols and tocotrienols are distributed to the skin and adipose tissue.[24-35]

The distribution and intracellular trafficking of vitamin E may be modulated by tocopherol regulatory proteins; three have been identified that specifically bind tocopherols.[35] Tocopherol transfer protein (TTP) is a cytosolic lipid-binding and transfer protein that has been found in rat brain, spleen, lung, and kidney, in some human brains, and in the mouse uterus. Tocopherol-associated protein (TAP), also a cytosolic lipid-binding and transfer protein, is found in bovine and human tissues; the greatest levels in human tissues were in the adult liver. The third protein, tocopherol-binding protein (TBP), is found in rat liver and heart, rabbit heart, bovine heart, and human placenta. Only TTP has been shown to influence plasma and tissue α-tocopherol concentrations. Relative affinities of purified TTP for α-tocopherol, as determined by competition between vitamin E analogs for transfer between liposomes and membranes *in vitro*, were 100% for RRR-α-tocopherol, 38% for β-tocopherol, 9% for γ-tocopherol, 2% for δ-tocopherol, 12% for α-tocotrienol, and 11% for SRR-α-tocopherol.

Tocopherols and tocotrienols were found in the skin of female hairless mice fed a chow containing 29.7 mg α-tocopherol, 10.3 mg γ-tocopherol , 3.1 mg α-tocotrienol, and 7.4 mg γ-tocotrienol per kg chow.[36] However, the distribution in the chow was different than that found in the skin; approximately 58% of the vitamin E in the chow was α-tocopherol and 20% was tocotrienols, but approximately 89% of the vitamin E found in the skin was α-tocopherol.

A procedure was developed that allowed for the simultaneous determination of tocopherols and tocotrienols, and the presence of these cogeners in tissues of the hairless mouse was determined.[37] The skin contained mostly α-tocopherol (5.4 nmol/g; 85.6%), but α-tocotrienol (0.24 nmol/g; 3.4%) and γ-tocotrienol (0.76 nmol/g; 10.4%) were also found. Tocotrienols were

also identified in the skin + subcutis, but to a lesser extent. The brain contained primarily α-tocopherol (5.4 nmol/g; 99.8%), and it contained no tocotrienols. The heart, kidneys, and liver also contained primarily α-tocopherol, but some tocotrienols were present, and the highest amount of α-tocopherol was found in the heart (24.2 nmol/g; 98.1%).

Groups of 10 female CF-1 mice were fed a basal diet (containing 75 IU/kg vitamin E) or basal diet supplemented with 0.3% a γ-tocopherol-rich mixture of tocopherols for 4 wks.[38] One gram of the mixture contained 900 mg total tocopherols with 13.0% of α-tocopherol, 1.5% β-tocopherol, 56.8% γ-tocopherol, and 24.3% δ-tocopherol. Pooled urine and fecal samples were collected for each group during the last 4 days of the study, and at study termination, blood and liver samples were collected. High pressure liquid chromatography/electrochemical detection (HPLC/EC) and liquid chromatography-electrospray ionization/ mass spectrometry (LC-ESI-MS) were used to analyze the major metabolites of the different tocopherols and tocotrienols in the samples. The major route of excretion was fecal; 18 tocopherol-derived metabolites and α-, γ-, and δ-tocopherol were identified in the fecal samples of the mice fed the tocopherol-diet. Short-chain degradation metabolites, particularly γ- and δ-carboxyethyl hydroxychromans and carboxymethylbutyl hydroxychromans were detected in urine, serum, and liver samples, and tocopherols were detected in serum and liver samples. The majority of the urinary metabolites were excreted as glucuronide conjugates.

The researchers also examined the metabolite profile of pooled samples of feces, blood, and urine from a study in which male CF-1 mice were fed a basal diet or the basal diet supplemented with 0.17% of a tocotrienol preparation for 21 wks; the preparation was a 65% oil suspension that contained 20.2% α-tocotrienol, 4.0% β-tocotrienol, 16.1% γ-tocotrienol, 24.3% δ-tocotrienol, 14.8% α-tocopherol, and 3.1% γ-tocopherol.[38] Twenty-four tocotrienol-derived side-chain degradation metabolites were identified in fecal samples, as were parent α-, γ-, and δ-tocotrienols; more short-chain than long-chain tocotrienols-derived metabolites were present in the feces. Short-chain degradation metabolites, particularly carboxyethyl hydroxychromans, carboxymethylbutyl hydroxychromans, and carboxymethylhexenyl hydroxychromans were detected in urine.

The metabolism of a tocopherol supplement was evaluated in humans.[38] Two male subjects were given six softgels containing γ-tocopherol-rich dietary supplements at 0 h, and six more softgels at 10 h. Each softgel contained 200 mg β-tocopherol, 78 mg δ-tocopherol, 133 mg α-tocopherol, and 2 mg tocotrienols. Fecal and urine samples were collected at 0, 12, 24, and 48 h, and blood samples were taken at 0 and 12 h. At 24 h, almost all the side-chain degradation products that were identified in mouse fecal samples (described previously) were found in human fecal samples, and the metabolite concentrations increased over time. γ-Tocopherol-derived metabolites were more prominent than δ- and α-derived metabolites at 24 h. The major metabolites found in the urine were carboxyethyl hydroxychromans and carboxymethylbutyl hydroxychromans.

Eight-day pregnant rats were dosed by gavage with 1 g/kg tocotrienol-rich fraction (1.23 mmol α-tocotrienol and 0.94 mmol tocopherol per g; isolated from palm oil) suspended in vitamin-E deficient corn oil for nine days.[39] The rats were killed on day 17 of gestation, and tissues were collected from the dams and the neonates. In the maternal brain, the α-tocopherol content and α-tocotrienol content changed by 0.1-fold and 5-fold, respectively. In the fetal brain, α-tocotrienol content increased more than 20-fold. Subsequent testing with lower doses of tocotrienols (5 mg/kg bw) over a longer time period (not specified) supported the finding that dietary tocotrienol was distributed to the brain.

Tocopherol Depletion by UV in the Skin

Tocopherol

Female C3H/HeN Tac mice were treated topically with 5.3 µmol *RRR*-α-tocopherol or with 500 nmol of [^{14}C]-α-tocopherol and 4800 nmol α-tocopherol, in 50 µl acetone, 15 min prior to ultraviolet B (UVB) irradiation; additional groups of mice were treated with [^{14}C]-α-tocopherol or α-tocopherol, in 50 µl acetone or dimethyl sulfoxide (DMSO) for 3 h prior to irradiation.[40] The dorsal skin was shaved, and the animals were irradiated with 2.6-2.9 J/m^2/s for 1 h (10 kJ/m^2) using Westinghouse FS-20 UV lamps. Approximately 80% of the lamp output was in the UVB range (290-320 nm). The researchers noted that the AIN 76 chow used in the study contained *d,l*-α-tocopheryl acetate. Depletion of α-tocopherol in the skin by UVB was quantified following application of 5.3 µM synthetic d_3-α-tocopherol in acetone or DMSO 3 h prior to irradiation. A 1-h exposure to UVB substantially depleted the level of d_3-α-tocopherol in both treatment groups, and approximately 40-60% of the applied α-tocopherol in the epidermis was consumed by UVB treatment.

With the 15-min tocopherol exposure prior to UV, the researchers found that α-tocopherol was most likely not absorbed into the epidermis prior to irradiation, and that photooxidation occurred on the skin surface. The vehicle that was used did not have an effect on the depletion of topically applied tocopherol, but it did have an effect on the products of photooxidation; more polar products occurred when DMSO was used as the vehicle.

Tocopherol Acetate

The hydrolysis of α-tocopheryl acetate to α-tocopherol was determined in non-irradiated and irradiated mouse skin.[41] Without UV, deuterium-labeled (d_3-) α-tocopheryl acetate in an inert cream (50 mg of a 5% cream, 5.3 µmol d_3-α-tocopheryl acetate) was applied to the shaved dorsal skin of female C3H/HeN Tac mice. With UV, either shaved mice were irradiated for 60 min with a total dose of 13 kJ/m^2 UVB, and then treated with 5.3 µmol d_3-α-tocopheryl acetate in either the inert cream, or, d_3-α-tocopheryl acetate was applied 15 min before UVB irradiation. All animals were killed 1, 3, 6, 24, or 48-h

after dosing, and the epidermis was isolated. At 24 and 48-h post-dosing with d_3-α-tocopheryl acetate in non-irradiated mice, d_3-α-tocopherol increased up to 10 times that of endogenous α-tocopherol. Application of d_3-α-tocopheryl acetate 60 min after irradiation resulted in a 40-fold increase in epidermal α-tocopherol. In both cases, hydrolysis was <1% of the total applied dose. Hydrolysis of α-tocopheryl acetate increased with increased UVB dose. Application of d_3-α-tocopheryl acetate prior to irradiation eliminated hydrolysis.

A dose-response effect of UVB on d_3-α-tocopheryl acetate hydrolysis was then examined. Shaved mice were exposed to UVB for 60, 90, or 120 min, corresponding to 13, 19.5, or 26 kJ/m^2, respectively, in a single dose or in multiple 30-min/day doses, and the mice were treated with 5.3 µmol d_3-α-tocopheryl acetate in the inert cream. The animals were killed 24 h after dosing, and the epidermis was isolated. UVB exposure, as a 30-min multiple day dose, potentiated UVB-induced hydrolysis of d_3-α-tocopheryl acetate to d_3-α-tocopherol.

The researchers also examined the effect of rate of absorption into the skin on hydrolysis by applying 5.3 µmol d_3-α-tocopheryl acetate in 50 µl DMSO, following the same procedure described earlier. In non-irradiated mouse skin, hydrolysis was maximal at 3-h post-treatment with d_3-α-tocopheryl acetate in DMSO, which was earlier than what was observed using the inert cream, suggesting that hydrolysis was limited by absorption in these mice. The researchers commented that although maximal absorption occurred earlier than when d_3-α-tocopheryl acetate was applied in an inert cream, the maximal capacity for hydrolysis was the same (i.e., ≈80 pmol d_3-α-tocopherol/mg epidermis). In mice irradiated for 1 h prior to dosing, a time-dependent increase in hydrolysis was observed at 6 ad 24-h post-treatment, which was similar to the response observed with the inert cream, suggesting that absorption is not the limiting factor in UVB-induced hydrolysis of d_3-α-tocopheryl acetate. These conclusions were supported by the fact that a 10-fold greater dose of d_3-α-tocopheryl acetate resulted in a 4-6 fold increase in d_3-α-tocopherol levels in non-irradiated mice, but only a 2-3 fold increase in irritated mice. Further testing by the researchers led to the suggestion that the esterase activity at the surface of the skin was capable of converting d_3-α-tocopheryl acetate to d_3-α-tocopherol.

Tocopherol Phosphate

One study found that following UV exposure, reduction of endogenous α-tocopherol in the skin was not inhibited by pre-treatment with α-tocopheryl phosphate.[42] Pretreatment of SKH-1 mouse skin cultures with 0.5% α-tocopheryl-6-*O*-phosphate produced a two- to three-times greater amount of endogenous α-tocopherol than that found in control skin, and the UV-induced reduction in cutaneous α-tocopherol was inhibited.

Dermal Penetration

In Vitro

The effect of vehicle on the dermal penetration of α-tocopherol was determined in micro-Yucatan pig skin.[43] The dermal penetration of 1% tocopherol was evaluated in several vehicles, and α-tocopherol permeated into the receptor fluid regardless of the vehicle used. The greatest permeation found in viable skin + the receptor was from an emulsion vehicle that contained 10% isopropyl myristate; 12.24% of the applied dose was found in the viable skin and receptor fluid. The lowest penetration, 6.47% applied dose, was observed with an alcoholic vehicle that was 96% SD alcohol.

The researchers also examined the kinetics of permeation and metabolism of α-tocopheryl acetate across micro-Yucatan pig skin.[44] Formulations containing a 5% tocopheryl acetate solution in isopropyl myristate, a 5% tocopheryl acetate emulsion, and a 1% solution of α-tocopherol in isopropyl myristate were studied, and permeation was measured at 2, 6, 12, and 24 h. Any presence of tocopheryl acetate in the receptor fluid was below the limit of detection. Tocopheryl acetate was converted to tocopherol within the viable skin tissue.

In vitro skin penetration data on tocopheryl acetate were obtained from a robust summary of data submitted to the European Chemical Agency (ECHA) as part of the REACH chemical registration process. These data are available on ECHA's website.[45] The *in vitro* skin absorption study, performed according to OECD Guideline 428, indicated that tocopheryl acetate in an alpha-hydroxy acid (AHA) vehicle (composition not defined further) penetrated into and through intact and stripped pig skin, and the total skin penetration rates were time- and formulation-dependent; the differences were not statistically significant. First, intact skin was exposed to 6 mg/cm^2 of 5% [^{3}H]tocopheryl acetate cream (300 µg active substance/ cm^2) in one of three AHA vehicles for 1, 6, or 18 h. After 1 h, 1.1-1.3% of the dose (based on penetration in the stratum corneum, remaining skin tissues layers, and chamber liquid) was absorbed. After 6 h, absorption was 2.6-4.2%, and after 18 h, 3.1-4.2% of the dose absorbed. Using stripped skin (stratum corneum removed), higher absorption rates through the remaining skin tissue was observed for all three formulations.

The dermal penetration of tocopheryl acetate in human skin samples was measured using four different vehicles, and the bio-conversion of tocopheryl acetate to tocopherol was determined.[5] Tocopheryl acetate was (1) delivered in a triglyceride oil (caprylic/capric acid triglyceride); (2) surfactant-solubilized in water (PPG-26-buteth-26 and PEG-40 hydrogenated castor oil); (3) encapsulated in soybean phosphatidylcholine liposomes; or (4) encapsulated in nanotopes™. The final concentration of tocopheryl acetate was 2%. A dose of 11 mg/cm^2 of each test formulation (corresponding to 220 µg/cm^2 tocopheryl acetate) was applied to skin samples under occlusive and non-occlusive conditions. The distribution of tocopherol acetate and tocopherol at the skin surface, in the horny layers, and in the underlying skin was determined after 8 h.

The recovery rate of tocopheryl acetate + tocopherol (vitamin E_{total}) exceeded 90% in each experiment. Tocopheryl acetate in the triglyceride oil was only deposited at the skin surface and in the horny layers. With the other three formulations, differences were found with occlusive versus non-occlusive application; more vitamin E_{total} was found in the underlying skin with the non-occlusive application. The conversion to free tocopherol only occurred in the underlying skin layer, and the percent bioconversion was greater with occlusive application. The distribution of vitamin E_{total} and the bioconversion of tocopheryl acetate to tocopherol from each formulation are summarized in Table 6.

An *in vitro* study using human cadaver skin was conducted to determine the absorption of tocopheryl acetate from various vehicles.[46] Modified Franz diffuser cells, and 95% degassed ethanol, as opposed to modified phosphate-buffered saline, was used in the receiver compartment. Sampling times ranged from 2-48 h. Tocopheryl acetate, as a 5% solution, was prepared in ethanol, isopropyl myristate, and mineral oil; 5% gel formulations were prepared in a 1% or 3% water-soluble polymer. Isopropyl myristate was the only vehicle that statistically significantly increased permeation through the skin. The calculated permeabilities (using a mathematical model) of the 5% tocopheryl acetate formulations were 1.0×10^{-4} cm/h with ethanol; 1.1×10^{-2} cm/h with isopropyl myristate; 1.4×10^{-4} cm/h with mineral oil; 2.1×10^{-4} cm/h with the 1% water-soluble polymer gel; and 4.7×10^{-4} cm/h with the 3% water-soluble polymer gel.

In Vivo

Tocopherol

Dermal application of 5 mg/cm^2 α-tocopherol to the backs of female hairless SKH1 mice for 24 h resulted in a 62-fold increase of α-tocopherol in the epidermis and a 22-fold increase in the dermis.[47]

Tocopheryl Acetate

An ointment containing 2% or 20% tocopheryl acetate was applied to the backs of five male Wistar rats for 24 h, and a 2 cm-diameter skin punch was then taken to determine the content of vitamin E in the skin.[48] (The ointment contained 15% squalane.) Application of 20% tocopheryl acetate, but not 2%, resulted in a statistically significant increase of vitamin E in the skin. Vitamin E increased by 2.9 µg/g skin with the 20% ointment, but only by 0.8 µg/g skin with the 2% ointment.

Tocopherols and Tocotrienols

A 5% w/v solution containing a tocotrienol-rich palm oil fraction (TRF) in polyethylene glycol (PEG)-400 was applied to the skin of four female hairless mice.[36] The distribution of four analogs in the TRF was approximately 35% α-tocopherol; approximately 10% γ-tocopherol; approximately 25% α-tocotrienols; and approximately 30% γ-tocotrienols. Four test sites were marked using polypropylene plastic rings, and 20 µl of the test mixture were applied to the area marked by two of the rings and 20 µl of PEG-400 were applied to the other two areas. After 2 h, the excess substance on the treated area was removed, first by rinsing three times with ethanol/water (95:5) and then twice with water alone. Half of the test sites were shielded, and the skin on the back of each animal was then irradiated for 29 min using an Oriel 1000-W solar simulator with an output of 2.8 mW/cm^2 of UVA and UVB light; this corresponded to 3 MED. The animals were then killed.

Baseline values of each analog in PEG-400-treated skin were determined first; approximately 8.5 nmol/g skin α-tocopherol, approximately 3 nmol/g skin γ-tocopherol, approximately 7.5 nmol/g skin α-tocotrienols, and approximately 7 nmol/g skin γ-tocotrienols were present in the skin. Dermal application of the TRF resulted in a 28-fold increase in α-tocopherol, an 80-fold increase in α-tocotrienol, a 130-fold increase in γ-tocopherol, and a 51-fold increase in γ-tocotrienol in the skin. The percent distribution of each of the analogs in the TRF mixture was compared with its percent distribution in skin. The percent distribution of each analog that penetrated the skin was significantly different from its distribution in the mixture; higher percentages of α-tocopherol, similar amounts of γ-tocopherol, and lower percentages of α- and γ-tocotrienols were found in the skin, compared to the distribution in the TRF mixture.

In the skin from the UV-irradiated sites, approximately 40% of the vitamin E remained in the TRF-treated skin; there were no significant differences in the degree of destruction of the various analogs. Approximately 80% of vitamin E remained in the PEG-treated skin. The researchers examined the effect of UV-irradiation on the breakdown of the analogs in the TRF solution itself; 86% α-tocopherol, 83% γ-tocopherol, 83% α-tocotrienol, and 84% γ-tocotrienol remained after irradiation.

The distribution of tocopherol and tocotrienol in the skin was also examined.[49] Forty µl of a 5% (w/v) solution α-tocopherol, α-tocotrienol, or γ-tocotrienol in PEG-400 was applied to the backs of SKH-1 hairless mice for 0.5, 1, 2, or 4 h using a 2 cm^2 polypropylene plastic ring, and the animals were killed after the skin was rinsed. Four animals were used per group. The concentration of each test article was measured in the skin layers. Application of all three analogs significantly increased the concentrations of vitamin E in the skin; application of the 5% α-tocopherol solution resulted in a 200- to 2000-fold increase in skin α-tocopherol content. The concentration of each analog in the skin increased with time, but the relationship between the analogs and the content of each skin layer did not change. When expressed per µ, the highest concentrations of vitamin E was found in the uppermost layer of the stratum corneum (5 µm), and the concentration of α-tocopherol was statistically significantly greater than that of α- or γ-tocotrienol. When the thickness of each skin layer was considered, the lower skin layers contained appreciable amounts of vitamin E. The largest fraction of vitamin E in the skin following topical application was found in the deeper subcutaneous layers; the papillary dermis (31-130 µm) and the dermis (131-530 µm) contained the major portions of vitamin E if the total amount of each analog in each of the layers was summed and expressed as a

percentage per its respective total. At 0.5 h, significant increases in vitamin E were found in the subcutaneous fat, suggesting rapid penetration of vitamin E through the skin.

Antioxidant Properties

From the original report on Tocopherols

Tocopherol is the major lipid-soluble chain-breaking antioxidant of membranes and an important cellular protectant against oxidative damage. It exerts antioxidant effects by trapping peroxyl radicals. Some researchers found that tocopherol was depleted from the skin upon exposure to UV light; they postulated that other antioxidants that can recycle tocopherol can also be depleted.[1]

Tocotrienols are reported to exert antioxidant effects by scavenging chain-propagating peroxyl radicals.[9]

Miscellaneous Effects

From the original report on Tocopherols

α-Tocopherol is a good inhibitor of nitrosation because its phenol ring is fully substituted. α-Tocopherol might inhibit formation of skin nitrosating agents from NO_2, but it did not inhibit nitrosamine production from skin nitrosating agents. Tocopheryl acetate altered the cellular response of rats to nitrite; it prevented nitrite-related mortality and the decrease in glutathione S-transferase activity.[1]

TOXICOLOGICAL STUDIES

Single Dose (Acute) Toxicity

From the original report on Tocopherols

In rats, the dermal LD_{50} is >3 g/kg for tocopherol and >2 g/kg for tocophersolan. The oral LD_{50} of tocopherol, tocopheryl acetate, tocopheryl nicotinate, tocopheryl succinate, and tocophersolan are greater than 4, 16, 10, 7, and 7 g/kg, respectively, in rats. In mice, the oral LD_{50} of tocopherol is >25 ml/kg and of tocopheryl acetate is >4 g/kg.[1]

Dermal

Tocopheryl Acetate

According to robust summary data submitted to ECHA, the dermal LD_{50} of tocopheryl acetate is > 3 g/kg bw in albino rats.[45] Five animals per group were dosed with 1 or 3 g/kg bw undiluted tocopheryl acetate in vegetable oil under an occlusive patch for 24 h. Slight erythema was observed 24-48 h after exposure. Slight abrasion was observed in one low-dose female, two high-dose females, and two high-dose males.

Tocopheryl Phosphate

The acute dermal toxicity of mixed tocopheryl phosphates (MTP) was determined in New Zealand rabbits; the dermal LD_{50} was greater than 1130 mg/kg bw MTP in female rabbits.[50] MTP is a mixture of α-tocopheryl phosphate and α-di-tocopheryl phosphate, and is produced by phosphorylating d-α-tocopherol with P_4O_{10}; the final composition typically contained α-tocopheryl phosphate, α-di-tocopheryl phosphate, and α-tocopherol in the ratio of 1:0.49:0.08 (w/w/w). (Unless indicated otherwise, this is the composition of MTP tested in various studies that are summarized throughout this report.) An aq. gel containing 1130 mg/kg bw MTP (918 mg/kg bw α-tocopherol equivalents) was applied to the clipped dorsal skin of five male and five female rabbits for 24 h using surgical gauze. The test site was washed upon patch removal, and evaluated for irritation at 24 h, 7 days, and 14 days. At 24 h, slight to well-defined erythema was observed in 4/5 males and all females, and slight to moderate edema was observed in 2/5 males and all females. Signs of irritation were not observed at days 7 and 14. All animals survived until study termination; no gross findings were observed at necropsy.

Oral

Tocopheryl Phosphate

The oral LD_{50} of MTP is >1130 mg/kg bw in Wistar rats.[50] Groups of five male and five female were dosed by gavage with 1130 mg/kg bw MTP (918 mg/kg bw α-tocopherol equivalents) in distilled water, and killed after 14 days. All animal survived until study termination.

Repeated Dose Toxicity

From the original report on Tocopherols

In rats, tocopherol was not toxic in a 60-day study and tocopheryl acetate was not toxic in an 8-wk feeding study. In a 90-day study, 7 of 10 male rats dosed orally with 2000 mg/kg d-α-tocopherol died in 9 to 11 wks because of internal hemorrhage; other signs of toxicity were observed in a dose-dependent manner. Rats fed ≤2% tocophersolan for 3 mos did not have any treatment-related effects. In a 2-yr study in which rats were fed ≤2000 mg/kg/day dl-α-tocopheryl acetate and supplemented with vitamin K, no significant treatment-related effects were observed. High doses of tocopherol and tocopheryl acetate have hemorrhagic activity. 1

Oral

Tocophersolan

Groups of five male Sprague-Dawley rats were dosed by gavage with an aqueous solution of 0.5, 1, or 2 g/k g tocophersolan once daily for 5 days, and the animals were then killed.[51] No signs of toxicity were observed, and all animals survived until

study termination. Heart, kidney, liver, and spleen weights were not affected by dosing with tocophersolan, and there were no dose-dependent or statistically significant changes in hematology, clinical chemistry, or urinalysis parameters.

Tocopheryl Phosphate

Groups of 10 male and 10 female Sprague-Dawley rats were fed a diet containing 0, 1, 3, or 5% MTP for 90 days; this MTP was composed of 72% α-tocopheryl phosphate and α-ditocopheryl phosphate, 13% α-tocopherol, 6% water, 7% phosphoric acid, and 2% other. [12] No clinical signs of toxicity, adverse effects on body weights, or test article-related mortality were reported. There were some statistically significant changes in hematology and clinical chemistry parameters, but generally these changes were dose-dependent and not considered toxicologically significant. No statistically significant, dose-dependent changes in organ weight were observed compared to controls. There were no gross changes observed at necropsy; changes in relative heart and epididymal organ weight were considered incidental. The only significant microscopic finding was the presence of foreign material in sinusoidal macrophages associated with mild inflammatory changes in the mesenteric lymph nodes in male and female treated animals that occurred in a dose-dependent manner; the foreign material was identified as tocopheryl phosphate. In the mid- and high-dose groups, but not the low-dose groups, the foreign material often had a crystalline appearance. Foreign material was also present in the small intestines of the mid- and high-dose animals. The NOAEL was 1% MTP in the diet.

Three repeated-dose oral toxicity studies of MTP were performed in rats, and no test article-related toxicity was observed.[50] The animals were dosed by gavage for 28 days in each of the studies. In the first study, 10-11 Sprague-Dawley rats/gender/group were dosed with 0, 368, 735, or 982 mg/kg bw/day MTP in a medium-chain triglyceride vehicle (corresponding to 0, 45.9, 229.5, and 458.8 mg/kg bw/day tocopherol equivalents, respectively). In the second study, groups of 4-5 Sprague-Dawley rats/gender/group were dosed with 0 or 869 mg/kg bw/day MTP (corresponding to 00 and 642 mg/kg bw/day tocopherol equivalents, respectively). And in the third study, groups of 5 Wistar rats/gender/group were dosed with 0, 56.5, 282.5 or 565 mg/kg bw/day MTP (corresponding to 0, 45.9, 229.5, and 458.8 mg/kg bw/day tocopherol equivalents, respectively). The animals were killed on day 29 in the first and third studies, and after a 14-day recovery period in the second study. No test-article related mortality, no signs of toxicity, and no microscopic or gross lesions were observed in any of the studies.

Tocotrienols

Groups of 10 male and 10 female F344/DuCrj rats were fed a diet containing 0, 0.19, 0.75 or 3% of a tocotrienols preparation for 13 wks; the preparation consisted of 21.4% α-tocotrienol, 3.5% β-tocotrienol, 36.5% γ-tocotrienol, 8.6% δ-tocotrienol, 20.5% α-tocopherol, 0.7% β-tocopherol, 1.0% γ-tocopherol, and 0.5% δ-tocopherol.[52] Another group was fed a diet containing 0.69% α-tocopherol. No remarkable changes in general appearance and no morality was reported. Body weight gain was decreased in the males fed the 3% tocotrienols diet as compared to the other groups, but feed consumption was similar to the other groups. Relative adrenal glands to body weights were dose-dependently increased in all treated males, and lung weights were statistically significantly decreased in all treated females. In the 3% tocotrienols group, statistically significant increases were observed in brain, heart, liver, kidneys, and testes weights of males and liver and spleen weights of females and statistically significant decreases in ovary and uterus weights were observed in females. Statistically significant changes were noted in various hematological parameters, and most of these changes were thought to have little toxicological significance; however, the statistically significant decrease in platelets in males, but not females, in a dose-dependent manner was thought to be a physiological response. Several hematology parameters were statistically significantly decreased in females of the 0.75 and 3% tocotrienols group. Effects on several serum biochemistry values also were considered of little toxicological significance. The NOAEL was 0.19% tocotrienols preparation in the diet; a no-observable effect level (NOEL) could not be determined.

Ocular Irritation

From the original report on Tocopherols

In ocular irritation studies, tocopherol was non-irritating in some tests and was a minimal or very slight ocular irritant in others. Tocopheryl acetate, tocopheryl nicotinate, and a mixture of dioleyl tocopheryl methylsilanol and oleic acid were not irritating to rabbit eyes. Tocophersolan was a slight ocular irritant. [1]

Tocopheryl Acetate

According to robust summary data submitted to ECHA, tocopheryl acetate was not irritating to rabbit eyes in one study, but it produced weak to moderate conjunctival irritation in another study.[45] Undiluted tocopheryl acetate was instilled into the conjunctival sac of three Vienna White rabbits, and the eyes were not rinsed. The eyes were scored at 1, 24, 48, and 72 h after instillation. Slight irritation was observed at 1-48 h, and the eyes were normal at 72 h.

In a modified Draize test, the same protocol was followed as above, and undiluted dl-α-tocopherol was instilled into the eyes of six rabbits; again, the eyes were not rinsed. Weak to moderate conjunctival irritation (i.e., redness) was observed, which subsided by day 7. No corneal changes were reported.

Tocopheryl Phosphate

MTP was not irritating to rabbit eyes.[50] One-tenth ml of an aq. gel containing 47.5 mg MTP (38.4 mg/kg bw α-tocopherol equivalents) was instilled into the conjunctival sac of one eye of one male and two female New Zealand rabbits; the contralateral eye served as a control. The eyes were scored for irritation 1, 24, 48, and 72 h after dosing.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

From the original report on Tocopherols

Oral administration of tocopherol ((up to 75 mg /day in the diet), tocopheryl succinate, and tocophersolan did not have reproductive or developmental effects in rats, and tocopheryl acetate (≤ 1.6 g/kg/day) generally did not have any reproductive or developmental effects in rabbits, hamsters, rats, or mice. Tocopherol and tocopheryl acetate had some effect on reducing the number of malformations observed in neonates from diabetic dams. Tocopherol did not have an effect on zinc deficiency-induced teratogenicity. In some studies, tocopheryl acetate potentiated the embryo-lethal effects of cortisone acetate. Tocopheryl succinate reduced some reproductive effects, but not all, induced by TCDD. [1]

GENOTOXICITY

From the original report on Tocopherols

Tocopherol, tocopheryl acetate, tocopheryl succinate, and a mixture of dioleyl tocopheryl methylsilanol and oleic acid were generally not mutagenic. The only effects observed were a dose-dependent increased elution rate of DNA in alkali in a DNA strand breakage assay and 50% inhibition in the incorporation of [^{3}H]-thymidine in a thymidine incorporation assay with tocopherol. Tocopherol has some anti-mutagenic activity and was able to modulate some mutagenic effects. Tocopheryl succinate also had some mutagenicity modulatory activity. Tocopherol and tocopheryl succinate generally did not affect UV-induced mutagenicity. [1]

In Vitro

Tocopherol

According to robust summary data submitted to ECHA, tocopherol was not genotoxic in a mammalian cell assay.[45] The genotoxic potential of d-γ-tocopherol (92.6% pure) was evaluated in a mammalian cell assay. Chinese hamster ovary (CHO) cells were exposed to 2.9 and 14.6 µg/ml (6.8 and 34 µM, respectively) d-γ-tocopherol for 5 h without metabolic activation.

Tocopheryl Acetate

According to robust summary data submitted to ECHA, tocopheryl acetate was not genotoxic in Ames tests or a chromosomal aberration assay.[45] Two Ames tests were performed; the first was performed with *Salmonella typhimurium* TA1535, TA97, TA98, TA100, and TA102, with and without metabolic activation, at test concentrations of 0, 50, 158, 500, 1580, and 5000 µg/plate *all-rac*- α-tocopheryl acetate in ethanol. The second was performed using *S. typhimurium* TA1535, TA1537, TA98, and TA100 at concentrations of 0, 20, 100, 500, 2500, and 5000 µg/plate tocopheryl acetate in DMSO, with and without metabolic activation. Vehicle and appropriate positive controls were used in each study. Tocopheryl acetate was not mutagenic.

In the chromosomal aberration assay, human peripheral lymphocytes were exposed to dl-α-tocopheryl acetate/*all-rac*-α-tocopheryl acetate in 0.5% ethanol, with and without metabolic activation. Without metabolic activation, cells were exposed to 200, 600, and 1800 µg/ml for 24 h or 75, 300, or 1200 µg/ml for 3 h. With metabolic activation, cells were exposed to 200, 600, and 1800 µg/ml for 5 h or 75, 300, or 1200 µg/ml for 3 h. A negative (vehicle) control and appropriate positive controls were used. Tocopheryl acetate was not genotoxic.

Tocopheryl Phosphate

MTP was not mutagenic in the Ames test nor genotoxic in a chromosomal aberration assay.[50] Two Ames test were conducted with *S. typhimurium* TA98, TA100, TA1535, ad TA1537 and *Escherichia coli* WP2 uvrA. The test concentrations were 0.9-2825 µg/plate aq. MTP (0.73-2294 µg tocopherol equivalents/plate) in the first study, and 88.3-2825 µg/plate MTP (71.7-2294 µg tocopherol equivalents/plate) in the second study. The tests were conducted with and without metabolic activation, and positive and negative controls gave valid results.

Two assays for chromosomal aberrations were conducted using CHO cells; the test article was suspended in 1% carboxymethyl cellulose. Concentrations of 13.4-40.8 and 15.5-37.9 µg/plate MTP (10.9-33.1 and 12.6-30.8 µg tocopherol equivalents/plate, respectively) were tested with metabolic activation, and concentrations of 26.1-40.8 and 3.7-67.8µg/plate and MTP (21.2-33.8 and 3.0-55.1 µg tocopherol equivalents/plate, respectively) were tested without metabolic activation in the two studies. The only difference in protocol between the two studies was that, in the second study without metabolic activation, the cells were exposed continuously for 20 h, as opposed to a 3-h exposure and 17-h recovery period.

Tocopheryl Succinate

Tocopheryl succinate was weakly positive in a sister chromatid exchange assay in the presence of metabolic activation, and was negative for genotoxicity in a chromosome aberration assay.[53] Two trials were performed. In the first trial, concentrations of 5, 7, 10, and 50 µg/ml D-α-tocopheryl succinate were tested without metabolic activation, and doses of 30, 100, 300, and 1000 µg/ml were tested with metabolic activation. In the second trial, concentrations of 15.1, 19.95, 25.2, and

30.2µg/ml D-α-tocopheryl succinate were tested without metabolic activation, and concentrations of 202, 298.2, 396, and 497µg/ml were tested with metabolic activation. Vehicle (DMSO) and appropriate positive controls were used.

In the chromosomal aberration assay, D-α-tocopheryl succinate concentrations of 39.8, 49.8, 60, and 75 µg/ml D-α-tocopheryl succinate were tested without metabolic activation, and doses of 400, 450, and 500 µg/ml were tested with metabolic activation.[54] In the second trial, concentrations of 24.9, 30.1, and 35 µg/ml D-α-tocopheryl succinate were tested without metabolic activation, and concentrations 4100, 5000,and 6000 µg/ml were tested with metabolic activation. Vehicle (DMSO) and appropriate positive controls were used.

CARCINOGENICITY

From the original report on Tocopherols

Tocopheryl acetate (≤ 2 g/kg/day) was not carcinogenic in a dietary study. Neoplasms developed in animals injected subcutaneously with tocopherol or tocopheryl acetate and soya oil; however, neoplasms were not seen in animals dosed with tocopherol or tocopheryl acetate only. Mixed results were found when studying the effects of tocopherol, tocopheryl acetate, and tocopheryl succinate on the modulation of carcinogenic effects of other agents. In most cases, there was an inhibition of the effect of the other agent; in some cases, no effect was seen. However, in one study, tocopherol acted as a complete tumor promoter, with an efficiency approaching a standard tumor promoter, the same promoter whose activity was inhibited by tocopherol in other studies. In a modulation study, tocopherol reduced spontaneous pulmonary tumorigenesis in A/J mice. [1]

In a dermal study in mice, tocopherol reduced photocarcinogenesis. However, dermally applied tocopheryl acetate and tocopheryl succinate were reported to enhance photocarcinogenesis. After oral administration, tocopherol appeared to reduce UV-induced lesions. Orally administered tocopheryl acetate reduced the incidence of skin cancer, but toxicity was observed.[1]

Oral

Tocopherol

Female Sprague-Dawley rats were given a single intraperitoneal (i.p.) injection of 50 mg/kg bw *N*-methyl-*N*-nitrosourea, and one week later, the animals were fed AIN-93M diet alone or supplemented with 0.1, 0.3,or 0.5% mixed tocopherols (57% γ-, 24% δ-, 13% α-, and approximately 0.5% β-tocopherol) for 9 wks, and the animals were then killed.[55] After 9 wks of supplementation with tocopherols, serum γ- and δ-tocopherol levels were statistically significantly increased. A dose-dependent inhibition in tumor growth was observed, and a statistically significant decrease in tumor burden and tumor multiplicity was reported at necropsy. Tumor burden was inhibited by 80% in the highest dose group. The researchers reported that γ- and δ-tocopherol, but not α-tocopherol, activated PPAR-γ.

A mixed tocopherol diet statistically significantly reduced azoxymethane (AOM)-induced colonic aberrant crypt foci in male F344 rats.[56] Male F344 rats were fed a modified AIN-76A diet at 5 wks of age, and then, at age 7 wks, were given subcutaneous (s.c.) injections of 15 mg/kg bw AOM once weekly for 2 wks. One day after the second injection of AOM, groups of animals were maintained on the AIN-76A diet alone or supplemented with 1000 ppm mixed tocopherols; the mixed tocopherols consisted of 58% γ-tocopherol, 21% δ-tocopherol, and 12% α-tocopherol. All animals were killed 8 wks after the second AOM injection. AOM-induced colonic aberrant crypt foci was reduced approximately 55% in the mixed tocopherol-fed rats, as compared to the controls.

Tocopheryl Succinate

The effect of tocopheryl succinate on benzo(*a*)pyrene (BaP)-induced forestomach tumors was studied in Kunming mice.[57] Thirty female mice were used per group. Negative and vehicle (corn oil) control groups were dosed with 1 g/kg bw succinic acid by gavage four times per wk for 4 wks. The vehicle controls and all other mice were dosed with 1 mg/animal B(a)P, two times per wk for 4 wks. The test groups were dosed with 1.25 or 2.5 g/kg bw tocopheryl succinate by gavage four times per wk, or with 20 mg/kg bw tocopheryl succinate via i.p. injection two times for wk, for 4 wks. The positive control group was dosed with B(a)P only. The animals were killed 11, 16, or 29 wks after the first dose of B(a)P.

The incidence of forestomach tumors was 100% in the positive controls, and there was an average of 5.4 tumors/mouse at wk 29. Similar results were observed in the vehicle-control group. With tocopheryl succinate, tumors were seen in only 81.8% and 76.9% of the mice dosed orally with 1.25 and 2.5 g/kg bw tocopheryl succinate, respectively, and only 50% of the mice dosed i.p. with tocopheryl succinate. The number of tumors per mouse was inhibited by 68.5% with 1.25 g/kg bw oral, 70.4% with 2.5 g/kg bw oral, and 79.6% with 20 mg/kg bw i.p. tocopheryl succinate. Tocopheryl succinate significantly affected total tumor volume per mouse. Total tumor volume per mouse was decreased by 63.5% and 81.1% with 1.25 and 2.5 g/kg bw tocopheryl succinate, respectively. With i.p. administration, total tumor volume was decreased by 84.1%.

Tocotrienols

Groups of 10 female FVB/N HER-2/neu transgenic female mice were dosed by gavage, three times per week, with 0, 50, or 100 mg/kg tocotrienols (10% γ- and 90% δ-) in olive oil to determine the effect of tocotrienols on the development of mammary tumors.[58] The appearance of tumors was significantly delayed in high-dose animals, and a statistically significant decrease in wk-26 tumor volume was also observed in this group. There were no statistically significant differences in the

incidence of metastasis or the kinetics of tumor incidence on tumor volume and multiplicity between the treated and control groups.

Photocarcinogenicity

Tocopherol

Female Skh-1 mice were exposed dorsally to 2250 J/m^2 UVB (equivalent to 1 MED) three times per week for 10 wks, and the animals were then dosed topically with vehicle (Surgilube®; n=20) or 5 mg *d*-α-tocopherol in vehicle for 15 wks with no additional UVB.[59] Topical treatment with tocopherol resulted in a trend toward increased tumor multiplicity, with 14.9% more tumors reported with tocopherol than with vehicle alone. A non-statistically significant increase in tumor burden (20.7%) was observed following treatment with tocopherol in vehicle when compared to vehicle only. Tumor growth rate was also increased. However, fewer tumors were malignant in animals dosed with tocopherol than in the controls.

Tumor Promotion

Dermal

Tocopherol

The ability of dermally-applied *dl*-α-tocopherol to induce tumor promotion was studied in groups of 24-27 female SENCAR mice.[60] Four promotion protocols were used; the effects of tocopherol with or without vitamin C were determined with each protocol. In each case, the test compounds were applied to dorsal skin that was clipped free of hair. The vehicles were acetone for tocopherol and 75% acetone/25% water for vitamin C. Each protocol and the corresponding groups are described:

- Protocol A: a single topical application of 10 nmol of the tumor initiator 7,12-dimethylbenz(a)anthracene (DMBA) was applied; 1 wk later, the proposed tumor promoter was applied topically 2x/wk. Test groups were exposed to 80 µmol tocopherol, with and without 80 µmol vitamin C.
- Protocol B: a single topical application of 10 nmol DMBA; 1 wk later, the proposed promoter was applied topically 2x/wk. Test group were exposed to 8 or 80 µmol tocopherol, with and without 80 µmol vitamin C.
- Protocol C: tocopherol was applied topically 2x/wk for 4 wks; a single topical application of 10 nmol DMBA followed; 1 wk later, 4 µg of the weak tumor promoter mezerein was applied topically 2x/wk. The test group was exposed to 80 µmol tocopherol.
- Protocol D: the proposed promoter or the promoter and ^{90}Sr/^{90}Y β-radiation were applied topically 2x/wk for 4 wks; a single topical application of 10 nmol DMBA followed; 1 wk later, 4 µg mezerein was applied topically 2x/wk. Test groups were exposed to 8 µmol tocopherol without vitamin C and to 80 µmol tocopherol with and without 80 µmol vitamin C.

Protocols A and C determined the number of tumors/animal (tumor multiplicity) 98 days after initiation with DMBA; protocols B and D determined tumor multiplicity 153 days after initiation with DMBA. With protocol A and B, tocopherol caused a statistically significant increase in tumors. Using protocol B, the low concentration of tocopherol did not produce tumors, with or without vitamin C.

Following protocols C and D, application of tocopherol prior to initiation with DMBA and then promotion with mezerein statistically significantly increased the tumor multiplicity. The increase in tumor multiplicity compared to controls was greater when measured after 98 days than after 153 days. β-Radiation amplified the effect of high-dose tocopherol; however, using protocol D, vitamin C did not. Tumor multiplicity in groups exposed to β-radiation → DMBA → mezerein, 8 µmol tocopherol → DMBA → mezerein, or tocopherol, 8 µmol + β-radiation → DMBA → mezerein was not statistically significantly different from the group exposed to DMBA → mezerein. Also, tumor multiplicity in the group exposed to tocopherol, 80 µmol + Vitamin C, 80 µmol → DMBA → mezerein was not significantly different from the group exposed to 80 µmol tocopherol → DMBA → mezerein. Pretreatment with tocopherol prior to DMBA reduced tumor latency.

Anti-Proliferative Effects/Pro-Apoptotic Effects

The effect of α- and γ-tocopherol and α- and γ-tocotrienols on proliferation and apoptosis was examined in rat normal hepatocyte (RLN-10) and hepatoma (dRLh-84) cells.[61] γ-Tocotrienols had the greatest effect in rat hepatoma cells; 50-100 µM γ-tocotrienols strongly decreased cell number in a dose-dependent manner after 24 h; γ-tocotrienols strongly suppressed proliferation of dRLh-84 cells, and 25 mM γ-tocotrienols induced DNA fragmentation in dRLh-84 cells. α-Tocotrienols also exerted effects, but to a lesser extent. α- and γ-Tocopherol did not affect cell number, proliferation, or DNA fragmentation. The researchers stated that the results suggested that caspase-8 activity was involved in the induction of apoptosis by tocotrienols.

DERMAL EFFECTS

Dermal Irritation and Sensitization

Non-Human

From the original report on Tocopherols

Tocopherol, 1%, was a weak primary skin irritant in rabbits in one study, and it was a weak cumulative irritant in guinea pigs in another study. Cosmetic formulations containing 2% dl-tocopherol, 12% vitamin E in wheat germ, and 32% mixed tocopherols in a wheat germ and vegetable oil base had mean cumulative irritation scores of 31, 7, and 12 (maximum possible score of 64), respectively, in rabbits. Tocopheryl acetate and tocopheryl nicotinate were generally not irritating to rabbit skin. A single dose of a mixture of dioleyl tocopheryl methylsilanol and oleic acid was not irritating to rabbits, but slight erythema was observed following multiple applications. The same was observed with 75% tocophersolan in guinea pigs.[1]

A mixture containing <0.1% tocopherol was not a sensitizer in an open epicutaneous test, whereas "higher concentrations" of tocopheryl acetate can cause sensitization in this test. However, tocopheryl acetate was not sensitizing in a guinea pig maximization test. Tocophersolan was not a sensitizer in a Buehler test.[1]

Tocopherol

dl-α-Tocopherol was a moderate sensitizer in a guinea pig maximization test in 20 test and 10 control female albino Dunkin Hartley guinea pigs.[62] Intradermal induction was conducted with 0.2% dl-α-tocopherol in light liquid paraffin or as an emulsion with Freund's Complete Adjuvant (FCA; epicutaneous induction was conducted with an occlusive patch of 25% tocopherol in ethanol. An occlusive 24-h challenge patch of the highest non-irritating concentration of tocopherol in ethanol was applied 2 wks after epicutaneous induction; based on a range-finding test, this concentration was determined to be 12.5%. Reactions were evaluated 24 and 48 h after patch removal. Three test animals had an erythema score of 1 at 24 h after patch removal. At 48-h after patch removal, an erythema score of 1 was reported for four animals, and a score of 2 was reported for three animals; all three of the animals that had a reaction at 24 h still had a reaction at 48 h, and for one of those animals the erythema score had increased to 2. None of the vehicle control animals reacted to tocopherol at challenge.

dl-α-Tocopherol was classified as having moderate sensitization potential in a local lymph node assay (LLNA).[63] Twenty-five µl tocopherol in 3:1 ethanol:diethyl phthalate was applied to the dorsum of the ears of CBA female mice for 3 days. The EC_3 was 7.4.

Tocopheryl Acetate

According to robust summary data submitted to ECHA, tocopheryl acetate is not irritating to rabbit skin. A 2.5 cm^2 semi-occlusive patch containing 0.5 ml undiluted tocopheryl acetate was applied to a shaved area on the back or the flank of two male and one female Vienna White rabbits, and no erythema or edema was observed. The test sites were scored 30-60 min after patch removal and at 24, 48, and 72 h after application. In a similar study using six New Zealand white rabbits, application of an occlusive patch containing 0.5 ml tocopheryl acetate to intact and abraded skin did not result in erythema or edema, and the PII was 0.

Tocopheryl Phosphate

MTP was not a dermal irritant in New Zealand rabbits.[50] A dose of 0.5 g/site of an aq. gel containing 88-101 mg/kg bw MTP (38.4 71-82 mg/kg bw α-tocopherol equivalents) was applied to a 10 cm^2 area of clipped dorsal skin of one male and two female rabbits. The semi-occlusive patch was removed after 4 h, and the test site was scored for irritation at 1, 24, 48, and 72 h after patch removal. The only observation was a barely perceptible erythema observed in the male at 60 min.

An LLNA was performed to evaluate the sensitization potential of MTP, and no evidence of sensitization was observed.[50] Groups of five female CBA/J mice were dosed with 25 µl of 5, 10, or 25% MTP in reverse osmosis water (corresponding to 1.13, 2.26, or 5.65 mg MTP, respectively); the test article was applied to the dorsal aspect of the ear daily for 3 consecutive days. On day 6, the animals were given a single intravenous injection of [H^3]thymidine, and then killed 5 h after the injection. Several negative controls and a positive control (25% hexylcinnamaldehyde in acetone/olive oil) were used.

Tocotrienols

Undiluted palm tocotrienol-rich fraction (TRF; composed of 50% tocotrienol/tocopherol complex, with 20% d-γ-tocotrienol, 5% d-δ-tocotrienol, 13% d-α-tocotrienol, and 12% d-α-tocopherol) was practically non-irritating to rabbit skin.[64] Undiluted TRF, 0.5 g, was applied to abraded and intact skin of six New Zealand albino rabbits for 24 h using an occlusive wrap; the test sites were then scored for irritation immediately and 48 h after removal of the test material. Sodium lauryl sulfate (SLS) was used as the positive control; an untreated control was also used. TRF induced slight to well-defined erythema in the six rabbits. The average primary irritation index (PII) for TRF was 1.0; the individual PIIs ranged from 0.8-1.2.

Human

From the original report on Tocopherols

Tocopherol and tocopheryl acetate were not irritants or sensitizers in clinical studies. Patients patch-tested by the North American Contact Dermatitis Group rarely reacted to tocopherol. A cosmetic line containing tocopheryl acetate introduced in Switzerland in 1992 resulted in a large number of outbreaks; positive patch tests with tocopheryl linoleate were seen. However,

the outbreaks were thought to be due to a metabolite or contamination of the product. Tocopheryl nicotinate was not an irritant or a sensitizer.

Tocopherol

The Mayo Clinic, Arizona, compared its positive patch-test reaction rate to tocopherol between June 1987-December 1997 to that observed during 1998-2007.[65] From 1987-1999, various concentrations of α-tocopherol in petrolatum were tested; these concentrations were not specified. In 2000-2005, patients were patch-tested with 10% α-tocopherol acetate in petrolatum; from 2005 on, undiluted α-tocopherol was used. During the period June 1987 – December 1997, 1136 patients were patch-tested with tocopherol; six patients (0.53%) had a positive patch-test reaction to tocopherol. A total of 1814 patients were patch-tested in 1998-2007; 11 patients had a positive reaction to α-tocopherol in petrolatum, and one reacted to undiluted tocopherol, for a positive reaction rate of 0.66%. The difference in positive reactions was not statistically significant.

The North American Contact Dermatitis Group (NACDG) patch-tested 4454 patients in 2005-2006.[66] Finn Chambers were applied for 48 h, and the test sites were read 48-72 h and 72-186 h after patching. The frequency rate of positive patch-test reactions to undiluted dl-α-tocopherol was 0.7%; this rate was significantly lower than it was in the 2003-2004 test period (1.1% in 5139 patients; p-value 0.036; risk ratio 0.63 (0.041-0.097)), as well as during the 1994-2004 time period (p-value 0.0245; risk ratio 0.64 (0.43-0.94)). However, the frequency of reactions was greater in 2005-2006 than it was in 2001-2002; in 2001-2002, 0.5% of the 4874 patients had positive reactions to tocopherol.

The reaction rate to undiluted DL-α-tocopherol was determined in 124 patients tested by the NACDG who had allergic reactions to at least one NACDG screening allergen that was associated with a sunscreen source; these 124 patients represented 0.52% of all patients patch-tested by the NACDG from 2001-2010.[67] DL-α-Tocopherol was the most frequent inactive ingredient allergen associated with a sunscreen source; six patients (4.8%) had a reaction to tocopherol.

Tocopheryl Acetate

A cuticle softener containing 36% tocopheryl acetate was essentially non-irritating in clinical testing.[68] A 24-h single-insult occlusive patch test was conducted in 19 subjects. One subject had a + reaction, and the PII was 0.03.

According to robust summary data submitted to ECHA, dl-α-tocopheryl acetate is not a sensitizer in humans.[45] In this study, 203 subjects were exposed to undiluted tocopheryl acetate during induction; 10 applications were made over a 2-wk period. The challenge was performed after a 2-wk non-treatment period, and the test substance was applied once daily for 3 days. The mean PII after induction was 0.076/subject; none of the subjects showed a higher irritation grade than 1. No positive reactions were reported after challenge.

Tocotrienols

At concentrations ≤5%, TRF was not an irritant in a patch test or a sensitizer in human repeated insult patch test (HRIPT); irritant reactions were observed at higher concentrations.[64] The patch test was performed by applying Finn chambers containing 0%, 1%, 2.5%, 5%, 7.5%, 10%, and 20% TRF in petrolatum to the backs of 30 subjects for 48 h. The test sites were evaluated 48 h and 96 h after application of the test material using the methods of the International Contact Dermatitis Research Group (ICDRG). No irritation reactions were observed with 1, 2.5, or 5% TRF at 48 or 96 h. However, reactions were observed upon patch removal with higher concentrations, ranging from doubtful erythema with 7.5% TRF to moderate-to-well-defined erythema (total skin reaction score of 9) with 20% TRF. These reactions subsided by the 96 h reading. SLS was highly irritating, with total skin reaction scores of 44 and 32 at the 48-h and 96-h readings, respectively.

An occlusive HRIPT of 2.5% and 5% TRF in petrolatum was conducted in 25 subjects. SLS was used as a positive control, and an untreated site as a negative control. The induction patches were applied for 24 h; the test site was evaluated 30 min after patch removal, and the site was then re-patched. A 2-wk non-treatment period followed the 21-day induction period, and then a 48-h challenge patch was applied to a previously unexposed site. Challenge readings were made 48 and 96 h after patch removal. Both 2.5% and 5% TRF had cumulative irritation scores that were lower than the negative control (4 and 7 for 2.5% and 5% TRF, respectively, compared to 14 for the negative control). After challenge, two subjects had transient reactions at the 48 h reading; no reactions were observed after 96 h.

Contact Allergy – Case Reports

Numerous case reports were presented in the original CIR report on tocopherol-containing products, and additional reports have been published since the original CIR report was issued (Table 7).[69-73]

Phototoxicity and Photoallergenicity

From the original report on Tocopherols

Tocopheryl acetate, 0.2 ml applied under an occlusive patch for 24 h prior to irradiation, was not phototoxic in a study in 11 subjects.[1]

Non-Human

Tocopheryl Acetate

According to robust summary data submitted to ECHA, dl-α-tocopheryl acetate was not photoallergenic in Himalayan guinea pigs.[45] Undiluted tocopheryl acetate was used at induction, and concentrations of 5, 50, 75, and 100% were used at challenge. For induction, undiluted test material was applied epicutaneously to an 8 cm^2 area on 20 guinea pigs, with intradermal injections of FCA. The test sites were then exposed to 1.8 J/cm^2 UVB and 10 J/cm^2 UVA. This procedure was repeated four times within 2 wks of the induction phase. A group of 10 control animals were treated with FCA only. The challenge was conducted 3 wks after the start of induction; the animals were exposed epicutaneously on both flanks with the test article at concentrations of 100%, 75%, 50% and 25% in ethanol. The treated sites were then either exposed to 10 J/cm UVA or were not exposed to irradiation, and then scored 24, 48 and 72 hrs after the challenge exposure.

A slight erythematous skin reaction was observed in two of the 20 test animals following challenge; no consistent or significant differences were detected between the irradiated and non-irradiated test sites, and the reactions were not clearly dependent on the test article concentration. It was stated that the reactions most likely resulted from cutaneous hyperirritability. No reactions were observed in the control group.

Photoprotective Effects

From the original report on Tocopherols

Tocopherol was generally found to inhibit UVB-induced lipid peroxidation. Dermal application of tocopheryl acetate also decreased lipid peroxidation, but oral administration was reported not have an effect. Both single and multiple applications of tocopherol inhibited 8-MOP photobinding to DNA/RNA and protein. A single application of tocopheryl acetate did not affect photobinding, but multiple applications protected..[1]

Application of tocopherol both prior to and after irradiation increased the MED. Tocopheryl acetate application after irradiation resulted in decreased skin thickness. In a study in which subjects were given a supplement containing tocopherol and ascorbic acid, a significant increase in MED was observed as compared to controls.[1]

Non-human and human studies have been conducted to examine whether tocopherols have a photoprotective effect (Table 8). Generally, the non-human studies have assessed the protection against oxidative damage in the skin. The human studies examined phototoxic effects and changes in MED.

Effect on Irritated Skin

Non-Human

Tocopheryl Acetate

The effect of tocopheryl acetate ointment containing 15% squalane on allergic contact dermatitis was investigated in male Wistar rats.[48] Allergic contact dermatitis was induced with 2,4-dinitrochlorobenzene (DNCB)-acetone, and 0.1 g tocopheryl acetate ointment was applied to the test site after the initiation of inflammation. The ointment was applied using a 2 cm diameter film, and the film was covered with gauze and a bandage. The tocopheryl acetate ointment inhibited allergic contact dermatitis in a dose-dependent manner, with significant inhibition of erythema observed with 20-40% tocopheryl acetate. An ointment with 2-10% tocopheryl acetate had an inhibitory effect on erythema. The inhibition was confirmed microscopically in keratinocytes from skin samples taken from the treated area.

The researchers then examined the effect of the tocopheryl acetate ointment on DNCB-acetone-induced irritant contact dermatitis on the backs of male Wistar rats; the ointment was applied in the same manner as described previously. The ointment with 20% tocopheryl acetate significantly reduced erythema,

The researchers also examined the effect of the ointment on irritant contact dermatitis in mice. Irritant contact dermatitis was induced in male ddY mice by applying phorbol 12-myristate 13-acetate (PMA)-acetone to the ears of each animal; 20 mg of the tocopheryl acetate ointment was then applied to both sides of the ear. The 20% tocopheryl acetate ointment significantly reduced ear swelling.

Tocotrienols

Tocotrienols reduced allergic dermatitis in mice.[74] Allergic dermatitis was induced in male NC/Nga mice using picryl chloride, with and without oral administration of 1 mg/day/animal tocotrienols in vitamin E-stripped corn oil for 1 wk prior to sensitization. Scratching behavior, dermal thickening, and serum histamine levels were statistically significantly reduced by tocotrienols administration. Subsequent studies concluded that tocotrienols significantly suppressed degranulation of mast cells and significantly reduced histamine release, and it also suppressed protein kinase C activity.

Effect on Barrier Function of Damaged Skin

Non-Human

Tocopheryl Acetate

Skin barrier function in male Wistar rats was damaged using a detergent and DNCB, and the effect of tocopheryl acetate on the damaged skin was evaluated.[48] The damaged test site was covered for 18 h with 0.1 g tocopheryl acetate ointment; ointments containing 2-40% tocopheryl acetate were used. Ointment containing 2% tocopheryl acetate had little effect on damaged skin. However, concentrations of 5-40% tocopheryl acetate statistically significantly reduced the increase in transepidermal water loss; the maximum effect was observed with 20% tocopheryl acetate. The 20% ointment also statistically significantly decreased the erythema intensity.

EPIDEMIOLOGY AND DIETARY SUPPLEMENTATION

Health claims exist in the literature regarding vitamin E supplementation;[75] however, the benefits of vitamin E supplements are still being debated. The original CIR safety assessment on tocopherol referred to numerous studies in which tocopherol appeared to have protective effect in carcinogenicity modulation studies.[1] However, as an example of possible negative effect of vitamin E, dietary supplementation with α-tocopherol was reported to significantly increase the risk of prostate cancer among healthy men in the Selenium and Vitamin E Cancer Prevention Trial.[76] While the CIR recognizes that there is a large literature on the effects of vitamin E supplementation, these articles are not included in this safety assessment because they are not relevant to the cosmetic use of the tocopherols.

SUMMARY

This re-review addresses the safety of tocopherols and tocotrienols as used in cosmetics. In 2002, the Panel published a review on tocopherol, concluding these ingredients are safe as used in cosmetics. Tocopherol is the component most commonly associated with vitamin E. However, tocotrienols is also a component of vitamin E, so it is appropriate to develop a report that includes all of these ingredients. This summary includes only information that has become available since the CIR safety assessment was issued on tocopherols, and all information on the tocotrienols and the tocopherols that have been added to this report.

Most of the tocopherols are reported to function in cosmetics as antioxidants or skin conditioning agents; tocotrienols is not reported to function as an antioxidant, instead it is listed as functioning as a light stabilizer, oral care agent, or skin conditioning agent. VCRP data obtained from the FDA in 2014 report that the frequency of use increased considerably for both tocopherol and tocopheryl acetate. The reported use of tocopherol increased from 1072 (1998 data) to 6635 uses (2014 data), and the reported use of tocopheryl acetate increased from 1322 (1998 data) to 9677 uses (2014 data). The use concentration of tocopherol, but not of tocopheryl acetate, has increased since the original assessment. According to the survey conducted by the Council in 2013, the concentration of use of tocopherol in leave-on products increased from 2% in 1999 to 5.4% in 2013. Tocotrienols is used in 433 formulations, with a reported maximum leave-on concentration reported of 0.12%.

Tocopherols and tocotrienols are distributed throughout the body, and the distribution and metabolism varies among the tissues. Tocopherol is the predominant form of vitamin E in human and animal tissues, and it has the highest bioavailability; natural vitamin E has approximately twice the systemic availability of synthetic tocopherol. The distribution and intracellular trafficking of vitamin E may be modulated by tocopherol regulatory proteins, but only one of the proteins, tocopherol transfer protein, has been shown to influence plasma and tissue α-tocopherol concentrations.

The structural differences between tocopherol and tocotrienols result in a difference in the penetration of these compounds into tissues. The presence of three unsaturated bonds in the carbon side chain allows tocotrienols to penetrate tissues with saturated fatty layers, such as the brain and the liver, more readily than tocopherol, which has a saturated carbon side chain. However, the tocotrienols are not as prevalent in the body as the tocopherols, and oral absorption of the tocotrienols has been reported to be incomplete. Orally administered tocopherols and tocotrienols are distributed in the skin and adipose tissue. Dermally applied tocopheryl acetate is hydrolyzed to tocopherol upon exposure to UV. Dermally-applied tocopherols do penetrate the skin.

Toxicity of dermally-applied (single-dose) tocopheryl acetate and tocopheryl phosphate and orally-administered tocophersolan (repeated dose) or tocopheryl phosphate (single and repeated-dose) is not remarkable. In rats fed a diet containing ≤3% tocotrienols for 13 wks, a statistically significant decrease in platelets in males, but not females, was interpreted to be a physiologic response.

Undiluted tocopheryl acetate was not irritating to rabbit eyes in one study, but it produced weak to moderate conjunctival irritation in another study. Undiluted mixed tocopheryl phosphates (MTP) was not irritating to rabbit eyes.

Numerous genotoxicity studies were conducted with tocopherol, tocopheryl acetate, MTP, and tocopheryl succinate. The only remarkable result was tocopheryl succinate with only a weak positive in a in a sister chromatid exchange assay in the presence of metabolic activation.

Topical treatment of Skh-1 mice with 5 mg d-α-tocopherol for 15 wks, following 10 wks of UVB irradiation, resulted in a trend toward increased tumor multiplicity in females compared to those mice exposed to vehicle only. A non-statistically significant increase in tumor burden was observed following treatment with tocopherol compared to controls. However, fewer tumors were malignant in animals dosed with tocopherol than in the controls. Mixed results were observed in a tumor promotion study; higher doses of tocopherol increased tumor multiplicity, and greater increases were seen after 98 days than after 153 days. In oral studies, tocopheryl acetate and tocopheryl succinate tended to decrease tumors in rodents. Tocotrienols delayed the onset of the appearance of tumors.

dl-α-Tocopherol was a moderate sensitizer in a guinea pig maximization test, and was classified as having moderate sensitization potential in an LLNA. In clinical patch-tests conducted by the Mayo Clinic in 1814 patients in the years 1998-2007, 11 patients had a positive reaction to α-tocopherol in petrolatum (concentrations of 10% or not specified), and one reacted to undiluted tocopherol, for a positive reaction rate of 0.66%. In testing conducted by the NACDG in 4454 patients between 2005-2006, the frequency rate of positive patch-test reactions to undiluted dl-α-tocopherol was 0.7%. In patients tested by the NACDG who had allergic reactions to at least one NACDG screening allergen that was associated with a sunscreen source (0.52% all patients patch-tested by the NACDG from 2001-2010), DL-α-tocopherol was the most frequent inactive ingredient allergen associated with a sunscreen source, with 6/124 patients (4.8%) reacting to tocopherol. Several case reports of contact dermatitis to tocopherol-containing products have been described.

Undiluted tocopheryl acetate was not irritating to rabbit skin, and tocopheryl acetate did not have photoallergenic effects in guinea pigs. A cuticle softener containing 36% tocopheryl acetate was essentially non-irritating in a single-insult occlusive patch test in 19 subjects, and undiluted tocopheryl acetate is not a sensitizer in humans.

An aq. gel containing 88-101 mg/kg bw MTP was not irritating to rabbit skin, nor was undiluted palm TRF. No evidence of sensitization was observed in an LLNA with MTP. In clinical testing, TRF was not an irritant in human subjects at concentrations up to 5%; however, reactions were observed at higher concentrations, ranging from doubtful erythema with 7.5% TRF to moderate-to-well-defined erythema (total skin reaction score of 9) with 20% TRF. TRF, 2.5 and 5%, was not a cumulative irritant or a sensitizer in an HRIPT in 25 subjects.

Tocopherol, tocopheryl acetate, and tocopheryl phosphate had some photoprotective effects in mice, and tocopherol and tocopheryl acetate were shown to have a photoprotective effect in humans. Tocopheryl acetate inhibited contact dermatitis in rats and reduced erythema, and tocotrienols reduced allergic dermatitis in mice.

The CIR recognizes that many articles on the effects of vitamin E supplementation can be found in the published literature; however, these articles are not included in this safety assessment because they are not relevant to the cosmetic use of the tocopherols.

DISCUSSION

The Expert Panel determined that the 2002 safety assessment on tocopherols should be expanded to include tocotrienols (a 2013 priority) and four additional tocopherols. Although data were not available on all of the ingredients, the Panel found the existing data on the tocopherols are sufficient to support the safety of this entire family of ingredients.

The Panel noted that the current reported maximum use concentration of tocopherol (i.e., 5.4%) is higher than what was reported in the original assessment (i.e., 2%), and that irritation and sensitization data at these higher concentrations are not available for tocopherol. The Panel discussed the issues of irritation and sensitization during both the original and current review of tocopherols, and in both instances, determined that the irritation and sensitization potentials of the ingredients included in this review were not of concern. In the 2002 safety assessment, the Panel was initially concerned with possible irritation and sensitization because of a large number of outbreaks reported in Switzerland with the release of a new line of cosmetics that contained tocopheryl linoleate; however, the researchers thought the outbreaks were due to either a contaminant or a metabolite. Other safety data in the 2002 report indicated that tocopherol was not an irritant or a sensitizer. Irritation and sensitization data that are available since the 2002 review was issued indicate that tocopheryl acetate is not an irritant or a sensitizer, and tocopheryl phosphate is not a sensitizer. Additionally, the Panel commented that although moderate sensitization potential was reported in a guinea pig maximization test of dl-α-tocopherol, dermal reactions to tocopherol in humans are rare, and as such, the North American Contact Dermatitis Group deleted this ingredient from its standard testing because of the extremely low incidence of reactions.

Tocopherol has some absorption in the UV Range. However, the Panel noted that according to animal and clinical testing, tocopheryl acetate was not photoallergenic or phototoxic.

During the original review of tocopherols, the Panel did carefully consider that the tumor promoting ability of tocopherol, tocopheryl acetate, and tocopheryl succinate had been extensively studied. In most studies, tocopherol is reported to inhibit tumor promotion, and studies published since the original tocopherol report seem to support this conclusion. The general experience of the Panel is that tocopherol is not a tumor promoter. Additionally, photocarcinogenicity testing with tocopherol did not raise any concerns for the Panel.

The Panel also noted that results of epidemiology and dietary supplementation studies are inconclusive; positive and negative results on the health effects of vitamin E have been observed with the use of vitamin E supplements. The Panel stated that the systemic exposure of vitamin E supplementation is much higher than that expected during cosmetic use; therefore, any adverse conclusions made during these studies did not cause concern for the cosmetic use of these ingredients.

Natural tocopherols and tocotrienols are plant-derived ingredients. However, these ingredients are specific, purified, highly-enriched lipid compounds, and often the tocopherols are produced synthetically. For these reasons, the usual concerns expressed during the discussion of botanical ingredients did not apply to the ingredients in this report.

Finally, the Panel discussed the issue of incidental inhalation exposure to tocopherols. The Panel stated that although there were no inhalation data available, the tocopherols are used at relatively low concentrations in products that could incidentally be inhaled, e.g., tocopheryl acetate is used at up to 5% in foot powders and sprays and up to 0.2% in aerosol hair spray formulations, and tocopherol is used at up to 1% in pump hair spray formulations. The Panel noted that in aerosol products, 95% – 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

CONCLUSION

The CIR Expert Panel concluded that the following 14 ingredients are safe in the present practices of use and concentration in cosmetics described in this safety assessment:

ascorbyl tocopheryl acetate*
ascorbyl tocopheryl maleate
dioleyl tocopheryl methylsilanol
potassium ascorbyl tocopheryl phosphate
sodium tocopheryl phosphate
tocopherol
tocophersolan
tocopheryl acetate
tocopheryl linoleate
tocopheryl linoleate/oleate
tocopheryl nicotinate
tocopheryl phosphate*
tocopheryl succinate
tocotrienols

*Not reported to be in current use. If these ingredients were to be used in the future, the expectation is that it would be used in product categories and at concentrations comparable to others in this group.

TABLES

Table1. Definitions, Structures, and Functions

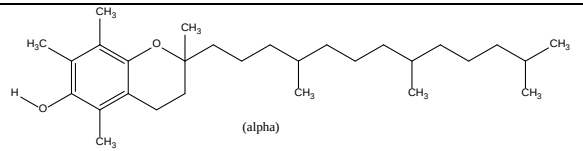
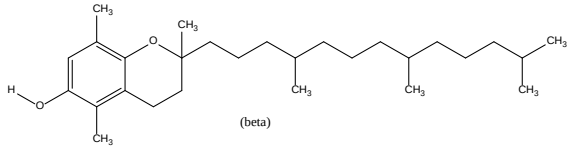
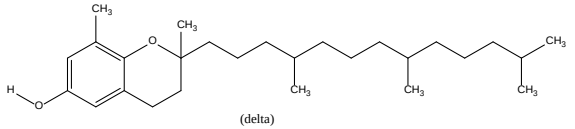
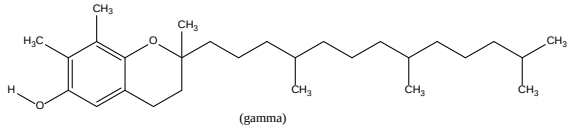
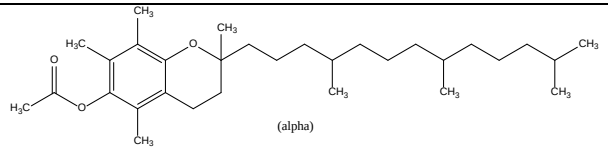
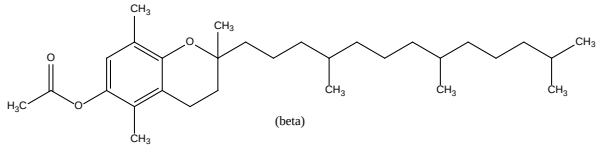
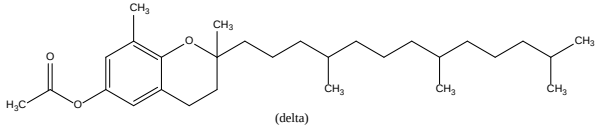
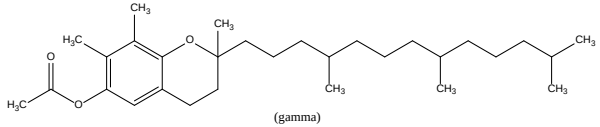
Ingredient/CAS No. [13]	Definition[13,*]	Structure[**]	Function(s)
Tocopherol 54-28-4 (gamma): 16698-35-4 (beta); 10191-41-0 (DL-); 2074-53-5 (DL-); 59-02-9 (D-); 119-13-1; 1406-18-4; 1406-66-2; 7616-22-0	consists of alpha-tocopherol, beta-tocopherol, delta-tocopherol and/or gamma-tocopherol and conforms to the formulae:	 (alpha)  (beta)  (delta)  (gamma)	antioxidant; fragrance ingredient; skin-conditioning agent - miscellaneous; skin-conditioning agent - occlusive
Tocopheryl Acetate 52225-20-4; 58-95-7; 7695-91-2	the ester of tocopherol and acetic acid	 (alpha)  (beta)  (delta)  (gamma)	antioxidant; skin-conditioning agent – miscellaneous

Table1. Definitions, Structures, and Functions

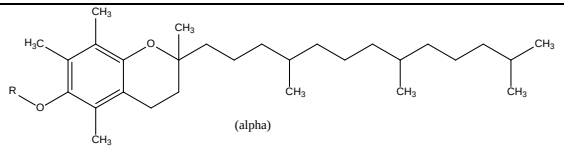
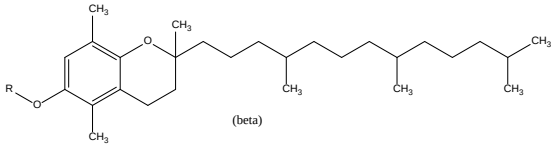
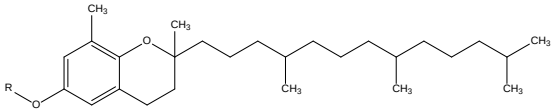
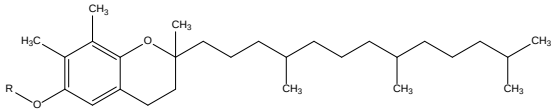
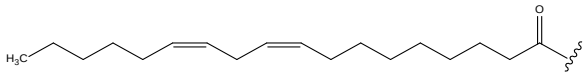
Ingredient/CAS No. [13]	Definition[13,*]	Structure[**]	Function(s)
Tocopheryl Linoleate 36148-84-2	the ester of tocopherol and linoleic acid	 (alpha)  (beta)  (delta)  (gamma) wherein R is 	antioxidant; skin-conditioning agent – miscellaneous

Table1. Definitions, Structures, and Functions

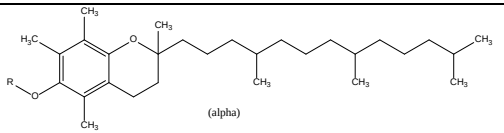
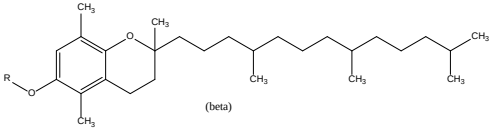
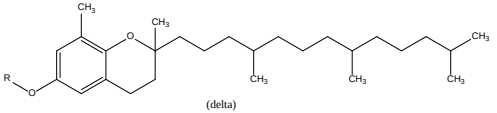
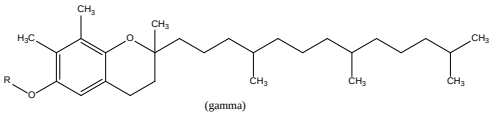
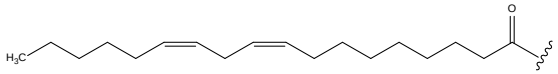
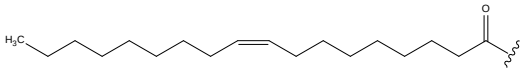
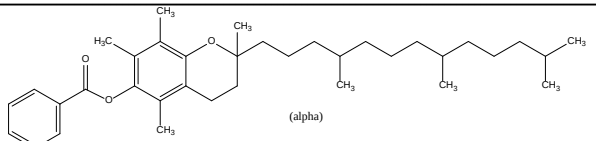
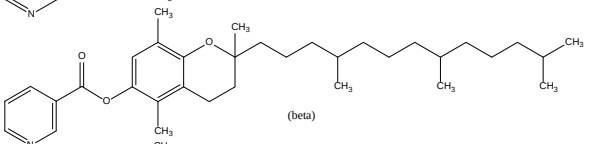
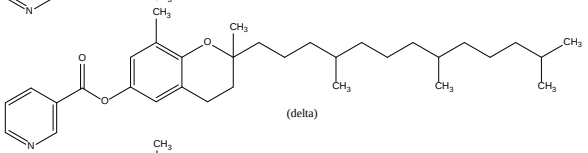
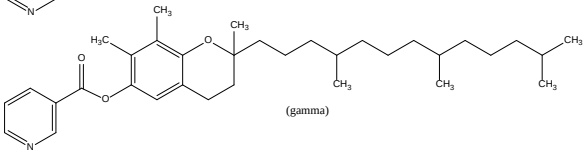
Ingredient/CAS No. [13]	Definition[13,*]	Structure[**]	Function(s)
Tocopheryl Linoleate/Oleate	the ester of tocopherol and a mixture of linoleic acid and oleic acid	 (alpha)  (beta)  (delta)  (gamma) wherein R is  or 	antioxidant; skin-conditioning agent – miscellaneous
Tocopheryl Nicotinate 16676-75-8; 43119-47-7; 51989-34-1; 86362-36-9	the ester of tocopherol and nicotinic acid	 (alpha)  (beta)  (delta)  (gamma)	antioxidant; skin-conditioning agent – miscellaneous

Table1. Definitions, Structures, and Functions

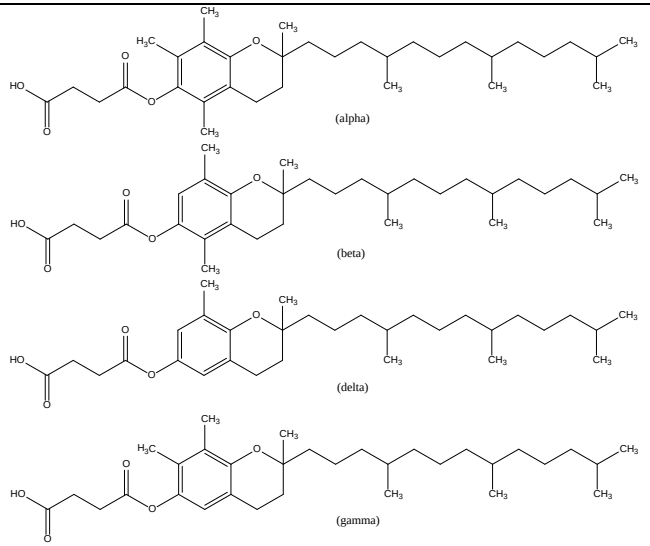
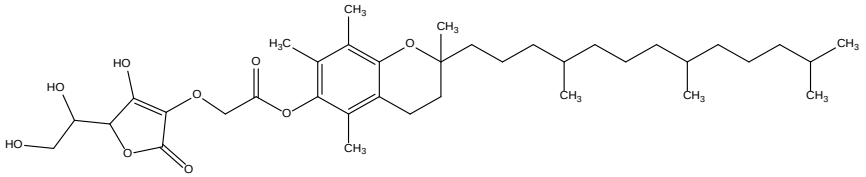
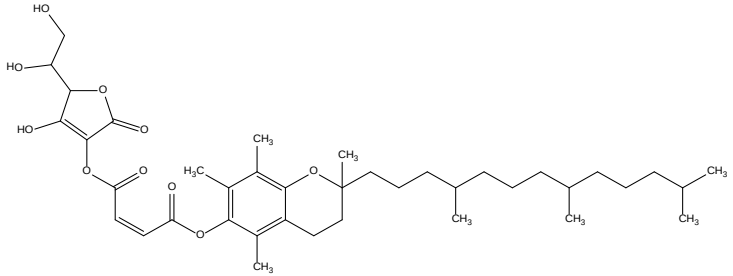
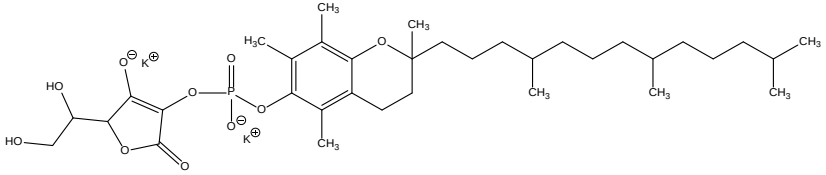
Ingredient/CAS No. [13]	Definition[13,*]	Structure[**]	Function(s)
Tocopheryl Succinate 17407-373-; 4345-03-3	the ester of tocopherol and succinic acid		antioxidant
Ascorbyl Tocopheryl Acetate	*the ascorbyl tocopheryl derivative with a glycolate linkage*; also, the organic compound that conforms to the formula:		skin conditioning agent - miscellaneous
Ascorbyl Tocopheryl Maleate	*the ascorbyl tocopheryl derivative with a maleate linkage*; the organic compound that conforms to the formula:		antioxidant; skin-conditioning agent – emollient
Potassium Ascorbyl Tocopheryl Phosphate 637764-10-4[77]	*the potassium salt of ascorbyl tocopheryl phosphate*; the organic compound that conforms to the formula:		antioxidant

Table1. Definitions, Structures, and Functions

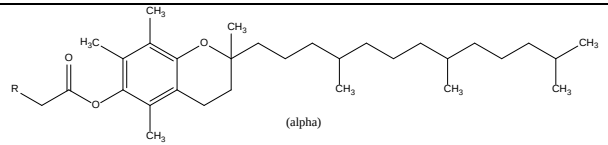
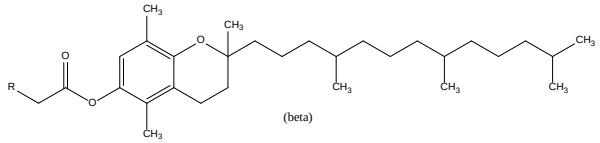
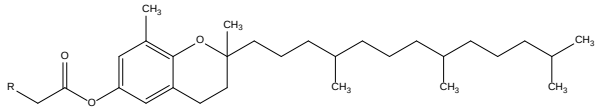
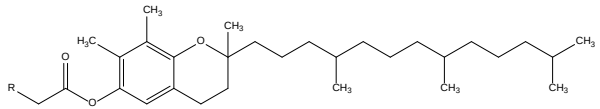
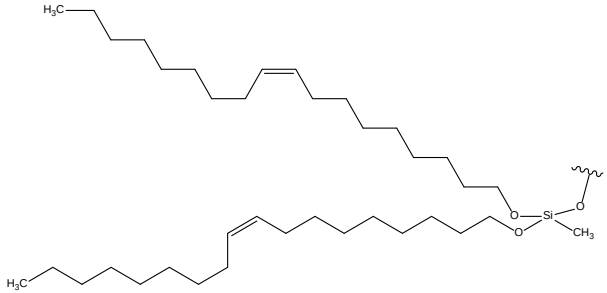
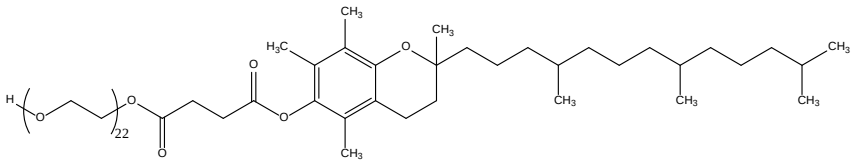
Ingredient/CAS No. [13]	Definition[13,*]	Structure[**]	Function(s)
Dioleyl Tocopheryl Methylsilanol	the dioleyl ether of tocopheryl acetate monoether with methylsilanetriol	 (alpha)  (beta)  (delta)  (gamma) wherein R is 	antioxidant; skin-conditioning agent – miscellaneous
Tocophersolan 30999-06-5 9002-96-4	*the PEG-22 ester of tocopheryl succinate*; also, the organic compound that conforms to the formula:		antioxidants

Table1. Definitions, Structures, and Functions

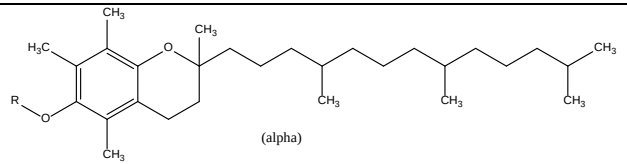
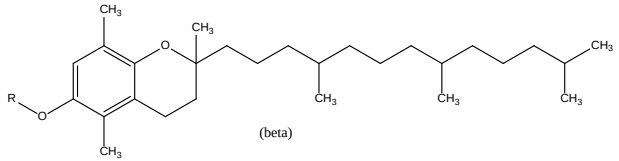
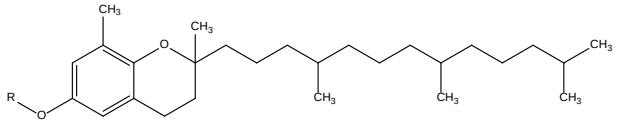
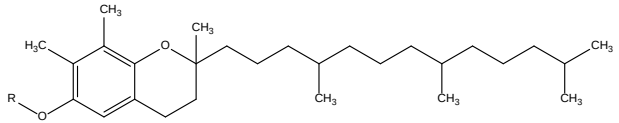
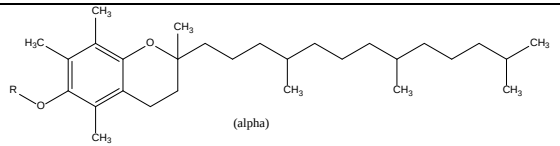
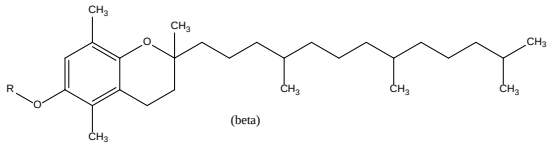
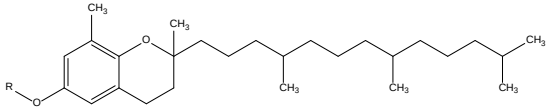
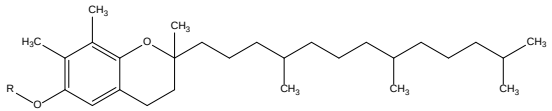
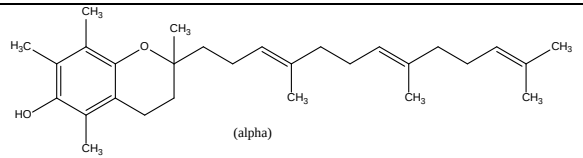
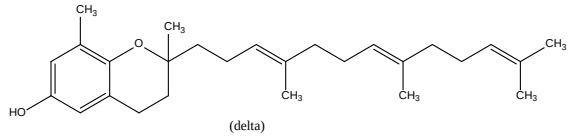
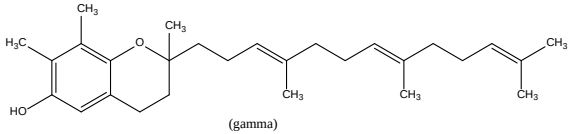
Ingredient/CAS No. [13]	Definition[13,*]	Structure[**]	Function(s)
Tocopheryl Phosphate 425429-22-7	*a complex mixture of tocopheryl phosphate ester*; also, a complex mixture of tocopherol and phosphoric acid.	 (alpha)  (beta)  (delta)  (gamma) wherein R is the residue of phosphoric acid or other tocopheryl phosphate moieties	hair conditioning agent; surfactant - cleansing agent; surfactant - emulsifying agent
Sodium Tocopheryl Phosphate 59981-60-1 (alpha)[77]	*the sodium salt of a complex mixture of tocopheryl phosphate esters*; also, the sodium salt of a complex mixture of esters of phosphoric acid and tocopherol	 (alpha)  (beta)  (delta)  (gamma) wherein R is the residue of sodium salt of phosphoric acid or other tocopheryl phosphate moieties	antioxidant; emulsion stabilizer; reducing agent; skin-conditioning agent - miscellaneous; surfactant - emulsifying agent; viscosity increasing agent - aqueous

Table1. Definitions, Structures, and Functions

Ingredient/CAS No. [13]	Definition[13,*]	Structure[**]	Function(s)
Tocotrienols	a mixture of alpha-, gamma- and delta-tocotrienols which conform to the following formulae:	 (alpha)  (delta)  (gamma)	light stabilizer; oral care agent; skin-conditioning agent - miscellaneous

* *Definitions in italic were generated by CIR*
**these structures were generated by CIR

Table 2. Physical and chemical properties

Property	Description	Reference
	Tocopherol	
molecular weight	α-tocopherol: 430.7 β-tocopherol: 416.7 γ-tocopherol :416.7 δ-tocopherol: 402.7	[37]
λ_{max}	α- tocopherol: 292 nm β- tocopherol: 296 nm γ- tocopherol: 298 nm δ- tocopherol: 298 nm	[37]
stability	readily oxidized upon exposure to atmospheric conditions or light	[5]
	Tocopheryl Acetate	
molecular weight	472.7	[78]
	Tocophersolan	
physical appearance and form	white to light brown, waxy solid	[79]
melting point	37-41° C	[79]
solubility	miscible in water	[79]
specific gravity	1.06 (at 45° C)	[79]
	Tocopheryl Phosphate	
solubility	water soluble	[80]
	Tocotrienols	
molecular weight	α-tocotrienol: 424.7 β- tocotrienol: 410.7 γ- tocotrienol: 410.7 δ- tocotrienol: 396.7	[37]
λ_{max}	α- tocotrienol: 292 nm β- tocotrienol: 295 nm γ- tocotrienol: 298 nm δ- tocotrienol: 292 nm	[37]

Table 3. Plants containing tocotrienols (µg/g) [3]

Plant Species	Tissue	α-	β-	γ-	δ-
Pinaceae	bark	n.d.	n.d.	n.d.	700
Pinus nigra pallasiana pyramidata	bark	n.d.	n.d.	n.d.	1600
Cinnamosma fragrans	bark	n.d.	n.d.	n.d.	700
Cinnamosma macrocarpa	bark	n.d.	n.d.	n.d.	1600
Bixa orellana	seed	18.7	1.84	534.7	977.9
Hevea brasiliesis.	latex	522.4	below detection	196.7	1869.6
Aesculus hippocastanum	seed	97	n.d.	626	336
Rosmarinus officinalis	seed	560.5	300.3	109.4	trace
Delphinium ajacis	seed	566	153	trace	n.d.
Litchi chinensis	seed	below detection	below detection	3.37	488
Elaeis guineensis	fruit	4-193	0-234	0-526	0-123
Cocos nucifera	seed	104.0	26.0	36.2	204
Zea mays	seed; young seedling	0-239	0.450	0-20	0-709

n.d. – none detected

Table 4. Current and historical frequency and concentration of use of tocopherols and tocotrienols according to duration and exposure

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2014[14]	**1998**[1]	**2013**[15]	**1999**[1]	**2014**[14]	**1998**[1]	**2013**[15]	**1999**[1]
	Tocopherol[**,#]				**Tocopheryl Acetate****			
Totals*	**6635**	**1072**	**0.0000009-5.4**	**0.001-2**	**9677**	**1322**	**0.00000001-36**	**0.001-36**
Duration of Use								
Leave-On	*5366*	*940*	*0.000003-5.4*	*0.001-2*	*7590*	*1024*	*0.00000001-36*	*0.001-36*
Rinse-Off	*1210*	*130*	*0.0000009-3*	*0.001*	*2014*	*268*	*0.000075-10*	*0.0001-25*
Diluted for (Bath) Use	*58*	*2*	*0.05-1*	*0.01-0.8*	*73*	*30*	*0.001-0.1*	*0.05-0.1*
Exposure Type								
Eye Area	986	121	0.00002-3	0.02-0.6	1014	77	0.000045-4.9	0.01-1
Incidental Ingestion	878	260	0.0005-2	0.05-0.9	935	85	0.0035-3	0.1-3
Incidental Inhalation-Spray	84 1174[a]; 894[c]	8 3[c]	0.000003-1 aerosol: 0.00002-0.0057 pump:0.0001-1	0.001-0.5	168 2322[a]; 1370[c]	48 3[c]	0.00000001- 0.5 aerosol: 0.00000001- 0.2 pump: 0.000001-0.2 0.5-5[c]	0.02-0.1
Incidental Inhalation-Powder	156 14[b]; 894[c]	56 3[c]	0.0005-0.02	0.02-0.6	233 33[b]; 1370[c]	29 3[c]; 10[b]	0.008-1.1 0.5-5[c]	0.02-0.1
Dermal Contact	5068	654	0.0000009-5.4	0.001-2	7384	907	0.000045-5	0.0001-25
Deodorant (underarm)	25[a]	2	not spray: 0.000015-0.8 aerosol: 0.0001-0.0005 pump: 0.0006	0.050.0	35[a]	NR	not spray: 0.1-0.5 aerosol: 0.001-0.1	0.2
Hair - Non-Coloring	401	87	0.000003-0.25	0.001-0.6	1046	252	0.00000001- 10	0.001-0.3
Hair-Coloring	168	9	0.0001-0.1	0.001	79	1	0.00013-0.05	0.001
Nail	31	3	0.00001-0.2	0.05-0.3	118	61	0.002-36	0.01-36
Mucous Membrane	1442	304	0.0000009-3	0.01-0.9	1822	185	0.0003-3	0.05-3
Baby Products	23	2	0.0002	1	56	9	0.001-0.1	0.001-0.1
	Tocopheryl Linoleate				**Tocopheryl Linoleate/Oleate**			
Totals*	**102**	**279**	**0.0001-1**	**0.1-2**	**4**	**NR**	**0.05-0.1**	**NR**
Duration of Use								
Leave-On	*98*	*273*	*0.0001-1*	*0.1-2*	*4*	*NR*	*0.05-1*	*NR*
Rinse-Off	*4*	*4*	*0.001-0.003*	*2*	*NR*	*NR*	*0.05*	*NR*
Diluted for (Bath) Use	*NR*	*2*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	11	64	0.05-0.2	0.1-2	NR	NR	NR	NR
Incidental Ingestion	10	33	0.05-0.5	0.1-2	1	NR	NR	NR
Incidental Inhalation-Spray	33[a]; 16[c]	NR	aerosol: 0.01	NR	2[c]	NR	NR	NR
Incidental Inhalation-Powder	10; 1[,c]	31	0.1	0.3-2	2[c]	NR	NR	NR
Dermal Contact	74	231	0.001-1	0.1-2	3	NR	0.05-1	NR
Deodorant (underarm)	NR	NR	NR	NR	1	NR	NR	NR
Hair - Non-Coloring	2	3	0.0001-0.01	NR	NR	NR	NR	NR
Hair-Coloring	NR	28	NR	NR	NR	NR	NR	NR
Nail	8	11	0.01	NR	NR	NR	NR	NR
Mucous Membrane	10	36	0.05-0.5	0.1-2	1	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	Tocopheryl Nicotinate				**Tocopheryl Succinate**			
Totals*	**42**	**3**	**0.001**	**0.0001-1**	**6**	**4[#]**	**0.00001-0.038**	**NR**
Duration of Use								
Leave-On	*39*	*1*	*0.001*	*0.01-0.2*	*6*	*#*	*0.00001-0.038*	*NR*
Rinse-Off	*3*	*2*	*0.1-1*	*0.0001-1*	*NR*	*#*	*0.00001- 0.0001*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*#*	*NR*	*NR*
Exposure Type								
Eye Area	8	NR	0.13	NR	1	#	0.00001-0.038	NR
Incidental Ingestion	11	NR	0.06-0.6	NR	NR	#	0.00008	NR
Incidental Inhalation-Spray	10[a]; 4[c]	NR	0.001-0.1	NR	2[c]	#	0.00001[a]	NR
Incidental Inhalation-Powder	4[c]	NR	NR	NR	2[c]	#	NR	NR
Dermal Contact	22	NR	0.05-1	0.1	6	#	0.00001-0.038	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	#	NR	NR
Hair - Non-Coloring	9	3	0.001-0.2	0.0001-1	NR	#	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	#	NR	NR
Nail	NR	NR	NR	NR	NR	#	NR	NR
Mucous Membrane	11	NR	0.06-0.6	NR	NR	#	0.00008- 0.0001	NR
Baby Products	NR	NR	NR	NR	NR	#	NR	NR

Table 4. Current and historical frequency and concentration of use of tocopherols and tocotrienols according to duration and exposure

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2014[14]	**1998[1]**	**2013[15]**	**1999[1]**	**2014[14]**	**1998[1]**	**2013[15]**	**1999[1]**
	Dioleyl Tocopheryl Methylsilanol				**Potassium Ascorbyl Tocopheryl Phosphate**			
Totals*	**6**	**12**	**0.014**	**NR**	**35**	**15**	**0.01-0.2**	**0.02**
Duration of Use								
Leave-On	*6*	*12*	*0.014*	*NR*	*35*	*13*	*0.01-0.2*	*0.02*
Rinse-Off	*NR*	*NR*	*NR*	*NR*	*NR*	*2*	*0.01*	*0.02*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	NR	NR	NR	NR	4	1	0.01-0.03	NR
Incidental Ingestion	5	4	0.014	NR	NR	NR	0.02	0.02
Incidental Inhalation-Spray	NR	NR	NR	NR	1; 17[a]; 9[c]	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	9[c]	NR	0.01	NR
Dermal Contact	NR	3	NR	NR	35	15	0.01-0.1	0.02
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	0.01-0.2	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	1	5	NR	NR	NR	NR	NR	NR
Mucous Membrane	5	4	0.014	NR	NR	NR	0.02	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	Tocophersolan							
Totals*	**41**	**2**	**NR**	**0.05-0.2**				
Duration of Use								
Leave-On	*41*	*1*	*NR*	*0.05-0.2*				
Rinse-Off	*NR*	*1*	*NR*	*NR*				
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*				
Exposure Type								
Eye Area	1	NR	NR	NR				
Incidental Ingestion	1	NR	NR	NR				
Incidental Inhalation-Spray	9[a]; 21[c]	NR	NR	NR				
Incidental Inhalation-Powder	21[c]	NR	NR	NR				
Dermal Contact	34	2	NR	0.05-2				
Deodorant (underarm)	NR	NR	NR	NR				
Hair - Non-Coloring	5	NR	NR	NR				
Hair-Coloring	NR	NR	NR	NR				
Nail	1	NR	NR	NR				
Mucous Membrane	1	NR	NR	NR				
Baby Products	NR	NR	NR	NR				

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses
**total VCRP data include listings under multiple names
[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[b] It is possible these products are powders, but it is not specified whether the reported uses are powders.
[c] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[#] in the 1998 data, some uses are for a tradename/mixture and not defined; for tocopherol, 50 uses are not defined; for tocopheryl succinate, 4 uses are not defined
NR – no reported use

Table 5. Frequency and concentration of use according to duration and type of exposure – added ingredients

	# of Uses[14]	*Max Conc of Use (%)*[15]	*# of Uses*[14]	*Max Conc of Use (%)*[16]	*# of Uses*[14]	*Max Conc of Use (%)*[15]
	Tocotrienols		**Ascorbyl Tocopheryl Maleate**		**Sodium Tocopheryl Phosphate**	
Totals*	**439**	**0.0015-0.12**	**41**	**0.000055-0.1**	**10**	**NR**
Duration of Use						
Leave-On	*190*	*0.015-0.12*	*38*	*0.0025-0.1*	*7*	*NR*
Rinse-Off	*238*	*0.0015*	*3*	*0.000055-0.005*	*3*	*NR*
Diluted for (Bath) Use	*11*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	6	0.019-0.039	9	0.02	2	NR
Incidental Ingestion	61	0.016-0.019	1	0.0025	NR	NR
Incidental Inhalation-Spray	50[c]; 28[c]	NR	26[a]	NR	1[a]; 3[c]	NR
Incidental Inhalation-Powder	28[c]	NR	NR	NR	3[c]	NR
Dermal Contact	363	0.0015-0.12	39	0.005-0.1	10	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	12	0.015	1	0.000055-0.0003	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	3	NR	NR	NR	NR	NR
Mucous Membrane	297	0.0015-0.019	1	0.0025-0.005	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 may not equal the sum of total uses.
[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[b] It is possible these products are powders, but it is not specified whether the reported uses are powders.
[c] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, there fore the information is captured in both categories
NR – no reported use

Table 6. Tocopherol and tocopheryl acetate recovery (mol %) and % bioconversion tocopheryl acetate to tocopherol in human skin *in vitro*

		Surface		**Horny Layer**		**Underlying Skin**[5]	
Formulation	**Vitamin E form**	**Non-Occlusive**	**Occlusive**	**Non-Occlusive**	**Occlusive**	**Non-Occlusive**	**Occlusive**
in triglyceride oil	tocopherol+acetate	79.2	74.2	12.6	17.0	0.4	--
	tocopherol	1.2	1.2	0.1	0.1	--	--
surfactant-solubilized in water	tocopherol+acetate	43.2	48.4	20.7	27.6	26.6	16.6
	tocopherol	1.3	1.4	0.9	1.3	9.3	8.1
	% conversion					*34.8*	*48.9*
soybean phosphatidylcholine liposomes	tocopherol+acetate	30.6	40.6	23.6	33.0	36.8	20.2
	tocopherol	1.3	1.9	1.3	1.7	14.5	10.5
	% conversion					*39.4*	*51.2*
nanotope	tocopherol+acetate	24.5	29.5	24.0	31.1	45.3	31.7
	tocopherol	1.12	1.5	1.1	1.5	19.5	15.69
	% conversion					*43.1*	*49.2*

Table 7. Case reports

Product	Case Report	Reference
moisturizing cream containing α--tocopherol	patient developed worsening of rosacea with pruritus and desquamation after use of cream; positive patch test results were observed with the moisturizing cream (++ at 48 h, 96 h, and 7 days); patch testing with the individual cream components resulted in a positive reaction to 0.5% α-tocopherol in pet.; a ROAT with the cream resulted in a positive result after 96 h; patch testing with 0.5% α-tocopherol in pet was negative in 10 controls	[69]
skin lotion containing 0.002% *D*-γ-tocopherol; ointment containing 5% *DL*-α-tocopherol acetate in pet.	patient developed itchy erythema after 7 mos using a lotion; rash spread after using the ointment; positive patch test to the lotion, the ointment, 5% *DL*-α-tocopherol acetate (all were ++ on days 2, 3, and 7), and to 2% pet. and 0.2% glycyrrhetinic acid (also ingredients in the ointment; + on day 2, ++ on days 3 and 7); patient did not react to 0.5% *DL*-α-tocopherol acetate; 5 controls did not react to *DL*-α-tocopherol acetate (or glycyrrhetinic acid)	[70]
vitamin E oil	eczematous dermatitis occurred on the eyelids and neck of a subject with xerotic skin after 4 wks of use; patch testing with an Italian baseline series and the oil resulted in a positive reaction to the oil only; a ++ reaction was observed on day 2 and +++ on day 3; a strong positive reaction was observed on day 3 in a ROAT on the volar forearm using the subject's own product; negative results were obtained in follow-up testing 1 mo. later; various concentrations and brands of tocopheryl acetate were used; negative results were obtained in a ROAT using a new sample of neat vitamin E oil.	[71]
vitamin E acetate lipogel	a patient developed eyelid and periocular dermatitis after using the lipogel; patch testing with the baseline series and the product resulted in positive results with the product only (+ on day 2 and ++ on day 3); ROATs on the volar forearm with the subject's product gave a positive result on day 4; however, additional patch tests performed with the active ingredient and excipients in the lipogel and various concentrations and brands of tocopheryl acetate produced negative result; negative results were also observed 1 month later in patch tests and ROATs performed using the vitamin E acetate lipogel	[71]
vitamin E lipogel (with cyclopentasiloxane)	a patient developed itching eczematous dermatitis on the eyelid after using the lipogel for 3 mos; the patient previously had used other vitamin E acetate products on other areas of the body with adverse effects; patch-testing with a baseline series and the patient's own lipogel produced positive reaction to the lipogel only (+ on day 2 and ++ on day 3); a ROAT on the volar forearm using the lipogel gave a strong positive result after 3 days additional patch tests performed 3 wks later with all the ingredients in the lipogel and with various concentrations of tocopheryl acetate produced negative results; however, an additional ROAT of a vitamin E acetate spray that contained only tocopheryl acetate and cyclopentasiloxane produced a positive reaction after 3 days, while a ROAT with pure tocopherol acetate oil did not; he researches concluded the reaction to the lipogel was the result of a compound allergy induced by the gel	[72]
cream containing DL-α-tocopheryl nicotinate	a patient developed itchy eruptions and well-defined edematous erythema after using the cream; patch testing was positive to the cream, 0.1% DL-α-tocopheryl nicotinate in pet. (+), and other components of the cream; patch tests with 1% D-α-tocopherol in pet. and 1% DL-α-tocopherol in pet. were negative; patch testing with 0.1% DL-α-tocopheryl nicotinate in pet in 3 subjects was negative	[73]

Abbreviations: pet. – petrolatum; ROAT - repeated open application test

Table 8. Photoprotective Effects

Test Article	Test Population	Dose/Conc Tested	UV Exposure/Source	Protocol	Results	Reference
			In Vitro			
α-tocopherol-6-*O*-phosphate	skin cultures from female hairless SKH mice	0-2% in distilled water	- 10-40 kJ/m^2 for 3-10 min -DNA-FIX apparatus (290-380 nm)	-skin cultures were exposed for up to 3 h -the effect of α-tocopheryl acetate in DMSO pretreatment was also evaluated	-TBARS were statistically significant reduced by tocopheryl phosphate; less of an effect was observed with tocopheryl acetate -tocopheryl phosphate and acetate decreased SBCs at 1, but not 20-40, kJ/m^2	[42]
			Non-Human			
			Topical			
TRF; contains tocopherols and tocotrienols; in PEG-400	female hairless mice	5%; 20 µl applied	- exposed for 29 min; 3 MED equiv. - Oriel 1000-W simulator, 2.8 mW/cm^2 UVA and UVB (290-400 nm)	- using 1 cm^2 polypropylene rings, TRF and PEG-400 were each applied to 2 sites for 2 h - half the sites were irradiated	- the concentrations of all forms of vitamin E were statistically significantly decreased in PEG-400 treated skin after irradiation, but the amounts of vitamin E statistically significantly increased in irradiated TRF-treated skin	[36]
α-tocopherol	female hairless SKH1 mice	5 mg/cm^2	- 25 J/cm^2 UVA + UVB; 10 MED equiv. -solar simulator model 14S (Solar Light Co.)	- tocopherol was applied on the backs and sides of the mouse 24 h prior to UV	- increased superoxide dismutase, reduced glutathione levels, and statistically significantly reduced the formation of epidermal lipid hydroperoxides, indicating protection of cutaneous tissue against oxidative damage	[47]
α-, γ-, or δ-tocopherol α-tocopheryl acetate - neutral cream vehicle	4 female C3H/HeNTac mice/group	50 mg of 1or 5% dispersions	- 3.6-3.7 J/m^2/s for 60 min -6 Westinghouse FS20 lamps; 80% of the output in UVB range	- the cream was applied to the shaved backs of animals 15 min before irradiation	- vitamin E compounds in a 5% dispersion inhibited thymidine dimer formation in epidermal DNA - α-tocopherol had the greatest effect; statistically significant decreases were observe with γ-, or δ-tocopherol, but the decrease with α-tocopheryl acetate was not statistically significant	[81]
RRR-α-tocopherol RRR-α-tocopheryl succinate	15 female Skh:2 hairless pigmented dark-eyed mice/group	5%	- UV exposure was increased gradually until reaching the maintenance dose of 0.24 J/cm^2/exposure 24 wks of exposure, for a total exposure of ~15 J/cm^2 - 4 Westinghouse FS40 bulbs; 265-440 nm	- 100 µl of an o/w emulsion of standard cosmetic base vehicle were applied 3x/wk for 1 wk prior to UV exposure and until study termination	-no toxic effects - both forms protected against blistering; α-tocopherol was most effective (73% of mice did not blister with α-tocopherol; 33% did not blister with the succinate) - both protected against pigmentation; tocopherol was more protective -both forms protected against tumors compared to untreated mice -time to tumor developmental was significantly delayed with tocopherol	[82]
tocopheryl acetate in acetone	hairless SKH-1 mice	2.6% solution (5.2 mg/0.2 ml)	- 90 mJ/cm^2 (1 MED); single dose of UV	- tocopheryl acetate was applied dermally either 30 min before or 30 min after UV - the effect on various reaction in the skin were measured	- cyclobutane pyrimidine dimers repair was increased 1.5- and 2-fold by tocopheryl acetate before and after UV, respectively - DNA synthesis was increased, but not in a statistically significant manner	[83]

Table 8. Photoprotective Effects

Test Article	Test Population	Dose/Conc Tested	UV Exposure/Source	Protocol	Results	Reference
tocopheryl acetate in acetone	hairless SKH-1 mice, 20/group	2.6% solution (5.2 mg/0.2 ml)	- initial dose of 90 mJ/cm^2 -dose was increased by 25% weekly until reaching 275 mJ/cm^2	- dermal application of tocopheryl acetate 30 min before or 30min after UV -mice were treated with maximum UV dose for 24 wks	tocopheryl acetate delayed tumor formation and yield until wk 20; by wk 24, tumor incidence was comparable for all groups	[83]
			Oral			
RRR-α-tocopheryl acetate	15 female Skh:2 hairless pigmented dark-eyed mice/group	62.5 IU/kg chow; oral intake of 0.276 IU/day	- maintenance dose: 0.24 J/cm^2/exposure 24 wks of exposure, for a total exposure of ~15 J/cm^2 - 4 Westinghouse FS40 bulbs; 265-440 nm		-no toxic effects -some protection against blistering (27% of mice did not blister) - protected against pigmentation - protected against tumors compared to untreated mice -time to tumor developmental was significantly delayed	[82]
tocopherols and tocotrienols	8 female hairless HR-1 mice/group	1.) vitamin E-free 2) 50 mg/kg α-tocopherol 3.) 229 mg/kg T-mix (see below)	- 180 mJ/cm^2 1x/day for 7 days - UVM-28 UV lamp	- animals were fed their respective diets for 6 wks - at 6 wks, half the animals were killed; half continued on their test diet and were irradiated	- sunburn was greatest in the vitamin E-free group, followed by the α-tocopherol group; weak sunburn was seen with T-mix - no significant differences in TEWL or skin hydration between the groups	[84]
tocopherols and tocotrienols	9 female hairless HR-1 mice/group	as above	- 180 mJ/cm^2 2x/wk	- all mice dosed topically with 390 nmol/0.1 ml acetone - 1 wk later, each group was started on its respective test diet and were irradiated	- incidence of papillomas appeared in wk 7 - α-tocopherol suppressed the incidence of papillomas in the final 4 wks - t-mix clearly suppressed the incidence of papillomas at wk 10	[84]
			Human			
			Topical			
10% tocopherols, 0.3% tocotrienols, and other cosmetic ingredients	30 subjects	2 mg/cm^2	- mean MED was 326.67 mJ/cm^2 - radiation dose was 2x the MED	- the test formulation was applied to a 2 cm^2 area of skin on the buttocks, and the site was irradiated 30 min after application of the formulation - test sites were evaluated immediately and 6 and 24 h after UVB exposure - an untreated and vehicle-, irradiated, controls were used	Phototoxic reactions were statistically significantly decreased at the test site than at the untreated site	[85]
o/w formulation containing 2.5% tocopheryl acetate and 5% vitamin C in 20% NEO-PCL autoemulsionable (cetearyl alcohol, decyl oleate, sodium cetearyl sulfate, sodium methylparaben, sodium propylparaben, and water) and 5% aq. propylene glycol	20 Caucasian subjects with Fitzpatrick's phototype II, II-III, or III			- subjects were exposed to 6 increasing doses of SSR (Oriel 16s – 300 W solar simulator; erythemal doses of 14.5-45.0 mJ/cm^2) on the back - effect on the cutaneous response was examined by applying the test formulation 20 min before or 0.5, 3, 6, 9, and 12 h after irradiation	- application prior to irradiation produced a 36.9% increase in MED - application after irradiation produced a 19.8% increase in MED - application of the vehicle only 20 min before and 0.5, 3, 6, 9,and 12 h after irradiation did not have a statistically significant effect on MED	[86]

Abbreviations: MED – minimal erythema dose; SBC – sunburn cells; SSR – simulated solar radiation; T-mix – consist of 21.8% α-tocopherol, 1.0% γ-tocopherol, 23.4% α-tocotrienol, 37.4% γ-tocotrienol; TBARS – thiobarbituric acid reactive substances; TEWL – transepidermal water loss; TRF – tocotrienol-rich fraction

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