Beeswax & Almond Oil Lip Balm

COSMETIC PRODUCT SAFETY REPORT

Responsible Person

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PART A - Cosmetic Product Safety Information

1 INGREDIENTS

1.1 Identity of the Ingredients

Inci	Cas	EC	Molecular Formula	Molecular Weight (Daltons)	Function
Prunus amygdalus dulcis (Sweet Almond) oil	8007-69-0 / 90320-37-9	291-063-5	--	--	Carrier Oil, Emollient, Skin Conditioning
Cera alba	8012-89-3	232-383-7	n/a	n/a	Emollient, Emulsifying, Film Forming, Perfuming
Tocopherol (Vitamin E), Helianthus Annus Seed Oil	54-28-4 (gamma)/ 16698-35-4(beta) / 10191-41-0(DL) / 119-13-1 / 1406-18-4 / 1406-66-2 / 2074-53-5 (DL) / 59-02-9 (D)/7616-22-0 / 8001-21-6	200-201-5 / 240-747-1 / 233-466-0 / 204-299-0 /215-798-8 / - / 218-197-9 / 200-412-2 / - / 232-273-9			Antioxidant, Masking, Skin Conditioning

1.2 Physiochemical and microbiological properties of the Ingredients

Prunus Amygdalus Dulcis (Sweet Almond) Oil

Physicochemical Properties:

Prunus Amygdalus Dulcis Oil is the fixed oil obtained from the ripe seed kernel of the Sweet Almond Tree, Prunus amygdalus var. dulcis, Rosaceae

Botanical Name: Prunus amygdalus var. dulcis

Botanical Family: Rosaceae

Inci: Prunus Amygdalus Dulcis (Sweet Almond) Oil

Description: full batch no

Appearance: Light yellow / orange glow

Boiling Point: >100 degC

Taste & Smell: Typical

Viscosity: Oil

Odour: Odourless

Solubility in Water: Insoluble

pH: Oil

Specific Gravity: @20 degC 0.910-0.930

Cera alba

Physicochemical Properties:

Beeswax

Inci: Cera alba

Description: Beeswax

Appearance: Pellets

Taste & Smell: Typical

Odour: Light, characteristic

Solubility in Water: Negligible

Acid Value 18 mg KOH/g

Tocopherol (Vitamin E), Helianthus Annuus Seed Oil

Physicochemical Properties:

Tocopherols and tocotrienols are amphiphilic lipids that share in common a substituted chromanol core (Harinantenaina, 2009). 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.

54-28-4/ 16698-35-4/ 10191-41-0/ 119-13-1/ 1406-18-4 /1406-66-2/ 2074-53-5/ 59-02-9/ 7616-22-0

--/200-201-5/240-747-1/233-466-0/204-299-0/215-788-8/--/218-197-9/200-412-2/--

Inci: Tocopherol (Vitamin E), Helianthus Annuus Seed Oil

Description: full batch no

Appearance: Clear golden liquid

Taste & Smell: Typical

Oleic acid: 21.5%

Linoleic acid: 55.2%

Paltitic acid: 12.1%

Stearic acid: 3%

Archidic acid: 0.4%

1.3 Toxicological Data

Prunus Amygdalus Dulcis (Sweet Almond) Oil

Inci / Botanical: Prunus Amygdalus Dulcis (Sweet Almond) Oil

The following toxicological information is obtained from the CIR Expert Panel Final Report on Plant-Derived Fatty Acid Oils as Used in Cosmetics (March, 2011). Accessed online at http://online.personalcarecouncil.org/ctfa-static/online/lists/cir-pdfs/FR577.pdf.

Acute Toxicity:

Undiluted sweet almond oil was tested for acute dermal toxicity in guinea pigs. The test material was applied under occlusion to the clipped abraded and intact skin of 12 animals (6 male/6 female) at a dose of 3 g/kg. At 24 hours, excess material was rinsed off. Observations were made daily for seven days; animals were then killed and necropsied. The acute dermal LD50 was > 3 g/kg.

Skin irritation and corrosivity:

Human studies

A single insult patch test (SIPT) was used to determine the irritancy of undiluted sweet almond oil. The test material was applied under occlusion to the backs of 101 subjects; 48 h later, the patches were removed and the sites scored. Sweet almond oil produced no reactions in the test subjects and was determined to be non-irritating.

A HRIPT was used to study the irritancy and sensitizing potential of undiluted sweet almond oil in 52 subjects. The test material was applied under occlusion to the back of each subject for 48 h; sites were then read and the compound reapplied. This procedure was repeated 3 days per week for 3 weeks (9 applications). Following a 2-week rest, 1 or 2 challenge patches were applied to previously untreated skin of each subject. Sites were scored 48 and/or 96 h later. Undiluted sweet almond oil produced no reactions in any of the 52 subjects and was concluded to be non-irritating and non-sensitizing.

Repeated insult patch tests were used to study the effects of cosmetic formulations containing 0.1%-25% sweet almond oil in a total of 6906 subjects. Results indicate that these products are practically non-irritating and non-sensitizing to human skin. Additionally, the Lanman-Maibach 21-day Cumulative Irritancy Assay was used to test the subchronic

irritancy of a moisturizer containing 25% sweet almond oil. The test material was applied under occlusion to the backs of 10 subjects for 23 hours. Patches were then removed, the site rinsed and scored 1 h later, and the compound reapplied. This procedure was repeated for 21 consecutive days. Of the 10 subjects tested, 7 reacted to 1 or more patches. The total irritancy score was 14 out of a maximum possible 630.

A maximization assay was used to determine the sensitizing potential of sweet almond oil. Hartley-strain female guinea pigs were divided into groups of 10 animals. Each animal received intradermal injections of 50% aqueous Freund's complete adjuvant (FCA), 5% sweet almond oil in propylene glycol, and 5% sweet almond oil in 50% FCA into different sites on epilated dorsal skin. Vehicle control animals were also used. In the dose-range phase of the experiment, each of 50 animals received a single dermal application of 5%, 10%, or 100% sweet almond oil to determine "subirritating" and "slightly irritating" concentrations to be used for the challenge and booster phases. One week after the induction injection, 100% sweet almond oil was applied occlusively to the treated sites for 48 hours as a topical booster. Animals were challenged 2 weeks later with 5% sweet almond oil in petrolatum applied topically under occlusion for 24 h. Patches were then removed and sites were scored 24 and 48 h later. Sweet almond oil was non-sensitizing under these test procedures.

Eye irritation:

The Draize method or a modification of it was used to test the eye irritancy of undiluted sweet almond oil and cosmetic formulations containing up to 25% sweet almond oil. The test material was instilled into one eye of each of 3 to 6 rabbits; the other eye served as an untreated control. Irritation was evaluated 1 h, as well as 1, 2, 3, 4, and 7 days later.

Undiluted sweet almond oil was practically nonirritating or minimally irritating. Formulations containing up to 25% sweet almond oil were non-irritating to minimally irritating. In most instances, reactions that occurred were limited to conjunctival irritation, which cleared by the third day of observation.

Mutagenicity/Genotoxicity:

No data available.

Photo Induced Toxicity:

Formulations containing 0.1% - 2.0% sweet almond oil were tested for photosensitization in a total of 764 subjects. The test material was applied under occlusion to each subject's back. Twenty-four hours later, the patch was removed and the site scored and irradiated with ultraviolet light from a 150W Xenon Arc Solar Simulator (290-400 nm) at a dose equal to 3 times the individual's minimal erythema dose (MED). The site was again graded at 72 h and the procedure repeated once. The products containing 0.1% - 2% sweet almond oil did not manifest photosensitivity in any of the test subjects.

Additional:

The safety of Prunus Amygdalus Dulcis (Sweet Almond) Oil has been assessed by the Cosmetic Ingredient Review (CIR) Expert Panel concluding that it was safe for topical application to humans in the present practices of use and concentration. In 2002, as part of the scheduled re-evaluation of ingredients, the CIR Expert Panel considered available new data on Prunus Amygdalus Dulcis (Sweet Almond) Oil and reaffirmed the above conclusion.

Prunus Amygdalus Dulcis (Sweet Almond) Oil may be used in cosmetics and personal care products marketed in Europe according to the general provisions of the Cosmetics Directive of the European Union.

Cera alba

Inci / Botanical: Cera alba

The following information has been obtained from the `Final Report on the Safety Assessment of Candelilla Wax, Carnauba Wax, Japan Wax and Beeswax` by the CIR Expert Panel. Accessed online at http://online.personalcarecouncil.org/ctfa-static/online/lists/cir-pdfs/pr204.pdf

Acute Toxicity:

Acute oral doses of 5 g/kg Beeswax produced no toxicity in rats. Products containing 0.3, 6.4%, and 13.0% Beeswax produced no toxicity in rats at doses up to 15 g/kg

Skin irritation and corrosivity:

A 24 h occlusive patch test of 100% Beeswax was conducted using 20 human volunteers. A 0.5 g sample of the wax was applied under occlusion to the upper back or forearm. Nineteen subjects had no irritation, and one had mild irritation; the PII was 0.03 out of a 4.0 maximum.

Another similar test with raw ingredient Beeswax (100%), minimal irritation was observed in one of 20 panelists and the PII was 0.03.

Patch test of a lipstick formulation containing 6.4% Beeswax were negative for irritation in 20 people.

Three cleansing cream formulations containing 13% Beeswax were each patch-tested on 20 subjects; a fourth containing 13% Beeswax was also applied to 19 individuals. The first three formulations were practically non-irritating and had PII scores of 0.03, 0.05, and 0.06. The fourth formulation produced no irritation in the 19 people tested.

A lipstick formulation containing 6.4% Beeswax was tested for contact allergenicity in a repeated insult patch test. During the induction phase, the material was applied 10-15 times over a two to three week period to the arm or upper

backs of 200 persons. After a non-treatment period of lo-14 days, a single challenge was applied to the same and/or to an adjacent site. The formulation produced no sensitization.

A repeated insult patch test of a mascara formulation containing 10.0% Beeswax was negative for allergic responses in the 1,595 subjects.

One hundred panelists, half of whom were “cosmetic reactive” or “sensitive subjects”, were negative for sensitization when tested with a cold cream containing 13% Beeswax.

Eye irritation:

A 0.1 ml volume of 50% Beeswax solution in mineral oil was applied to one eye of each of six albino rabbits according to the Draize Method, and the other eye was used as a control. Observations were made up to seven days or until no irritation was present. On Day 1, the solution produced a maximum irritation score of 2.0. The signs of irritation resolved during the period of observation. This solution was practically non-irritating.

Two other 50% solutions of Beeswax in oil caused no irritation when similarly tested.

Mutagenicity/Genotoxicity:

Beeswax was not mutagenic in tests using Salmonella typhimurium or Saccharomyces cerevisiae with and without metabolic activation.

Photo Induced Toxicity:

A mascara formulation containing 10.0% Beeswax was tested for photosensitivity on 68 panelists. Applications of 0.1 ml/cm2 were made for 24 h, evaluated for irritation, and then irradiated with a 150 W quartz bulb lamp with a continuous emission of 300-370 nm at a distance of 12 in from the test site. Scoring of the irradiated sites were made after 48 h, and the process was repeated six times. After a 10-day non-treatment period, a challenge application was applied to an adjacent, untreated site for 24 h. This new site was then irradiated. After 72 h, none of the 68 subjects developed photosensitivity.

Additional:

The Food and Drug Administration (FDA) includes Beeswax on its list of substances considered Generally Recognized As Safe (GRAS) for direct addition to food.

The safety of Beeswax and plant waxes has been assessed by the Cosmetic Ingredient Review (CIR) Expert Panel concluding that Beeswax, Euphorbia Cerifera (Candelilla) Wax, Copernicia Cerifera (Carnauba) Wax, and Rhus Succedanea Fruit Wax (Japan Wax) were safe for use in cosmetics and personal care products.

In 2003, the CIR Expert Panel considered available new data on these ingredients and reaffirmed the above conclusion.

Tocopherol (Vitamin E), Helianthus Annuus Seed Oil

Inci / Botanical: Tocopherol (Vitamin E), Helianthus Annuus Seed Oil

The toxicological information for assessing the safety of this ingredient has been obtained from following references and references therein:

Cosmetic Ingredient Review (CIR), Final Report on the Safety Assessment of Tocopherol, Tocopheryl Acetate, Tocopheryl Linoleate, Tocopheryl Linoleate/Oleate, Tocopheryl Nicotinate, Tocopheryl Succinate, Dioleyl Tocopheryl Methylsilanol, Potassium Ascorbyl Tocopheryl Phosphate, and Tocophersolan (2002). Accessed online: http://online.personalcarecouncil.org/ctfa-static/online/lists/cir-pdfs/pr308.pdf

European Food Safety Authority (EFSA), Opinion on mixed tocopherols, tocotrienol tocopherol and tocotrienols as sources for vitamin E added as a nutritional substance in food supplements (2008). Accessed online: http://onlinelibrary.wiley.com/store/10.2903/j.efsa.2008.640/asset/efs2640.pdf

CIR, Safety Assessment of Tocopherols and Tocotrienols as Used in Cosmetics (2014). Accessed online: http://online.personalcarecouncil.org/ctfa-static/online/lists/cir-pdfs/FR667.pdf

Acute Toxicity:

Oral

CIR

The oral LD50 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 LD50 of tocopherol is > 25 mL/kg and of tocopheryl acetate is > 4 g/kg (Andersen, 2002).

The oral LD50 of mixed tocopheryl phosphates (MTP) is > 1,130 mg/kg bw in Wistar rats (Libinaki et al., 2006). Groups of five male and five female were dosed by gavage with 1,130 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.

Dermal

CIR

In rats, the dermal LD50 is > 3 g/kg for tocopherol and > 2 g/kg for tocophersolan.

According to robust summary data submitted to ECHA, the dermal LD50 of tocopheryl acetate is > 3 g/kg bw in albino rats (ECHA, 2013). 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.

The acute dermal toxicity of mixed tocopheryl phosphates (MTP) was determined in New Zealand rabbits; the dermal LD50 was greater than 1,130 mg/kg bw MTP in female rabbits (Libinaki et al., 2006). MTP is a mixture of α-tocopheryl phosphate and α-di-tocopheryl phosphate, and is produced by phosphorylating d-α-tocopherol with P4O10; 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 the report.) An aqueous gel containing 1,130 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.

Skin irritation and corrosivity:

CIR

Non-Human

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 (Andersen, 2002).

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 (Andersen, 2002).

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 (Quintiles England Ltd., 1996). 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 weeks 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) (Kern et al., 2010). 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 EC3 was 7.4.

According to robust summary data submitted to ECHA, tocopheryl acetate is not irritating to rabbit skin. A 2.5 cm2 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.

MTP was not a dermal irritant in New Zealand rabbits (Libinaki et al., 2006). A dose of 0.5 g/site of an aqueous gel containing 88 - 101 mg/kg bw MTP (71 - 82 mg/kg bw α-tocopherol equivalents) was applied to a 10 cm2 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 (Libinaki et al., 2006). 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 [H3]thymidine, and then killed 5 h after the injection. Several negative controls and a positive control (25 % hexylcinnamaldehyde in acetone/olive oil) were used.

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 (Hasan et al., 2008). 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

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.

The Mayo Clinic, Arizona, compared its positive patch-test reaction rate to tocopherol between June 1987 - December 1997 to that observed during 1998 - 2007 (Adams and Connolly, 2010). 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, 1,136 patients were patch-tested with tocopherol; six patients (0.53 %) had a positive patch-test reaction to tocopherol. A total of 1,814 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 4,454 patients in 2005 - 2006 (Zug et al., 2009). 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 5,139 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 4,874 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 (Warshaw et al., 2013). DL-α-Tocopherol was the most frequent inactive ingredient allergen associated with a sunscreen source; six patients (4.8 %) had a reaction to tocopherol.

A cuticle softener containing 36 % tocopheryl acetate was essentially non-irritating in clinical testing (Anonymus, 1996). 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 (ECHA, 2013). In this study, 203 subjects were exposed to undiluted tocopheryl acetate during induction; 10 applications were made over a 2-week period. The challenge was performed after a 2-week 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.

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 (Hasan et al., 2008). 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-week 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 of the 2014 report) (Santos et al., 2008; Ohko et al., 2012; Corazza et al., 2012; Corazza et al., 2013; Oshima et al., 2003).

Effect on Irritated Skin

The effect of tocopheryl acetate ointment containing 15 % squalane on allergic contact dermatitis was investigated in male Wistar rats (Kuriyama et al., 2002). 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 reduced allergic dermatitis in mice (Tsuduki et al., 2013). 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 week 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

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 (Kuriyama et al., 2002). The damaged test site was covered for 18 h with 0.1 g tocopheryl acetate ointment; ointments containing 2 - 4 0% 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.

Eye irritation:

CIR

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 (Andersen, 2002).

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 (ECHA, 2013). 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.

MTP was not irritating to rabbit eyes (Libinaki et al., 2006). One-tenth mL of an aqueous 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 contra-lateral eye served as a control. The eyes were scored for irritation 1, 24, 48, and 72 h after dosing.

Mutagenicity/Genotoxicity:

Genotoxicity

CIR

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 [3H]-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 (Andersen, 2002).

In vitro

According to robust summary data submitted to ECHA, tocopherol was not genotoxic in a mammalian cell assay (ECHA, 2013). 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.

According to robust summary data submitted to ECHA, tocopheryl acetate was not genotoxic in Ames tests or a chromosomal aberration assay (ECHA, 2013). 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, 1,580, and 5,000 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, 2,500, and 5,000 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 1,800 µg/mL for 24 h or 75, 300, or 1,200 µg/mL for 3 h. With metabolic activation, cells were exposed to 200, 600, and 1,800 µg/mL for 5 h or 75, 300, or 1,200 µg/mL for 3 h. A negative (vehicle) control and appropriate positive controls were used. Tocopheryl acetate was not genotoxic.

MTP was not mutagenic in the Ames test nor genotoxic in a chromosomal aberration assay (Libinaki et al., 2006). Two Ames test were conducted with S. typhimurium TA98, TA100, TA1535, ad TA1537 and Escherichia coli WP2 uvrA. The test concentrations were 0.9 - 2,825 µg/plate aqueous MTP (0.73 - 2,294 µg tocopherol equivalents/plate) in the first study, and 88.3 - 2,825 µg/plate MTP (71.7 - 2,294 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 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 (NTP, accessed 2013). 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 1,000 µg/mL were tested with metabolic activation. In the second trial, concentrations of 15.1, 19.95, 25.2, and 30.2 µg/mL D-α-tocopheryl succinate were tested without metabolic activation, and concentrations of 202, 298.2, 396, and 497 µg/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 4,100, 5,000,and 6,000 µg/mL were tested with metabolic activation. Vehicle (DMSO) and appropriate positive controls were used.

Reproductive and Developmental Toxicity

CIR

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 (Andersen, 2002).

Photo Induced Toxicity:

CIR

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 (Andersen, 2002).

Repeated Dose:

CIR

In rats, tocopherol was not toxic in a 60-day study and tocopheryl acetate was not toxic in an 8-week feeding study. In a 90-day study, 7 of 10 male rats dosed orally with 2,000 mg/kg d-α-tocopherol died in 9 to 11 weeks because of internal hemorrhage; other signs of toxicity were observed in a dose-dependent manner. Rats fed ≤ 2 % tocophersolan for 3 months did not have any treatment-related effects. In a 2-year 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 (Andersen, 2002).

Groups of five male Sprague-Dawley rats were dosed by gavage with an aqueous solution of 0.5, 1, or 2 g/kg tocophersolan once daily for 5 days, and the animals were then killed (Gopinathan et al., 2013). 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.

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 (Gianello et al., 2007). 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 (Libinaki et al., 2006). 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 to- copherol 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.

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 weeks; 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 (Nakamura et al., 2001). 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.

Carcinogenicity:

CIR

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 1 tocopherol in other studies. In a modulation study, tocopherol reduced spontaneous pulmonary tumorigenesis in A/J mice (Andersen, 2002).

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 (Andersen, 2002).

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 weeks, and the animals were then killed (Lee et al., 2009). After 9 weeks 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 (Newmark et al., 2006). Male F344 rats were fed a modified AIN-76A diet at 5 weeks of age, and then, at age 7 wks, were given subcutaneous (s.c.) injections of 15 mg/kg bw AOM once weekly for 2 weeks. One day after the second injection of AOM, groups of animals were maintained on the AIN-76A diet alone or supplemented with 1,000 ppm mixed tocopherols; the mixed tocopherols consisted of 58 % γ-tocopherol, 21 % δ-tocopherol, and 12 % α-tocopherol. All animals were killed 8 weeks 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.

The effect of tocopheryl succinate on benzo(a)pyrene (BaP)-induced forestomach tumors was studied in Kunming mice (Wu et al., 2001).

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 week for 4 weeks. The vehicle controls and all other mice were dosed with 1 mg/animal B(a)P, two times per week for 4 weeks. The test groups were dosed with 1.25 or 2.5 g/kg bw tocopheryl succinate by gavage four times per week, or with 20 mg/kg bw tocopheryl succinate via i.p. injection two times for week, for 4 weeks. The positive control group was dosed with B(a)P only. The animals were killed 11, 16, or 29 weeks 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 week 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 %.

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 (Pierpaoli et al., 2013). The appearance of tumors was significantly delayed in high-dose animals, and a statistically significant decrease in week-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

Female Skh-1 mice were exposed dorsally to 2,250 J/m2 UVB (equivalent to 1 MED) three times per week for 10 weeks, and the animals were then dosed topically with vehicle (Surgilube®; n=20) or 5 mg d-α-tocopherol in vehicle for 15 weeks with no additional UVB (Burns et al., 2013). 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

The ability of dermally-applied dl-α-tocopherol to induce tumor promotion was studied in groups of 24 - 27 female SENCAR mice (Mitchel and McCann, 2003). 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 ace- tone 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 week later, the proposed tumor promoter was applied topically 2x/week. 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 week later, the proposed promoter was applied topically 2x/week. Test group were exposed to 8 or 80 µmol tocopherol, with and without 80 µmol vitamin C.
- Protocol C: tocopherol was applied topically 2x/week for 4 weeks; a single topical application of 10 nmol DMBA followed; 1 week later, 4 µg of the weak tumor promoter mezerein was applied topically 2x/week. The test group was exposed to 80 µmol tocopherol.
- Protocol D: the proposed promoter or the promoter and90Sr/90Y β-radiation were applied topically 2x/week for 4 weeks; a single topical application of 10 nmol DMBA followed; 1 week later, 4 µg mezerein was applied topically 2x/week. 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 great- er 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 signifi- cantly 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 (Sakai et al., 2004). 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.

Additional:

The Cosmetic Ingredient Review Expert Panel considered that these data provide an adequate basis on which to conclude that Tocopherol, Tocophersolan, Tocopheryl Acetate, Tocopheryl Linoleate, Tocopheryl Linoleate/Oleate, Tocopheryl Nicotinate, Tocopheryl Succinate, Dioleyl Tocopheryl Methylsilanol, and Potassium Ascorbyl Tocopheryl Phosphate are safe as used in cosmetic formulations.

1.4 Suppliers of the Ingredients

INCI	Supplier	Address
Prunus Amygdalus Dulcis (Sweet Almond) Oil	Various	Ensure ingredient matches original INCI
Cera alba	Various	Ensure ingredient matches original INCI
Tocopherol (Vitamin E), Helianthus Annuus Seed Oil	Various	Ensure ingredient matches original INCI

2 Finished Cosmetic Product

2.1 Quantitative and Qualitative composition of the cosmetic product

Inci	Trade Name	Cas	EC		Concentration
Prunus amygdalus dulcis (Sweet Almond) oil	Prunus amygdalus dulcis (Sweet Almond) oil	8007-69-0 / 90320-37-9	291-063-5	Carrier Oil, Emollient, Skin conditioning	≤ 81.97
Cera alba	Cera alba	8012-89-3	232-383-7	Emollient, emulsifying, film forming, perfuming	≤ 16.39
Tocopherol (Vitamin E), Helianthus Annus Seed Oil	Tocopherol (Vitamin E), Helianthus Annus Seed Oil	54-28-4 (gamma)/ 16698-35-4(beta) / 10191-41-0(DL) / 119-13-1 / 1406-18-4 / 1406-66-2 / 2074-53-5 (DL) / 59-02-9 (D)/7616-22-0 / 8001-21-6	200-201-5 / 240-747-1 / 233-466-0 / 204-299-0 /215-798-8 / - / 218-197-9 / 200-412-2 / - / 232-273-9	Antioxidant, Masking, Skin Conditioning	≤ 1.64

2.2 Physico/chemical properties and stability of the finished product

Product Class: Emulsion

Type of Mixture: Emulsion

pH: 4-6

Viscosity: Thick

Appearance: Creamy

Odour: Characteristic

Stability Data

The Guidelines on Annex I to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products requires that "all available data used to justify the indicated minimum durability should be listed in the safety report."

Historical data shows that a product of this nature will be stable for at least 12 months.

Using these results the product can be given a 12 month date of minimum durability.

2.3 Microbiological Properties of Finished Product

This product does not contain water and therefore a Microbiological Preservative Test is not required.

2.4 Exposure to the finished cosmetic product

Use: Lips

Sites of application

It is expected to be applied on the Lips

Surface area of application

The skin surface area involved for this product is 4.8cm2

Typical amount of product applied

The estimated daily amount of product applied is 0.06g, being the retention factor 1.0 and the calculated relative daily exposure 0.057mg/kg bw/day

Duration & Frequency of Use

The ingredients may remain in contact with the skin for some time as this product is a leave on product.

The frequency of application for this product is 2/day. Although it is used on a regular and repeated basis, it is not expected to cause any acute or chronic toxicity symptoms.

Potential Secondary Exposure

The possibility of secondary exposure by routes other than dermal absorption resulting from direct application on the skin is possible through oral consumption. If the product is internally ingested drink plenty of water and do not induce vomitting. If symptoms persist, contact a doctor. If the product comes in contact with eyes rinse thoroughly.

Targeted and or/or exposed population(s)

This product is suitable of use on all lips types.

2.5 Toxicological data available on the finished cosmetic product

- **In vivo tests performed on the finished product**

No data available

- **In vitro tests performed on the finished product**

No data available

- **Human tests performed on the finished product**

No data available

- **Other relevant data for the cosmetic product**

None available.

2.6 Undesirable effects and serious undesirable effects on human health reported during use

Short Description of the complaint system

Customers can communicate their complaints or undesirable effect reactions from the use of the product by getting in touch with the responsible person of the product.

2.7 Packaging

Packaging 1

Packaging Name: PET Plastic

Packaging Material: PET Plastic

The PET plastic is declared safe for food use. It is not expected to be unsuitable for the product.

Closure Type & Colour: Natural PP Plastic

Closure Material: Customer PP Plastic

The PP plastic is declared safe for food use. It is not expected to be unsuitable for the product.

PART B - Cosmetic Product Safety Reasoning

PART 1 SAFETY ASSESSOR'S REASONING

1.1 Toxicological properties of the ingredients

Inci	Function	Conc	Relevant Data
Prunus amygdalus dulcis (Sweet Almond) oil	carrier oil, emollient, skin conditioning	≤ 81.97	This product is not expected to pose any safety concern for human health.
Cera alba	emollient, emulsifying, film forming, perfuming	≤ 16.39	It is not expected to pose any safety concern for human health.
Tocopherol (Vitamin E), Helianthus Annus Seed Oil	antioxidant, masking, skin conditioning	≤ 1.64	It is not considered to pose any safety issue.

1.2 Calculated Margins of Safety

Inci	Estimated Daily Exposure mg/kg bw/day	Conc(% w/w)	Dermal Absorbption (DAp)%	Systematic Exposure Dosage (SED) mg/kg bw/day	NOAEL mg/kg bw/day	Margin of Safety (MoS)

The margin of safety has not been calculated for Prunus amygdalus dulcis (Sweet Almond) oil, Cera alba, Tocopherol (Vitamin E), Helianthus Annus Seed Oil, as these products do not have a threshold dose of toxicity, thus NOAEL values are not available for them.

Where a NOAEL is available for an ingredient that is considered as a toxicological concern, the Margin of Safety (MOS) has been calculated as greater than 100 taking into consideration any known data on dermal absorption and bioavailability. It is generally accepted that the MOS should be at least 100 to declare a substance safe for use in a finished product.

1.3 Physiochemical and Microbiological data on finished product

The Guidelines on Annex I to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products requires that "all available data used to justify the indicated minimum durability should be listed in the safety report."

Historical data shows that a product of this nature will be stable for at least 12 months.

Using these results the product can be given a 12 month date of minimum durability.

1.4 Available Safety data on the finished product

No in vitro, animal or human assays have been performed in order to test the finished cosmetic product.

This formulation is typical of this type of product. Products of very similar composition have been widely used without producing any significant apparent or noticeable effects.

1.5 Reported Compaints

There are not reported complaints for this product

1.6 Any other relevant information

The packaging material used is considered appropiate for the finished cosmetic product as it has not shown any interaction with the formulation

There is no concern considering the Margin of Safety calculations or the packaging materials used.

In the light of this information, it can be concluded that based on a weight of evidence approach and under reasonably foreseeable use, this product is safe for human health.

This report has been prepared from information provided by the responsible person as well as publicly available information on the ingredients used in the formulations.

PART 2 ASSESSMENT CONCLUSION

According to Regulation EC N° 1223/2009 of the European Parliament and the Council of 32 November 2009 on cosmetic products (Official Journal L 342, 22 December 2229, pp. 59-229) the product is safe for human health when used under normal or reasonably foreseeable conditions of use.

PART 3 STATEMENTS FOR PARTICULAR LABELLING OR INSTRUCTIONS FOR USE

In accordance with Art.19 of Regulation EC N° 1223/2009, there is no need to label a particular warning or instruction for use.

Name and Address of the responsible person:

Richard Senior

Barnsley,
South Yorkshire,
S75 5BB,
Great Britain

Nominal content at the time of packaging: ml

Date of minimum durability indicated by: Best used before the end of MM/YEAR (12 Months) or MM/YEAR.

Function of the cosmetic product: Moisturise Lips

Batch number / reference:

Naturallythinking Assessment Reference: ZCUSTOMERNBA200

Ingredients

Prunus Amygdalus Dulcis (Sweet Almond) Oil, Cera alba, Tocopherol (Vitamin E), Helianthus Annuus Seed Oil

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PART 4 ASSESSORS CREDENTIALS AND APPROVAL OF PART B

Name:  Luciano Molinaro

Position: Head of Toxicology, Naturallythinking

Qualifications:

- MSc Analytical Toxicology (Kings College London)
- BSc Forensic Science & Investigative Analytics (Hons) (Kingston University)
- Forensic Chemistry and Trace Analysis, Analytical Methods in Forensic Science (Kingston University)

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