Final Report of the Cosmetic Ingredient Review Expert Panel

Safety Assessment of Glyceryl Dilaurate, Glyceryl Diarachidate, Glyceryl Dibehenate, Glyceryl Dierucate, Glyceryl Dihydroxystearate, Glyceryl Diisopalmitate, Glyceryl Diisostearate, Glyceryl Dilinoleate, Glyceryl Dimyristate, Glyceryl Dioleate, Glyceryl Dipalmitate, Glyceryl Dipalmitoleate, Glyceryl Distearate, Glyceryl Palmitate Lactate, Glyceryl Stearate Citrate, Glyceryl Stearate Lactate, and Glyceryl Stearate Succinate

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Cosmetic Ingredient Review
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Final Report on the Safety Assessment of Glyceryl Dilaurate, Glyceryl Diarachidate, Glyceryl Dibehenate, Glyceryl Dierucate, Glyceryl Dihydroxystearate, Glyceryl Diisopalmitate, Glyceryl Diisostearate, Glyceryl Dilinoleate, Glyceryl Dimyristate, Glyceryl Dioleate, Glyceryl Dipalmitate, Glyceryl Dipalmitoleate, Glyceryl Distearate, Glyceryl Palmitate Lactate, Glyceryl Stearate Citrate, Glyceryl Stearate Lactate, and Glyceryl Stearate Succinate

Abstract: Glyceryl Dilaurate, Glyceryl Diarachidate, Glyceryl Dibehenate, Glyceryl Dierucate, Glyceryl Dihydroxystearate, Glyceryl Diisopalmitate, Glyceryl Diisostearate, Glyceryl Dilinoleate, Glyceryl Dimyristate, Glyceryl Dioleate, Glyceryl Dipalmitate, Glyceryl Dipalmitoleate, Glyceryl Distearate, Glyceryl Palmitate Lactate, Glyceryl Stearate Citrate, Glyceryl Stearate Lactate, and Glyceryl Stearate Succinate are cosmetic ingredients that function as skin conditioning agents - emollients in cosmetics. These chemicals are also called diacylglycerols or diglycerides. Glyceryl diesters are a mixture of 1,2-, 2-3, and 1-3-diglycerides. They are not stable and are easily isomerized under acidic, basic, or thermal conditions. It was estimated that 10-15% 1,2 diester (optically and physiologically active isomer) is present in one glyceryl diester as sold, with 10-15% 2,3 diester (optically active, physiologically non-active isomer) and 70-80% 1,3 diester (not optically or physiologically active) comprising the remainder of the product. Glyceryl Dilaurate, Glyceryl Diisostearate, Glyceryl Dioleate Glyceryl Distearate, and Glyceryl Stearate Lactate reportedly are used in a variety of product types. are used in cosmetics (over 100 uses total); the remaining ingredients are not reportedly in current use. Concentration of use data indicate most uses involve concentrations less than 10%, although Glyceryl Diisostearate is reported to be used at concentrations up to 43% in lipstick. Glyceryl diesters have been approved for use as indirect food additives. Glyceryl diesters are intermediates in the metabolism of triglycerides to free fatty acids and glycerol. Glyceryl diesters exhibited little acute oral toxicity in rats. Glyceryl diesters were non-irritating to the eyes and were only a mild skin irritant in rabbits. In guinea pigs, these ingredients did not produce sensitization, phototoxicity, or photoallergenicity. Various 1,2-diacylglycerols have been demonstrated to induce protein kinase C, produce dermal hyperplasia, and act as a tumor promoter. These effects depend on the extent of exposure (high doses needed), the frequency of exposure (multiple exposures per day over many days are needed), the length of the fatty acid chains on the glyceryl backbone (less than 14 is most effective), and the saturation of those chains (most effective if one fatty acid is unsaturated and the other saturated). In clinical tests, glyceryl diesters were non-irritating to mildly irritating. and produced no sensitization or photosensitization. Case reports do exist, however, in which individuals have had sensitization reactions to products containing glyceryl diesters. Because of the potential that 1,2-diesters in these ingredients could act as tumor promoters, the Cosmetic Ingredient Review Expert Panel concluded that the available data were insufficient to assure their safety in cosmetic products. The Panel particularly cited the high 1,2-diester content, the high use concentration in lipstick products, and the frequency of application of these products (i.e., several times daily). The following are needed in order to arrive at a conclusion on the safety of Glyceryl Diesters in cosmetic products: (1) dose-response study (topical application of samples of cosmetic grade Glyceryl Dilaurate in a model formulation) to determine whether the concentrations of 1,2-diesters found in Glyceryl Diesters and the frequency of application induce hyperplasia, and (2) if 1,2-diester concentrations in cosmetic grade Glyceryl Dilaurate induce hyperplasia, then a long-term, dermal tumor UV initiation-promotion study will be needed.

INTRODUCTION

The report addresses the safety of the following glyceryl diesters (glycerin/aliphatic acid diesters) in cosmetics:

Glyceryl Dilaurate
Glyceryl Diarachidate
Glyceryl Dibehenate
Glyceryl Dierucate
Glyceryl Dihydroxystearate
Glyceryl Diisopalmitate
Glyceryl Diisostearate
Glyceryl Dilinoleate
Glyceryl Dimyristate
Glyceryl Dioleate
Glyceryl Dipalmitate
Glyceryl Dipalmitoleate
Glyceryl Distearate
Glyceryl Palmitate Lactate
Glyceryl Stearate Citrate
Glyceryl Stearate Lactate
Glyceryl Stearate Succinate

These ingredients are commonly referred to as diacylglycerols. The term diglycerides historically was also used to describe these diesters.

The glyceryl diesters included in this review are used as skin-conditioning agents - emollients in cosmetic products.

Not all glyceryl diesters are in current use. Of those in current use, safety test data are available on: Glyceryl Diisostearate, Glyceryl Dilaurate, Glyceryl Dioleate, Glyceryl Distearate, and Glyceryl Palmitate Lactate. Glyceryl Stearate Lactate is in current use, but no safety test data are available. For most toxicologic endpoints, the data likely can be extrapolated from one glyceryl diester to another. As will be discussed, this may not be true for tumor promotion potential.

Other related assessments of safety have been done. For example, glycerin and glycerides are classified as generally recognized as safe (GRAS) food ingredients (Informatics., Inc., 1973; Federation Of American Societies For Experimental Biology, 1975).

OTHER COSMETIC INGREDIENT SAFETY ASSESSMENTS

The Cosmetic Ingredient Review (CIR) found Isostearic Acid safe in the present practices of use (Elder, 1983). Oleic, Lauric, Palmitic, Myristic, and Stearic Acids were found safe in the present practices of use and concentration (Elder, 1987). The available data were insufficient to support the safety of Arachidonic Acid (Elder, 1993). Lactic Acid was found safe for use with qualifications (Andersen, 1998). Glyceryl Stearate and Stearate SE were found safe for topical application in the present practices of use and concentration (Elder, 1982).

CIR found the following glyceryl monoesters safe in the present practices of use and concentration, except that the available data were insufficient to support the safety of Glyceryl Arachidonate (CIR, 2001a):

Glyceryl Laurate
Glyceryl Laurate SE,
Glyceryl Laurate/Oleate
Glyceryl Adipate
Glyceryl Alginate
Glyceryl Arachidate
Glyceryl Behenate
Glyceryl Caprate
Glyceryl Caprylate
Glyceryl Caprylate/Caprate
Glyceryl Citrate/Lactate/ Linoleate/Oleate
Glyceryl Cocoate
Glyceryl Collagenate
Glyceryl Erucate
Glyceryl Hydrogenated Rosinate
Glyceryl Hydrogenated Soyate
Glyceryl Hydroxystearate
Glyceryl Isopalmitate
Glyceryl Isostearate
Glyceryl Isostearate/Myristate
Glyceryl Isostearates
Glyceryl Lanolate,
Glyceryl Linoleate,
Glyceryl Linolenate,
Glyceryl Montanate,
Glyceryl Myristate,
Glyceryl Isotridecanoate/ Stearate/Adipate
Glyceryl Oleate SE
Glyceryl Oleate/Elaidate
Glyceryl Palmitate
Glyceryl Palmitate/Stearate
Glyceryl Palmitoleate
Gyceryl Pentadecanoate
Glyceryl Polyacrylate
Glyceryl Rosinate
Glyceryl Sesquioleate
Glyceryl/Sorbitol Oleate/Hydroxystearate
Glyceryl Stearate/Acetate
Glyceryl Stearate/Maleate
Glyceryl Tallowate
Glyceryl Thiopropionate
Glyceryl Undecylenate

Likewise, CIR found the following glyceryl triesters to be safe as used (CIR, 2001b):

Trilaurin
Triarachidin
Tribehenin
Tricaprin
Tricaprylin
Trierucin
Triheptanoin
Triheptylundecanoin
Triisononanoin
Triisopalmitin
Triisostearin
Trilinolein
Trimyristin
Trioctanoin
Triolein
Tripalmitin
Tripalmitolein
Triricinolein
Tristearin
Triundecanoin
Glyceryl Triacetyl Hydroxystearate
Glyceryl Triacetyl Ricinoleate
Glyceryl Stearate Diacetate

CHEMISTRY

CHEMICAL AND PHYSICAL PROPERTIES

Properties of Glyceryl Diesters (Glyceryl Dipalmitate, Glyceryl Distearate, and Glyceryl Diisostearate) that were identified in the published literature are included in Table 1.

Glyceryl Dilaurate

Glyceryl Dilaurate (CAS No. 27638-00-2) is the diester of glycerin and lauric acid that conforms generally to the formula shown in Figure 1 (Pepe et al., 2002).

According to the Scientific & Technical Information Network (STN) International (1997a) and Pepe et al. (2002), other

names for Glyceryl Dilaurate include:

- Dilaurin;
- Dodecanoic Acid, Diester with 1,2,3-Propanetriol
- Didodecanoyl Glyceride;
- Dilauroyl Glyceride;
- Glycerine Dilaurate; and
- Glycerol Dilaurate

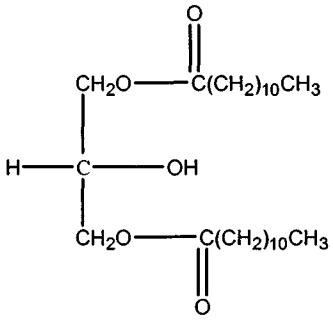

Figure 1. Glyceryl Dilaurate

Glyceryl Diarachidate

Glyceryl Diarachidate (CAS Nos. 89648-24-8 and 127039-55-8) is the diester of glycerin and arachidic acid (q.v.) that generally conforms to the formula shown in Figure 2 (Pepe et al., 2002).

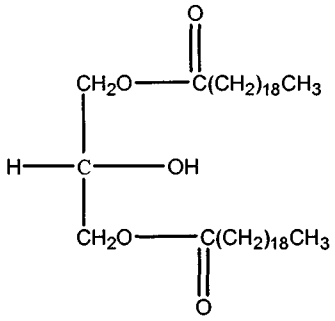

Figure 2. Glyceryl Diarachidate

According to Pepe et al. (2002), other names for Glyceryl Diarachidate include:

1. Eicosanoic Acid, Diester with 1,2,3-Propanetriol;
2. Eicosanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester;
3. Glyceryl Diarachate; and
4. 1-(Hydroxymethyl)-1,2-Ethanediyl Eicosanoate

Glyceryl Dibehenate

Glyceryl Dibehenate (CAS No. 99880-64-5) is the diester of glycerin and behenic acid (q.v.) that generally conforms to the formula shown in Figure 3 (Pepe et al., 2002).

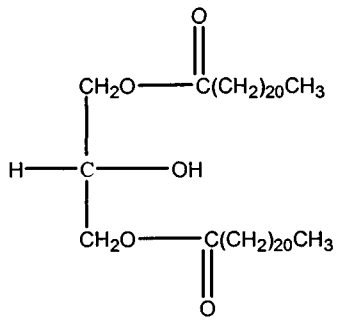

Figure 3. Glyceryl Behenate

Docosanoic Acid, Diester with 1,2,3-Propanetriol is another name for this chemical (Pepe et al., 2002).

According to Pepe et al. (2002), Glyceryl Dibehenate is present in a trade mixture, Compritol 888 ATO, that also contains Tribehenin and Glyceryl Behenate. The International Nomenclature Cosmetic Ingredient (INCI) name for this mixture is Glyceryl Dibehenate/Tribehenin/Glyceryl Behenate. Data on the chemical and physical properties of Compritol 888 ATO are included in Table 2.

The proportions of Glyceryl Dibehenate and Tribehenin in Compritol 888 ATO were not specified; however, the mixture reportedly consists of 1-mono- glycerides (12 to 18%), free glycerol (< 1.0%), water (< 0.5%), and behenic acid (> 80%) (Gattefossé, 1998a,b).

Glyceryl Dierucate

Glyceryl Dierucate is the diester of glycerin and erucic acid (q.v.) that generally conforms to the formula shown in Figure 4 (Pepe et al., 2002).

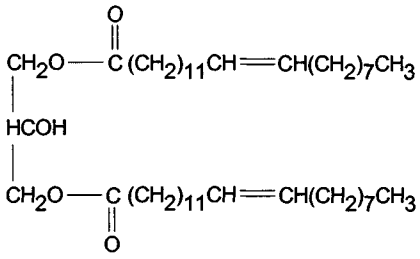

Figure 4. Glyceryl Dierucate

Docosenoic Acid, Diester with 1,2,3-Propanetriol is another name for this chemical (Pepe et al., 2002).

Glyceryl Dihydroxystearate

Glyceryl Dihydroxystearate (CAS No. 122546-20-7) is the diester of glycerin and hydroxystearic acid (q.v.) that generally

Table 1. Properties of Glyceryl Diesters

Property	Ingredient			
	Glyceryl Dioleate (Aarhus Oliefabrik, 1997)	Glyceryl Dipalmitate (Lide and Frederikse, 1993)	Glyceryl Distearate (Lide and Frederikse, 1993)	Glyceryl Diisostearate (Cosmetic, Toiletry, and Fragrance Association [CTFA] 1998)
Form	Beige, waxy solid (at 20°C) with bland odor	Crystals obtained from ethanol and chloroform as solvents	Needles or plates obtained from ether, chloroform, and ligroin as solvents	Pale yellow, oily liquid with faint, characteristic odor
Molecular Weight	---	568.92	625.03	---
Density at 50°C	0.89 - 0.92 g/ml	---	---	---
Solubility	Insoluble in water	Soluble in ether	Soluble in ether	---
Vapor Pressure at 200°C	< 0.01 mm Hg	---	---	---
Melting Point	---	72 - 74°C	79.1°C	---
Slip Melting Point	35 - 39°C	---	---	---
Flash Point	> 200°C	---	---	---
Acid Value	2	---	---	Not more than 10
Saponification Value	172 - 183	---	---	175 to 195
Iodine Value	55 - 66	---	---	---
Hydroxyl Value	140 - 190	---	---	50 to 130
Peroxide Value (meq/kg)	2	---	---	---
Loss on Drying	---	---	---	Not more than 1%
Residue on Ignition	---	---	---	Not more than 0.5%

Table 2. Properties of Compritol 888 ATO (Gattefossé, 1998a,b)

Property	Specification/Typical Values
Form	powder
Color (Gardner scale)	< 5, white-yellow
Odor	faint
Solubility (at 20°C)	soluble in chloroform and methylene chloride when heated;
	insoluble in water, n-hexane, ethanol, and mineral oils
Melting point	69 - 74°C
Drop point	69 - 74°C
Boiling point	> 150°C
Flash Point	> 150°C
Acid Value	< 4.0 mgKOH/g
Iodine Value	< 3g I_2/100 g
Saponification Value	145 to 165 mg KOH/g
Peroxide Value	< 6.0 meq O_2/kg

conforms to the formula shown in Figure 5 (Pepe et al., 2002).

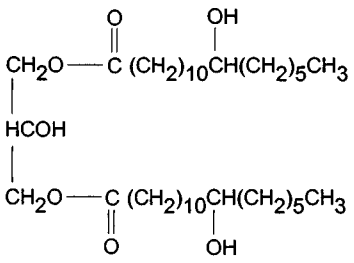

Figure 5. Glyceryl Dihydroxystearate

According to Pepe et al. (2002), other names for this chemical include:

- 12-Hydroxyoctadecanoic Acid, 2-Hydroxy-1,3-Propanediyl;
- 2-Hydroxy-1,3-Propanediyl 12-Hydroxyoctadecanoate;
- Hydroxystearic Acid, Diester with 1,2,3-Propanetriol; and
- Octadecanoic Acid, 12-Hydroxy-,2-Hydroxy-1,3-
- Propanediyl Ester.

Glyceryl Diisopalmitate

Glyceryl Diisopalmitate is the diester of glycerin and a branched chain 16-carbon aliphatic acid that generally conforms to the formula shown in Figure 6 (Pepe et al., 2002).

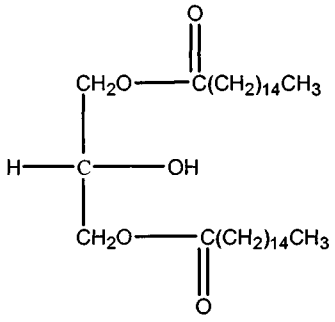

Figure 6. Glyceryl Diisopalmitate

Isohexadecanoic Acid, Diester with 1,2,3-Propanetriol is another name for this chemical (Pepe et al., 2002).

Glyceryl Diisostearate

Glyceryl Diisostearate (CAS No. 68958-48-5) is the diester of glycerin and isostearic acid that generally conforms to the formula shown in Figure 7 (Pepe et al., 2002).

Isooctadecanoic Acid, Diester with 1,2,3-Propanetriol is another name for this chemical (Pepe et al., 2002).

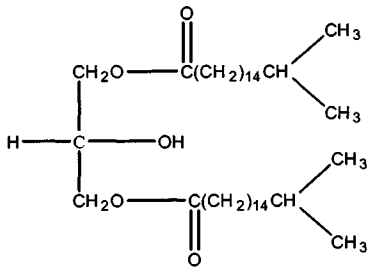

Figure 7. Glyceryl Diisostearate

Glyceryl Dilinoleate

Glyceryl Dilinoleate (CAS No. 30606-27-0) is the diester of glycerin and linoleic acid that generally conforms to the formula shown in Figure 8 (Pepe et al., 2002).

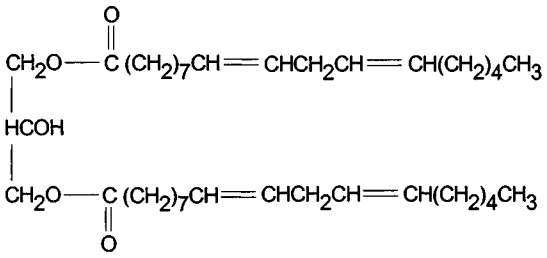

Figure 8. Glyceryl Dilinoleate

9,12-Octadecadienoic Acid, Diester with 1,2,3-Propanetriol is another name for this chemical (Pepe et al., 2002).

Glyceryl Dimyristate

Glyceryl Dimyristate (CAS No. 53563-63-6) is the diester of glycerin and myristic acid that generally conforms to the formula shown in Figure 9 (Pepe et al., 2002).

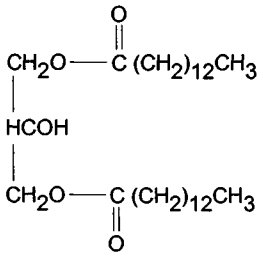

Figure 9. Glyceryl Dimyristate

Tetradecanoic Acid, Diester with 1,2,3-Propanetriol is another name for this chemical (Pepe et al., 2002).

Glyceryl Dioleate

Glyceryl Dioleate (CAS No. 25637-84-7) is the diester of glycerin and oleic acid that generally conforms to the following formula (Pepe et al., 2002):

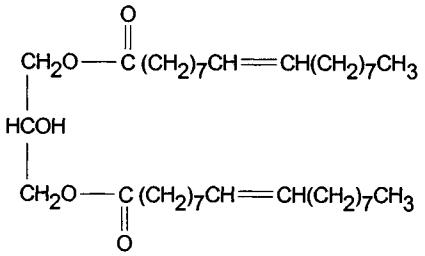

Figure 10. Glyceryl Dioleate

9-Octadecenoic Acid, Diester with 1,2,3-Propanetriol is another name for this chemical (Pepe et al., 2002).

According to a chemical supplier, Glyceryl Dioleate is a mixture consisting of diglycerides (49%), monoglycerides (36%), and triglycerides (15%) (Aarhus Oliefabrik, 1997).

Glyceryl Dipalmitate

Glyceryl Dipalmitate (CAS No. 26657-95-4) is the diester of glycerin and palmitic acid that generally conforms to the formula shown in Figure 11(Pepe et al., 2002).

Hexadecanoic Acid, Diester with 1,2,3-Propanetriol is another name for this chemical (Pepe et al., 2002).

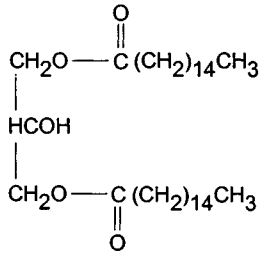

Figure 11. Glyceryl Dipalmitate

Glyceryl Dipalmitoleate

Glyceryl Dipalmitoleate is the diester of glycerin and palmitoleic acid that generally conforms to the formula shown in Figure 12 (Pepe et al., 2002).

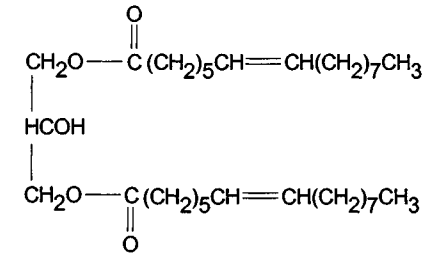

Figure 12. Glyceryl Dipalmitoleate

Palmitoleic Acid, Diester with 1,2,3-Propanetriol is another name for this chemical (Pepe et al., 2002).

Glyceryl Palmitate Lactate

Glyceryl Palmitate Lactate is the lactic acid ester of glyceryl palmitate that conforms to the formula shown in Figure 13 (Pepe et al., 2002).

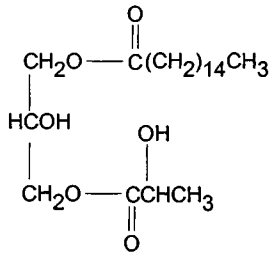

Figure 13. Glyceryl Palmitate Lactate

According to a chemical supplier, Glyceryl Palmitate Lactate is a lactic acid ester of mono-diglyceride made from edible, refined palmetic acid. (Danisco Ingredients, 1999a).

Glyceryl Distearate

Glyceryl Distearate (CAS No. 1323-83-7) is the diester of glycerin and stearic acid that conforms to the formula shown in Figure 14 (Pepe et al., 2002).

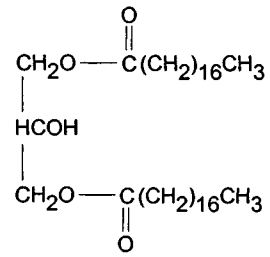

Figure 14. Glyceryl Distearate

Octadecanoic Acid, Diester with 1,2,3-Propanetriol is another name for Glyceryl Distearate (Pepe et al., 2002).

Glyceryl Stearate Citrate

Glyceryl Stearate Citrate (CAS No. 39175-72-9) is the citric acid ester of glyceryl stearate (q.v.) that conforms to the formula shown in Figure 15 (Pepe et al., 2002).

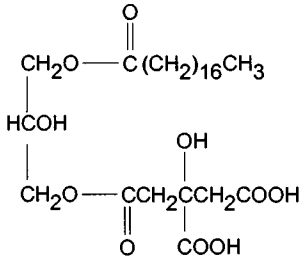

Figure 15. Glyceryl Stearate Citrate

Huls America, Inc. (no date) characterized Glyceryl Stearate Citrate as a partially neutralized ester of monoglycerides and diglycerides of saturated, edible fatty acids with citric acid. It contains 1-mono-glyceride (10-30%) and free glycerol (max 3%).

According to Pepe et al. (2002) two other names for Glyceryl Stearate Citrate are:

- 2-Hydroxy-1,2,3-Propanetricarboxylic Acid, Monoester with 1,2,3-Propanetriol Monooctadecanoate; and
- 1,2,3-Propanetricarboxylic Acid, 2-Hydroxy-,Monoester with 1,2,3-Propanetriol Monooctadecanoate.

Glyceryl Stearate Lactate

Glyceryl Stearate Lactate is the lactic acid ester of glyceryl stearate (q.v.) that conforms to the formula shown in Figure 16 (Pepe et al., 2002).

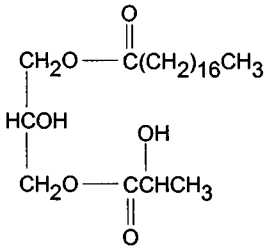

Figure 16. Glyceryl Stearate Lactate

Glyceryl Stearate Succinate

Glyceryl Stearate Succinate is the succinic acid ester of glyceryl stearate (q.v.) (Pepe et al., 2002). Using the same convention as in the previous formulae, likely conforms to that shown in Figure 17.

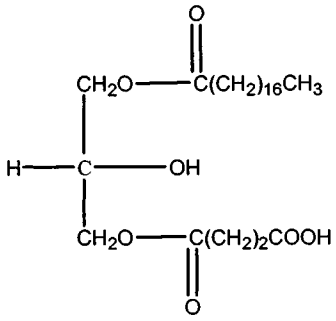

Figure 17. Glyceryl Stearate Succinate

ANALYTICAL METHODS

Glyceryl Dilaurate

Capillary supercritical fluid chromatography has been used in the analysis of Glyceryl Dilaurate (Giron et al., 1992).

METHODS OF PRODUCTION

Methods of production for most glyceryl diesters were not available.

Glyceryl Dioleate

Aarhus Oliefabrik (1997) stated that the raw materials for the production of Glyceryl Dioleate are fully refined non-lauric vegetable oils. Glyceryl Dioleate is said to consist of C16-C18 glycerides manufactured from food grade raw materials. The oils are further processed using special hydrogenation and fractionation techniques. The end products are produced by reacting selected mixtures of the partly hydrogenated, partly fractionated oils and fats with vegetable-derived glycerine to yield partial glycerides. In the final stage of the production process, the products are purified by deodorization.

COMPOSITION

DeGroot (1972) reported that mixtures of 1,2- and 1,3-diglycerides stored at temperatures below their melting points undergo a rather rapid isomerization of practically all of the 1,2-diglycerides to 1,3-isomers. Prior to storage, the mixtures consisted of 47 to 75% 1,3-diglycerides and 25 to 53% 1,2-diglycerides. Storage temperatures and the duration of storage for the individual mixtures ranged from 26 to 65°C and 2 to 210 days, respectively. The resulting mixtures (after storage) contained 90 to 98% 1,3-diglycerides and 2 to 10% 1,2-diglycerides. This solid-phase isomerization was characterized as resulting in a substantial increase in the yield of saturated and unsaturated mono- and mixed-acid 1,3-diglycerides.

According to another source, 1,2- and 1,3-diglycerides are not stable and are easily isomerized under acidic, basic, or thermal conditions to an equilibrium of approximately 40% of the 1,2- and 60% of the 1,3-isomer (Serdarevich, 1967).

Enkelund (1999) provided an interpretation of the above data. He stated that, at high temperature, the equilibrium mixture has a ratio of 1,3/1,2-diglycerides of approximately 75%:25%. At lower temperatures, the equilibrium will, depending on the constituent fatty acids, temperature, and time, favor the 1,3-diester. The 1,2-diester content will be lowered to the range of 2-10%. In practice, this means that during cooling, packaging, and storing of the ingredient, most of the 1,2-diglycerides are isomerized to the 1,3-isomer. The process is favored by the cosmetic producer's handling, as the material will normally be heated to 60 - 75°C when mixing the material with other lipids and then cooled to room temperature.

More recent information was provided by International Specialty Products (ISP). Based on the industrial chemical processes that are used to produce the glyceryl fatty acid esters, some diesters will be present in any monoester material (on the order of 10%) and diesters will also be present in triester material (on the order of 5%). Likewise, monoesters and triesters will be present in any diester material (ISP, 2001)..

An important question about the composition of glyceryl diesters is the position on the glycerine backbone of the fatty acid or other group. ISP (2001) estimated that between 30 and 50 percent of the diester composition will be as 1,2-diester, and, the remainder, 1,3-diester. Aarhus Oliebabrik (2001), however stated that the content of 1,2 isomers in [glyceryl diester] products such as Cremeol FR-36 (now Cegesoft FR 36) and Cremeol FR-57 (now Cegesoft FR 57) is unlikely to be more than a few percent and under no circumstances would it be 20-50%. Neither report indicated the presence of 2,3-diesters.

ISP (2002) performed analytical testing to determine the percentage of 1,2 diester fraction (1,2-dilauryl-s,n-glycerol) in Emulsynt GDL, glycerol laurate, material on an as sold basis. They estimated that 10-15% 1,2 diester (characterized as optically and physiologically active isomer) is present in the product as sold, with 10-15% 2,3 diester (characterized as optically active, physiologically non-active isomer) and 70-80% 1,3 diester (characterized as not optically or physiologically active) comprising the remainder of the product.

Glyceryl Palmitate Lactate

According to a chemical supplier, the composition of Glyceryl Palmitate Lactate has been defined as follows: free glycerol (max. 1%); lactic acid (15%); and palmitic acid content, of total fatty acids (> 90%) (Danisco Ingredients, 1996). In a more recent description, the specifications for Glyceryl Palmitate Lactate were: palmitic acid [min. 90%], lactic acid content [13-16%), iodine value [max. 1.5], saponification value [245-265], acid value [max. 4], free glycerol [max. 1%], dropping point [≈ 50°C], and form [pellets] (Danisco Ingredients, 1999a). This source also stated that Glyceryl Palmitate Lactate contains 0.03% glycerol and 0.82% free fatty acid, and that the ratio of 1,2-(mono)glycerol diester to total (mono)glycerol diester is 30.6 (Danisco Ingredients, 1999 a, b).

Glyceryl Stearate Lactate

Glyceryl Stearate Lactate contains glycerol (0.24%) and free fatty acid (0.16%), and the ratio of 1,2-(mono)glycerol diester to total (mono)glycerol diester is 33.9 (Danisco Ingredients, 1999 a, b).

IMPURITIES

Glyceryl Dioleate

The final stage in the processing of oil (deodorization - mentioned in preceding paragraph) effectively removes pesticide residues and lower boiling residues such as residues of halogenated solvents and aromatic solvents. Thus, reportedly, the following impurities are not present at concentrations above the detection limits of the methodology used in any of the deodorized oils and fats produced by Aarhus Oliefabrik: organochlorine pesticides; halogenated solvents and aromatic solvents; PCBs, organic solvents (e.g., ethanol and acetone), metals (heavy metals included), aflatoxin, ash, radioactive material, and microbial activities (Aarhus Oliefabrik, 1998).

Glyceryl Dibehenate

Sulfated ashes (< 0.10%) and heavy metals (< 10 ppm) are impurities in a trade mixture (Compritol 888 ATO) containing Glyceryl Dibehenate (Gattefossé 1998 a,b).

Glyceryl Diisostearate

Glyceryl Diisostearate contains not more than 20 ppm heavy metals and not more than 2 ppm arsenic (CTFA, 1998).

Glyceryl Palmitate Lactate

Specifications for heavy metal impurities in Glyceryl Palmitate Lactate are as follows: arsenic (As) [max. 3 mg/kg], lead (Pb) [max. 5 mg/kg], mercury (Hg) [max. 1 mg/kg], cadmium (Cd) [max. 1 mg/kg], and heavy metals (as Pb) [max. 10 mg/kg] (Danisco Ingredients, 1999a). Glyceryl Palmitate Lactate has been described as containing heavy metals (as Pb) at < 10 mg/kg (Danisco Ingredients, 1996).

Glyceryl Stearate Citrate

Reportedly, Glyceryl Stearate Citrate is free of polyethylene glycol and its monomers (Huls America, Inc., no date).

STABILITY/REACTIVITY

Glyceryl Dibehenate

A trade mixture (Compritol 888 ATO) containing an unspecified concentration of Glyceryl Dibehenate reacts with strong acids and oxidizing agents (dangerous reactions) (Gattefossé, 1998b).

Glyceryl Dioleate

Glyceryl Dioleate is not explosive and autoflammability is not possible. Prooxidants and oxidation promoting conditions should be avoided. Glyceryl Dioleate is not known to have any hazardous decomposition products. Water and carbon dioxide are its thermal decomposition products (Aarhus Oliefabrik, 1997).

USE

COSMETIC

The glyceryl diesters included in this review function as skin conditioning agents - emollients in cosmetic products (Pepe et al., 2002).

Frequency of use data provided by the Food and Drug Administration (FDA) in 2002 indicate that only three of the 17 ingredients in this safety assessment are being used in cosmetics, Glyceryl Dilaurate, Glyceryl Diisostearate, and Glyceryl Dioleate (FDA, 2002). The reported uses as a function of product type are given in Table 3. Where uses in a product were reported to FDA, column one also gives the total number of such product types; e.g. Glyceryl Dilaurate is used in 1 of 324 foundations.

As shown in Table 3, concentration of use data provided by CTFA in 1999 confirm that Glyceryl Dilaurate, Glyceryl Diisostearate, and Glyceryl Dioleate are in current use, but also indicate that Glyceryl Distearate and Glyceryl Stearate Lactate are currently used in cosmetics (CTFA, 1999a).

Cosmetic products containing Glyceryl Dilaurate, Glyceryl Diisostearate, and Glyceryl Dioleate are applied to most areas of the body, and could come in contact with the ocular and nasal mucosae. These products could be used on a daily basis, and could be applied frequently over a period of several years.

Of the 17 ingredients reviewed in this report, none are included among the substances listed as prohibited from use in cosmetic products marketed in the European Union (European Economic Community, 2001).

According to the Ministry or Health, Labor and Welfare (MHLW), Glyceryl Diesters reviewed in this report are not included on the list of ingredients that must not be combined in cosmetic products or on the list of restricted ingredients for cosmetic products that are marketed in Japan (MHLW, 2001a,b).

NONCOSMETIC

The safety of mono- and diglycerides in food has been reviewed by the Food Protection Committee of the National Academy of Sciences - National Research Council Food and Nutrition Board (National Academy of Sciences, 1960). The Food Protection Committee concluded that there appears to be no reason to question the safety of mono-, di-, or triglycerides of lauric acid (i.e., Glyceryl Laurate, Glyceryl Dilaurate, or Glyceryl Trilaurate [Trilaurin]) as food additives. This conclusion was based on the following: (1) Lauric acid glycerides are found in important foods, such as human and cow's milk, at concentrations of 3-6% and in large quantities in coconut oil, without recognized toxic effects; (2) Lauric acid glycerides undergo the usual metabolic changes of the higher fatty acids; (3) When lauric acid glycerides are fed in diets containing a variety of glycerides, there is no evidence of a specific toxic or harmful effect. Soni et al. (2001) reported that diacylglycerol oil has been approved for use in cooking oil in Japan.

Glyceryl Diesters have been approved for use as components of adhesives, paper and paperboard, and other materials that come in contact with food (i.e., indirect food additive uses): (21 CFR 175.105; 176.210, and 177.2800).

Glyceryl Dilaurate has been detected in pharmaceutical excipients (Giron et al., 1992).

BIOLOGICAL ACTIVITY

METABOLISM

Glyceryl diesters (diglycerides) are intermediates in the metabolism of triglycerides to free fatty acids and glycerol. Triglyceride digestion begins in the intestinal tract. Initially, the triglyceride is hydrolyzed enzymatically to α,β-diglyceride, which is then hydrolyzed to β-monoglyceride in the lumen of the intestines. These hydrolytic reactions occur at an oil-water interface. Approximately 28% of the β-monoglyceride is isomerized to α-monoglyceride, and approximately 75% of the α-monoglyceride is further hydrolyzed to free glycerol. Free glycerol enters the intestinal wall independent of the lipids, and there is no further use of it in terms of lipid absorption. The free fatty acids and glycerol are available for the resynthesis of

Table 3. Product Formulation Data on Glyceryl Diesters.

Product Category (Number of Formulations Reported to FDA, 2002)	Number of Formulations Containing Ingredient (FDA, 2002)	Current Concentration of Use (CTFA, 1999a)
	Glyceryl Dilaurate	
Eye Shadow	-	2%
Foundations (324)	1	-
Lipstick (962)	1	-
Makeup Bases (141)	3	-
Other Makeup Preparations (201)	1	-
Bath Soaps and Detergents (421)	3	0.02%
Cleansing (775)	3	-
Body and Hand [Exc. Shave] (840)	13	2-4%
Moisturizing (905)	5	-
Night (200)	2	5%
Paste Masks [Mud Packs] (271)	2	5%
Suntan Gels, Creams, and Liquids (131)	1	-
2002 Total Uses/Concentration Range for Glyceryl Dilaurate	**35**	**0.02 - 5%**
	Glyceryl Diisostearate	
Perfumes (235)	1	18%
Lipstick (962)	93	43%
Nail Polish and Enamel (123)	1	
Body and Hand [Exc. Shave] (840)	1	-
Moisturizing (905)	1	-
2002 Total Uses/Concentration Range for Glyceryl Diisostearate	**97**	**18 - 43%**
	Glyceryl Dioleate	
Foundations	-	2%
Face and Neck [Exc. Shave]	-	0.8%
Moisturizing	-	2%
Other Skin Care Preps (725)	1	-
2002 Total Uses/Concentration Range for Glyceryl Dioleate	**1**	**0.8 - 2%**
	Glyceryl Distearate	
Eye Lotion	-	0.02-0.5%
Mascara	-	0.003%
Shampoos [Noncoloring]	-	6%
Foundations	-	7%
Lipstick	-	7%
Cuticle softeners	-	0.02%
Nail Creams and Lotions	-	0.02%
Cleansing	-	0.0003%
Face and Neck [Exc. Shave]	-	0.2-0.5%

Table 3 (continued). Product Formulation Data on Glyceryl Diesters.

Product Category (Number of Formulations Reported to FDA, 2002)	Number of Formulations Containing Ingredient (FDA, 2002)	Current Concentration of Use (CTFA, 1999a)
Glyceryl Distearate (continued)		
Body and Hand [Exc. Shave] Preparations	-	7%
Moisturizing	-	0.2%-7%
Skin Fresheners	-	0.00003%
2002 Total Uses/Concentration Range for Glyceryl Distearate	-	**0.00003 - 7%**
Glyceryl Stearate Lactate		
Deodorants [underarm]	-	5%
Face and Neck [Exc. Shave]	-	5%
Moisturizing	-	5%
2002 Total Uses/Concentration Range for Glyceryl Stearate Lactate	-	**5%**

triglycerides. β-monoglycerides are not hydrolyzed because of their transfer to a water-soluble phase and, also, because of enzyme specificity. However, they can be acylated directly to triglyceride (Mattson and Volpenhein, 1964).

PERCUTANEOUS ABSORPTION

Information on the percutaneous absorption of Glyceryl Diesters included in this review was not found in the published literature. However, C log P (octanol/water partition coefficient) values that were calculated for Glyceryl Dilaurate (10.4) and Glyceryl Distearate (16.7) indicate that these two Glyceryl Diesters would not be well- absorbed (FDA, 2000).

SKIN PENETRATION ENHANCEMENT

Glyceryl Dilaurate

Aungst et al. (1986) evaluated the effect of Glyceryl Dilaurate or Glyceryl Laurate on the penetration of Naloxone-HCl across cadaver skin using Franz diffusion cells. Naloxone is a potent opioid antagonist used for the reversal of narcosis. Naloxone concentrations in the reservoir were determined by HPLC using UV detection. Glyceryl Dilaurate was evaluated at a concentration of 10% in propylene glycol. The average flux through human cadaver skin (10 experiments) for Naloxone alone was 1.6 ± 0.4 $\mu g/cm^2 \cdot h^{-1}$. In the presence of Glyceryl Dilaurate, average Naloxone flux increased to 18.7 ± 1.8 $\mu g/cm^2 \cdot h^{-1}$ (3 experiments). In the presence of urea (10% in propylene glycol), the average Naloxone flux was 0.4 ± 0.1 $\mu g/cm^2 \cdot h^{-1}$ (3 experiments).

EFFECT ON ENZYME ACTIVITY

Because of the established link between prostate cancer and high dietary fat intake, Niederpruem et al. (1995) evaluated the effect of Glyceryl Dilaurate, various fatty acids, and their derivatives on 5α-reductase activity *in vitro*. Prostate gland tissue specimens (human) were used. 5α-reductase catalyzes the reduction of testosterone to dihydrotestosterone, which controls cellular division in the prostate gland. The authors reported that it has been suggested that the modulation/inhibition of this enzyme could prevent carcinogenesis in the prostate gland. Results indicated that the inhibitory effect of lauric acid on 5α-reductase activity was totally lost by esterification to Glyceryl Dilaurate.

EFFECT ON SIGNAL TRANSDUCTION

According to Lee and Severson (1994), the generation of intracellular second messengers is a common mechanism of signal transduction for external stimuli such as hormones, neurotransmitters, growth factors, and drugs (agonists) that interact with plasma membrane receptors. The established role of phospholipid turnover in signal transduction mechanisms is based on the observations that agonist-induced hydrolysis of a minor phospholipid in the plasma membrane, phosphatidylinositol 4,5-bisphosphate (PIP_2), in a reaction catalyzed by a phosphoinositide-specific phospholipase C (PI-PLC) enzyme generated the following two intracellular second

messengers: (1) inositol 1,4,5-triphosphate (IP_3), responsible for the mobilization of Ca^{2+} from intracellular stores and (2) diacylglycerol, responsible for the activation of protein kinase C (PKC).

As stated by Lévy et al. (1994) these isoenzymes transduce mitotic signals induced by growth factors. PKC α, as well as other isoenzymes in this family, are activated by Ca^{2+}, phosphatidylserine, and diacylglycerols. Diacylglycerols are considered to be hydrophobic anchors that may recruit PKC to the membrane, leading to an increase in the enzyme's membrane affinity and to the activation of PKC.

Sánchez-Piñera et al. (1999) studied the lipid activation of PKC α by comparing the activation capacity of different 1,2-diacylglycerols and 1,3-diacylglycerols incorporated into mixed micelles or vesicles. The authors noted that PKC isoenzymes are a large family of serine/threonine kinases that are involved in cellular signaling. PKC consists of a family of at least ten isozymes that phosphorylate serine and threonine residues. Classical PKC isozymes (α, β_I, β_{II}, γ) are dependent on Ca^{2+} and phospholipids and are activated by diacylglycerol.

Study results indicated that unsaturated 1,2-diacylglycerols were more potent activators of protein kinase C α than saturated 1,2-diacylglycerols when 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS)/Triton X-100 mixed micelles and pure POPS vesicles were used. These differences were not observed when 1-palmitoyl -2-oleoyl-sn-glycero-3-phosphocholine (POPC)/POPS (4:1 molar ratio) vesicles were used. Additionally, 1,2-diacylglycerols had a considerably greater activating capacity than 1,3-diacylglycerols in POPS Triton X-100 mixed micelles and in POPC/POPS vesicles. However, the difference between 1,2- and 1,3-diacylglycerols was smaller when pure POPS vesicles were used. That is, both were able to activate PKC α practically to the same extent. Nevertheless, saturated diacylglycerols induced significant activation of PKC α in Triton X-100 micelles and in pure POPS vesicles in this study (Sánchez-Piñera et al., 1999).

Unlike the preceding study, a very low capacity for 1,3-diacylglycerol-induced activation was demonstrated in an earlier study in which sonicated phosphatidylserine vesicles were used (Nomura et al., 1986). Unsonicated preparations of multilamellar vesicles were used in the Sánchez-Piñera et al. (1999) study.

PKC, CELL GROWTH, AND PROLIFERATION

Studies (Fisher et al., 1993; Reynolds et al., 1993) have shown that diacylglycerols, intracellular ligands for PKC, were elevated in psoriatic lesions, and that this was accompanied by alterations in PKC isoform levels and activity.

As reported by Mischak et al. (1993), studies with NIH 3T3 cells indicate that different PKC isozymes fulfill different functions in the cell. The 3T3 cells were transfected with vectors containing the individual PKC isozymes delta and epsilon, and cell lines that overexpress the isozymes were obtained. The overexpression of PKC delta induced morphological alteration, inhibited cell growth, and decreased cell density at confluency. PKC epsilon expression did not affect cell morphology; however, it did cause enhanced cell growth and increased cell density at confluency. Blumberg et al. (1994) stated that these findings in 3T3 cells demonstrate that PKC may function as an oncogene.

According to Huckle and Earp (1994), Angiotensin II has trophic or mitogenic (increases rate of cell division) effects on a variety of target tissues, including adrenal cortical cells and vascular smooth muscle cells. As reported by Lee and Severson (1994), activation of the type 1 Angiotensin II receptor rapidly increases intracellular concentrations of inositol phosphates, notably inositol 1,4,5-triphosphate (IP_3), and 1,2-diacylglycerol (DAG) in adrenal cortical cells, hepatocytes, and vascular smooth muscle. The type 1 Angiotensin II receptor is responsible for all known physiologic actions of Angiotensin II. Angiotensin II, an octapeptide, is well known as an acute regulator of vasomotor tone and fluid homeostasis. Angiotensin II has many characteristics of the 'classical' peptide growth factors such as EGF/TGFα, PDGF, and IGF-1. Characteristics of these growth factors include the regulation of growth in a self-contained autocrine/paracrine fashion, and the ability to stimulate tyrosine phosphorylation, to activate MAPKs (mitogen activated protein kinases), and to increase expression of nuclear proto-oncogenes. In vascular smooth muscle, hydrolysis via the lipase pathway is the predominant metabolic fate of diacylglycerol. The activation of PKC in vascular smooth muscle modulates agonist-stimulated phospholipid turnover, produces an increase in contractile force, and regulates cell growth and proliferation.

As reported by Blobe et al. (1994), studies have indicated that the sustained activation or inhibition of PKC (diacylglycerol-activated isoenzyme) activity *in vivo* may play a critical role in the regulation of long-term cellular events such as proliferation, differentiation, and tumorigenesis. Many of the signals transduced by PKC are mitogenic and the result of growth factors (e.g., platelet-derived growth factor [PDGF] and epidermal growth factor [EGF]). For example, PDGF binds to its high affinity receptor (PDGFR) and activates this receptor's intrinsic tyrosine kinase to mediate the initiation of DNA synthesis and other cellular effects. It is important to note that PKC has been linked directly to the pathogenesis of several human cancers, including skin, colon, and breast cancer. In the epithelium, long-term changes in PKC activity, either through the action of phorbol esters or by specific changes in PKC isoenzyme activities, either leads to growth of melanocytes, the differentiation of keratinocytes, or to cellular transformation.

In colon cancer, PKC acts as a tumor suppressor. Thus, decreasing the PKC activity can result in cellular transformation. In breast cancer, an increase in PKC activity appears to correlate with enhanced oncogenicity.

Lévy et al. (1994) reported increased PKC activity in hyperplastic pituitary cells has also been reported. An increase in diacylglycerol content parallels the increase in PKC activity.

Wang and Smart (1999) stated that the overexpression of PKCα in the epidermis of transgenic mice increases the expression of specific proinflammatory mediators and induces cutaneous inflammation, but has little to no effect on epidermal differentiation, proliferation, or 12-O-tetradecanoylphorbol-13-acetate (TPA) tumor promotion.

DIACYLGLYCEROLS AND PKC

In a study reported by Hansen et al. (1990) involving female CD-1 mice, PKC was linked to epidermal hyperplasia and tumor promotion. In this study, a tumor-promoting dosing regimen consisting of multiple applications of 5 or 10 µmol *sn*-1,2-didecanoylglycerol, a model *sn*-1,2-diacylglycerol and complete tumor promoter, twice daily for one week caused more than a 60% decrease in cytosolic and particulate PKC activity and marked epidermal hyperplasia.

However, two applications of 10 µmol *sn*-1,2-didecanoylglycerol for one week, a nonpromoting dosing rate, caused a decrease in cytosolic PKC activity, increased particulate PKC activity, but did not induce hyperplasia. These data, together with other study results, demonstrated to the authors that the down-regulation of epidermal PKC is associated with and may be a permissive event for epidermal hyperplasia and tumor promotion (Hansen et al., 1990).

COMPETITIVE BINDING TO PKC BY DIACYLGLCEROL ANALOGUES

Sharkey et al. (1984) reported that the phorbol ester receptor in brain may be the same as the Ca^{2+} - phospholipid-dependent kinase (PKC). Assays were performed using the mouse brain cytosolic phorbol ester aporeceptor, which requires phospholipids for activity. In the presence of phosphatidylserine, 1,2-diolein inhibited the specific binding of [^{3}H]phorbol 12,13-dibutyrate ([^{3}H]PBt_2) in a dose-dependent fashion. Other short-chain saturated diacylglycerol derivatives, 1,3-dicaprylin and 1,3-dicaproin, also inhibited ([^{3}H]PBt_2) binding. However, the long-chain saturated derivatives 1,2-dipalmitin and 1,2-distearin were much less active.

König et al. (1985) also reported that diacylglycerol stimulates protein kinase C in a manner that is similar to that of the phorbol esters and inhibits phorbol ester binding.

Sharkey and Blumberg (1985), stated that the diacylglycerol, 1,2-diolein inhibits binding of the phorbol ester, [20-^{3}H]phorbol 12,13-dibutyrate to PKC. These authors also reported the inhibition of phorbol ester binding to reconstituted PKC by the diglyceride, glycerol 1-myristate 2-acetate (Sharkey and Blumberg, 1986).

Wender et al. (1986) performed computer modeling of the diterpene diester, phorbol 12-myristate 13-acetate and other structural classes of tumor promoters. The authors stated that the results indicated a marked similarity in the relative positions of certain heteroatoms and hydrophobic groups. For phorbol esters, this mapping consists of the C-4, C-9, and C-20 hydroxyl groups and a hydrophobic region that is filled by a long-chain acyl functionality attached to the C-12 or the C-13 positions. Diacylglycerols, thought to be the endogenous activators of the major phorbol ester receptor PKC, fit this model in a stereo-specific fashion.

Lee et al. (1996a, b) and Sharma et al. (1996) also stated that PKC interaction with diacylglycerol analogues is stereospecific.

Caloca et al. (1999) reported that the binding of diacylglycerol and related analogs occurs at C1 domains (also called cysteine-rich regions or zinc fingers) that are present in PKC isozymes.

According to Lee et al. (1993) and Bögi et al. (1998), phorbol esters, potent tumor promoters, function as ultrapotent analogues of 1,2-diacylglycerol.

DIACYLGLYCEROLS AND UV LIGHT

Punnonen and Yuspa (1992) reported that the irradiation of cultured murine keratinocytes with UVB light (270 -380 nm; 20 to 120 J/m^2) induced a dose-dependent increase in cellular concentrations of diacylglycerols. The turnover of other phospholipids (e.g., phosphatidylcholine and phosphatidylethanolamine) was unaffected. These results were reported at 24 h post-irradiation. The UV light-induced increase in cellular diacylglycerols (specific chemical names not stated) was accompanied by changes in diacylglycerol kinase (DAG-kinase). Cytosolic DAG-kinase activity and the activity of DAG-kinase in the membrane fraction were decreased and increased, respectively. The authors suggested that irradiation with UVB light increases diacylglycerol concentrations via changes in de novo metabolism that involve a DAG-kinase pathway. Furthermore, it was suggested that elevated diacylglycerol may influence signal-transduction pathways that are mediated by cellular lipids and contributes to keratinocyte responses to UV light.

Agin et al. (1991) studied the effect of topically applied diacylglycerols, 1,2-dioctanoyl-sn-glycerol (DOG) and 1-oleyl-2-acetyl-sn-glycerol (OAG), on melanogenesis using female

Skh HR-2 pigmented, hairless mice (ages = 4 to 6 weeks). Each diacylglycerol was dissolved in acetone and applied (120 µl doses, using micropipette) to the total exposed area of dorsal skin, approximately 25 cm^2. Additionally, some of the groups were then irradiated with a 0.8 minimum erythemal dose of UVA/UVB light. The light source was a Kodacel 401-filtered FS-20 lamp bank that delivered 134 mJ/cm^2 of UVB light (290-320 nm) and 146 mJ/cm^2 of UVA light (320-400 nm). Four different test protocols (experiments) incorporating this application procedure and study results were conducted. At the end of each treatment, dorsal skin was removed and stained for melanin and L-dopa. The epidermis was then separated and prepared for microscopic examination.

In the first experiment, four groups of 10 mice were treated with 0.18, 0.88, 1.75, and 3.5 µmol DOG in acetone, respectively, on three consecutive days per week (Mondays, Wednesdays, and Fridays) for three weeks. A fifth group was treated with acetone (vehicle) only. A marked, dose-dependent increase in dopa-positive areas in the whole epidermis was noted, having reached a maximum at a dose of 1.75 µmol. There were no visual signs of skin irritation at either of the doses administered. Few highly stained dopa-positive melanocytes were observed in untreated control epidermal tissue. The analysis of sectioned skin for melanin deposition revealed that treatment with DOG induced negligible enhancement of melanin deposition.

In the second experiment, four groups of 10 mice were treated with either vehicle alone or 1.75 µmol DOG in acetone twice weekly (Tuesday and Thursday). Additionally, the animals either were or were not irradiated three times weekly (Mondays, Wednesdays, and Fridays) for three weeks. Treatment with DOG alone did not significantly affect the dopa-positive area and melanin deposition. UV light alone induced a 5.4-fold increase in the area that was dopa-positive. However, compared to controls, when DOG treatment was combined with UV exposure, the dopa-positive area increased ten-fold. The sum of DOG treatment alone + UV light treatment alone amounted to an increase in the dopa-positive area that was less than that observed following the combined treatment, suggesting a synergistic effect. For melanin deposition, a 7.2-fold increase over that observed in the presence of UV light alone was reported. Hematoxylin and eosin and Mowry's staining of skin sections treated with DOG or DOG + UV light revealed no skin irritation or mucopolysaccharide deposition that was in excess of that anticipated from exposure to UV light alone. Additionally, DOG alone did not induce hyperplasia of the stratum corneum or epidermis.

In the third experiment, diacylglycerol or vehicle was applied to groups of ten mice on five consecutive days (Monday through Friday). Beginning on the following Monday, three of the groups were irradiated on Mondays, Wednesdays, and Fridays for two weeks. A fourth group of animals (no irradiation) served as diacylglycerol-treated controls. Mice in a fifth group (no irradiation) were euthanized two days after the last application of OAG. The topical application of OAG for one week, followed by a two-week non-treatment period without UV irradiation, resulted in a six-fold increase in melanin deposition. The six-fold increase refers to increased melanin deposition over that noted after one week of OAG treatment only. Furthermore, the extent of melanin deposition was 50% of that noted after two weeks of UV irradiation alone:

When one week of OAG treatment was followed by two weeks of UV irradiation, a 37-fold increase in melanin deposition (compared to results for OAG only) with no lag time was observed. This increase in melanin deposition was 3-fold higher when compared to the results for UV irradiation alone, indicating that OAG pretreatment acts synergistically with UV light exposure. It was determined that this synergistic effect with UV light was more pronounced when melanization was used as the endpoint.

In the fourth experiment, five groups of ten mice were treated as follows over a period of five consecutive days (Monday through Friday): Group 1 (vehicle), Group 2 (0.18 µmol OAG), Group 3 (0.58 µmol OAG), Group 4 (1.75 µmol OAG) and Group 5 (1.75 µmol DOG). All groups were irradiated on Mondays, Wednesdays, and Fridays for two weeks. The effect of OAG in combination with UV irradiation was comparable to that of DOG + UV irradiation. However, it is important to note that OAG treatment increased the dopa-positive area and melanin deposition more effectively than did DOG. Specifically, 0.58 µmol of OAG induced the same effect as 1.75 µmol of DOG.

The authors reported that the results of the preceding study suggest that a protein kinase C-dependent process is involved in melanogenesis, because diacylglycerols are known to activate protein kinase C (Agin et al., 1991).

Allan et al. (1995) studied the effects of 1-oleyl-2-acetyl-sn-glycerol (OAG) and its analogues [OAG (sn) and OAG (racemic)], 1,2 dioctanoyl-sn-glycerol (diC_8), and 1,2-dipalmitoyl-sn-glycerol (diC_{16}) on melanin production in vivo using four groups of outbred, pigmented guinea pigs (12 to 20 weeks old; 4 to 6/group). Test substances were applied to skin (1 cm^2) that had been shaved and treated with a depilatory. Sites were irradiated using two Sylvania FS40UVB bulbs. The irradiance (2.6×10^{-4} W/cm^2) was measured through a quartz filter using an IL700A research radiometer fitted with a UVB probe (filter UVB, 290 ± 5 nm). The animals were exposed daily for ten days to 70 mJ/cm^2 for 3 minutes (12 seconds equals 0.6 minimal erythemal dose).

Animals in Group 1 were treated with the vehicle (propylene glycol) alone and four separate concentrations of OAG (20, 30,

40, and 60 mg/ml) once daily for five days. Group 2 animals were treated with vehicle only and five separate concentrations of OAG (10, 20, 30, 40, and 60 mg/ml) either once or twice daily for five days. Animals in Group 3 were treated with 50 mg/ml OAG(sn)OAG(rac), diC_8, diC_{16}, and vehicle alone twice daily for five days. In Group 4, animals were treated with OAG (25 mg/ml) three times daily at one site and with the estimated 0.6 minimal erythemal dose daily for ten days at a second site. Sites were evaluated weekly according to the following grading scale: 0 (no change from baseline color) to +4 (profound even darkening). Punch biopsies were obtained from test and control application sites and specimens examined microscopically. For statistical analysis of the data, analysis of variance (ANOVA) was performed.

In Group 1, erythema and scale were observed in most, but not all, sites treated with OAG. However, pigmentation developed at all test sites, and was classified as dose-dependent ($p < 0.001$). The following results are based on a computer-image analysis that was performed on sections obtained from untreated, vehicle-treated, and sites treated with 60 mg/ml OAG. Compared to untreated or vehicle control skin sites, a significant increase (280% increase; $p < 0.00001$) in melanin content was reported for OAG-treated skin sites. The difference in melanin content between untreated and vehicle-treated skin was not significant.

Cutaneous reactions in Group 2 were similar to those observed in Group 1, with the exception that the 10 mg/ml dose of OAG induced less inflammation. Mild erythema was observed in one of the four animals dosed with 10 mg/ml OAG. Additionally, the degree of pigmentation induced by OAG in Group 2 was consistent with the dose-response pattern ($p < 0.02$) that was noted in Group 1. The following results are based on a computer-image analysis of sections from biopsies obtained from each test site in a representative animal. A linear relationship between the concentration of OAG applied and the percent epidermal area covered by melanin was noted. These results indicated a dose-dependent increase in pigmentation ($p < 0.00001$). Additionally, compared to the vehicle control, 60 mg/ml OAG induced a 250% increase in epidermal melanin content.

In Group 3, mild erythema was observed at some of the sites treated with OAG. Erythema was not observed at vehicle control sites or sites treated with diC_{16}. On days 19 to 24, peak pigmentation (scores of +1 to +2) was noted in all five animals at sites treated with OAG(sn), OAG (racemic), and diC_8 (Allan et al., 1995). The increase in skin pigmentation at these sites was statistically significant ($p < 0.005$), compared to the complete lack of increased pigmentation at sites treated with vehicle or diC_{16}. The following results are based on a computer-image analysis of sections from biopsies. Compared to the control, OAG(sn), OAG(rac), and diC_8 induced a significant increase in pigmentation ($p < 0.0005$). DiC_{16} did not induce a significant change in pigmentation.

In group 4, even pigmentation (scores of +2 to +3) was observed in all six animals at sites treated with OAG as well as those treated with UVB, but not at vehicle-treated sites. The results of an image analysis of biopsy cross sections from a representative animal indicated a 367% increase in melanin content at the OAG-treated site (compared to vehicle control) and a 397% increase in melanin content at the UVB- irradiated site (compared to untreated control). These results indicated that simulation of melanin production by OAG treatment and UVB irradiation was comparable.

The authors stated that results indicate that topically applied diacylglycerols can induce an increase in epidermal pigmentation, presumably via protein kinase C activation. The increase in epidermal pigmentation in this study was long-lasting, and, both clinically and histologically, was comparable to ultraviolet-induced tanning. The effects of OAG treatment on skin histology were also evaluated in this study. Except for the presence of acanthosis, hematoxylin and eosin-stained sections of OAG-treated skin at 19 to 25 days after the final application were similar to vehicle or untreated control sections. (Allan et al., 1995).

ANTIMICROBIAL ACTIVITY

Glyceryl Dilaurate

No antimicrobial activity was observed in a study in which various bacterial strains (*Streptococcus pyogenes* included) were incubated with Glyceryl Dilaurate (Conley and Kabara, 1973).

Kabara et al. (1977) observed that Glyceryl Dilaurate had no effect on the antimicrobial activity of Glyceryl Laurate in the bacterial strain *Streptococcus pyogenes*. In this assay, bacterial cultures were treated with 5 µg/ml Glyceryl Laurate + 5 µg/ml Glyceryl Dilaurate, 10 µg/ml Glyceryl Laurate + 10 µg/ml Glyceryl Dilaurate, 5 µg/ml Glyceryl Laurate, and 10 µg/ml Glyceryl Dilaurate, respectively. Bacterial growth was monitored by changes in the optical density of the medium at 550 nm.

ANIMAL TOXICOLOGY

ACUTE ORAL TOXICITY

Glyceryl Dilaurate

MB Research Laboratories, Inc. (1991a) evaluated the acute oral toxicity of Glyceryl Dilaurate using ten male Wistar Albino rats (weights = 211-279 g). The test substance was melted in a water bath and administered orally (single dose of 5 g/kg) to each animal using a syringe and dosing needle. The animals were observed at 3 to 4 h post-dosing and then daily for 14 days. Necropsy was not performed. None of the animals died, and all were in good health throughout the study.

It was concluded that the LD50 was greater than 5.0 g/kg.

Glyceryl Dibehenate

The Institut Français de Recherches et Essais Biologiques (1980) evaluated a trade mixture (Compritol 888 ATO) containing an unspecified concentration of Glyceryl Dibehenate in an acute oral toxicity study using five female and five male OFA SPF rats (Sprague-Dawley originated; body weights = 163 g). The mixture was diluted with hydroxycellulose gel to a concentration of 25%, and then administered to each animal by esophageal intubation. Each animal received a single dose of 5 g/kg (dose volume = 20 ml/kg). Dosing was followed by a 14-day observation period. No deaths were reported.

Glyceryl Stearate Citrate

An acute oral LD50 of > 2 g/kg has been reported for Glyceryl Stearate Citrate. Neither the species of animals tested nor details concerning the test protocol were included (Huls America, Inc., no date).

ACUTE DERMAL TOXICITY

Glyceryl Stearate Citrate

An acute dermal LD50 of > 2 g/kg has been reported for Glyceryl Stearate Citrate. Neither the species of animals tested nor details concerning the test protocol were included (Huls America, Inc., no date).

CHRONIC ORAL TOXICITY

Glyceryl Dilaurate

In a study by Fitzhugh et al. (1960), 24 albino rats (males and females, Osborne-Mendel strain) were fed a diet consisting of Trilaurin (8%), Glyceryl Dilaurate (45%), and Glyceryl Laurate (40 to 45%) at a concentration of 25% in the diet for two years. Thus, the individual glyceryl esters were fed at effective dietary concentrations of ~2% (Trilaurin), ~11.25% (Glyceryl Dilaurate), and ~10% to 11.25% (Glyceryl Laurate). The 24 control rats were fed hydrogenated cottonseed oil at a concentration of 25% in the diet.

After 26 or 52 weeks of feeding, no significant difference in weight gain between test and control animals was noted. However, due to the higher caloric intake, weight gain was increased in the test group. Additionally, no significant difference in the total number of deaths between test and control groups was noted at the end of the two-year study. No gross pathology attributable to feeding of the mixture was observed at necropsy. The only effect that was attributable to feeding, slight excess of hepatic cell fatty change (compared to controls), was noted at microscopic examination. A lesser and questionably significant degree of intrahepatic bile duct proliferation was also noted (Fitzhugh et al., 1960).

OCULAR IRRITATION

Glyceryl Dilaurate

MB Research Laboratories, Inc. (1991b) evaluated the ocular irritation potential of Glyceryl Dilaurate using six New Zealand albino rabbits. The undiluted test substance (0.1 ml) was instilled into the conjunctival sac of one eye of each animal. The eye lids were held together briefly after instillation. Untreated eyes served as controls. Reactions, recorded on days 1, 2, and 3, were scored according to the Draize scale (0 to 110). Slight conjunctival irritation was observed in two of the six treated eyes, and had cleared by day 2. Glyceryl Dilaurate was non-irritating to the eyes of rabbits.

Glyceryl Dibehenate

Pharmakon Europe (1995) evaluated a trade mixture (Compritol 888 ATO) containing an unspecified concentration of Glyceryl Dibehenate in an ocular irritation study using three male New Zealand White rabbits . A 5% (W/W) suspension of the mixture in kernel oil (0.1 ml) was instilled into the inferior conjunctival sac of the right eye of each animal. After instillation, the upper and lower eyelids were held together for approximately 10 sec to prevent loss of the test substance; eyes were not rinsed. The conjunctiva, iris, and cornea were examined at 1 h and 1, 2, and 3 days post-instillation. Ocular lesions were evaluated according to the Draize scale and mean ocular irritation indices calculated for each examination period. The maximum ocular irritation index (MaOI) was the highest of the four mean ocular irritation indices recorded. Ocular lesions were observed at 1 h, but had cleared by day 3. Compritol 888 ATO was classified as a slight ocular irritant (MaOI = 8.67, at 1 h post-instillation).

Glyceryl Stearate Citrate

Glyceryl Stearate Citrate did not induce ocular irritation in the Draize test. Details concerning the test protocol and study results were not included (Huls America, Inc., no date).

SKIN IRRITATION

Glyceryl Dilaurate

The Product Safety Labs (1987) evaluated the skin irritation potential of undiluted Glyceryl Dilaurate using six male New Zealand albino rabbits. The test substance (0.5 ml) was applied to one abraded site and one intact site (2 cm^2 area, clipped free of hair) within an area encompassing dorsal and ventral surfaces from the scapular to pelvic area. The sites were covered with an occlusive patch, and the entire trunk (patches included) was wrapped with an elastic cloth. At 24 h

post-application, the patches were removed and sites wiped to remove any residual test material. Reactions were scored according to the following scales: 0 (no erythema) to 4 (severe erythema to slight eschar formation) and 0 (no edema) to 4 (severe edema, raised more than 1 mm and extending beyond the area of exposure). Well defined erythema was observed at all test sites (24 and 72 h post-application, abraded and intact skin), except for one intact site in which the reaction diminished to barely perceptible erythema at 72 h. The test substance was classified as a mild primary irritant (average primary irritation score = 2).

In another study, MB Research Laboratories, Inc. (1991c) evaluated the skin irritation potential of Glyceryl Dilaurate was evaluated using six new Zealand Albino rabbits. The test substance was melted in a water bath and then applied (0.5 ml) to an abraded site and an intact site on the back of each animal. Test sites were covered with occlusive patches, secured with adhesive tape and wrapped with plastic, for 24 h. After patch removal, reactions were scored according to the following scales: 0 (no erythema) to 4 (severe erythema to slight eschar formation) and 0 (no edema) to 4 (severe edema, raised more than 1 mm and extending beyond the area of exposure). The primary irritation index (PII) was calculated by adding the mean values (6 rabbits) for erythema/eschar and edema on intact and abraded skin at 24 and 72 h (total of 8 values) and dividing the sum by 4. Glyceryl Dilaurate was classified as a non-irritant (PII = 0).

Glyceryl Stearate Citrate

Glyceryl Stearate Citrate induced slight skin irritation in the Draize test. Details concerning the test protocol and study results were not included (Huls America, Inc., no date).

SKIN SENSITIZATION

Glyceryl Stearate Citrate

The Safepharm Laboratories (1981) evaluated the skin sensitization potential of Glyceryl Stearate Citrate in a modification of the Magnusson-Kligman guinea pig sensitization test using ten female albino guinea pigs of the Dunkin-Hartley strain. The test methodology involved a two-stage induction procedure followed by a topical challenge two weeks later. The test concentrations of Glyceryl Stearate were as follows: intradermal induction (5% in liquid paraffin), topical induction (25% in petroleum jelly), and topical challenge (25% in petroleum jelly). During the first phase of induction (day 0), three pairs of intradermal injections were made simultaneously to skin (in shoulder region) that had been clipped free of hair as follows: Freund's complete adjuvant (0.1 ml), 5% Glyceryl Stearate Citrate (0.1 ml), and 5% Glyceryl Stearate emulsified in Freund's complete adjuvant.

The second phase of induction (day 7) involved the topical application of 25% Glyceryl Stearate Citrate to the injection site (at one week post-injection) using a patch made of filter paper. The patch was secured with an elastic adhesive bandage for 48 h. During the challenge phase (day 21), 25% Glyceryl Stearate Citrate was applied (under occlusive patch) to shaved skin of the right flank. Petroleum jelly (vehicle) was applied to shaved skin of the left flank according to the same procedure. Patches were secured with an elastic adhesive bandage, and another bandage of the same type was wrapped around the trunk of the animal. At 24 h post-application, patches were removed. Challenge reactions were scored at 24 and 48 h according to the following scale: 0 (no reaction) to 3 (intense redness and swelling). An additional 5 guinea pigs served as the controls, and they were exposed to the test substance vehicles according to the same induction procedure indicated for test animals. The challenge phase (same procedure consisted of the application of 25% Glyceryl Stearate to the flank.

Sensitization was not induced in any of the animals tested (Safepharm Laboratories, 1981).

Glyceryl Diisostearate

The Shiseido Research Center (1977) evaluated the contact allergenicity of Glyceryl Diisostearate in a maximization test using nine Hartley guinea pigs. On day 1 of the induction phase, the animals were injected intradermally (shaved nuchal region) with the following: 0.1 ml of emulsified Freund's complete adjuvant (E-FCA), from equal volumes of FCA and distilled water; 0.1 ml of 25% Glyceryl Diisostearate; and 0.1 ml of 50% Glyceryl Diisostearate emulsified with FCA. At week 1 post-injection, 50 mg of 10% sodium lauryl sulfate (in petrolatum) was applied to the nuchal region. On the following day, 0.2 ml of 100% Glyceryl Diisostearate was applied occlusively for 48 h. The five control guinea pigs were injected intradermally with distilled water (intradermal injection phase) and then treated with "four points" of 0.1 ml of E-FCA at the same time that test animals received occlusive patch applications.

During the challenge phase (3 weeks after the first induction), Glyceryl Diisostearate concentrations of 5, 20, 50, and 100% in ethanol (0.1 ml each) were applied topically to the flank (clipped free of hair) of test and control animals. Reactions were scored at 24 and 48 h post-application according to the following scale: 0 (no reaction) to 4 (severe erythema [beet redness] to slight eschar formation (injuries in depth) and severe edema [raised more than 1mm and extending beyond area of exposure).

Glyceryl Diisostearate did not induce sensitization at any of the challenge concentrations tested (Shiseido Research Center, 1977).

PHOTOTOXICITY/PHOTOALLERGENICITY

Data on the phototoxicity/photoallergenicity of glyceryl diesters were not available. As noted earlier, however, glyceryl triesters and glyceryl monoesters do contain glyceryl diesters. Therefore, data on the phototoxicity/photoallergenicity of a glyceryl triester, Triisostearin, and a glyceryl monoester, Glyceryl Isostearate, are included in this review.

Triisostearin

Unichema International (1997) evaluated the phototoxicity and photoallergenicity potential of Triisostearin using 20 albino guinea pigs. The back and sides of each animal were divided into the following six treatment areas: test material + UVA, test material + UVB, test material alone, positive control (8-methoxypsoralen) + UVA, UVB alone, and UVA alone. Doses of the test material and positive control (dose for each = 0.02 ml/cm^2) were applied 30 min prior to irradiation. UV irradiations were performed using Philips tubes (TL 20W/09 for UVA and TL 20W/12 UV for UVB). Cutaneous reactions were evaluated at 24 h post-treatment. With or without UV irradiation, Triisostearin did not induce significant cutaneous reactions. The positive control (8-methoxypsoralen) induced major reactions.

Glyceryl Isostearate

Unichema International (1997) evaluated the phototoxicity and photoallergenicity potential of Glyceryl Isostearate using 20 albino guinea pigs. The back and sides of each animal were divided into the following six treatment areas: test material + UVA, test material + UVB, test material alone, positive control (8-methoxypsoralen) + UVA, UVB alone, and UVA alone. Doses of the test material and positive control (dose for each = 0.02 ml/cm^2) were applied 30 min prior to irradiation. UV irradiations were performed using Philips tubes (TL 20W/09 for UVA and TL 20W/12 UV for UVB). Cutaneous reactions were evaluated at 24 h post-treatment. Glyceryl Isostearate did not induce significant cutaneous reactions with or without UV irradiation. The positive control (8-methoxypsoralen) induced severe reactions.

CARCINOGENICITY AND TUMOR PROMOTION

Glyceryl Distearate

In a study reported by Setala et al. (1961), the character of ultrastructural changes in mouse epidermis caused by Glyceryl Distearate was evaluated with and without initiation. Randomly-bred mice of a tumor-resistant strain were used. Glyceryl Distearate (0.0066 M in acetone) was administered six times per week (Sundays excluded). The test substance was dropped onto the skin and the theoretical single dose of Glyceryl Distearate at the cutaneous surface was 0.16 mg. In initiation experiments, 9,10-dimethyl-1,2-benzanthracene (DMBA) in light paraffin was used. The solution was carefully dropped onto the center of the treatment area on the back and spread with a glass rod. Post-treatment with Glyceryl Distearate was started after 30 days. Hair within the treatment area was cut at one-week intervals. In experiments without initiation, skin biopsies were obtained from five animals on days 2, 6, 10, 16, 30, and 60 after the beginning of the study. In experiments with initiation, the first skin biopsy was taken on day 30 and 2 days after initiation.

In the absence of initiation, Glyceryl Distearate (0.0066 M in acetone) induced a moderate hyperplastic response. The stratum corneum was thicker and coarser than normal (biopsy taken on day 30). Glyceryl Distearate did not cause cellular or nuclear atypia, and the pilo-sebaceous apparatus was intact. Epidermal alterations were accompanied by inflammation of the dermis. With initiation, there was an increase in the total cell count.

Ultrastructurally, Glyceryl Distearate, in the absence of initiation, induced hyperplasia of the interfollicular epidermis (IFE). In the presence of initiation, few and sparse alterations were observed in the early stages of the treatment period. Later, a definite hyperplasia of the interfollicular epidermis was noted. Whether or not Glyceryl Distearate increased the DMBA tumor incidence was not stated. (Setala et al., 1961).

Glyceryl Dioleate

Semba and Inui (1991) evaluated the inhibitory effects of free radical scavengers (superoxide dismutase, catalase, and mannitol) on diacylglycerol-induced transformation. Glyceryl Dioleate promoted transformation in 3-methylcholanthrene (3-MC)-initiated BALB/3T3 A31-1-1 cloned cells *in vitro*. In cultures treated with Glyceryl Dioleate (10 μg/ml), the mean number of transformed foci per dish (25 dishes total) was 1.0 ± 0.2. In control cultures treated with DMSO (control), the mean number of transformed foci per dish (60 dishes total) was 0. The mean number of transformed foci per dish (40 dishes total) in 3-MC (0.5 μg/ml)-treated cultures was 0.2 ± 0.1. Superoxide dismutase, catalase, and mannitol had significant inhibitory effects ($p < 0.01$) on Glyceryl Dioleate-induced transformation. The results of this study suggest that activation of protein kinase C alone is insufficient, and that the generation of reactive oxygen accompanied by activation of the enzyme is essential to the promotion process in BALB/3T3 cells.

Glyceryl Stearate

As noted earlier glyceryl triesters and glyceryl monoesters do contain glyceryl diesters. Therefore, data on the phototoxicity/photoallergenicity of a glyceryl monoester, Glyceryl Stearate, are included in this review.

Saffioti and Shubik (1963) tested the tumor promoting activity of Glyceryl Stearate on the clipped dorsal skin of Swiss mice. One week after a single application of 9,10-dimethylbenz(a)anthracene (DMBA) (1-1.5% in mineral oil), 5% Glyceryl Stearate (in acetone) was applied to the skin twice weekly. No tumors developed; slight epidermal hyperplasia at the site of application was noted.

Other Diacylglycerols

Smart et al. (1986) reported that sn-1,2-didecanoylglycerol (2.6 μmol), sn-1,2-dioctanoylglycerol (2.6 μM), and sn-1,2-dioleylglycerol (2.6 μmol) stimulated epidermal DNA synthesis after topical application to CD-1 mice. However, 1,3-didecanoylglycerol (2.6 μmol) had little or no effect. The increased epidermal DNA synthesis induced by sn-1,2-dioleoylglycerol or TPA was inhibited when either was administered simultaneously with fluocinolone acetonide, a potent inhibitor of tumor promotion.

In another study, Smart et al. (1988) reported that the topical application of 2.5 μM sn-1,2-didecanoylglycerol to female CD-1 mice and a tumor-promoting dose of 1 nmol TPA induced ornithine decarboxylase activity to the same extent. However, topical application of 2.5 μM sn-1,2-didecanoylglycerol did not cause morphological changes in the epidermis following a single application or applications twice a week for four weeks. Topical application of 2.5 μM sn-1,2-didecanoylglycerol also did not function as a complete tumor promoter when applied twice weekly for 28 weeks. More frequent applications of sn-1,2-didecanoylglycerol (5 μmol twice a day for 5 days) induced epidermal hyperplasia that was comparable to that of 5 nmol TPA twice weekly for four weeks.

Smart et al. (1989) demonstrated that, after frequent, repetitive treatment, the synthetic lipid second messenger, sn-1,2-didecanoylglycerol is a complete tumor promoter in DMBA-initiated mouse skin. In this study, an application frequency and dose of sn-1,2-didecanoylglycerol that induced epidermal hyperplasia were used. One week after treatment with 200 nmol DMBA, the mice (CD1 females) were dosed with 2 or 5 μmol sn-1,2-didecanoylglycerol for five days per week over a period of 20 or 24 weeks. sn-1,2-didecanoylglycerol functioned as a complete tumor promoter in a dose-dependent manner. It was stated that it is likely that rapid metabolism of sn-1,2-diacylglycerols necessitates frequent application for their effectiveness as complete tumor promoters.

Mills et al. (1993), using transgenic TG.AC mice, found that diacylglycerols are implicated in the clonal expansion of mutated Ha-ras-containing cells. The authors concluded that these data support a mechanism that an increase in endogenous Diacylglycerol could contribute to the clonal expansion of cells containing Ha-ras oncogene.

Smart (2001) noted, in studies on the tumor promoting activity of 1,2-diacylglycerols, that the structure of the particular diacylglycerol, its dose, and the exposure regimen by which the dose is delivered all play a role in tumor promotion. In particular, only a specific 1,2-diacylglycerol configuration was active and that the other configuration (also known as 2,3-diacylglycerol) was not active in tumor promotion.

Findings relating to 1,2-diacylglycerol-induced activation of PKC are summarized as follows: (1) If the fatty acid in the 1 or 2 position is unsaturated, then the diacylglycerol can activate PKC. 1,2-Diacylglycerols with two saturated fatty acids are less effective (Mori et al., 1982). (2) The stereospecificity of diacylglycerols is critical in terms of their biological activity. 1,2-sn-, but not 2,3-sn-diacylglycerols activate PKC (Nomura et al., 1986). (3) The activities of different diacylglycerols (i.e., activation of PKC) have been compared. The most important observation was that the activity of saturated 1,2-diacylglycerols was lost when the fatty acid moiety in the structure was a long-chain fatty acid. This effect was not only observed in *in vitro* kinase assays, but also in a cellular model (platelets). When the length of the acyl chain was greater than 14 carbons, the activity was very low or absent. However, the issue of unsaturated 1,2-diacylglycerols potently activating PKC, even if the acyl chain is long, was raised by Go et al. (1987).

Given the relationship between chain length/structure of fatty acid moiety and PKC activation, Table 4 contains a list of the 1,3-diacylglycerols included in this safety assessment, along with a description of the fatty acid moiety (unsaturated or not) and its chain length.

Diacylglycerol Oil

Soni et al. (2001) evaluated the chronic toxicity of diacylglycerol oil in a two-year feeding study using four groups of 60, four-week-old male and female SPF rats of the Crj:CD(SD) strain. A randomized diacylglycerol, manufactured through the esterification of fatty acids, is the main constituent of diacylglycerol oil. The primary components of this oil are 1,3- and 1,2-diacylglycerol (ration of 7:3). Other components include 20% triacylglycerol and 5% monoacylglycerol.

The four groups used in the study were defined as follows: low dose group (2.65% diacylglycerol oil + 2.65% edible oil composed of rapeseed oil, corn oil, high linoleic safflower oil, and high oleic safflower oil [equivalent fatty acid composition to diacylglycerol oil ≈ 0.89 g/ kg body weight/day - males and 1.18 g/kg body weight/day - females), high dose group (5.3% diacylglycerol oil [≈ 1.77 g/kg body weight/ day- males and 2.35 g/kg body weight/ day - females]), control group 1 (5.3% edible oil composed of rapeseed, corn, high linoleic safflower, and high oleic safflower oils [fatty acid composition equivalent to diacylglycerol oil), and control group 2 (5.3% edible oil

Table 4. Diacylglycerol Fatty Acid Moieties

Ingredient	Fatty Acid (FA) Moiety Saturation	Fatty Acid Chain Length
Glyceryl Dilaurate	No unsaturated FA	12 carbons
Glyceryl Diarachidate	No unsaturated FA	20 carbons
Glyceryl Dibehenate	No unsaturated FA	22 carbons
Glyceryl Dierucate	2 unsaturated FA	22 carbons
Glyceryl Dihydroxystearate	No unsaturated FA	18 carbons
Glyceryl Diisopalmitate	No unsaturated FA	16 carbons
Glyceryl Diisostearate	No unsaturated FA	18 carbons
Glyceryl Dilinoleate	2 unsaturated FA	18 carbons
Glyceryl Dimyristate	No unsaturated FA	14 carbons
Glyceryl Dioleate	2 unsaturated FA	18 carbons
Glyceryl Dipalmitate	No unsaturated FA	16 carbons
Glyceryl Dipalmitoleate	2 unsaturated FA	16 carbons
Glyceryl Palmitate Lactate	No unsaturated FA	16 carbons (palmitic) 3 carbons (lactic)
Glyceryl Distearate	No unsaturated FA	18 carbons
Glyceryl Stearate Citrate	No unsaturated FA	18 carbons (stearic) 5 carbons (citric)
Glyceryl Stearate Lactate	No unsaturated FA	18 carbons (stearic) 3 carbons (lactic)
Glyceryl Stearate Succinate	No unsaturated FA	18 carbons (stearic) 4 carbons (succinic)

composed of rapeseed and soybean oils). The animals were observed for general signs and mortality throughout the study.

At weeks 30 and 77, ten rats per sex from each group were anesthetized i.p. with sodium phenobarbital, and blood was collected from the abdominal aorta for clinical hematological and chemical analysis; urinalyses were also performed on ten rats per sex from each group. The remaining animals were killed at the end of week 105 and necropsied. Mortalities for the four groups were as follows: low dose group (54 rats: 24 males, 30 females), high dose group (58 rats: 29 males, 29 females), control 1 (50 rats: 28 males, 22 females), and control 2 (58 rats: 27 males, 31 females).

Histopathological examination was performed on a variety of tissues from animals that were killed at the end of 30 and 77 weeks of feeding. For rats killed after 105 weeks of feeding, only the mammary gland was examined microscopically.

No statistically significant differences in cumulative survival rate or treatment-related clinical signs of toxicity were observed in animals killed at 30, 77, or 105 weeks. Additionally, no treatment-related statistically significant differences in body weight were reported for these animals. A statistically significant difference in body weight (limited to days 148 to 162) occurred between low dose males and control group 2. Feed consumption also differed significantly from controls, on an intermittent basis, in high- and low- dose males. However, this finding was not considered toxicologically significant.

Hematology, blood chemical analysis, or urinalysis findings, for rats in low or high dose groups indicated no consistent pattern that was indicative of a treatment-related effect. Statistically significant changes in several parameters were reported. For example, compared to control group 2, activated partial thromboplastin time (APTT) in high-dose male rats, dosed for 30 weeks, was significantly prolonged. Additionally, compared to control groups 1 and 2, the platelet count in high- and low-dose females, dosed for 77 weeks, was significantly

decreased. However, it is important to note that all significant differences regarding the parameters evaluated were considered incidental because they were either limited to one sex or one collection interval and lacked a dose response, or were not supported by any other changes in related clinical parameters.

At gross examination, changes in organ weight were found to be significant when results for test and control animals were compared after 30 weeks of feeding. Compared to control group 2, absolute weights of the thyroid, heart, kidney, and liver and relative liver weight in the low-dose males were significantly lower. A comparison of control groups 1 and 2 with high-dose males revealed no significant differences in organ weight. No significant changes in weight of these organs (heart, thyroid, kidney, liver) were noted after 77 weeks of feeding. None of the observed changes in the weight of these organs were considered treatment-related.

There were statistically significant differences in organ weights in high-dose males and females or low-dose females after 77 weeks of feeding (e.g. absolute and relative brain weight, relative spleen weight in males, and absolute and relative heart weights in females) and at 105 weeks (absolute thyroid weight in high-dose males). However, these differences were not considered toxicologically significant or treatment-related, in that they were either limited to one sex, one collection period, or were not associated with a dose-response.

Compared to controls, neither gross nor microscopic findings indicated an increase in the incidence of non-neoplastic changes in rats that received high or low doses of diacylglycerol oil. The incidence of either benign or malignant epithelial mammary gland tumors was not different from that noted in the control group. Benign mammary gland tumors in dead female rats of the high dose group were more frequent. However, it was concluded that the increased incidence was not related to feeding with diacylglycerol oil for the following reasons: (1) No dose-related increase in the incidence of mammary tumors was observed; (2) Compared to control group 1 or the low-dose exposure group, the mammary tumor incidence in the high-dose group was not significantly increased; (3) The number of rats with benign tumors in the high-dose group was within the range of values reported in the published literature.

The only statistically significant differences (compared to control group 2, but not control group 1) among the groups tested were the increased incidence of adenocarcinoma in rats that died early, and the significantly higher total incidence (compared to control group 2, but not control group 1) of benign and malignant tumors combined for high-dose rats that died early and those killed at 105 weeks. The neoplastic findings were not dose-related (Soni et al., 2001).

It was concluded that no treatment-related effects of diacylglycerol oil were observed in rats at dietary concentrations up to 5.3% in the diet over a two-year period (Soni et al., 2001).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Studies on the reproductive and developmental toxicity of glyceryl diesters were not found in the published literature.

CLINICAL ASSESSMENT OF SAFETY

SKIN IRRITATION

Glyceryl Dilaurate

CTFA (1977) reported that the skin irritation potential of an eye shadow containing 1.5% Glyceryl Dilaurate was evaluated in a single insult (24 hours), occlusive patch test using 20 subjects (ages not stated). The control group consisted of 20 subjects (ages not stated) tested with an eye shadow that did not contain Glyceryl Dilaurate. Volatiles on the patch were allowed to evaporate over a period of ~15 to 30 minutes, and each patch was hydrated prior to application. Reactions were scored according to the following scale: 0 (no evidence of any effect) to 4 (Severe: deep red erythema with vesiculation or weeping with or without edema). Skin irritation was not observed in the test or control group.

CTFA (1999b) reported the skin irritation potential of a foundation containing 1.5% Glyceryl Dilaurate evaluated using 20 subjects (ages not stated) according to the procedure in the preceding study. The control group consisted of 20 subjects (ages not stated) tested with a foundation that did not contain Glyceryl Dilaurate. Three of the 20 subjects tested with the Glyceryl Dilaurate foundation had a barely perceptible reaction (±, minimal faint uniform or spotty erythema). Reactions were not observed in the remaining 17 subjects in this group. The distribution of reactions in the control group was as follows: ± reaction (4 subjects), 1 reaction (2 subjects) ,1+ reaction (1 subject), and 0 reaction (13 subjects). A 1 reaction (mild) was defined as pink, uniform erythema covering most of the contact site, and a 2 reaction (moderate) was defined as pink-red erythema, visibly uniform in the entire contact area. The 1+ reaction was classified as an intermediate reaction. Primary irritation indexes for the test and control foundation were 0.08 and 0.28, respectively.

Glyceryl Dibehenate

The Institut D'espertise Clinique (1996) evaluated a trade mixture (Compritol 888 ATO) containing an unspecified concentration of Glyceryl Dibehenate in a skin irritation test using ten volunteers (8 females, 2 males). The test substance

(10% in kernel stone oil; volume = 0.02 ml) was applied to the back under an occlusive patch (Finn Chamber on Scanpor) for a period of 48 h. Kernel stone oil, patch tested according to the same procedure, served as the negative control. At ~ 30 min post-removal, reactions (erythema and edema) were scored according to the following scales: 0 (no erythema) to 4 (purpuric erythema) and 0 (no edema) to 3 (severe edema [1 mm thick at least] on a surface greater than the application area). Additional numerical scales were used for the evaluation of dryness and desquamation, papules, vesicles, bullae, pustules, detergent effect, and reflectivity.

Slight erythema (hardly visible) was observed in two of the ten subjects tested. An observation described as "a slightly worn aspect of the skin" was reported for two additional subjects. Compritol 888 ATO (10%) did not induce pathological irritation or significant cutaneous intolerance, and, therefore, the investigators concluded that the test substance was well tolerated (Institut D'espertise Clinique, 1996).

SKIN IRRITATION AND SENSITIZATION

Glyceryl Dilaurate

Ivy Laboratories (1997) evaluated the skin sensitization potential of an eye shadow containing 1.5% Glyceryl Dilaurate (see preceding section on Skin Irritation for additional results) in a maximization test using 29 healthy adult volunteers (19 females, 10 males; age range = 18 to 60 years old). Twenty-five subjects completed the study. Three subjects were released due to lack of compliance, and one was released due to intolerance to sodium lauryl sulfate. During induction and challenge phases, patches were applied to the upper outer arm, the volar forearm, or to the back of each subject.

According to the induction procedure, 0.25% aqueous Sodium Lauryl Sulfate (~0.1 ml) was applied under an occlusive patch that was fastened to the skin with occlusive tape for 24 hours. After patch removal, the eye shadow containing 1.5% Glyceryl Dilaurate was applied (same site), and the site was covered with occlusive tape for 48 hours (or 72 hours - weekend application). If skin irritation was not observed at the time of patch removal, the SLS patch was reapplied (same site) for 24 hours. Patch removal was followed by reapplication of another induction patch with the eye shadow. This sequence of SLS pre-treatment, followed by application of the eye shadow was repeated for a total of five induction exposures. If skin irritation was observed during induction, SLS pre-treatment was eliminated and eye shadow only was applied.

The challenge phase was initiated after a 10-day non-treatment period. Following one hour of pre-treatment with 5.0% aqueous SLS (under occlusive patch, new site), the subjects were challenged with a single application of the eye shadow at the same site. The new site was on the opposite arm, forearm, or side of the back. The challenge patch remained in place for 48 hours, and sites were graded according to the following scale at 1 and 24 hours post-removal: 0 (not sensitized) to 3 (strong sensitization: large vesiculo-bullous reaction). Neither contact allergy nor any other adverse reaction was observed in any of the 25 subjects who completed the study. It was concluded that the eye shadow containing 1.5% Glyceryl Dilaurate does not possess a detectable contact-sensitizing potential, and, thus, is not likely to cause contact sensitivity reactions under normal use conditions (Ivy Laboratories, 1997).

Ivy Laboratories (1999) also evaluated the skin sensitization potential of a foundation containing 1.5% Glyceryl Dilaurate according to the procedure in the preceding maximization test. The study group consisted of 28 healthy adult volunteers (19 females, 9 males; ages = 18 to 47 years old). Twenty-seven subjects completed the study. One subject was lost to follow-up. Neither contact allergy nor any other adverse reaction was observed in any of the 27 subjects who completed the study. It was concluded that the foundation containing 1.5% Glyceryl Dilaurate does not possess a detectable contact-sensitizing potential, and, thus, is not likely to cause contact sensitivity reactions under normal use conditions.

Glyceryl Dioleate

Inveresk Research International Limited (1991) evaluated the skin irritation and sensitization potential of Glyceryl Dioleate in a human repeat insult patch test using 86 volunteers. The test laboratory stated that 82 subjects completed the study and that the reasons for withdrawal were not test substance-related.

The test substance (50% w/w in liquid paraffin, 0.3 ml) was applied to Webril pads located down the center line of a piece of Blenderm adhesive tape, and the patch strip was applied to the lateral surface of the upper arm. A patch scheme comprised two patch strips, each with three Webril pads. The subjects were also patch tested with liquid paraffin (100%), which served as the negative control. During the three-week induction phase, patches were applied to the same test site for 24 h on Mondays, Wednesdays, and Fridays. Reactions were scored prior to each subsequent patch application according to the following scale: 0 (no visible reaction) to 5 (bullous reaction). At week 6, challenge patches were applied to both arms of each subject (original site and new test site, respectively). Challenge reactions were scored at 48 h and 96 h post-application according to the same grading scale.

Glyceryl Dioleate induced minimal irritation (mild erythematous reaction in 7 subjects) during induction. However, the results of a statistical comparison indicated that reactions at test sites were not significantly more irritating when compared to control sites. No reactions indicative of sensitization were observed during the challenge phase (Inveresk Research International Limited, 1991).

Glyceryl Palmitate Lactate

Danisco Ingredients (1996) evaluated the skin irritation and sensitization potential of Glyceryl Palmitate Lactate (50% w/v in liquid paraffin) using 91 healthy volunteers (males and females, 18 to 65 years old) in a modified Draize repeat insult patch test. The study was classified as single-blind. Patches consisted of a 5 cm wide strip of Scanpor® tape to which ten "Finn-chambers" were fixed in pairs. The test substance was heated to 60°C and 0.02 g was placed directly into individual Finn chambers; 0.02 ml liquid paraffin was dispensed onto a filter disc in the chamber. Liquid paraffin was the negative control.

The 91 volunteers were divided into two groups, Group 1 (39 subjects) and Group 2 (52 subjects). In Group 1, the first set of induction patches was applied to the upper back for 23 h and subsequent patch applications were 47 h in duration. The patch test schedule for these subjects was as follows: days 1, 2, 4, 7, 9, 11, 14, 16 and 18. In Group 2, 47 h induction patch applications were made according to the following schedule: days 1, 3, 5, 8, 10, 12, 15, 17, and 19. In both groups, induction patches were applied to the same site, unless a reaction stronger than mild erythema was observed. Reactions were scored after patch removal according to the following scale: 0 (no visible reaction) to 5 (bullous reaction). During the challenge phase, initiated on day 35, patches were applied to new sites on the upper back for 47 h.

Challenge reactions were scored at 1 and 49 h after patch removal. Classification of either test substance as irritating was based on 10% of the induction scores defined as > 1 (mild erythema). Sensitization was defined as a rapid response to challenge patch application, characterized by severe erythema and edema (usually with papules and/or vesicles).

According to the test laboratory, 17 of the 91 subjects withdrew for reasons unrelated to patch testing; 74 subjects (64 women, 10 men) completed the study.

Glyceryl Palmitate Lactate (50% w/v in liquid paraffin) did not induce skin irritation or sensitization (Danisco Ingredients, 1996).

PHOTOTOXICITY/PHOTOALLERGENICITY

Studies on the phototoxicity/photoallergenicity of the glyceryl diesters were not identified in the published literature. As noted earlier, however, glyceryl monoesters do contain glyceryl diesters. Therefore, data on the phototoxicity and photo-allergenicity of a glyceryl monoester, Glyceryl Rosinate are included in this review.

Glyceryl Rosinate

Biosearch, Inc. (1992a) reported a study in which the phototoxicity of a lipstick containing 1.0% Glyceryl Rosinate, as supplied, was evaluated using ten volunteers (17 to 55 years old). All subjects were in good health and free of any visible skin disease or anomaly in the area of skin designated for patch testing. Subjects on medication (especially medications suspected of causing photobiological reactions or medications with the potential for modifying the inflammatory response) were excluded. The subjects were classified as Fitzpatrick skin types I, II, and III. The degree of skin pigmentation did not significantly influence responses to UV light or interfere with the scoring of skin reactions. The test substance was applied (approximately 20 mg/site) to two sites on the back of each subject and spread to cover the areas uniformly.

One of the test sites was irradiated with 0.5 MED (minimal erythemal dose, in seconds) of UVA and UVB light (continuous spectrum in UVA and UVB regions, 290 - 400 nm) between 30 and 60 min after application of the test substance. The MED was defined as the shortest exposure time at which erythema was first observed 20 ± 4 h after exposure. Irradiation with UVA and UVB light was followed by exposure to a total of 14 Joules/cm^2 of UVA. A 2 mm thick WG-345 Schott filter was interposed to eliminate UVB (290-320 nm) radiation from the ultraviolet source. Reactions were scored at 24, 48, and 72 h post-irradiation according to the following scale: 0 (no visible erythema) to 3 (severe erythema [very intense redness]).

The second site to which the test substance had been applied was not irradiated and served as an irritation control. A third site served as the untreated, irradiated control.

Skin irritation was not observed (Score = 0) at control or irradiated sites in either of the ten subjects tested. The lipstick containing 1.0% Glyceryl Rosinate did not elicit a phototoxicity response (Biosearch, Inc.,1992a).

Biosearch, Inc. (1992b) also reported a study in which the photoallergenicity of a lipstick containing 1% Glyceryl Rosinate, as supplied, was evaluated using 26 volunteers (17 to 55 years old). Four of the original 30 subjects withdrew for personal reasons. All subjects were in good health and free of any visible skin disease or anomaly in the area of skin designated for patch testing. Subjects on medication (especially medications suspected of causing photobiological reactions or medications with the potential for modifying the inflammatory response) were excluded. Skin types were variable and the degree of skin pigmentation did not significantly influence responses to UV light or interfere with the scoring of skin reactions. During the induction phase, each of the subjects received six applications of the test substance over a period of three weeks. For each application, approximately 0.15 g of the test substance was placed on an occlusive patch that was applied to the back for 24 h. Patches were applied on Tuesdays and Thursdays.

After patch removal, each site was exposed to 2.0 MED's of UVB radiation and 4 Joules/cm^2 of UVA radiation. The subjects were instructed to keep the back covered throughout the study to avoid exposure to natural or artificial sunlight. The challenge phase was initiated 18 days after the last induction exposure. Challenge patches containing the test substance were applied to two new, adjacent test sites for 24 h. After patch removal, reactions were scored according to the scale indicated in the preceding study.

One of the test sites was then exposed to a combination of 0.5 MED of UVB and 4 Joules/cm^2 of UVA light. The other site was not exposed to UVA light and served as the irritation control. The UV light control site was defined as an additional site that was not exposed to the test substance, but was irradiated with 0.5 MED of UVB and 4 Joules/cm^2 of UVA light. Challenge sites were scored at 24, 48, and 72 h post-irradiation.

No reaction (Score = 0) was observed at control or test sites on any of the 26 volunteers tested . The lipstick containing 1.0% Glyceryl Rosinate did not elicit a photoallergic response (Biosearch, Inc., 1992b).

CASE REPORTS

Glyceryl Diisostearate

Hayakawa et al. (1987) reported chelitis in an 18-year-old girl with a history of what was described as lip cream dermatitis. The patient was patch tested with Glyceryl Diisostearate, an ingredient of one of the lipsticks used, as well as Glyceryl Diisostearate impurities. Both Glyceryl Diisostearate (in petrolatum) and purified Glyceryl Diisostearate (no glyceryl monoisostearate peak in the gas chromatograph) were tested in this study. The results of an analysis of Glyceryl Diisostearate by gas chromatography were as follows: Glyceryl Diisostearate (65.89%), glyceryl triisostearate (29.05%), glyceryl monoisosterate (0.43%), and isostearic acid (0.21%). Patch tests were conducted using Finn chambers that were secured with Scanpor tape for 48 h. Reactions were scored according to International Contact Dermatitis Research Group (ICDRG) recommendations. A strong positive reaction (++) to Glyceryl Diisostearate (35.5% in petrolatum) was observed at 48 and 72 h. Purified Glyceryl Diisostearate induced + and ++ reactions at 48 and 72 h, respectively. All three Glyceryl Diisostearate impurities induced ++ reactions (48 and 72 h) at the following test concentrations in petrolatum: Glyceryl Monoisostearate (0.01%), isostearic acid (10%), and Glyceryl Triisostearate (27%). In an analysis of Glyceryl Triisostearate by gas chromatography, a peak for glyceryl monoisostearate was not observed.

Tanaka et al. (1993) observed itchy, facial erythema in a 35-year-old female, following application of a foundation containing 1.77% Glyceryl Diisostearate. The reaction cleared within a few days after withdrawal of the product and application of a mild, topical corticosteroid. In patch tests with individual ingredients of the product, a positive (+) reaction to 1.77% Glyceryl Diisostearate in petrolatum was found. The investigators stated that allergic contact dermatitis to this ingredient was extremely rare, and also noted that the patient also had sensitization reactions to lipsticks containing 35.5% Glyceryl Diisostearate. The following three chemical impurities were detected when Glyceryl Diisostearate was analyzed by gas chromatography: glyceryl monoisostearate (0.43%), glyceryl triisostearate (29.05%), and isostearic acid (0.21%). A strong positive reaction was observed when the patient was tested with 0.01% glyceryl monoisostearate.

SUMMARY

The safety of 17 glyceryl diesters (a.k.a. diacylglycerols or diglycerides) in cosmetics is reviewed in this report: Included are: Glyceryl Dilaurate, Glyceryl Diarachidate, Glyceryl Dibehenate, Glyceryl Dierucate, Glyceryl Dihydroxystearate, Glyceryl Diisopalmitate, Glyceryl Diisostearate, Glyceryl Dilinoleate, Glyceryl Dimyristate, Glyceryl Dioleate, Glyceryl Dipalmitate, Glyceryl Dipalmitoleate, Glyceryl Distearate, Glyceryl Palmitate Lactate, Glyceryl Stearate Citrate, Glyceryl Stearate Lactate, and Glyceryl Stearate Succinate.

Only information on the manufacturing process for Glyceryl Dioleate was available. For this glyceryl diester, the raw materials are fully refined non-lauric vegetable oils. The oils are further processed using special hydrogenation and fractionation techniques. The end products are produced by reacting selected mixtures of the partly hydrogenated, partly fractionated oils and fats with vegetable-derived glycerine to yield partial glycerides. In the final stage of the production process, the products are purified by deodorization. Deodorization effectively removes pesticide residues and lower boiling residues such as residues of halogenated solvents and aromatic solvents.

1,2- and 1,3-diglycerides are not stable and are easily isomerized. While there are various reports of the relative proportion of the 1,2 form, the 2,3 form, and the 1,3 form, the most recent analytical data demonstrate that 10-15% 1,2 diester (optically and physiologically active isomer) is present in the product as sold, with 10-15% 2,3 diester (optically active, physiologically non-active isomer) and 70-80% 1,3 diester (not optically or physiologically active) comprising the remainder of the product.

Glyceryl diesters are used as skin conditioning agents - emollients in cosmetics. Reports to FDA in 2001 indicate that of the 17 ingredients in this safety assessment, Glyceryl Dilaurate, Glyceryl Diisostearate, and Glyceryl Dioleate are used in cosmetics (133 uses total). Concentration of use data received from the cosmetics industry in 1999 confirm the use of the above three glyceryl diesters, but also indicate use of

Glyceryl Stearate Lactate and Glyceryl Distearate. Current concentrations of use in cosmetics were: Glyceryl Dilaurate (up to 5%), Glyceryl Diisostearate (up to 43%), Glyceryl Dioleate (up to 2%), Glyceryl Distearate (up to 7%), and Glyceryl Stearate Lactate (up to 5%).

Glyceryl diesters have been approved by FDA for use as indirect food additives. Reportedly, Glyceryl Dilaurate has been detected in pharmaceutical excipients.

Diglycerides are intermediates in the metabolism of triglycerides to free fatty acids and glycerol.

Various studies indicate that the sustained activation or inhibition of PKC (protein kinase C; diacylglycerol-activated isoenzyme) *in vivo* may play a critical role in the regulation of long-term cellular events such as proliferation, differentiation and tumorigenesis. *In vitro* results indicate that the capacity for activation of PKC α is considerably greater for 1,2-diacylglycerols than for 1,3-diacylglycerols. However, mixed results have been reported in other *in vitro* experiments. Practically the same degree of activation of PKC α was induced by 1,2- and 1,3-diacylglycerols in one experiment and a very low capacity for 1,3-diacylglycerol-induced activation of PKC α was demonstrated in another. The activation of PKC α was dependent on the components of the micelle in which PKC was incorporated.

Exposure of murine keratinocytes to UVB radiation increases cellular diacylglycerol concentrations via changes in *de novo* metabolism that involve a diacylglycerol kinase pathway. Elevated diacylglycerol may influence signal-transduction pathways that are mediated by cellular lipids, and contribute to keratinocyte responses to UV light.

The topical application of diacylglycerols increased pigmentation in skin of outbred pigmented guinea pigs and female Skh HR-2 pigmented hairless mice. The increase in epidermal pigmentation was a protein kinase C-dependent process. Treatment of the hairless mice with a diacylglycerol or the diacylglycerol + UVA/UVB light revealed no skin irritation or mucopolysaccharide deposition in excess of that anticipated from exposure to UV light alone.

An acute oral LD50 of > 5 g/kg (Glyceryl Dilaurate) was reported in rats. The oral administration of a trade mixture containing an unspecified concentration of Glyceryl Dibehenate did not cause death in rats.

A mono- di-, and triglyceride mixture including 45% Glyceryl Dilaurate did not induce any deleterious effects when fed to male and female albino rats at a dietary concentration of 25% (effective concentration of Glyceryl Dilaurate = 11.25%) for two years.

Undiluted Glyceryl Dilaurate was non-irritating to the eyes of albino rabbits. A trade mixture containing an unspecified concentration of Glyceryl Dibehenate was classified as a slight ocular irritant in a test involving rabbits. Glyceryl Stearate Citrate did not induce ocular irritation in the Draize test.

Glyceryl Dilaurate (undiluted) was classified as a mild primary irritant in a study involving albino rabbits. in which the test site was covered with occlusive patches for 24 h. In another 24 h occlusive patch test, Glyceryl Dilaurate (melted in a water bath) was nonirritating to abraded and intact skin sites of albino rabbits. In a Draize skin irritation test using albino rabbits, Glyceryl Stearate Citrate induced slight skin irritation. However, results were negative for this ingredient in the Magnusson-Kligman guinea pig maximization test. The animals were challenged with 25% Glyceryl Stearate Citrate in petroleum jelly for 48 h; sites were covered with patches made of filter paper. In another guinea pig maximization test, Glyceryl Diisostearate did not induce sensitization at concentrations ranging from 5 to 100% in ethanol.

Triisostearin (C18) did not induce significant cutaneous reactions in a study evaluating the phototoxicity and photoallergenicity potential of this ingredient in guinea pigs. No significant cutaneous reactions, with or without UV irradiation, were found when the phototoxicity and photoallergenicity potential of Glyceryl Isostearate was evaluated using guinea pigs.

In the absence of initiation, Glyceryl Distearate (in acetone) induced a moderate hyperplastic response in randomly-bred mice of a tumor-resistant strain. With DMBA initiation, an increase in the total cell count was observed. Whether Glyceryl Distearate increased the DMBA tumor incidence was not stated. In a glyceryl monoester study, a single application of DMBA to the skin was followed by 5% Glyceryl Stearate (in acetone) twice weekly. No tumors developed and slight epidermal hyperplasia at the site of application was noted.

Glyceryl Dioleate induced transformation in 3-methylcholanthrene-initiated BALB/3T3 A31-1-1 cloned cells *in vitro*.

In a study involving female CD-1 mice, PKC was linked to epidermal hyperplasia and tumor promotion. A tumor-promoting dosing regimen that consisted of multiple applications of 5 or 10 μmol *sn*-1,2-didecanoylglycerol, a model *sn*-1,2-diacylglycerol and complete tumor promoter, twice daily for one week caused more than a 60% decrease in cytosolic and particulate PKC activity and marked epidermal hyperplasia. However, applications of 10 μmol *sn*-1,2-didecanoylglycerol twice weekly for one week, a non-tumor-promoting dosing regimen, caused a decrease in cytosolic PKC activity, increased particulate PKC activity and did not induce epidermal hyperplasia.

A study indicated that the overexpression of PKCα in the

epidermis of transgenic mice increased the expression of specific proinflammatory mediators and induced cutaneous inflammation, but had little to no effect on epidermal differentiation, proliferation, or TPA tumor promotion. Other studies have shown that Diacylglycerols, intracellular ligands for PKC, were elevated in psoriatic lesions, and that this was accompanied by alterations in PKC isoform levels and activity.

The increased epidermal DNA synthesis induced by sn-1,2-dioleylglycerol or TPA after topical application to CD-1 mice was inhibited when either was administered simultaneously with fluocinolone acetonide, a potent inhibitor of tumor promotion. A single application or applications twice a week for four weeks of sn-1,2-dioleoylglycerol did not cause morphological changes in the epidermis or function as a complete tumor promoter when applied twice weekly for 28 weeks. More frequent applications of sn-1,2-didecanoylglycerol (5 μmol twice a day for 5 days) induced epidermal hyperplasia that was comparable to that resulting from application of 5 nmol TPA twice weekly for four weeks.

It has been demonstrated that the synthetic lipid second messenger, sn-1,2-didecanoylglycerol is a complete tumor promoter in DMBA-initiated mouse skin.

In studies on the tumor promoting activity of 1,2-diacylglycerols, it was noted that the structure of the particular diacylglycerol, its dose, and the exposure regimen by which the dose is delivered all play a role in tumor promotion. In particular, only a specific 1,2-diacylglycerol configuration was active and that the other configuration (also known as 2,3-diacylglycerol) was not active in tumor promotion.

Compared to one of the two control groups in a chronic oral study using groups of 60 SPF rats, a statistically significant increase in the number of either benign or malignant epithelial mammary gland neoplasms was observed in female rats fed a basal diet containing 5.3% diacylglycerol oil, but not in the remaining dose group fed a basal diet containing 2.65% diacylglycerol oil. These changes were not considered biologically significant because of the absence of a dose response and because the tumor incidences in the two control groups were not similar. No treatment-related gross or microscopic changes were observed in other tissues that were evaluated.

Findings relating to 1,2-diacylglycerol-induced activation of PKC are summarized as follows: (1) If the fatty acid in the 1 or 2 position is unsaturated, then the diacylglycerol can activate PKC. 1,2-Diacylglycerols with two saturated fatty acids are less effective (Mori et al., 1982). (2) The stereospecificity of diacylglycerols is critical in terms of their biological activity. 1,2-sn-, but not 2,3-sn-diacylglycerols activate PKC. (3) The activities of different diacylglycerols (i.e., activation of PKC) have been compared. The most important observation was that the activity of saturated 1,2-diacylglycerols was lost when the fatty acid moiety in the structure was a long-chain fatty acid. This effect was not only observed in *in vitro* kinase assays, but also in a cellular model (platelets). When the length of the acyl chain was greater than 14 carbons, the activity was very low or absent. However, the issue of unsaturated 1,2-diacylglycerols potently activating PKC, even if the acyl chain is long, was raised.

An eye shadow containing 1.5% Glyceryl Dilaurate did not induce skin irritation in a single insult, 24 hour patch test involving 20 subjects. Mild skin irritation reactions to a foundation containing 1.5% Glyceryl Dilaurate were observed in 7 of 20 subjects tested according to the same procedure. A trade mixture containing an unspecified concentration of Glyceryl Dibehenate did not induce pathological irritation or significant cutaneous intolerance in a 48 h occlusive patch test involving 8 female and 2 male subjects.

In a maximization test involving 25 subjects, it was concluded that an eye shadow containing 1.5% Glyceryl Dilaurate did not possess a detectable contact-sensitizing potential, and, thus, is unlikely to cause contact sensitivity reactions under normal use conditions. The same conclusion was made for a foundation containing 1.5% Glyceryl Dilaurate in a maximization test involving 27 subjects. Sensitization was not induced in any of 82 subjects patch tested with 50% w/w Glyceryl Dioleate (in liquid paraffin) in a repeated insult, occlusive patch test. Mild erythema was observed in seven subjects during induction. Glyceryl Palmitate Lactate (50% w/v in liquid paraffin) did not induce skin irritation or sensitization in 74 subjects patch tested (Finn chambers) in a modified Draize repeat insult patch test.

Phototoxicity was not induced in a group of ten healthy volunteers tested with a lipstick containing 1.0% Glyceryl Rosinate. Similarly, photoallergenicity was not induced in a group of 26 healthy volunteers patch tested (occlusive patches) with the same product in a repeated insult patch test.

A 35-year-old female subject had sensitization reactions to a foundation containing 1.77% Glyceryl Diisostearate, 1.77% Glyceryl Diisostearate in petrolatum (+ reaction), and lipsticks containing 35.5% Glyceryl Diisostearate. In another case report, an 18-year-old girl with a history of lip cream dermatitis was patch tested (48 h test, Finn chambers) with 35.5% Glyceryl Diisostearate in petrolatum. A strong positive reaction was observed at 48 and 72 h.

DISCUSSION

The available safety test data indicate that glyceryl diesters do not present any significant acute toxicity risk. Nor are these ingredients irritating, sensitizing, or photosensitizing. While no data are available regarding reproductive or developmental toxicity, there is no reason to suspect any such toxicity.

The Panel noted that these nominally glyceryl-1,3-diesters contain significant levels of 1,2-diesters. There is a concern that. 1,2-diesters induce hyperplasia. The Panel also noted that, in the relevant studies, the terminology is "1,2-diacylglycerol" and not glycerol-1,2-diester. Data regarding the induction of protein kinase C and the tumor promotion potential of 1,2-diacylglycerols increased the level of concern. The Panel did note that these compounds are more likely to cause these effects when the fatty acid chain length is ≤ 14 carbons, when one fatty acid is saturated and one is not, and when given at high doses, repeatedly.

While most of the glyceryl diesters have fatty acid chains longer than 14 carbons and none have mixed saturated/unsaturated fatty acid moieties, the significant 1,2-diester content, the high use concentration in lipstick products (up to 43%), and the frequency of application of these products (i.e., several times daily) suggests reason for concern over the potential for 1,2-diester-induced hyperplasia after repeated applications of cosmetic products.

Section 1, paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be enough to justify a determination of safety." Thus, the Expert Panel determined that the following data are needed in order to arrive at a conclusion on the safety of Glyceryl Diesters in cosmetic products.

Dose-response study (topical application of samples of cosmetic grade Glyceryl Dilaurate in a model formulation) to determine whether the concentrations of 1,2-diesters found in Glyceryl Diesters and the frequency of application induce hyperplasia. If 1,2-diester concentrations in cosmetic grade Glyceryl Dilaurate induce hyperplasia, then a long-term, dermal tumor UV initiation-promotion study will be needed.

CONCLUSION

The Expert Panel concludes that the available data are insufficient to support the safety of Glyceryl Dilaurate, Glyceryl Diarachidate, Glyceryl Dibehenate, Glyceryl Dierucate, Glyceryl Dihydroxy-stearate, Glyceryl Diisopalmitate, Glyceryl Diisostearate, Glyceryl Dilinoleate, Glyceryl Dimyristate, Glyceryl Dioleate, Glyceryl Dipalmitate, Glyceryl Dipalmitoleate, Glyceryl Distearate, Glyceryl Palmitate Lactate, Glyceryl Stearate Citrate, Glyceryl Stearate Lactate, and Glyceryl Stearate Succinate for use in cosmetic products.

REFERENCES

Aarhus Oliefabrik. 1997. Technical memorandum. Cremeol FR™. Fractionated vegetable glycerides. Unpublished data submitted by CTFA, February 2, 1999. 21 pages.[1]

Aarhus Oliefabrik. 1998. Monitoring Report. Oil products from Aarhus Oliefabrik A/S. Unpublished data submitted by CTFA, February 2, 1999. 23 pages.[1]

Aarhus Oliefabrik 2001. Letter on Glyceryl Diesters from Jorgen Eriksen to Dr. Carol Eisenmann. Unpublished data submitted by CTFA, June 26, 2001. 1 page[1]

Agin, P.P., J.D. Dowdy, and M.E. Costlow. 1991. Diacylglycerol-induced melanogenesis in Skh-2 pigmented hairless mice. *Photodermatol. Photoimmunol. Photomed.* 8:51-56.

Allan, A.E., M. Archambault E. Messana, and B.A. Gilchrest. 1995. Topically applied diacylglycerols increase pigmentation in guinea pig skin. *J. Invest. Dermatol.* 105:687-692.

Andersen, F.A., ed. 1998. Final report on the safety assessment of Glycolic Acid; Ammonium, Calcium, Potassium, and Sodium Glycolate; Lactic Acid; Ammonium, Calcium, Potassium, Sodium, TEA-Lactate; Methyl, Ethyl, Isopropyl, and Butyl Lactate and Lauryl, Myristyl, and Cetyl Lactate. *Iint. J. Toxicol.* 17(S1):1-242

Aungst, B.J., N.J. Rogers, and E. Shefter. 1986. Enhancement of naloxone penetration through human skin *in vitro* using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. *Int. J. Pharm* .33:225-34.

Biosearch, Inc. 1992a. Human phototoxicity test. Pure Gloss 3402-35 (lipstick, as supplied) containing 1% glyceryl rosinate. Unpublished data submitted by CTFA. 8 pages.[1]

Biosearch, Inc. 1992b. Human photoallergy test. Pure Gloss 3402-35 (lipstick, as supplied) containing 1% glyceryl rosinate. Unpublished data submitted by CTFA. 9 pages.[1]

Blobe, G.C., L.M. Obeid, and Y.A. Hannun. 1994. Regulation of protein kinase C and role in cancer biology. *Cancer Metastasis Rev* 13:411-431.

Blumberg, P.M., G. Acs, L.B. Areces, M.G. Kazanietz, N.E. Lewin, and Z. Szallasi. 1994. Protein kinase C in signal transduction and carcinogenesis. *Prog. Clin. Biol. Res.* 387:3-19.

Bögi, K., P.S. Lorenzo, S. Szállási, P. Ács, G.S. Wagner, and P.M. Blumberg. 1998. Differential selectivity of ligands for the C1a and C1b phorbol ester binding domains of proetin kinase Cdelta: possible correlation with tumor-promoting activity. *Cancer Res.* 58:1423-1428.

Caloca, M.J., M.L. Garcia-Bermejo, P.M. Blumberg, et al. 1999. Beta2-chimaerin is a novel target for diacylglycerol: binding properties and changes in subcellular localization mediated by ligand binding to its C1 domain. *Proc. Natl. Acad .Sci. USA* 96:11854-11859.

Conley, A.J. and J.J. Kabara. 1973. Antimicrobial action of esters of polyhydric alcohols. *Antimicrob. Agents Chemother.* 4:501-6.

[1]Available for review from the Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC, 20036, U.S.A.

Cosmetic Ingredient Review (CIR). 2001a. Final report on the safety assessment of Glyceryl Laurate, Glyceryl Laurate SE, Glyceryl Laurate/Oleate, Glyceryl Adipate, Glyceryl Alginate, Glyceryl Arachidate, Glyceryl Arachidonate, Glyceryl Behenate, Glyceryl Caprate, Glyceryl Caprylate, Glyceryl Caprylate/Caprate, Glyceryl Citrate/Lactate/Linoleate/Oleate, Glyceryl Cocoate, Glyceryl Collagenate, Glyceryl Erucate,Glyceryl Hydrogenated Rosinate, Glyceryl Hydrogenated Soyate, Glyceryl Hydroxystearate, Glyceryl Isopalmitate, Glyceryl Isostearate, Glyceryl Isostearate/Myristate, Glyceryl Isostearates, Glyceryl Lanolate, Glyceryl Linoleate, Glyceryl Linolenate, Glyceryl Montanate, Glyceryl Myristate, Glyceryl Isotridecanoate/Stearate/Adipate, Glyceryl Oleate SE, Glyceryl Oleate/Elaidate, Glyceryl Palmitate, Glyceryl Palmitate/Stearate, Glyceryl Palmitoleate, Gyceryl Pentadecanoate, Glyceryl Polyacrylate, Glyceryl Rosinate, Glyceryl Sesquioleate, Glyceryl/Sorbitol Oleate/Hydroxystearate, Glyceryl Stearate/Acetate, Glyceryl Stearate/Maleate, Glyceryl Tallowate, Glyceryl Thiopropionate, and Glyceryl Undecylenate.. Washington:CIR.[1]

Cosmetic Ingredient Review (CIR). 2001b. Final report on the safety assessment of Trilaurin, Triarachidin, Tribehenin, Tricaprin, Tricaprylin, Trierucin, Triheptanoin, Triheptylundecanoin, Triisononanoin, Triisopalmitin, Triisostearin, Trilinolein, Trimyristin, Trioctanoin, Triolein, Tripalmitin, Tripalmitolein, Triricinolein, Tristearin, Triundecanoin, Glyceryl Triacetyl Hydroxystearate, Glyceryl Triacetyl Ricinoleate, and Glyceryl Stearate Diacetate. Washington:CIR.[1]

Cosmetic, Toiletry, and Fragrance Association (CTFA). 1997. Clinical evaluation report: Human patch test. 1.5% glyceryl dilaurate was used in the product, 08634-10 (eyeshadow). Unpublished data submitted by CTFA. 1 page.[1]

CTFA. 1998. Concentration of use data and specifications from Japanese references. Unpublished data submitted by CTFA, July 9, 1998. 4 pages.[1]

CTFA. 1999a. Use concentration data on gyceryl diesters from industry survey. Unpublished data submitted by CTFA, July 26, 1999. 2 pages.[1]

CTFA. 1999b. Clinical evaluation report: Human patch test. 1.5% glyceryl dilaurate was used in the product, 13023-12 (foundation). Unpublished data submitted by CTFA. 1 page.[1]

Danisco Ingredients. 1996. A modified Draize repeat insult patch test in healthy volunteers to investigate the irritation and sensitization potential of nine cosmetic ingredients of edible quality and a control (liquid paraffin) following repeated cutaneous patch applications (Report No. DANF001). Unpublished data submitted by Danisco Ingredients, February, 1999. 70 pages.[1]

Danisco Ingredients. 1999a. Impurity analysis for glycerol monoesters. Unpublished data submitted by CTFA, August 25, 1999. 20 pages.[1]

Danisco Ingredients. 1999b. Impurity analysis for glycerol monoesters. Unpublished data submitted by CTFA, September 9, 1999. 2 pages.[1]

De Groot, W. Th. 1972. Acyl migration solid phase isomerization of 1,2-diglycerides to 1,3-isomers. *Lipids* 7:626-628.

Elder, R.L., ed. 1982. Final report on the safety assessment of Glyceryl Stearate and Glyceryl Stearate/SE. *J. Amer. Coll. Toxicol.* 1(4):169-192.

Elder, R.L., ed. 1983. Final report on the safety assessment of Isostearic Acid.. *J. Amer. Coll. Toxicol.* 2(7):61-74.

Elder, R.L., ed. 1987. Final report on the safety assessment of Oleic Acid, Lauric Acid, Palmitic Acid, Myristic Acid, and Stearic Acid. *J. Amer. Coll. Toxicol.* 6(3):321-401.

Elder, R.L., ed. 1993. Final report on the safety assessment of Arachidonic Acid. *J. Amer. Coll. Toxicol.* 12(5):481-559.

Enkelund, J. 1999. Information about 1,2-diester levels in glyceryl diesters. Unpublished data submitted by CTFA, July 29, 1999. 2 pages.[1]

European Economic Community (EEC). 2001. EEC Cosmetics Directive 76/768/EEC, as amended, Annexes I through VII. Brussels:EEC.

Federation Of American Societies For Experimental Biology. 1975. Evaluation of the health aspects of glycerin and glycerides as food ingredients. NTIS Report No. PB 254 536.

Fisher, G.J., A. Tavakkol, K. Leach, et al. 1993. Differential expression of protein kinase C isoenzymes in normal and adult psoriatic adult human skin: reduced expression of protein kinase C-βII in psoriasis. *J. Invest. Dermatol.* 101:553-559.

Fitzhugh, O.G., P.J. Schouboe, and A.A. Nelson. 1960. Oral toxicities of lauric acid and certain lauric acid derivatives. *Toxicol. Appl. Pharmacol.* 2:59-67.

Food and Drug Administration (FDA). 2000. C log P values calculated for glyceryl dilaurate and glyceryl disteate. Personal communication with Dr. Robert Bronaugh, FDA.[1]

FDA. 2002. Frequency of use of cosmetic ingredients. *FDA database.* Washington:FDA

Gattefossé. 1998a. Data sheet. Glyceryl dibehenate/tribehenin/glyceryl behenate (Compritol 888 ATO). Unpublished data submitted by CTFA, March 10, 1999, 2 pages.[1]

Gattefossé. 1998b. Glyceryl dibehenate/tribehenin/glyceryl behenate (Compritol 888 ATO). Material safety data sheet according to 91/155 EC. Unpublished data submitted by CTFA, March 10, 1999, 4 pages.[1]

Giron, D., R. Link, and S. Bouissel. 1992. Analysis of mono-, di- and triglycerides in pharmaceutical excipients by capillary supercritical fluid chromatography. *J. Pharm. Biomed. Anal.* 10:821-30.

Go, M., K. Sekiguchi, H. Nomura, U. Kikkawa, and Y. Nishizuka. 1987. Further studies on the specificity of diacylglycerol for protein kinase C activation. *Biochem. Biophys. Res. Commun.* 144:598-605

Hansen, L.A., N.A. Monteiro-Riviere, and R.C. Smart. 1990. Differential down-regulation of epidermal protein kinase C by 12-O-tetradecanoylphorbol-13-acetate and diacylglycerol: Association with epidermal hyperplasia and tumor promotion. *Cancer Res* 50:5740-5745.

Hayakawa, R., K. Matsunaga, M. Suzuki, Y. Arima, and Y. Ohkido. 1987. Lipstick dermatitis due to C18 aliphatic compounds. *Contact Dermatitis* 16:215-219.

Huckle, W.R. and H.S. Earp. 1994. Regulation of cell proliferation and growth by angiotensin II. *Prog. Growth Factor Res.* 5:177-194.

Huls America, Inc. No date. Product information on glyceryl citrate/lactate/linoleate/oleate, glyceryl stearate citrate, glyceryl cocoate, and glyceryl caprylate. Unpublished data submitted by CTFA, 15 pages[1]

Informatics, Inc. 1973. GRAS (Generally Recognized as Safe) Food Ingredients - Glycerine and Glycerides. NTIS Report No. PB-221 227.

Institut D'expertise Clinique. 1996. Verification of the good epicutaneous local tolerance of a cosmetic test article, after a single application to the skin of the back and under occlusive patch for 48 hours, in 10 healthy adult volunteers: "single patch test" (Report No. R60127D). Unpublished data submitted by CTFA, March 10, 1999, 26 pages.[1]

Institut Français de Recherches et Essais Biologiques. 1980. Etude de toxicité aiguë par voie orale chez le rat (Acute oral toxicity study in the rat). Unpublished data submitted by CTFA, March 10, 1999, 4 pages.[1]

International Specialty Products (ISP). 2001. Comments on the composition of glyceryl mono-, di-, and triesters, by Karl Enters and Mark Rerek. Minutes from the 79th CIR Expert Panel meeting, held on June 4-5, 2001.[1]

ISP. 2002. Analytical testing to determine the percentage of 1,2 diester fraction (1,2-dilauryl-s,n-glycerol) in Emulsynt GDL, glycerol laurate, material on an as sold basis. Letter from E. Karl Enters to Gerald McEwen. Unpublished data submitted by CTFA, June 7, 2002, 2 pages.[1]

Inveresk Research International Limited. 1991. Vegetable fats for cosmetic use: A human repeat insult patch test (Report No. 6908). Unpublished data submitted by CTFA, February 2, 1999, 37 pages.[1]

Ivy Laboratories. 1997. The determination of the contact-sensitizing potential of one material by means of the maximization assay (Final Report). Sample: Eye shadow (08634-10). Unpublished data submitted by CTFA, 11 pages.[1]

Ivy Laboratories. 1999. An evaluation of the contact-sensitization potential of a topical coded product in human skin by means of the maximization assay (Final Report). Sample: Foundation (13023-12). Unpublished data submitted by CTFA, 10 pages.[1]

Kabara, J.J., R. Vrable, and M.S.F. Lie Ken Jie. 1977. Antimicrobial lipids: natural and synthetic fatty acids and monoglycerides. *Lipids* 12:753-9.

König, B., P.A. DiNitto, and P.M. Blumberg. 1985. Stoichiometric binding of diacylglycerol to the phorbol ester receptor. *J. Cell Biochem.* 29:37-44.

Lee, J., V.E. Marquez, P.M. Blumberg, K.W. Krausz, and M.G. Kazanietz. 1993. Conformationally constrained analogues of diacylglycerol (DAG) – II. Differential interaction of delta-lactones and gamma-lactones with protein kinase C (PK-C). *Bioor. Med. Chem.* 1:119-123.

Lee, M.W. and D.L. Severson. 1994. Signal transduction in vascular smooth muscle: diacylglycerol second messengers and PKC action. *Am. J. Physiol.* 267:C659-678.

Lee, J., S. Wang, G.W.A. Milne, R. Sharma, N.E. Lewin, P.M. Blumberg, and V.E. Marquez. 1996a. Conformationally constrained analogues of diacylglycerol . 11. Ultrapotent protein kinase C ligands based on a a chiral 5-disubstituted tetrahydro-2-furanone template. *J. Med. Chem.* 39:29-35.

Lee, J., R. Sharma, S. Wang, G.W.A. Milne, N.E. Lewin, Z. Szallasi, P.M. Blumberg, C. George, and V.E. Marquez. 1996b. Conformationally constrained analogues of diacylglycerol. 12. Ultrapotent protein kinase C ligands based on a chiral 4,4-disubstituted heptono-1,4-lactone template. *J. Med. Chem.* 39:36-45.

Lévy, L., V. Alvaro, C. Dubray, and D. Joubert. 1994. Ca(2+)-dependent protein kinase C isoforms in rat pituitary hyperplasia: effect of *in vivo* treatment with quinagolide. *Eur. J. Pharmacol.* 16:327-334.

Lide, D.R. and H.P.R. Frederikse, eds. 1993. *CRC Handbook of Chemistry and Physics. A Ready-Reference Book of Chemical and Physical Data.* 74th ed. Boca Raton:CRC Press, 3-260 to 3-261, 3-502 to 3-504.

Mattson, F.H. and R.A. Volpenhein. 1964. The digestion and absorption of triglycerides. *J. Biol. Chem.* 239: 2772-2777.

MB Research Laboratories, Inc. 1991a. Single dose oral toxicity in rats (Project No. MB 91-425 A). Unpublished data submitted by CTFA, 6 pages.[1]

MB Research Laboratories, Inc. 1991b. Eye irritation in albino rabbits (Project No. MB 91-425 D). Unpublished data submitted by CTFA, 11 pages.[1]

MB Research Laboratories, Inc. 1991c. Primary dermal irritation in albino rabbits (Project No. MB 91-425 C). Unpublished data submitted by CTFA, 7 pages.[1]

Mills, K.J., S.H. Reynolds, and R.C. Smart. 1993. Diacylglycerol is an effector of the clonal expansion of cells containing activated Ha-*ras* genes. *Carcinogenesis* 14:2645-2648.

Ministry of Health, Labor and Welfare (MHLW). 2001a. Unofficial translation of MHW Ordinance No. 331, Attached Table 1 [Negative List]. Ministry of Health Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 2-2,1-chome, Kasumigaseki, Chiyoda-ku, Tokyo 100-8045, Japan.

MHLW. 2001b. Unofficial translation of MHW Ordinance No. 331, Attached Table 2 [Restricted List]. Ministry of Health Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 2-2,1-chome, Kasumigaseki, Chiyoda-ku, Tokyo 100-8045, Japan.

Mischak, H., J. Goodnight, W. Kolch et al. 1993. Overexpression of protein kinase C-delta and -epsilon in NIH 3T3 cells induces opposite effects of growth, morphology, anchorage dependence, and tumorigenicity. *J. Biol. Chem.* 268:6090.

Mori, T., Y. Takai, B. Yu, J. Takahashi, Y. Nishizuka, and T. Fujikura. 1982. Specificity of the fatty acyl moieties of diacylglycerol for the activation of calcium-activated, phospholipid-dependent protein kinase. *J. Biochem.* 91:427-431.

National Academy of Sciences (NAS). 1960. The safety of mono- and diglycerides for use as intentional additives in food (NAS publication no. 251). Washington:NAS.

Niederpruem, H., H. Schweikert, J.W. Thueroff, and K.S. Zaenker. 1995. Inhibition of steroid 5 alpha-reductase activity by aliphatic fatty acids. Candidates for chemoprevention of prostate cancer. *Ann. NY Acad. Sci.* 768:227-30.

Nomura, H., A. Katsuhiko, K. Sekiguchi, U. Kikkawa, and Y. Nishizuka. 1986. Stereospecificity of diacylglycerol for stimulus-response coupling in platelets. *Biochem. Biophys. Res. Commun.* 140:1143-1151.

Pepe, R.C., J.A. Wenninger, G.N. McEwen, Jr., eds. 2002. *International Cosmetic Ingredient Dictionary and Handbook.* 9th ed. Washington:CTFA, 675-677, 683, 686-687.

Pharmakon Europe. 1995. Compritol 888 ATO - Ocular irritatiion test in the rabbit (O.I.) (Report No. 40195). Unpublished data submitted by CTFA, March 10, 1999, 31 pages.[1]

Product Safety Labs. 1987. Emulsifier 287-93A. FHSA primary dermal irritation test in rabbits (Study No. T-6855). Unpublished data submitted by CTFA, 6 pages.[1]

Punnonen, K. and S.H. Yuspa. 1992. Ultraviolet light irradiation increases cellular diacylglycerol and induces translocation of diacylglycerol kinase in murine keratinocytes. *Invest. Dermatol.* 99:221-226.

Reynolds, N.J., H.S. Talwar, J.J. Baldassare, P.A. Henderson, J.T. Elder, J.J. Voorhees, and G.J. Fisher. 1993. Differential induction of phosphatidylcholine hydrolysis, diacylglycerol formation and protein kinase C activation by epidermal growth factor and transforming growth factor-α in normal human skin fibroblasts and keratinocytes [published erratum appears in *Biochem. J.* (1993) 295:903] *Biochem. J.* 294:535-544.

Safepharm Laboratories. 1981. Determination of the contact sensitization potential of product Imwitor 370 (Glyceryl Stearate Citrate) (Experiment No. 310/8102). Unpublished data submitted by CTFA, May 4, 1999, 20 pages.[1]

Saffiotti, U. and P. Shubik. 1963. Studies on promoting action in skin carcinogenesis. *Natl. Cancer Inst. Monogr.* 10:489-507.

Sánchez-Piñera, P., V. Micol, S. Corbalán-Garcia, and J.C. Gómez-Fernández. 1999. A comparative study of the activation of protein kinase C α by different diacylglycerol isomers. *Biochem. J.* 337:387-395.

Scientific & Technical Information Network (STN) International. 1997. Properties of esters of glycerin and lauric acid. *Registry database file.* Columbus:STN International.

Semba, M. and N. Inui. 1991. Inhibitory effects of radical scavengers on diacylglycerol-promoted transformation in Balb/3t3 cells. *Toxicol. Lett.* 56:299-303.

Serdarevich, B. 1967. Glyceride isomerizations in lipid chemistry. *J. Am. Oil Chem. Soc.* 44:381-393.

Setala, K., L. Stjernvall, M. Nyholm, L. Merenmies, and E.E. Miskanen. 1961. Mechanism of experimental tumorigenesis 7. Ultrastructural changes in mouse epidermis caused by glyceryl stearate-type tumor enhancers in acetone and in water. *Acta Pathologica et Microbiologica Scandinavica* 51:305-320.

Sharkey, N.A. and P.M. Blumberg. 1985. Kinetic evidence that 1,2-diolein inhibits phorbol ester binding to protein kinase C via a competitive mechanism. *Biochem. Biophys. Res. Commun.* 133:1051-1056.

Sharkey, N.A. and P.M. Blumberg. 1986 Comparison of the activity of phorbol 12-myristate 13-acetate and the diglyceride glycerol 1-myristate 2-acetate. *Carcinogenesis* 7:677-679.

Sharkey, N.A., K.L. Leach, and P.M. Blumberg. 1984. Competitive inhibition by diacylglycerol of specific phorbol ester binding. *Proc. Natl. Acad. Sci. USA* 81:607-610.

Sharkey, N.A., K.L. Leach, and P.M. Blumberg. 1986. Comparison of the activity of phorbol 12-myristate 13-acetate and the diglyceride glycerol 1-myristate 2-acetate. *Carcinogenesis* 7:677-679.

Sharma, R., J. Lee, S. Wang, G.W.A. Milne, N.E. Lewin, P.M. Blumberg, and V.E. Marquez. 1996. Conformationally constrained analogues of diacylglycerol. 10. Ultrapotent protein kinase C ligands based on a racemic 5-disubstituted tetrahydro-2-furanone template. *J. Med. Chem.* 39:19-28.

Shiseido Research Center. 1977. Safety data of glyceryl diisostearate. Unpublished data submitted by CTFA, June 7, 1999, 4 pages.[1]

Smart, R.C. 2001. Minutes from the 81st CIR Expert Panel meeting, held on November 29-30, 2001.[1]

Smart, R.C., M-T. Huang, and A.H. Conney. 1986. sn-1,2-diacylglycerols mimic the effects of 12-O-tetradecanoylphobol-13-acetate *in vivo* by inducing biochemical changes associated with tumor promotion in mouse epidermis. *Carcinogenesis* 7:1865-1870.

Smart, R.C., M-T. Huang, N.A. Monteiro-Riviere, C-Q. Wong, K.J. Mills, and A.H. Conney. 1988. Comparison of the effect of sn-1,2-didecanoylglycerol and 12-O-tetradecanoylphorbol-13-acetate on cutaneous morphology, inflammation and tumor promotion in CD-1 mice. *Carcinogenesis* 9:2221-2226.

Smart, R.C., K.J. Mills, L.A. Hansen, and A.H. Conney. 1989. Synthetic lipid second messenger *sn*-1,2-didecanoylglycerol: A complete tumor promoter in mouse skin. *Cancer Research* 49:4455-4458.

Soni, M.G., H. Kimura, and G.A. Burdock. 2001. Chronic study of diacylglycerol oil in rats. *Food and Chemical Toxicology* 39:317-329.

Tanaka, M., S. Shimizu, and S. Miyakawa. 1993. Contact dermatitis from glyceryl di-isostearate. *Contact Dermatitis* 29:41-42.

Unichema International. 1997. Isostearate esters of glycerol and polyglycerol : Safety evaluation. Unpublished data submitted by CTFA, May 4, 1998, 8 pages.[1]

Wang, H-Q. and R.C. Smart. 1999 Overexpression of protein kihnase C-α in the epidermis of transgenic mice results in striking alterations in phorbol ester-induced inflammation and COX-2, MIP-2, and TNF-α expression but not tumor promotion. *Journal of Cell Science* 112:3497-3506.

Wender, P.A., K.F. Koehler, N.A. Sharkey, and M.L. Dell'Aquila. 1986. Analysis of the phorbol ester pharmacophore on protein kinase C as a guide to the rational design of new classes of analogs. *Proc. Natl. Acad. Sci. USA* 83:4214-4218.