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[1]

Triesters of glycerin and aliphatic acids, known generically as glyceryl triesters and specifically as Trilaurin, etc., are used in cosmetic products as occlusive skin-conditioning agents and/or nonaqueous viscosity-increasing agents. Hundreds of glyceryl triesters are used in a wide variety of cosmetic products at concentrations ranging from a few tenths of a percent to 46%. Glyceryl triesters are also known as triglycerides; ingested triglycerides are metabolized to monoglycerides, free fatty acids, and glycerol, all of which are absorbed in the intestinal mucosa and undergo further metabolism. Dermal absorption of Triolein in mice was nil; the oil remained at the application site. Only slight absorption was seen in guinea pig skin. Tricaprylin and other glyceryl triesters have been shown to increase the skin penetration of drugs. Little or no acute, subchronic, or chronic oral toxicity was seen in animal studies unless levels approached a significant percentage of caloric intake. Subcutaneous injections of Tricaprylin in rats over a period of 5 weeks caused a granulomatous reaction characterized by oil deposits surrounded by macrophages. Dermal application was not associated with significant irritation in rabbit skin. Ocular exposures were, at most, mildly irritating to rabbit eyes. No evidence of sensitization or photosensitization was seen in a guinea pig maximization test. Most of the genotoxicity test systems were negative. Tricaprylin, Trioctanoin, and Triolein have historically been used as vehicles in carcinogenicity testing of other chemicals. In one study, subcutaneous injection of Tricaprylin in newborn mice produced more tumors in lymphoid tissue than were seen in untreated animals, whereas neither subcutaneous or intraperitoneal injection in 4- to 6-week-old female mice produced any tumors in another study. Trioctanoin injected subcutaneously in hamsters produced no tumors. Trioctanoin injected intraperitoneally in pregnant rats was associated with an increase in mammary tumors in the offspring compared to that seen in offspring of untreated animals, but similar studies in pregnant hamsters and rabbits showed no tumors in the offspring. One study of Triolein injected subcutaneously in rats showed no tumors at the injection site. As part of an effort to evaluate vehicles used in carcinogenicity studies, the National Toxicology Program conducted a 2-year carcinogenicity study in rats given Tricaprylin by gavage. This treatment was associated with a statistically significant dose-related increase in pancreatic acinar cell hyperplasia and adenoma, but there were no acinar carcinomas, the incidence of mononuclear leukemia was less, and nephropathy findings were reduced, all compared to corn oil controls. Overall, the study concluded that Tricaprylin did not offer significant advantages over corn oil as vehicles in carcinogenicity studies. Trilaurin was found to inhibit the formation of neoplasms initiated by dimethylbenzanthracene (DMBA) and promoted by croton oil. Tricaprylin was not teratogenic in mice or rats, but some reproductive effects were seen in rabbits. A low level of fetal eye abnormalities and a small percentage of abnormal sperm were reported in mice injected with Trioctanoin as a vehicle control. Clinical tests of Trilaurin at 36.3% in a commercial product applied to the skin produced no irritation reactions. Trilaurin, Tristearin, and Tribehenin at 40%, 1.68%, and 0.38%, respectively, in commercial products were also negative in repeated-insult patch tests. Tristearin at 0.32% in a commercial product induced transient, mild to moderate, ocular irritation after instillation into the eyes of human subjects. Based on the enhancement of penetration of other chemicals by skin treatment with glyceryl triesters, it is recommended that care be exercised in using them in cosmetic products. On the basis of the available data, the following 23 glyceryl triesters are considered safe as used in cosmetics: 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. Some of these are not currently in use, but would be considered safe if used at concentrations similar to those glyceryl triesters that are in use as cosmetic ingredients.

Received 19 September 2001; accepted 11 October 2001.

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Wilbur Johnson, Jr., Senior Scientific Analyst and Writer. Address correspondence to him at Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

International Journal of Toxicology, 20(Suppl. 4):61–94, 2001

1091-5818/01 $12.00 + .00

INTRODUCTION

The safety of glyceryl triesters, triesters of glycerin and aliphatic acids, is reviewed in this report. These ingredients are used mostly as skin-conditioning agents—occlusive and/or viscosity-increasing agents—nonaqueous in cosmetic products.

Of the ingredients reviewed in this safety assessment, toxicity data are available only on Trilaurin, Triarachidin, Tribehenin, Tricaprylin, Trierucin, Triisostearin, Trilinolein, Trioctanoin, Triolein, Tripalmitin, and Tristearin. Only 11 ingredients in this safety assessment are being used in cosmetics, including Tribehenin, Triisononanoin, Triisostearin, Trilaurin, Trilinolein, Trimyristin, Trioctanoin, Tripalmitin, Tristearin, Glyceryl Triacetyl Hydroxystearate, and Glyceryl Triacetyl Ricinoleate.

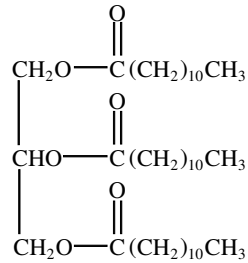
Information is available on the constituent chemicals and on related cosmetic ingredients. Glycerin is classified as a generally recognized as safe (GRAS) food ingredient based on a literature review published in 1973 (Informatics, Inc., 1973). The Cosmetic Ingredient Review (CIR) Expert Panel concluded that the glyceryl triesters Caprylic/Capric Triglyceride (Elder 1980), and Trihydroxystearin (CIR 1997) are safe in the present practices of use and concentration in cosmetics.

CHEMISTRY

Chemical and Physical Properties

Physical and chemical properties of Trilaurin and other glyceryl triesters are included in Table 1. Trilaurin has a saponification value of 261 (Mattson, Baur, and Beck 1951). Descriptions/specifications for cosmetic grade glyceryl triesters are included in Table 2.

Trilaurin (CAS No. 538-24-9) is the triester of glycerin and lauric acid that conforms generally to the formula (Wenninger and McEwen 1997):



It has also been defined as a crystalline glyceride synthesized from palm-nut, coconut, and bayberry oils (Grant 1972). Other names (Wenninger and McEwen 1997; Scientific & Technical

TABLE 1
Physical and chemical properties of Glyceryl Triesters

Properties	Trilaurin*	Tricaprylin**	Trimyristin**	Triolein**	Tripalmitin**	Tristearin**
Form	Needles (obtained from alcohol as solvent)	—	Polymorphic (crystallized from ethanol and diethyl ether)	Polymorphic	Needles obtained from ethanol as solvent	—
Molecular weight	638.97	470.70	768.28	885.47	807.35	891.51
Dipole moment	2.59 D					
Boiling point(s)	15°C, 35°C, and 46.4°C	233.1°C	311°C	235–240°C	310–320°C	—
Melting point	36°C	10°C (stable); −22°C (unstable)	56.5°C (stable); 32°C (unstable)	—	66°C (stable); 44.7°C (unstable)	55°C (α); 73°C (β)
Density	0.8986 at 55°C	0.9540 (density of liquid at 20°C relative to density of water at 4°C)	0.08848 (density of liquid at 60°C relative to density of water at 4°C)	0.8988 g/ml at 60°C	0.8752 (density of liquid at 70°C relative to density of water at 4°C)	0.8559 (density of liquid at 90°C relative to density of water at 4°C)
Refractive index	1.4404 at 60°C	1.4482 at 20°C	1.4428 at 60°C	1.4621 at 40°C	1.4381 at 80°C	1.4395 at 80°C
Solubility	Insoluble in water; soluble in alcohol, ether, chloroform, and petroleum ether; very soluble in acetone and benzene	Soluble in ethanol, diethyl ether, benzene, chloroform, and ligroin (volatile, flammable fraction of petroleum)	Soluble in ether, acetone, benzene, and chloroform	Soluble in ether, chloroform, and petroleum ether	Soluble in ether, benzene, and chloroform	Soluble in acetone

*STN International (1997b, 1997c).
**Lide and Frederikse (1993).

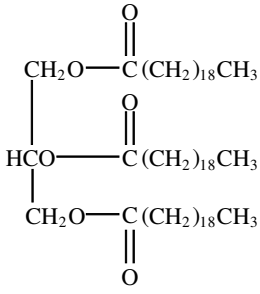
TABLE 2
Descriptions/specifications of Glyceryl Triesters

Properties	Triolein (Nikitakis and McEwen 1990)	Triundecanoin (Nikitakis and McEwen 1990)	Triisostearin (CTFA 1998a)
Form	Colorless to yellowish oily liquid with a slight characteristic odor and taste	Colorless to slightly amber liquid or white to off-white, waxy solid, depending on ambient temperature	Light yellow, oily substance with a faint, characteristic odor
Acid value	5 maximum	10 maximum	Not more than 3
Saponification value	192 to 202	265 to 290	185 to 210
Hydroxyl value	10 maximum	25 maximum	Not more than 30
Moisture	0.3% maximum	—	—
Loss on drying	—	—	Not more than 1%
Residue on ignition	—	—	Not more than 0.5 g
Identification	Positive: Close match to a standard IR spectrum with no indication of foreign materials	Positive: Close match to a standard IR spectrum with no indication of foreign materials	—
Solubility	Practically insoluble in water; slightly soluble in alcohol; soluble in chloroform, ether, and carbon tetrachloride	Soluble in petroleum ether, chloroform, and hot alcohol; insoluble in water	—

Information Network [STN] International 1997a) for this chemical include:

- Dodecanoic Acid, 1,2,3-Propanetriyl Ester
- Glyceryl Trilaurate
- 1,2,3-Propanetriol Tridodecanoate
- Laurin, Tri-
- Glycerin Tridodecanoate
- Glycerin Trilaurate
- Glycerol Trilaurate
- Glyceryl Tridodecanoate
- Lauric Acid Triglyceride
- Lauric Acid Triglycerin Ester
- Lauric Triglyceride
- Tridodecanoin
- Tridodecanoyl Glycerol
- Trilauroylglycerol

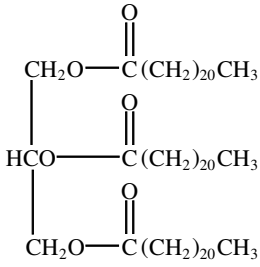
Triarachidin (CAS No. 620-64-4) is the triester of glycerin and arachidic acid (q.v.) that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for Triarachidin include:

- Eicosanoic Acid, 1,2,3,-Propanetriyl Ester
- Glyceryl Triarachidate
- 1,2,3-Propanetriol Trieicosanoate

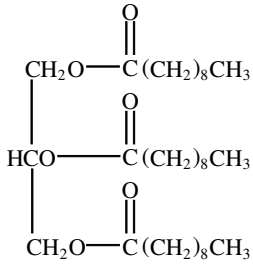
Tribehenin (CAS No. 18641-57-1) is a cream-colored solid with a faint odor (Unichema International 1992). It is the triester of glycerin and behenic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names for Tribehenin include:

- Behenic Acid, 1,2,3-Propanetriyl Ester
- Docosanoic Acid, 1,2,3-Propanetriyl Ester
- Glyceryl Tribehenate
- 1,2,3-Propanetriol Tridocosanoate (Wenninger and McEwen 1997)

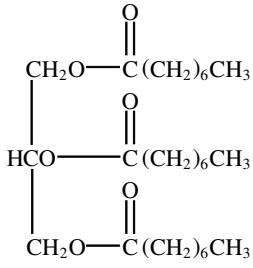
Tricaprin (CAS No. 621-71-6) is the triester of glycerin and capric acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for this chemical include:

- Decanoic Acid, 1,2,3-Propanetriyl Ester
- 3,6,9,12,15,18,21,24,27,30-Decaoxadotriacontan -l-ol, 32-[4-(1,1,3,3,-Tetramethylbutyl)Phenoxy]-
- Glyceryl Tricaprate
- Glyceryl Tridecanoate
- 1,2,3-Propanol Tridecanoate

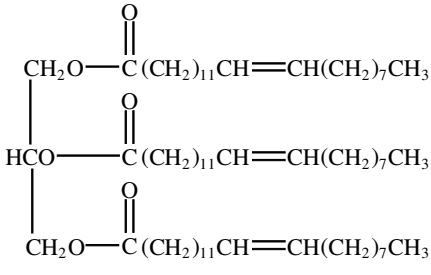
Tricaprylin (CAS No. 538-23-8) is the triester of glycerin and caprylic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Unichema International 1996; Wenninger and McEwen 1997) for Tricaprylin include:

- Caprylic Acid, 1,2,3-Propanetriyl Ester
- Glyceryl Tricaprylate
- Octanoic Acid, 1,2,3-Propanetriyl Ester
- 1,2,3-Propanetriol Trioctanoate
- Glycerol Trioctanoate

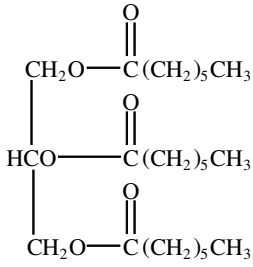
Trierucin (CAS No. 2752-99-0) is the triester of glycerin and erucic acid that conforms to the following formula:



Other names (Wenninger and McEwen 1997) for this chemical include:

- 13-Docosenoic Acid, 1,2,3-Propanetriyl Ester
- Glyceryl Trierucate
- 1,2,3-Propanetriol Tri(13-Docosenoate)

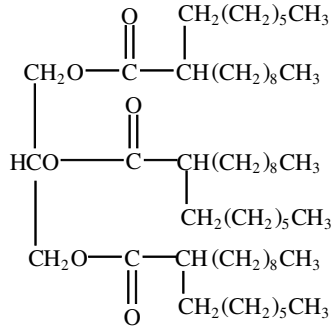
Triheptanoin (CAS No. 620-67-7) is the triester of glycerin and heptanoic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for Triheptanoin include:

- Glyceryl Triheptanoate
- Heptanoic Acid, 1,2,3-Propanetriyl Ester
- 1,2,3-Propanetriol Triheptanoate

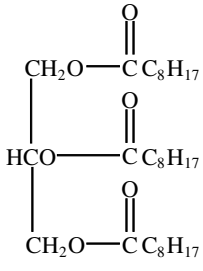
Triheptylundecanoin (CAS No. 105214-66-2) is the triester of glycerin and heptylundecanoic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for this chemical include:

- Glyceryl Triheptylundecanoate
- 2-Heptylundecanoic Acid, 1,2,3-Propanetriyl Ester
- 1,2,3-Propanetriol Triheptylundecanoate
- Triheptylundecanoic Acid, 1,2,3-Propanetriyl Ester
- Undecanoic Acid, 2-Heptyl-, 1,2,3-Propanetriyl Ester

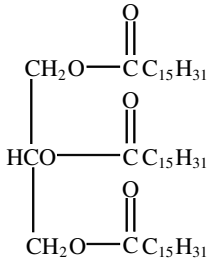
Triisononanoin is the triester of glycerin and a branched-chain nonanoic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for this chemical include:

- 1,2,3-Propanetriol Triisononoate
- Isononanoic Acid, 1,2,3-Propanetriyl Ester

Triisopalmitin (CAS No. 68957-79-9) is the triester of glycerin and a 16-carbon branched-chain aliphatic acid that conforms to the following formula (Wenninger and McEwen 1997):



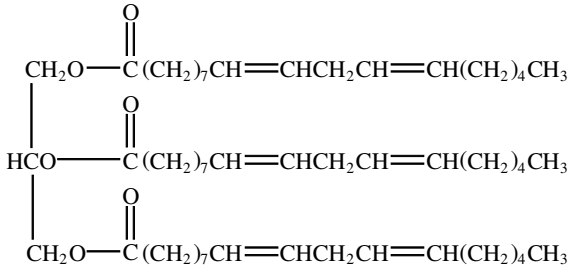
Other names (Wenninger and McEwen 1997) for Triisopalmitin include:

- Glyceryl Triisopalmitate
- Isohexadecanoic Acid, 1,2,3-Propanetriyl Ester
- 1,2,3-Propanetriol Triisohexadecanoate
- Triisohexadecanoic Acid, 1,2,3-Propanetriyl Ester

Triisostearin (CAS No. 26942-95-0) is the triester of glycerin and isostearic acid (q.v.). Other names (Wenninger and McEwen 1997) for this chemical include:

- Glyceryl Triisostearate
- Isooctadecanoic Acid, 1,2,3-Propanetriyl Ester
- 1,2,3-Propanetriol Triisooctadecanoate

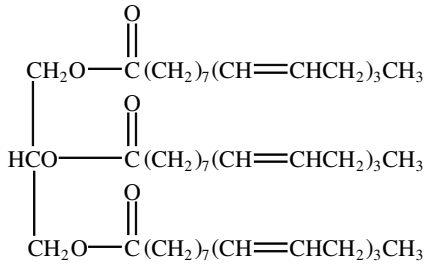
Trilinolein (CAS No. 537-40-6) is the triester of glycerin and linoleic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for this chemical include:

- Glyceryl Trilinoleate
- Linoleic Acid, 1,2,3-Propanetriyl Ester
- 1,2,3-Propanetriol Trilinoleate
- 9,12-Octadecadienoic Acid, 1,2,3-Propanetriyl Ester
- 1,2,3-Propanetriol Tri(9,12-Octadecaienoate)

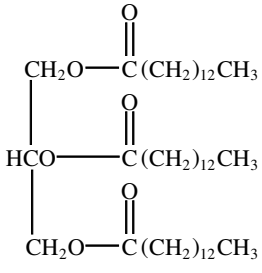
Trilinolenin (CAS No. 14465-68-0) is the triester of glycerin and linolenic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for Trilinolenin include:

- Glyceryl Trilinolenate
- Linolenic Acid, 1,2,3-Propanetriyl Ester
- 9,12,15-Octadecatrienoic Acid, 1,2,3-Propanetriyl Ester
- 1,2,3-Propanetriyl Linolenate
- 1,2,3-Propanetriyl-9,12,15-Octadecatrienoate

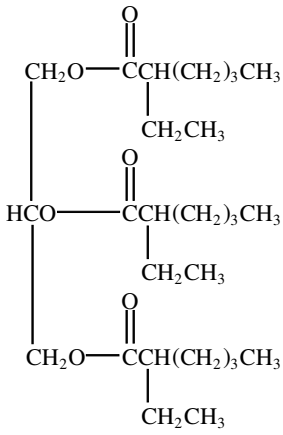
Trimyristin (CAS No. 555-45-3) is present in many vegetable fats and oils, notably in coconut oil and nutmeg butter (Budavari 1989). It is the triester of glycerin and myristic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for this chemical include:

- Glyceryl Trimyristate
- 1,2,3-Propanetriol Tritetradecanoate
- Tetradecanoic Acid, 1,2,3-Propanetriyl Ester

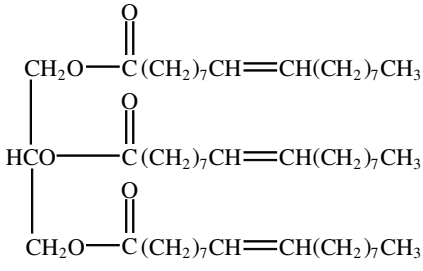
Trioctanoin (CAS No. 7360-38-5) is a colorless to pale yellow, transparent oily liquid with negligible odor (Unichema International 1996). It is the triester of glycerin and 2-ethylhexanoic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for Trioctanoin include:

- 2-Ethylhexanoic Acid, 1,2,3-Propanetriyl Ester
- Glyceryl Tri(2-Ethylhexanoate); Glyceryl Trioctanoate
- Hexanoic Acid, 2-Ethyl-, 1,2,3-Propanetriyl Ester
- Octanoic Acid, 1,2,3-Propanetriyl Ester

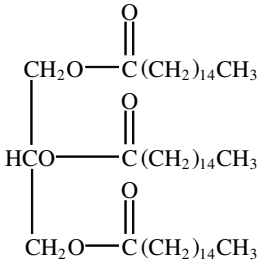
Triolein (CAS No. 122-32-7) is the predominating constituent in expressed almond oil, lard, and many of the more fluid animal oils and those of vegetable origin (Gennaro 1990). It is the triester of glycerin and oleic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for this chemical include:

- Glyceryl Trioleate
- 9-Octadecenoic Acid, 1,2,3-Propanetriyl Ester
- 1,2,3-Propanetriol Tri(9-Octandecenoate)

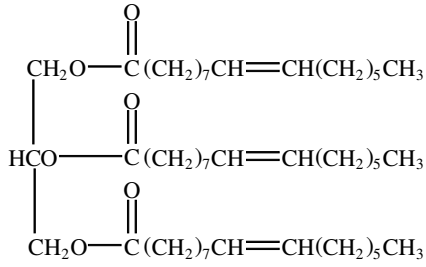
Tripalmitin (CAS No. 555-44-2) predominates in palm oil and coconut oil (Gennaro 1990). It is the triester of glycerin and palmitic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for Tripalmitin include:

- Glyceryl Tripalmitate
- 1,2,3-Propanetriol Trihexadecanoate
- Hexadecanoic Acid, 1,2,3-Propanetriyl Ester

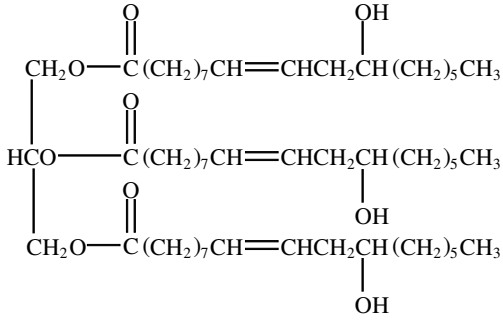
Tripalmitolein (CAS Nos. 20246-55-3 and 129784-33-4) is the triester of glycerin and palmitoleic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for this chemical include:

- Glyceryl Tripalmitoleate
- 9-Hexadecenoic Acid, 1,2,3-Propanetriyl Ester
- Palmitoleic Acid, 1,2,3-Propanetriyl Ester
- 1,2,3-Propanetriol Tri(9-Hexadecenoate)
- 1,2,3-Propanetriol Tripalmitoleate

Triricinolein (CAS No. 2540-54-7) constitutes approximately 80% of castor oil (Lewis 1993). Triricinolein is the triester of glycerin and ricinoleic acid that conforms to the following formula (Wenninger and McEwen 1997):

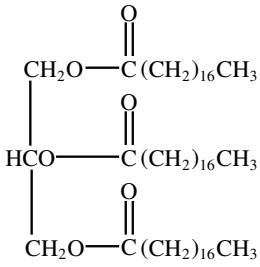


Other names (Wenninger and McEwen 1997) for Triricinolein include:

- Glyceryl Triricinoleate
- 12-Hydroxy-9-Octadecenoic Acid, 1,2,3-Propanetriyl Ester
- 9-Octadecenoic Acid, 12-Hydroxy-, 1,2,3-Propanetriyl Ester
- 1,2,3-Propanetriol Tri(12-Hydroxy-9-Octadecenoate)

Tristearin (CAS No. 555-43-1) is present in many animal and vegetable fats, especially the hard ones (e.g. cacao butter and tallow) (Budavari 1989). It is the triester of glycerin and

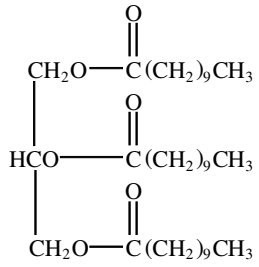
stearic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for this chemical include:

- Glyceryl Tristearate
- Octadecanoic Acid, 1,2,3-Propanetriyl Ester
- 1,2,3-Propanetriol Trioctadecanoate

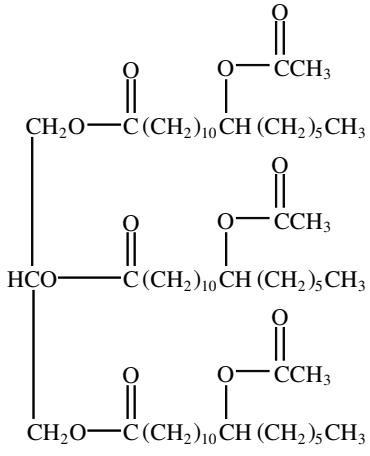
Triundecanoin (CAS No. 13552-80-2) is the triester of glycerin and undecanoic acid that conforms to the following formula (Wenninger and McEwen 1997):



Other names (Wenninger and McEwen 1997) for Triundecanoin include:

- Glyceryl Triundecanoate
- Undecanoic Acid, 1,2,3-Propanetriyl Ester

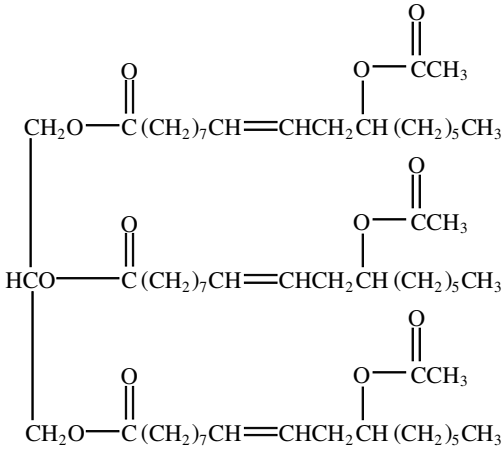
Glyceryl Triacetyl Hydroxystearate (CAS No. 27233-00-7) is the triester of glycerin and acetyl hydroxystearic acid that conforms to the following formula (Wenninger and McEwen 1997):



Two other names (Wenninger and McEwen 1997) for this chemical are:

- Octadecanoic Acid, (Acetyloxy)-1,2,3-Propanetriyl Ester
- Octadecanoic Acid, 12-Hydroxy-, 1,2,3-Propanetriyl Ester

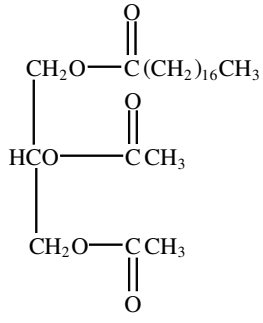
Glyceryl Triacetyl Ricinoleate (CAS No. 101-34-8) is the triester of glycerin and acetyl ricinoleic acid that conforms to the following formula (Wenninger and McEwen, 1997):



Two other names (Wenninger and McEwen 1997) for Glyceryl Triacetyl Ricinoleate are:

- 9-Octadecenoic Acid, 12-(Acetyloxy)-,1,2,3-Propanetriyl Ester
- 1,2,3-Propanetriyl 12-(Acetyloxy)-9-Octadecenoate

Glyceryl Stearate Diacetate is the organic compound that conforms to the following formula (Wenninger and McEwen 1997):



Glyceryl Diacetate Monostearate and Glyceryl Monostearate Acetate are other names for this chemical (Wenninger and McEwen 1997).

Analytical Methods

Trilaurin has been analyzed/identified using the following methods: infrared (IR) spectroscopy (Deman and Deman 1982);

mass spectroscopy (STN International 1997b); nuclear magnetic resonance (NMR) spectroscopy (STN International 1997c); capillary supercritical fluid chromatography (Giron, Link, and Bouissel 1992); nonaqueous reverse phase, high-performance liquid chromatography (Fabien, Craske, and Wootton 1993); and high-performance size-exclusion chromatography (Lubke, Le Quere, and Barron 1996).

Trimyristin has been analyzed by thin-layer chromatography (Frank et al. 1971).

Tricaprin has been analyzed by gas-liquid chromatography (Mingrone et al. 1995).

Tricaprylin has been analyzed by IR and NMR spectroscopy (NTP 1994).

Tribehenin has been analyzed by IR spectroscopy (Abramovici et al. 1991).

Tripalmitin has been analyzed using the thin-layer chromatography/flame-ionization detection system (Rao, Riley, and Larkin 1985) and liquid chromatography–mass spectrometry (Erdahl and Privett 1977).

Trilinolein, Trilinolenin. Triolein, and Tristearin have been analyzed by gas-liquid chromatography (Watts and Dils 1968). Additionally, Triolein has been analyzed using reverse-phase high-performance liquid chromatography (Castilho, Silveira, and Pena 1989) and thin-layer chromatography (Padley 1969). Trilinolein has also been analyzed by reversed phase high performance liquid chromatography (Castilho, Silveira, and Pena 1989).

Methods of Production

Trilaurin may be produced by reacting glycerol with lauric acid or glycerol with lauroyl chloride (reagent: pyridine or quinoline). The reaction of lauric acid with glycerine is another method of production (STN International 1997c).

Triolein may be prepared by the esterification of oleic acid (Budavari 1989).

Tripalmitin can be prepared from glycerol and palmitic acid in the presence of either Twitchell reagent or trifluoroacetic anhydride (Budavari 1989).

Tristearin may be prepared from stearic acid and glycerol in the presence of Al_2O_3 (Budavari 1989).

Triundecanoin is produced by esterification of undecanoic acid and glycerine. The undecanoic acid is produced from castor oil, which is hydrolyzed to fatty acids and subjected to thermal degradation and fractionation. The resulting undecenoic acid is transformed to undecanoic acid and reesterified to the glycerol moiety. Deodorization, the final step, is accomplished using steam to remove components that give rise to unwanted flavors and odors (Karlshamns Sweden AB 1997).

Impurities

Triisostearin contains not more than 20 ppm heavy metals and not more than 2 ppm arsenic (CTFA 1998a).

Triundecanoin contains no impurities or residues of catalysts or solvents. 1,4-Dioxane, ethylene oxide, free amines, and nitrosamines are not added or formed during the production process. Furthermore, volatile compounds are effectively removed, by the deodorization process, below detection limits (0.1 ppm). The deodorization process also has removed any organochlorine or organophosphorus pesticides that may be present in the crude oil used in the production process. It is also important to note that the total content of polycyclic aromatic hydrocarbons (PAHs), if present in the crude oil, is reduced below 10 ppb. Additionally, aflatoxins, if present in the raw materials, are reduced below detection limits (0.5 ppb) by neutralization and bleaching (Karlshamns Sweden AB 1997). The specifications for heavy metals are as follows: As (<0.1 ppm), Cd (<0.001 ppm), Pb (<0.1 ppm), Hg (<0.01 ppm), Cr (<0.05 ppm), Ni (<0.1 ppm), Cu (<0.1 ppm), and Fe (<1.5 ppm) (Karlshamns Sweden AB 1997).

Reactivity

Tripalmitin, Tristearin, and Triolein

Glycerol chlorohydrins and their esters have been identified as products of the hydrolysis of Tripalmitin, Tristearin, and Triolein with hydrochloric acid. Column chromatography and IR, NMR, and mass spectrometry were the analytical methods used. The main reaction products were the corresponding diesters of 3-chloro-propane-1,2-diol, followed by monoesters of 3-chloropropane-1,2-diol and esters of 1,3-dichloropropan-2-ol (Davidek et al. 1980).

After Triolein was heated under simulated deep-fat frying conditions (at 185°C for 72 hours), thermally oxidized Triolein was converted into methyl esters by transesterification using sodium methoxide as a catalyst. The methyl esters were fractionated by urea exclusion. The urea adduct-forming ester, methyl oleate (89.2%), predominated (Paulose and Chang 1978).

Triolein (major skin lipid) was irradiated with 300-nm ultraviolet (UV) light, and the conditions for exposure approximated those at the skin surface exposed to sunlight. Using gas chromatography, the irradiated samples were analyzed for the presence of acrolein, formaldehyde, and acetaldehyde. The maximum amount of acrolein (1.05 nmol/mg Triolein) was formed after 6 hours of irradiation. Maximum amounts of formaldehyde (6 nmol/mg Triolein) and acetaldehyde (2.71 nmol/mg Triolein) were formed after 12 hours of irradiation (Nihati-Shirkhodaee and Shibamoto 1992).

USE

Purpose in Cosmetics

Information on the functions of glyceryl trIesters in cosmetics is summarized in Table 3. The glyceryl triesters are used mostly as skin-conditioning agents—occlusive and/or viscosity-increasing agents—nonaqueous (Wenninger and McEwen 1997).

TABLE 3
Functions of Glyceryl Triesters in cosmetics (Wenninger and McEwen 1997)

Ingredient	Function(s)
Trilaurin	Skin-conditioning agent—occlusive and/or viscosity-increasing agent
Triarachidin	as above
Tribehenin	as above
Tricaprin	as above
Tricaprylin	as above
Trierucin	as above
Triheptanoin	as above
Triheptylundecanoin	as above
Triisononanoin	as above
Triisopalmitin	as above
Triisostearin	as above
Trilinolein	as above
Trimyristin	as above
Triolein	as above
Tripalmitin	as above
Tripalmitolein	as above
Triricinolein	as above
Tristearin	as above
Trioctanoin	Hair conditioning agent; skin-conditioning agent—occlusive
Triundecanoin	as above
Glyceryl Triacetyl Hydroxystearate	Skin-conditioning agent—emollient
Glyceryl Triacetyl Ricinoleate	as above
Glyceryl Stearate Diacetate	Skin-conditioning agent—occlusive; viscosity-increasing agent—nonaqueous

Frequency of use data submitted to the Food and Drug Administration (FDA) in 1998 indicate that only 12 of the 23 ingredients in this safety assessment are being used in cosmetics. These include: Tricaprylin, Tribehenin, Triisononanoin, Triisostearin, Trilaurin, Trilinolein, Trimyristin, Trioctanoin, Tripalmitin, Tristearin, Glyceryl Triacetyl Hydroxystearate, and Glyceryl Triacetyl Ricinoleate. FDA frequency of use data on these ingredients are summarized in Table 4 (FDA 1998).

Concentration of use values are no longer reported to FDA by the cosmetics industry (FDA 1992). However, current concentration of use data received from the cosmetics industry are included in Table 5.

International Use

Eleven of the 23 ingredients reviewed in this report are listed in the *Japanese Comprehensive Licensing Standards of Cosmetics by Category* (*CLS*) (Rempe and Santucci 1997). The following ingredients, which conform to the specifications of the *Japanese Cosmetic Ingredients Codex*, have precedent for use without restriction in all *CLS* categories: Trilaurin, Tribehenin, Trioctanoin, Trimyristin, Tripalmitin, and Tristearin.

Noncosmetic Use

Trilaurin has been detected in pharmaceutical excipients (Giron, Link, and Bouissel 1992).

Tricaprylin has been used as an energy source for burn patients and for patients having difficulty digesting long-chain fatty acids (NTP 1994).

Tristearin has been approved for use as a direct food additive (21 CFR 172.811). Additionally, the following Glyceryl Triesters have been approved for use as components of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food (i.e., use as indirect food additives): Trilaurin, Trimyristin, Triolein, Tripalmitin, Tristearin (21 CFR 177.2800), and Glyceryl Triacetyl Hydroxystearate (21 CFR 178.3505). The following noncosmetic uses of Tristearin have been reported: soap, candles, candies, adhesive pastes, metal polishes, waterproofing paper, textile sizes, leather stuffing, and manufacture of stearic acid (Lewis 1993).

TABLE 4
Product formulation data on Glyceryl Triesters (FDA 1998)

Product category	Total no. of formulations in category	Total no. containing ingredient
Trilaurin		
Eyebrow pencil	91	6
Eyeliner	514	128
Eye shadow	506	9
Other eye makeup preparations	120	2
Other fragrance preparations	148	1
Blushers (all types)	238	1
Foundations	287	25
Lipstick	790	14
Other makeup preparations	135	2
Cleansing skin care preparations (creams, lotions, powders, sprays)	653	2
Body and hand skin care (excluding shaving) preparations (creams, lotions, powders, sprays)	796	4
Moisturizing skin care preparations (creams, lotions, powders, sprays)	769	1
Night skin care preparations (creams, lotions, powders, sprays)	188	1
Other skin care preparations (creams, lotions, powders, sprays)	692	1
1998 Trilaurin totals		**197**
Trilinolein		
Night skin care preparations (creams, lotions, powders, sprays)	188	1
Other skin care preparations (creams, lotions, powders, sprays)	692	1
1998 Trilinolein totals		**2**
Trimyristin		
Eye shadow	506	1
Blushers (all types)	238	4
Face powders	250	5
1998 Trimyristin totals		**10**
Tripalmitin		
Cleansing skin care preparations (creams, lotions, powders, sprays)	653	1
1998 Tripalmitin totals		**1**
Tristearin		
Eyeliner	514	21
Eye shadow	506	1
Other fragrance preparations	148	2
Foundations	287	1
Lipstick	790	4
Cleansing skin care preparations (creams, lotions, powders, sprays)	653	3
Face and neck (excluding shaving) skin care preparations (creams, lotions, powders, sprays)	263	1
Body and hand skin care (excluding shaving) preparations (creams, lotions, powders, sprays)	796	4
Moisturizing skin care preparations (creams, lotions, powders, sprays)	769	3
Paste masks (mud packs)	255	1
Other skin care preparations (creams, lotions, powders, sprays)	692	5
1998 Tristearin totals		**46**
Triisostearin		
Eye shadow	506	1
Lipstick	790	4
1998 Triisostearin totals		**5**
Triisononanoin		
Face and neck (excluding shaving) skin care preparations (creams, lotions, powders, sprays)	263	2
Body and hand skin care (excluding shaving) preparations (creams, lotions, powders, sprays)	796	4
Night skin care preparations (creams, lotions, powders, sprays)	188	1
Other skin care preparations (creams, lotions, powders, sprays)	692	1
1998 Triisononanoin totals		**8**

(Continued on next page)

TABLE 4
Product formulation data on Glyceryl Triesters (FDA 1998) (*Continued*)

Product category	Total no. of formulations in category	Total no. containing ingredient
Tribehenin (Glyceryl Tribehenate)		
Eyebrow pencil	91	1
Other eye makeup preparations	120	2
Hair conditioners (noncoloring)	636	4
Blushers (all types)	238	8
Foundations	287	12
Makeup bases	132	2
Other makeup preparations	135	6
Body and hand skin care (excluding shaving) preparations (creams, lotions, powders, sprays)	796	3
Moisturizing skin care preparations (creams, lotions, powders, and sprays)	769	3
Other suntan preparations	38	1
1998 Tribehenin totals		**42**
Tricaprylin (Glyceryl Tricaprylate)		
Eyebrow pencil	91	2
Eyeliner	514	1
Eye shadow	506	1
Other eye makeup preparations	120	1
Tonics, dressings, and other hair-grooming aids (noncoloring)	549	3
Face powders	250	2
Foundations	287	24
Lipstick	790	15
Makeup bases	132	1
Makeup fixatives	11	1
Other makeup preparations	135	1
Nail polish and enamel removers	34	1
Cleansing skin care preparations (creams, lotions, powders, and sprays)	653	2
Body and hand skin care (excluding shaving) preparations (creams, lotions, powders, and sprays)	796	5
Foot powders and sprays	35	1
Moisturizing skin care preparations (creams, lotions, powders, and sprays)	769	6
Paste masks (mud packs)	255	1
Other skin care preparations (creams, lotions, powders, and sprays)	692	1
Suntan gels, creams, and liquids	136	1
1998 Tricaprylin totals		**70**
Trioctanoin (Glycerol Tris-(2-Ethylhexanoate))		
Eye shadow	506	2
Mascara	167	1
Blushers (all types)	238	1
Foundations	287	1
Lipstick	790	6
Other makeup preparations	135	2
Cleansing skin care preparations (creams, lotions, powders, and sprays)	653	2
Body and hand skin care (excluding shaving) preparations (creams, lotions, powders, and sprays)	796	3
Moisturizing skin care preparations (creams, lotions, powders, and sprays)	769	4
Other skin care preparations (creams, lotions, powders, and sprays)	692	5
1998 Trioctanoin totals		**27**
Glyceryl Triacetyl Hydroxystearate		
Tonics, dressings, and other hair grooming aids	549	1
Lipstick	790	2
1998 Glyceryl Triacetyl Hydroxystearate totals		**3**
Glyceryl Triacetyl Ricinoleate		
Lipstick	790	31
Other skin care preparations (creams, lotions, powders, and sprays)	692	1
1998 Glyceryl Triacetyl Ricinoleate totals		**32**

TABLE 5
Use concentration data on Glyceryl Triesters (CTFA 1998a,* 1998b,** 1998c,*** 1999)

Product type	Maximum use concentrations
Trilaurin	
Other bath preparations	1%
Eyebrow pencil	20%
Eyeliner	5%–36%
Eye shadow	0.003%–46%
Foundations	2%
Lipstick	0.2%–46%
Lip liner	46.3%**
Makeup fixatives	0.8%
Face and neck creams, lotions, powders, and sprays (excluding shaving preparations)	0.4%–3%
Body and hand creams, lotions, powders, and sprays (excluding shaving preparations)	3%
Moisturizing creams, lotions, powders, and sprays	3%
Night creams, lotions, powders, and sprays (excluding shaving preparations)	0.9%
Tribehenin	
Deodorants (underarm)	3%–6%
Suntan gels, creams, and liquids	3%
Eye cream	0.32%*** (not a maximum value)
Lip cream	0.38%*** (not a maximum value)
Hand cream	0.38%*** (not a maximum value)
Tricaprylin	
Blushers (all types)	5%
Face powders	5%
Foundations	0.5%–2%
Other makeup preparations	10%
Face and neck creams, lotions, powders, and sprays (excluding shaving preparations)	2%
Body and hand creams, lotions, powders, and sprays (excluding shaving preparations)	2%
Indoor tanning preparations	2%
Triheptanoin	
Lipstick	12%
Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	15%
Triisononanoin	
Lipstick	25%
Triisostearin	
Lipstick	36%
All product types	<40%*
Trimyristin	
Eye shadow	2%
Powders (dusting and talcum) (excluding aftershave talc)	1%
Blushers (all types)	1%
Face powders	1%
Trioctanoin	
Eyebrow pencil	10%
Eyeliner	17%
Eye shadow	2%–5%
Hair conditioners	0.2%
Tonics, dressings, and other hair-grooming aids	1%
Blushers (all types)	3%
Foundations	7%–14%
Lipstick	46%

(Continued on next page)

TABLE 5

Use concentration data on Glyceryl Triesters (CTFA 1998a,* 1998b,** 1998c,*** 1999) (*Continued*)

Product type	Maximum use concentrations
Makeup bases	4%
Nail polish and enamel	3%
Aftershave lotions	0.2%
Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	50%
Face and neck creams, lotions, powders, and sprays (excluding shaving preparations)	6%
Body and hand creams, lotions, powders, and sprays (excluding shaving preparations)	3%
Moisturizing creams, lotions, powders, and sprays	2%-5%
Night creams, lotions, powders, and sprays (excluding shaving preparations)	8%
Paste masks (mud packs)	0.1%
Suntan gels, creams, and liquids	2%
Indoor tanning preparations	4%
Tripalmitin	
Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	2%
Tristearin	
Eyeliner	2%
Eye pencils	~2%***
Foundations	0.1%–3%
Glyceryl Triacetyl Hydroxystearate	
Lipstick	9%
Glyceryl Triacetyl Ricinoleate	
Lipstick	8%

BIOLOGICAL PROPERTIES

Absorption, Distribution, and Metabolism

The following summary of triglyceride absorption, metabolism, and distribution is included in the NTP (National Toxicology Program) report on the comparative toxicology of corn oil, safflower oil, and tricaprylin (NTP 1994); Johnson et al. (1990) is the original source:

> In the small intestine, most triglycerides are split into monoglycerides, free fatty acids, and glycerol, which are absorbed by the intestinal mucosa. Within the epithelial cells, resynthesized triglycerides collect into globules along with cholesterol and phospholipids and are encased in a protein coat as chylomicrons. Chylomicrons are transported in the lymph to the thoracic duct and eventually to the venous system. The chylomicrons are removed from the blood as they pass through the capillaries of adipose tissue. Fat is stored in adipose cells until it is transported to other tissues as free fatty acids which are used for cellular energy or incorporated into cell membranes. When ^{14}C-labeled long-chain triglycerides are administered intravenously, 25% to 30% of the radiolabel is found in the liver within 30 to 60 minutes, with less than 5% remaining after 24 hours. Lesser amounts of radiolabel are found in the spleen and lungs. After 24 hours, nearly 50% of the radiolabel has been expired in carbon dioxide, with 1% of the carbon label remaining in the brown fat. The concentration of radioactivity in the epididymal fat is less than half that of the brown fat.

In addition to the preceding information, there are also data indicating that, after absorption, long-chain saturated fatty acids are transported mainly via the intestinal lymph as triglycerides. Fatty acids with 10 or less carbon atoms are transported mainly from the intestine via the portal blood vessels. There are also data indicating that unsaturated long-chain fatty acids are absorbed mainly via the lymph vessels (Bergstrom, Blomstrand, and Borgstrom 1954).

It is also important to note that there are data indicating a difference in the rate of metabolism of long- versus medium-chain triglycerides. (Medium-chain triglycerides is the term used to describe one form of neutral lipid, triglyceride that contains fatty acid molecules with a chain length varying from 6 to 12 carbon atoms.) Specifically, in one experiment, ^{14}C-Trioctanoin (8 carbons in fatty acid chain) and ^{14}C-Tripalmitin (16 carbons) were injected into isolated intestinal loops of rats. At 15 minutes after ^{14}C-Trioctanoin injection, mostly all of the lipid remaining in the luminal contents (92%) was present as fatty acid. However, after the injection of ^{14}C-Tripalmitin, only 29% of the residual ^{14}C-labeled lipid had been hydrolyzed to fatty acid (Greenberger, Rodgers, and Isselbacher 1966).

In Vivo Studies

Trilaurin

Three rats were placed under light anesthesia and dosed with olive oil (dose = 0.12 ml per 100 g of body weight) containing Glyceryl Trilaurate-1-C^{14} (0.5 μC$_i$). Doses were administered by gastric probe after 15 to 18 hours of fasting. Cumulative $^{14}CO_2$ elimination curves of the percentage of radioactive carbon dioxide relative to the dose ingested indicated that the amount of $^{14}CO_2$ exhaled over a period of 7 hours was 73%. Furthermore,

the absorption rate for Glyceryl Trilaurate-1-C^{14} was 5.4% per minute and its utilization rate was 0.91% per minute (Metais, Bach, and Warter 1967). Studies conducted prior to 1970 have indicated that triglycerides (e.g., Trilaurin) are metabolized to mono- and diglycerides in the body during the process of fat digestion. Pancreatic enzymes are primarily responsible for hydrolysis of the triglyceride to monoglyceride and free fatty acid, which are absorbed into the intestinal wall and used to resynthesize triglycerides (Kabara 1984). According to another source, the triglycerides are hydrolyzed by intracellular lipases to yield fatty acids and glycerol. Glycerol is converted directly to glucose, whereas the fatty acids are metabolized into two-carbon units that contribute to the formation of citric acid (Informatics, Inc. 1973).

Tricaprin

After Tricaprin was fed to white mice, capric acid (15%) was found in the depot fat along with caprylic acid (trace amounts), lauric acid (as high as 25%), and myristic acid (as high as 17.5%) (Powell 1932).

Tricaprylin

Tricaprylin and Triolein are normal body constituents found in fat cells and in chylomicrons (Bryson and Bischoff 1969).

Triolein, Tripalmitin, and Tristearin

Suzuki et al. (1978) evaluated the percutaneous absorption of Glyceryl Tri-(oleate-1-^{14}C) (a.k.a. ^{14}C-Triolein) using male hr/hr strain hairless mice (average weight = 25 g) and male Hartley guinea pigs (average weight = 340 g). In each experiment, the radioactive oil was applied (0.01 ml on 2.0 cm diameter Japanese papers backed with Lumirror film) to dorsal skin. The oil was applied either undiluted or in a hydrophilic ointment. The hydrophilic ointment had the following composition: 5% ^{14}C-labelled oil, 30% white petrolatum, 15% stearyl alcohol, 12% propylene glycol, 2% sodium lauryl sulfate, and 36% distilled water. Mice were killed at 1, 6, 24, and 48 hours post application, and guinea pigs were killed at 6 and 24 hours post application. Whole body autoradiography was the technique used in the experiment with hairless mice and microautoradiography was used in the experiment with guinea pigs.

As determined by whole body autoradiography, ^{14}C-Triolein (undiluted or in hydrophilic ointment) did not penetrate into the body organs of mice. The oil remained localized at the application site at 48 hours post application. The results of the microautoradiography study using guinea pigs are summarized as follows: After 6 hours, the silver grains were distributed from the stratum corneum to the sebaceous glands. After 24 hours, the grains had spread up to the hair bulges and concentrated considerably in the sebaceous glands. The grains were also observed slightly in the dermis under the basal layer and around the hair follicles and the sebaceous glands (Suzuki et al. 1978).

Rats were fed an emulsion diet (via stomach tube) consisting of 95 parts Triolein (Glycerol Trioleate) and 5 parts Glycerol 1-^{14}C-Trioleate. The percentage of administered Glycerol 1-^{14}C-Trioleate that was identified in the lymph in 24 hours was 88% (Mattson and Volpenhein 1972). In an earlier study four male rats (weights $\approx$ 250 g) were dosed orally with [1-^{14}C]Triolein. The percentage of radioactivity that was absorbed in 24 hours ranged from 57% to 92% (mean = 78.2%). The percentage of absorbed activity that was recovered in the lymph fat from the thoracic duct ranged from 51% to 83% (mean = 65.5%) (Bergstrom, Blomstrand, and Borgstrom 1954).

After a single dose of [1-^{14}C]Triolein was administered intravenously into fasted rats, a high rate of uptake was noted within the first hour in the following organs: liver, myocardium, gastric mucosa, and diaphragm. However, after 24 hours, radioactivity in these tissues had decreased markedly. A similar pattern of distribution was noted in mice; however, large amounts of radioactivity were also noted in the brown fat, white adipose tissue, and spleen, even after 24 hours (Becker and Bruce 1985). In an earlier study, a ^{14}C-oleic acid–labeled Triolein emulsion was administered intravenously to rats. Following injection, an initial rapid drop in the serum concentration of Triolein was noted ($t_{1/2} = 4.5$ minutes). Approximately 95% of the administered dose disappeared within 30 minutes. Radioactivity was increased in the liver (maximum at 10 to 20 minutes), followed by a subsequent decline. As much as 8% of the administered radioactivity was detected in the epidermal fat pads (Procter & Gamble Company 1973).

In another study, rats were fed Triolein in which the fatty acids occupying specific positions in the glyceride molecule had been labeled. The recovery of labeled glycerol and oleic acid and the location of labeled acid in the triglyceride molecules of the lymph were determined. It was concluded that approximately 75% of the glycerol of dietary triglyceride was absorbed as monoglyceride, and 75% of the fatty acids of dietary triglyceride were absorbed as free acids (Mattson and Volpenhein 1964).

The absorption of [I-^{14}C]Tristearin was evaluated using groups consisting of six to seven male Wistar rats (weights = 200 to 250 g). The rats were prepared either with an external bile fistula or a sham operation (control group), and then allowed to recover for 6 to 12 hours. Weighed doses of [I-^{14}C]Tristearin were fed in a pellet of bran. Doses of 25, 50, 100, and 200 mg were administered to four groups, respectively. The rats were killed after 16 hours and lipid from the stomach, small gut, and colon (with feces) was extracted. Absorption was expressed as the percentage of the dose that had left the stomach. Only rats in which 80% or more of the dose had left the stomach were used. Tristearin absorption was classified as poor at all administered doses. Significantly lower absorption of Tristearin was noted only in the 200 mg dose group ($p < .02$, $n = 6$) (Hamilton, Webb, and Dawson 1969). Results for [1-^{14}C]Triolein and [1-^{14}C]Tripalmitin are summarized below:

When groups of rats were dosed with [I-^{14}C]Triolein according to the same procedure, the absorption of 25 mg and

200 mg doses was almost complete (95% and 96%, respectively) (Hamilton et al. 1969).

Sham operated rats (group of seven) were fed 25 mg doses of [I-^{14}C]Tripalmitin in bran pellets. Absorption was expressed as the percentage of that which left the stomach. The mean percentage absorption reported was 70% ± 5% (Hamilton, Webb, and Dawson 1969).

Following the oral administration of a Glycerol-tri(1-^{14}C)-Palmitate (Tripalmitin) emulsion to pregnant female Wistar rats during late gestation, total radioactivity in the plasma increased more rapidly in 20-day pregnant rats than in either 19-day pregnant rats or virgin controls. Four hours after administration of the tracer (peak of plasma radioactivity) most of the plasma radioactivity corresponded to ^{14}C lipids in triglyceride-rich lipoproteins. Also, at 4 hours post administration, the estimated recovery of administered radioactivity in total white adipose tissue, mammary glands, and plasma lipids was greater in pregnant rats than in virgin rats (Argiles and Herrera 1989).

Trilinolein and Trioctanoin

The metabolism of Trilinolein in the rat testis was evaluated using adult male Sprague-Dawley rats (weights = 200 to 225 g). The rats were injected intratesticularly with a 1-^{14}C-Trilinolein emulsion (50 μl). Groups of two to four animals were killed at the following intervals, after which testes were excised: ¼, ½, 1, 3, 6, 12, 24, 36, and 48 hours. Results indicated that radioactive 1-^{14}C-linoleic acid was released from 1-^{14}C-Trilinolein and incorporated throughout the lipid classes. The specific activities and pattern of distribution of the radioactivity indicated that the transformation of linoleic acid between the triglyceride, diglyceride, and fatty acid pools was an equilibrium process. Furthermore, linoleic acid released from 1-^{14}C-Trilinolein was converted to higher polyunsaturated fatty acids that were incorporated throughout the lipid classes, and was catabolized. Evidence for linoleic acid catabolism was the finding of radioactivity in palmitic acid (Nakamura and Privett 1969).

The absorption of intraduodenally administered carboxyl-^{14}C-Glyceryl Trioctanoate was evaluated using 20 normal dogs (controls), 9 pancreatectomized dogs, and 4 pancreatectomized dogs with a thoracic duct fistula. In the 20 control dogs, 26.7% ± 5.2% of the administered radioactivity was recovered as expired $^{14}CO_2$ in 150 minutes. After pancreatectomy, $^{14}CO_2$ recovery rates diminished significantly. Using thin-layer and gas chromatography of lymph collected from four pancreatectomized dogs, results indicated that no 8-carbon fatty acids or glycerides were present. Labeled and unlabeled Trioctanoin were administered to the four pancreatectomized dogs. The researchers stated that the results of these experiments indicate that Trioctanoin absorption was retarded in the absence of pancreatic lipase, but that fractional amounts of the lipid were absorbed via the portal route (Schwabe et al. 1967).

The utilization and distribution of radioactive lipid emulsions were evaluated using three groups of 12 male Sprague-Dawley rats (weights = between 190 and 250 g). The medium-chain triglyceride (MCT), Trioctanoin (glycerol tri-[1-^{14}C]octanoate), and the long-chain triglyceride (LCT), Trilinolein (glycerol tri-[1-^{14}C]linoleate), were the radioactive triglycerides that were used. The composition of the typical LCT molecule includes fatty acids of 16 and 18 carbons in length with trace amounts of larger fatty acids. The fatty acids of the triglyceride fractions of emulsions prepared from MCTs are usually 8 to 10 carbons in length. Group 1 was fed [^{14}C]MCT, and group 2 was fed a 75%:25% (vol:vol) admixture of [^{14}C]MCT:unlabeled LCT lipid emulsion. Group 3 was fed [^{14}C]LCT. Radioactivity (monitored over a 24-hour period) was detected in expired CO_2 and the following body tissues: liver, brain, lungs, heart, muscle, kidneys, epididymal fat, duodenum, plasma, and urine. Study results indicated that the MCT was oxidized more rapidly and completely than the LCT. Approximately 90% of the MCT was converted to CO_2 within 24 hours, compared to 45% of LCT. Following the simultaneous administration of MCT and LCT, the metabolism of MCT was slowed, but remained more rapid than the metabolism of LCT. Additionally, removal of MCT from the blood was more rapid, and tissue radioactivity was lower. The investigators noted medium-chain fatty acids are metabolized more rapidly and completely than long-chain fatty acids because they enter the mitochondria of the liver, heart, and kidneys for oxidation without first being converted to a carnitine transport form (Johnson et al. 1990).

In Vitro Studies

Triolein

The in vitro percutaneous absorption of Triolein through full thickness Skh-1 hairless mouse skin was evaluated using one-chambered static diffusion cells. Skin samples from the mid-dorsal region were mounted on the diffusion cells with the dermal side in contact with the receptor fluid (phosphate-buffered saline [PBS]) and the epidermis open to the atmosphere. Triolein was applied as 10-μl aliquots of 40 μg/ml solutions in ethanol. Gentamicin was added to the receptor fluid to control bacterial growth in this compartment. The receptor fluid was modified during the study by adding other chemicals. The extent of percutaneous absorption, expressed as the mean and standard deviation ($n = 6$) of the percentage of the applied radioactivity recovered in the receptor fluid after 24 hours, of Triolein in PBS + gentamicin was 0.3% ± 0.2%. The percutaneous absorption of Triolein was greatest (4.7% ± 0.8%) in the presence of PBS + gentamicin + bovine serum albumin and least in the presence of PBS + gentamycin + PEG-20 oleyl ether (0.2% ± 0.1%). Thus, the chemical composition of the receptor fluid significantly affected the extent of absorption of Triolein (Moloney 1988).

The metabolism of Triolein in vitro was evaluated using isolated perfusion of a rat liver in tandem with an isolated rat hind end. This permitted the study of lipid transfer between the two. In the absence of added Triolein, a net removal of free fatty acids was demonstrated in both tissue beds when fatty acid

gradients across tissue beds were measured. Following the addition of 100 mg of Triolein (as [^{3}H]-glycerol-[^{14}C]triolein) to either reservoir in the system, an appreciable net production of free fatty acid was noted for the hind end gradient at 30 minutes. This hind-end free fatty acid efflux amounted to more than one third of the catabolism of Triolein. In the presence of Triolein, a fatty acid influx similar to the hind end–generated efflux was noted. Furthermore, after the introduction of radioactive Triolein into the peripheral (hind) end of the system, a significantly greater (compared to injection in liver reservoir) fraction of the recovered lipid ^{14}C radioactivity was detected in the liver tissue. The percentage of recovered lipid detected in peripheral tissues (i.e., muscle, subcutaneous adipose tissue, and epididymal adipose tissue) after 90 minutes of perfusion was similar regardless of the site of Triolein injection into the system. Most of the ^{14}C radioactivity was identified in subcutaneous adipose tissue (Schirmer et al. 1983).

Other Glyceryl Triesters

Hydrolysis of the following synthetic glyceryl triesters by hepatic triacylglycerol lipase in plasma from ICR mice has been demonstrated in vitro: Tricaprylin, Tricaprin, Trilaurin, Trimyristin, Tripalmitin, Tristearin, and Triolein (Masuno and Okuda 1986).

Skin Penetration Enhancement

Tricaprylin and Other Triglycerides

The skin penetration enhancement of drugs by Tricaprylin has been demonstrated in vivo using Wistar rats (Lee et al. 1993) and in vitro using hairless female mice (Lee et al. 1993; Goto et al. 1993). In the study by Goto et al. (1993), the drug permeation ratio in the presence of triglycerides increased in the following order: Tricaprylin (C8) > Triolein (C18) > Tributyrin (C4) > Triacetin (C2).

Cardiac Effects

Trilinolein

Reportedly, Trilinolein reduced infarct size and suppressed ventricular arrhythmias in vivo in male Sprague-Dawley rats (weights = 200 to 300 g) subjected to coronary ligation. Trilinolein was administered intravenously at doses ranging from 10^{-11} to 10^{-7} g/kg. Complete suppression of all ventricular arrhythmias was noted at a dose of 10^{-7} g/kg. The pretreatment of rats with 10^{-7} g/kg 15 minutes prior to the 4-hour coronary ligation resulted in significant reduction of infarct size (Chan et al. 1995).

Effect on Phagocytosis

Tricaprin

Outbred, specific pathogen free–male mice were injected intravenously with a Tricaprin suspension at intervals ranging from 30 minutes to 16 days. The animals were then killed and samples of liver and spleen were prepared for light and electron microscopy. Control mice were injected intravenously with colloidal carbon, and samples of the liver and spleen were examined microscopically at intervals ranging from 15 minutes to 24 hours after injection. Compared to controls, the injection of Tricaprin was followed by an increase in the size of Kupffer cells and the number of lysosomes within them was increased. Additionally, the liver had a heavier and more homogenous distribution of carbon within the lobule, indicating increased phagocytic activity. Microscopically and macroscopically, no differences were found between the spleens of test and control mice. No changes of toxicity were found in the liver or spleen (Stuart and Smith 1975).

Triolein

The effect of Triolein on the monocyte-macrophage system were evaluated using four groups of Wistar female rats (average body weight = 150 ± 20 g). Dosing with Triolein was according to the following schedule: single intravenous (IV) injection with 50 mg/100 g (group A); two IV doses of 25 mg/100 g, separated by 24 hours (group B); single IV dose of 16.7 mg/100 g (group C); and single IV dose of 25 mg/100 g (group D). Phagocytic activity indices were determined over a period of 7 days (at intervals of 24 hours after the last IV injection) by measuring the rate of clearance of 8 mg of colloidal carbon in 1% calf-skin gelatin per 100 g body weight. Compared to untreated controls, the overall phagocytic activity of group A increased 100% within 24 to 48 hours after dosing. In group B, the overall phagocytic activity increased 500% within 24 hours after the second dose. Results for group C indicated a fourfold increase in carbon clearance within 24 hours. This degree of phagocytic stimulation was twice as great as that induced by a single IV dose of triolein (50 mg/100 g) in group A. Results for group D indicated a greater degree of phagocytic stimulation than a dose of 50 mg/100 g (group A); however, in group D, a period of 72 hours was required for reaching peak activity (Altura and Hershey 1970). Triolein has been described as one of the most potent stimulants of macrophages (Mouton et al. 1975).

Pharmacological Effects

Trilaurin

The effect of Trilaurin in the diet on plasma apolipoprotein A-I (apo A-I) and high-density lipoprotein (HDL) cholesterol concentrations was evaluated in two experiments using Watanabe (WHHL) and New Zealand white (NZW) rabbits. In the first experiment, two WHHL rabbits (6 months old) were fed a chow diet supplemented with Trilaurin (6.5% *w/w*) for 4 days. Significant increases in plasma HDL cholesterol and apo A-I concentrations were noted after feeding (250% and 200% of the baseline value for HDL cholesterol and apo-A-I concentrations, respectively). Concentrations of both substances returned to baseline after Trilaurin was removed from the diet. In the second experiment, three WHHL rabbits and three NZW rabbits (10 months old) were fed a chow diet supplemented with Trilaurin (6.5% *w/w*) for 1 week. HDL cholesterol and apo

A-I concentrations increased 50% and 62%, respectively, in WHHL rabbits , and 43% and 31%, respectively, in NZW rabbits ($p < .01$) (Carlson and Kottke 1991).

Tricaprylin

The pharmacological activity of Tricaprylin was evaluated using dogs. Dosing with Tricaprylin resulted in loss of spontaneous movement and a slight increase in the response of the ileum. No effects were observed on the following: respiration, blood pressure, the isolated heart and uterus, electrocardiogram (ECG), auricular vessel, and duration of anesthesia. Effects on behavior and the isolated ileum were noted (Ohta et al. 1970).

Triolein

Following intravenous injection of a 10% Triolein emulsion (4- and 7-ml/kg doses) into cats after bilateral vagotomy, a rise in blood pressure in the pulmonary artery and a slight secondary fall in blood pressure were noted. The elevation in blood pressure had a high degree of tachyphylaxis. These effects were not observed when injections were repeated (Oro and Wretlind 1961).

Effect on Enzyme Activity

Trilaurin

The effect of Trilaurin and various fatty acids and derivatives on 5α-reductase activity in vitro was evaluated because of the established link between prostate cancer and high dietary fat intake. Prostate gland tissue specimens (human) were used. 5α-Reductase catalyzes the reduction of testosterone to dihydrotestosterone, which controls cellular division in the prostate gland. 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 as a result of esterification to Trilaurin (Niederpruem et al. 1995).

Effect on Glucose Production

Tricaprylin

The effect of Tricaprylin on glucose production was evaluated using the isolated perfused rat liver. Tricaprylin (1 mM) stimulated glucose production in the presence of lactate, galactose, or alanine. Tricaprylin (1 mM) also induced a twofold increase in ketogenesis (Ingebretsen and Wagle 1974).

Antioxidant Activity

Trilinolein, Triolein, and Tristearin

The antioxidant activity of Trilinolein, Triolein, and Tristearin has been demonstrated using enhanced chemiluminescence (method of measuring oxygen-derived free radicals) in a reaction mixture consisting of a human polymorphonuclear leucocyte cellular suspension. Trilinolein had the strongest antioxidant activity, followed by Triolein and then Tristearin (Chan et al. 1996).

TOXICOLOGY

To facilitate the comparison of results as a function of the molecular size of the ingredient, the length of the fatty acid carbon chain is included in parentheses after the ingredient name.

Acute Oral Toxicity

Tribehenin (C22)

An acute oral LD_{50} of 5 g/kg (mice) has been reported for Tribehenin. Details concerning the test protocol and study results were not reported (Registry of Toxic Effects of Chemical Substances 1998).

A 40% suspension of Tribehenin in corn oil did not induce toxicity in acute oral toxicity studies involving rats. Details concerning the test protocol and study results were not included (Unichema International 1992).

Tricaprylin (C8)

Acute oral LD_{50} values for Tricaprylin were 34.2 and 29.6 g/kg in male and female mice, respectively. The researchers concluded that Tricaprylin induced very low acute toxic effects in mice (Ohta et al. 1970).

In another experiment in the preceding study, acute oral LD_{50} values for Tricaprylin were 34.2 and 33.3 g/kg in male and female rats, respectively. The researchers concluded that Tricaprylin induced very low acute toxic effects in rats (Ohta et al. 1970).

Trioctanoin (C8)

The acute oral toxicity of Trioctanoin (a.k.a. Glycerol Tris(2-Ethylhexanoate)) was evaluated using ten male, Ichikawaken mice of the ddY strain (weight range = 21.8 to 25.2 g). Each animal received an oral dose of 50 ml/kg, and the LD_{50} was calculated at the end of a 1-week observation period. Suppression of spontaneous movement was observed immediately after test substance administration, and some excretion of the administered dose was observed 20 to 30 minutes later. At 1 to 2 hours post administration, the hair appeared completely wet. On the day after dosing, suppression of spontaneous movement was described as slight and gradual recovery followed. The appearance of the hair also returned to normal. None of the animals died, and it was concluded that the LD_{50} was >50 ml/kg (Sanitary Laboratory Kanagawa Prefecture 1975a).

Tristearin (C18)

The acute oral toxicity of Tristearin was evaluated using ten Sprague-Dawley rats (males and females; weights = 150 to 300 g). None of the animals died during the 14-day period after dosing. The LD_{50} was >20.0 g/kg. At necropsy, none of the animals had gross lesions (Safepharm Limited 1980).

Triisostearin (C18)

A single oral dose (2 g/kg body weight) of Triisostearin did not result in any harmful effects in rats. Details concerning

the test protocol were not provided (Unichema International 1997a).

Acute Intravenous Toxicity

Tricaprylin (C8)

The acute intravenous toxicity of Tricaprylin was evaluated using six groups of 10 mice (strain not stated; weights = 13 to 29 g). Tricaprylin (25% emulsion) was injected into the tail vein of each animal. Motor uneasiness developed immediately after injection, and was followed by spasms in the hind legs, respiratory distress, urination, lateral recumbency, as well as froth at the nose. A mean acute IV LD_{50} of 3,700 ± 194 mg/kg was reported (Wretlind 1957).

A minimum lethal intravenous dose of 4 g/kg for a Tricaprylin emulsion was reported for two groups of male and female mice respectively, and two groups of male and female rats, respectively. The researchers concluded that Tricaprylin induced very low acute toxic effects when administered intravenously to mice and rats (Ohta et al. 1970).

Triolein (C18)

Triolein was injected intravenously into two mongrel dogs (weights = 10 to 15 kg). Injection was repeated at 30, 60, 120, 180, and 240 minutes and after 24 hours. The animals were killed and lungs were prepared for gross and microscopic examination. No changes in the following parameters were noted: lung compliance, arterial gases, platelet counts, prothrombin times, activated partial thromboplastin times, serum lipase, or plasma lactate. Mild tachypnea was noted after 4 hours but not after 24 hours. Hypotension was not observed in either animal. Focal areas of hemorrhage (not extensive) were observed at gross and microscopic examination. Surfactant activity was reduced in hemorrhagic areas. No alterations in any of the parameters studied were reported for the three saline-treated control dogs (Baker, Kuenzig, and Peltier 1969).

Acute Intraperitoneal Toxicity

Tricaprylin (C8)

The minimum lethal dose for Tricaprylin in two groups of male and female mice, respectively, and in two groups of male and female rats (number of animals not stated), respectively was >27.8 g/kg. The authors concluded that Tricaprylin induced very low toxicity when administered intraperitoneally to mice and rats (Ohta et al. 1970).

Acute Subcutaneous Toxicity

Tricaprylin (C8)

The minimum lethal dose of Tricaprylin was determined to be >27.8 g/kg in two groups of male and female mice, respectively, and in two groups of male and female rats, respectively (number of animals not stated). The researchers concluded that Tricaprylin induced very low acute toxic effects when administered subcutaneously to mice and rats (Ohta et al. 1970).

Short-Term Oral Toxicity

Trilaurin (C12), Tristearin (C18), and Triolein (C18)

The short-term oral toxicity of Trilaurin, Tristearin, and Triolein was evaluated using four groups of ten weanling rats, respectively. Each glyceryl ester was administered orally at a concentration of 25% in the diet for a period of 10 weeks. Equal gains in body weight were reported for all groups tested. No lesions were found at necropsy or microscopic examinations that were attributable to administration of test diets (Procter & Gamble Company 1950).

In another study, five weanling albino rats were fed 5% Tristearin in the diet daily for 37 days. Another group of five rats was fed a control diet (no Tristearin). The animals ranged in weight from 62 to 66 g. Growth rates for the two groups were identical. At necropsy, no abnormalities were found that were related to the administration of Tristearin. No significant differences in organ weight were found between the two groups (Hodge 1954).

Tricaprylin (C8)

The short-term oral toxicity of Tricaprylin was evaluated using groups of male and female Wistar rats (7 to 10 per group). The groups were dosed for 31 days with 2, 5, or 10 ml/kg. Compared to controls dosed with distilled water, statistically significant ($.01 < p < .05$) differences in the following clinical chemistry and hematological parameters were noted: urea nitrogen (mg/dl) significantly lower in groups of female rats dosed with 5 or 10 ml/kg, GOT (glutamic oxaloacetic transaminase) activity and erythrocyte counts ($\times 10^4/mm^3$) significantly lower in males dosed with 2 ml/kg, GPT (glutamic pyruvic transaminase) activity significantly lower in females dosed with 10 ml/kg, and leukocyte counts ($\times 10^2/mm^3$) significantly higher in females dosed with 10 ml/kg. Glucose concentration (mg/dl) was significantly greater ($p < .01$) in males dosed with 2 ml/kg (Ohta et al. 1970). Results for changes in organ weights are summarized below:

Compared to distilled water controls, the following statistically significant changes in organ weight were noted: significant reduction in heart weight ($.01 < p < .05$) in males dosed with 2, 5, or 10 ml/kg; significant reduction in spleen weight ($.01 < p < .05$) in males dosed with 5 ml/kg or 10 ml/kg; significant reduction in right and left kidney weights ($p < .01$) in males dosed with 5 ml/kg or 10 ml/kg; significant reduction in left testis weight in 2 ml/kg ($.01 < p < .05$), 5 ml/kg ($p + .01$), and 10 ml/kg ($.01 < p < .05$) dose groups; and significantx reduction in right testis weight in 2 ml/kg ($.01 < p < .05$) and 10 ml/kg ($p < .01$). At microscopic examination, no lesions were found in either test or control groups (Ohta et al. 1970).

Short-Term Subcutaneous Toxicity

Tricaprylin (C8)

Tricaprylin was used as a vehicle control in a study evaluating the short-term subcutaneous toxicity of monocrotaline pyrrole. Ten male and ten female rats of the SPF CSE strain (average

weight = 1200 g) were injected with 0.25 ml Tricaprylin twice a week for 5 weeks (total of 10 injections in right flank, same site). The animals were killed in pairs (one male, one female) 24 hours after the first injection and all subsequent injections. Subcutaneous tissue at the injection site was removed and prepared for histological examination. Initially, subcutaneous injections of Tricaprylin produced a granulomatous reaction characterized by numerous oil deposits surrounded by several layers of macrophages, accompanied by mononuclear cells and a few polymorphs. Additionally, from the third injection until the end of the experiment, fibrous tissue formed around the oil globules and fibroblasts (together with other chronic inflammatory cells) were observed between strands of collagen (Hooson and Grasso 1976).

Short-Term Parenteral Toxicity

Triolein (C18)

Over a period of 1 hour, Triolein (dose = 30% of the LD_{50}, where LD_{50} equals 1.5 times the weight of the animal in kilograms) was infused into the ascending aorta of each of six spontaneously breathing dogs. Four of the six dogs were made hypoxic with a 10% oxygen–90% nitrogen air supply. An additional six dogs were ventilated with a respirator during Triolein infusion. Three control dogs received a slow saline infusion into the ascending aorta. The respiratory rate increased in spontaneously breathing dogs, and two of the four dogs made hypoxic during infusion had a respiratory arrest. Atelectasis, hemorrhage, and interstitial edema were observed in the lungs at biopsy and postmortem specimens of test animals, but not in control animals. Severe intravascular accumulation of fat was noted in sections of the brain, heart, and kidneys stained for fat. However, the accumulation of fat was small in the lungs (Shaffer et al. 1976).

Chronic Oral Toxicity

Trilaurin (C12)

Two groups of rats were fed a mixture consisting of Trilaurin (8%), Glyceryl Dilaurate (45%), and Glyceryl Laurate (40% to 45%) at a concentration of 25% in the diet for 2 years. Thus, the individual glyceryl esters were fed at effective dietary concentrations of ~0.0002% (Trilaurin), ~0.0011% (Glyceryl Dilaurate), and ~0.0010% to 0.0011% (Glyceryl Laurate). Control rats were fed hydrogenated cottonseed oil at a concentration of 25% in the diet. No differences were found in growth rate and lesions between test and control rats. A slight excess of hepatic cell fatty change (compared to controls) was the only microscopic finding in both groups fed the mixture. Details concerning the test protocol and study results were not included (Unichema International 1997b).

Trierucin (C22)

The chronic oral toxicity of Trierucin (Glyceryl Trierucate) was evaluated using 18 male Wistar rats (3 weeks old; weights not stated). The rats were fed a basal diet consisting of 30 cal % Trierucin for 24 weeks. Six and 12 animals were killed by decapitation after 1 week and 24 weeks of dosing, respectively. Six animals were killed (after week 1) to determine any effects on cardiac morphology. Cardiac features were assessed using frozen sections. The remaining 12 rats were killed (after week 24) to determine any effects on renal morphology. Renal morphology was studied using sections obtained from formalin-fixed tissues. Weekly growth and organ weight (heart and kidneys) determinations were also recorded for the remaining 12 rats. Severe cardiac lipidosis was observed in all six rats killed after one week of dosing. Additionally, cardiac lesions (lipidosis and/or focal fibrosis) were observed in the remaining 12 rats that were killed after 24 weeks of dosing. It is important to note that the cardiac lipidosis observed in this group was less severe, compared to rats killed after six weeks. Cardiac fibrosis was observed in all 18 rats. Tubular dilatation, proteinaceous casts or interstitial foci of fibrosis were observed in kidneys from all rats killed after 24 weeks (Abdellatif and Vles 1973).

Tricaprylin (C8)

The chronic oral toxicity of Tricaprylin was evaluated using groups of male Wistar rats (8 to 12 per group). The groups were dosed with Tricaprylin for 26 weeks. Compared to rats dosed with distilled water, significant reductions in GOT activity and hemoglobin concentration were noted in rats dosed with 10 ml/kg. Statistically significant increases in organ weight ($.01 < p < .05$) were reported for the liver (2 ml/kg dose group) and adrenal glands (2-ml/kg and 10-ml/kg dose groups) (Ohta et al. 1970). In another chronic oral toxicity experiment, Tricaprylin caused few lesions in the kidneys, myocardium, and the aorta of Wistar rats (Ohta et al. 1970).

Ocular Irritation

Trilaurin (C12)

The ocular irritation potential of an eyeliner containing 36.3% Trilaurin was evaluated using six rabbits. The test substance was instilled three times in each animal; eyes were not rinsed. Reactions were scored according to the Draize scale (0 to 110). The eyeliner was classified as mildly irritating (Draize score = 2) (CTFA 1984a).

Tribehenin (C22)

Little or no ocular irritation was reported following the instillation of a 20% solution of Tribehenin (in liquid paraffin) into the conjunctival sac of the eyes of rabbits. Details concerning the test protocol and study results were not included (Unichema International 1992).

The ocular irritation potential of an eye enhancer (eye area cosmetic product) containing 0.32% Tribehenin was evaluated using the in vitro chorioallantoic membrane vascular assay (CAMVA). The eye enhancer (cream) was diluted and then tested at concentrations of 10%, 25%, 50%, and 75% using four

groups of 10-day-old fertilized, Dekalb chicken eggs (4 eggs/group), respectively. Undiluted eye enhancer was also tested using an additional group of 10 eggs. According to the protocol, chorioallantoic membranes of the five groups were dosed with the test substance at the end of a 10-day incubation period. The eggs were also incubated for an additional 30 minutes, after which the membranes were observed for signs of vascular hemorrhage, capillary injection, or ghost vessels. The RC_{50} (concentration that elicits a positive response in 50% of the treated eggs) was then determined. The incidence of positive responses (capillary injection and hemorrhage) per test group was as follows: 2 at 10%, 4 at 25%, 6 at 50%, and 10 at 75%. The undiluted cream did not induce positive responses. Based on the RC_{50} value of 35 (confidence limits = 18% to 67%) that was determined, the eye enhancer was not considered an ocular irritant (CTFA No date a, 1998c). A hand cream containing 0.38% Tribehenin also was not considered an ocular irritant when tested in two CAMVAs according to the same test procedure. Details concerning the study results were not included (CTFA No date a, 1998c).

Trioctanoin (C8)

In the Draize test, undiluted Trioctanoin did not induce ocular irritation when instilled (0.05 ml) into the conjunctival sac of the eyes of rabbits. Details concerning the test protocol were not included (Unichema International 1996).

Triisostearin (C18)

Triisostearin was not classified as an ocular irritant in rabbits. No reactions were found in the cornea or iris. Minor irritation reactions that were noted did not persist beyond the 24-hour assessment. Details concerning the test protocol were not provided (Unichema International 1997a).

Skin Irritation

Tribehenin (C22)

The skin irritation potential of Tribehenin (20% solution in liquid paraffin) was evaluated using six albino rabbits. The test solution was applied (0.5 ml, under surgical gauze) to abraded and intact skin sites that had been clipped free of hair. Patches were secured with adhesive tape and the entire trunk of each animal was wrapped with an impervious material (e.g., rubberized cloth), which remained in place for 24 hours. At the time of patch removal (at 24 hours) and 48 hours later, reactions were scored according to the following scales: 0 (no erythema) to 4 (severe erythema [beet redness] to slight eschar formation) and 0 (no edema) to 4 (severe edema, raised more than 1 mm and extending beyond area of exposure). The primary irritation index (PII) was calculated after all scores had been determined. Tribehenin (20% in liquid paraffin) induced mild skin irritation when applied (under occlusive patches) to intact or abraded skin of rabbits for 24 hours (PII = 0.3) (Huntingdon Research Centre 1977).

Trilaurin (C12)

The skin irritation potential of an eyeliner containing 36.3% Trilaurin was evaluated in a single occlusive patch test using nine rabbits. The test substance (0.1 ml) was applied topically under a Whatman filter disc that was secured with Blenderm tape. The entire trunk of each animal was wrapped with a nonabsorbent binder, adhesive tape, and masking tape. The test substance remained in contact with intact skin (clipped free of hair) for 24 hours. Reactions were scored at 2 and 24 hours after patch and test substance removal according to the following Draize scale: 0 (no erythema) to 4 (severe erythema [beet redness] to slight eschar formation [injuries in depth]); 0 (no edema) to 4 (severe edema [raised more than 1 mm and extending beyond area of exposure]). Neither erythema nor edema was observed in any of the rabbits at 2 or 24 hours after patch application. The test substance was classified as nonirritating (CTFA, No date b, 1984b).

Trioctanoin (C8)

The skin irritation potential of Trioctanoin was evaluated using three female Ichikawaken rabbits. The undiluted test substance was applied simultaneously to two sites on shaved skin of the back, by intracutaneous injection (0.1 ml; site at left of spine) and patch application (0.1 ml; site at right of spine), respectively. Liquid paraffin served as the control, and was applied according to the same procedure. Reactions were scored at 3, 24, 48, and 72 hours post administration according to the Draize method. Skin irritation was expressed in terms of the degree of erythema, the area of skin affected, and the area index. Trioctanoin (undiluted) did not induce skin irritation in rabbits when injected intracutaneously or applied topically (Sanitary Laboratory Kanagawa Prefecture 1975b).

Triisostearin (C18)

Undiluted Triisostearin was evaluated in a skin irritation test involving three New Zealand albino rabbits. The test substance was applied (0.5 ml) to a semiocclusive patch that was placed on intact skin of the right flank. Patches were secured with a hypoallergenic, microporous adhesive strip, and the trunk was wrapped with an elastic band (secured with adhesive tape). The dressings were removed at the end of a 4-hour contact period. At ~5 hours and at 24, 48, and 72 hours post application, reactions were scored according to the following scales: 0 (no erythema) to 4 (severe erythema [crimson red] with or without eschar [deep injuries] and lesions showing a serious cutaneous reaction such as a burn, a necrosis) and 0 (no edema) to 4 (severe edema [more than 1 mm thick and extending beyond the area of exposure] showing a serious cutaneous reaction such as a burn). The PII was calculated after all scores had been recorded. Slight erythema was noted in two rabbits at the time of patch removal and persisted to day 5 (both rabbits) and day 6 (1 rabbit) post application. A very slight loss of skin suppleness with reactional dryness was also noted. Edema was not observed. It was concluded that Triisostearin may be considered a nonirritant when

applied to the skin of rabbits (PII = 0.46) (Biogir S.A. Conseil Recherche 1989).

Skin Sensitization

Tribehenin (C22)

Tribehenin did not induce sensitization in a Magnusson-Kligman guinea pig maximization test. Details concerning the test protocol and study results were not included (Unichema International 1992).

Trioctanoin (C8)

The skin sensitization potential of Trioctanoin was evaluated using the Magnusson and Kligman (1969) guinea pig maximization test. The test concentrations used during the study were as follows: 1% (intradermal injection induction), 100% (topical application induction, occlusive patch), and 25% (topical application challenge, occlusive patch). Sensitization was induced in guinea pigs by intradermal injections of the test substance and Freund's complete adjuvant. The induction process was supplemented 6 to 7 days later by application of the test substance to the shoulder injection sites under occlusion for 48 hours. The animals were challenged with the test substance using a 24-hour occlusive patch; further challenges were made at weekly (or longer) intervals as required. A slight response was observed in two guinea pigs during the first challenge. No reactions were noted during the second challenge. Trioctanoin was classified as a non-sensitizer (Environmental Safety Laboratory 1990).

Phototoxicity and Photoallergy

Triisostearin (C18)

The phototoxicity and photoallergenicity potential of Triisostearin was evaluated 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 minutes 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 hours post treatment. With or without UV irradiation, Triisostearin did not induce significant cutaneous reactions. The positive control (8-methoxypsoralen) induced major reactions (Unichema International 1997a).

GENOTOXICITY

Tricaprylin (C8)

The mutagenicity of Tricaprylin in the following *Salmonella typhimurium* strains was evaluated using the Ames preincubation test procedure with and without metabolic activation: TA97, TA98, TA100, and TA1535. Tricaprylin was mutagenic only in strain TA1535 (with metabolic activation) at doses >6666 μg/plate (Zeiger et al. 1996).

The mutagenicity of Tricaprylin was evaluated in a dominant lethal study using T-stock and (C3H × C57BL)F_1 female mice. In the first experiment, 44 T-stock females were used. Forty-seven T-stock females and 37 (C3H × C57BL)F_1 females were used in the second experiment. In both experiments, a single intraperitoneal dose (0.2 ml) of Tricaprylin was administered to female mice of both strains. Over a period of six days post dosing, the females were mated with (C3H × C57BL)F_1 males. Tricaprylin did not induce dominant lethal mutations in female germ cells (Generoso, Cain, and Hughes 1985).

Tricaprylin was used as a vehicle control in a host-mediated mutagenicity assay. The induction of recombinations (mitotic gene conversion), strongly correlated with the induction of mutations, was evaluated. Male BDII rats (weights = 200 g) each received an oral dose of Tricaprylin (3 ml), after which 10^9 to 10^{10} yeast cells (*Saccharomyces cerevisiae* strain D4-RDII) were injected into the intraperitoneal cavity. Strain D4-RDII requires adenine and tryptophan for growth, and gene conversion creates cells that no longer require these two ingredients for growth. The animals were then killed by cervical dislocation and yeast cells withdrawn and cultures incubated for 8 hours. No difference in the spontaneous frequency of convertants of yeasts injected intraperitoneally was found when vehicle control cultures and yeast suspensions that were not injected into rats were compared (Siebert, Bayer, and Marquardt 1979).

Tricaprylin served as the solvent control in a study evaluating the mutagenicity of polyaromatic hydrocarbons in the following three assays: chromosome aberrations assay, micronucleus test, and sister chromatid exchanges assay. Chinese hamsters (between 8 and 20 weeks old) were used. Test procedures and study results are summarized below (Bayer 1978):

In the chromosome aberrations assay, Tricaprylin (1 ml) was injected intraperitoneally into Chinese hamsters and animals were killed 24 hours later. Bone marrow from both femurs was used for chromosome preparations. Chromosome preparations from untreated animals served as controls. The results were pooled because there were no differences between preparations from treated and untreated animals. Of the 3564 bone marrow cells, the incidence of chromosome aberrations was 1.36% (1.3% gaps and 0.06% breaks). The researchers noted that this control value corresponds to the control value achieved in another laboratory (1.33% gaps and breaks) (Bayer 1978).

In the micronucleus test, Tricaprylin (1 ml) was injected intraperitoneally into Chinese hamsters. The animals were killed 30 hours later and bone marrow smears prepared. The number of micronucleated polychromatic erythrocytes was determined. Because no differences were found between preparations from treated (12 hamsters) and untreated animals (12 hamsters), the results were pooled. In each of the 12 animals, at least 2000 erythrocytes were counted and the polychromatic to normochromatic cells ratio was 1:3.93. The control value for micronucleated polychromatic cells was 5.08% (Bayer 1978).

In a sister-chromatid exchanges (SCE) assay, one group of eight Chinese hamsters was pretreated with bromodeoxyuridine (BUdR) and fluorodeoxyuridine (FUdR), and then injected intraperitoneally with 1 ml of Tricaprylin. The eight untreated hamsters were pretreated with BudR and FUdR. Bone marrow was prepared according the procedure used in the chromosome aberrations assay. It was noted that the crucial prerequisite for the in vivo SCE test is the inhibition of thymidine kinase and the incorporation of BudR into the DNA. Because no differences were found between preparations from treated and untreated animals, the results were pooled. Based on pooled results, a control value of 3.2 ± 0.07 SCEs per cell (500 cells studied) was determined (Bayer 1978).

Trilaurin (C12), Triolein (C18), and Tristearin (C18)

The mutagenicity of Trilaurin, Triolein, and Tristearin was evaluated using *S. typhimurium* strains TA1535, TA100, TA1537, TA1538, and TA98, with and without metabolic activation, according to the procedure of Ames, McCann, and Yamasaki (1975). At a test concentration of 4 mg per plate, Trilaurin (in DMSO) and Tristearin (in DMSO) were not mutagenic, with or without metabolic activation, in any of the strains tested. The same was true for Triolein at a test concentration of 1 mg per plate. The test concentrations of 1 mg/plate and 4 mg/plate represented the greatest doses tested due to limits of solubility, which did not allow testing at concentrations great enough to cause lethality. Based on the results of this test, Trilaurin, Triolein, and Tristearin were not mutagenic (Nestmann et al. 1980).

In the micronucleus test procedure by Schmid (1976), the chromosome breaking effects (indicated by appreciable formation of micronucleated polychromatic erythrocytes) of methylmethanesulfonate, benzo(a)pyrene, and chloramphenicol in bone marrow cells were reduced in the presence of Trilaurin. Trilaurin also had an effect on the germ cell genotoxicity of these three chemicals in the dominant lethal test, performed according to the procedure of Generoso (1985). In the presence of Trilaurin, the fertility index of virgin female mice mated with male mice treated with the three genotoxins was increased, and the percentages of dead implants and females with resorptions were reduced. Methylmethanesulfonate, benzo(a)pyrene, and chloramphenicol are known to reduce the fertility index and increase the percentage of dead implants and the number of females with resorptions in the dominant lethal test that was used. It was concluded that Trilaurin was antigenotoxic in bone marrow cells as well as germ cells (Nolasco and Lim-Sylianco 1993).

Triisostearin (C18)

The mutagenicity of Triisostearin (test concentrations up to 5000 μg/plate) with and without metabolic activation was evaluated in the Ames test using the following *S. typhimurium* strains: TA1535, TA1537, TA98, and TA100. Triisostearin was not mutagenic and did not induce toxicity in any of the strains tested (Unichema International 1997a).

Trioctanoin (C8)

The mutagenicity of Trioctanoin was evaluated using the spot test (gene mutation test). The basis of the spot test is the detection of mutant clones of pigment cells arising in mouse embryos, heterozygous for a number of coat color genes, after somatic mutation induced in utero. After fertilization had occurred (time zero) female mice were dosed intraperitoneally (dose = 2.0 ml; vehicle not stated) with the test substance for 17 days. The day of gestation was stated as day 10.25. Trioctanoin was classified as mutagenic in this test (Styles and Penman 1985).

Trioctanoin was not mutagenic when tested at concentrations up to 5000 μg/plate in a bacterial mutagenicity assay using *S. typhimurium* strains. Additionally, no clastogenic activity was observed in an in vitro cytogenetic test assay. In both experiments, details concerning the test protocol were not provided (Unichema International 1996).

Trioctanoin served as a vehicle control in a micronucleus test using 15 male Sch:ICR Swiss mice (8 to 9 weeks old). After intraperitoneal dosing with Trioctanoin (0.5 ml/dose), the three groups of five mice were killed at 30, 42, and 54 hours post dosing, respectively. Slides of bone marrow cells (from femur) were prepared, and the percentage of polychromatic erythrocytes (PCEs) that contained micronuclei determined. One thousand PCEs were scored per mouse. The percentage of PCEs containing micronuclei varied from 0.04 to 0.22 for control mice in two separate experiments. There was no untreated control group for comparative purposes (Lockard et al. 1982).

Trioctanoin also served as the vehicle control in an in vivo SCE assay using eight male Sch:ICR mice (10 to 14 weeks old). Twenty-two hours after intraperitoneal (IP) dosing with Trioctanoin (0.5 ml per mouse), the mice were killed by cervical dislocation. Seven of the eight animals that survived were used. Slides of bone marrow cells (from femur) were prepared, and a total of 175 cells was examined. The numbers of SCEs were counted in 25 metaphase cells from each mouse; each metaphase had 36 to 42 chromosomes. Another control group of five mice, dosed with water (0.2 ml/mouse), was also used in the study. A mean of 5.3 SCEs/cell was reported for mice dosed with Trioctanoin. The five mice dosed with water had a mean of 4.8 ± 0.4 SCEs/cell (total of 125 cells examined) (Lockard et al. 1982).

The intrarectal administration of Trioctanoin (50 mM in saline) into male Sprague-Dawley rats increased the incorporation of tritiated deoxythymidine (^{3}[H]dThd) into colonic DNA. Compared to the control group, there was a $299\% \pm 82\%$ increase in ^{3}[H]dThd incorporation. The researchers noted that many of the chemicals that increase colonic ^{3}[H]dThd incorporation also are known to enhance colonic tumorigenesis (Bull et al. 1983).

Triolein (C18)

In an in vitro differential DNA repair assay using *Escherichia coli*, Triolein reduced nitrosamine-induced DNA damage in the 1 to 10 μg/ml dose range, and in an in vitro liquid preincubation

assay, prevention of the genotoxic activity of nitrosamines by Triolein was also demonstrated (Knasmüller et al. 1993).

CARCINOGENICITY

Tricaprylin (C8)

Tricaprylin served as the vehicle control in a study evaluating the tumorigenicity of manufactured gas plant residue. The vehicle control group and an untreated control group both consisted of 30 female A/J mice (6 weeks old). Both groups of mice were fed NIH-07 pellet diet. Each vehicle control mouse was injected intraperitoneally with Tricaprylin (0.25 ml; single injection). The mice were killed by cervical dislocation at 260 days post injection. The lungs and stomach were removed from each animal and examined microscopically for tumors. Lung tumors were observed in 37% of vehicle controls and in 23% of untreated control mice. Pulmonary adenomas predominated. When the two groups were compared, values for the mean number of tumors per mouse were not significantly different. Gastric tumors involving the squamous portion were not observed in either group (Weyland et al. 1995).

The carcinogenicity of Tricaprylin also was evaluated using three groups of 60 male F344/N rats (average weights $\approx$ 145 g). The three groups received 2.5, 5, and 10 ml of Tricaprylin/kg body weight by gavage 5 days per week for 2 years. Sixty untreated rats (average weight = 146 g) served as controls. Groups of rats were also dosed with corn oil and safflower oil according to the same procedure to evaluate the carcinogenicity of these two oils. Untreated control groups were also used. Groups of 50 rats (instead of 60) were used for the corn oil experiment. After a period of 15 months, 10 rats from each group were selected for interim hematologic evaluations. Rats found in a moribund state, selected for the 15-month interim evaluations or surviving to the end of the 2-year study, were killed by CO_2 asphyxiation. Necropsy and histopathologic evaluation were performed on all animals. The numbers of rats that survived to study termination are listed as follows: 2.5 ml/kg group (30 rats), 5 ml/kg group (31 rats), and 10 ml/kg group (23 rats), and untreated-control group (31 rats). Compared to untreated controls, statistically significant differences in hematocrit (%), hemoglobin (g/dl), and erythrocytes ($10^6/\mu$l) were noted in the 10 ml/kg dose group (15-month interim evaluation). Results relating to incidences of neoplasia are summarized below (NTP 1994).

In addition to untreated controls, 50 saline control rats were used to determine whether 10 ml of gavage fluid/kg could affect the exocrine pancreas. The incidences of exocrine pancreatic hyperplasia (5/50) and adenoma (1/50) were essentially identical to the incidences of hyperplasia and exocrine pancreatic adenoma in the corn oil, safflower oil, and Tricaprylin untreated control groups. The incidence of skin neoplasms was greater in untreated controls (skin tumor incidence = 7 of 50 rats) than in saline controls (skin tumor incidence = 1 of 50 rats). Skin neoplasms included papillomas, trichoepitheliomas, keratoacanthomas, squamous cell carcinomas, and basal cell carcinomas. This finding was not considered biologically significant because no statistically significant differences were found between saline controls and corn oil or safflower oil untreated control groups (NTP 1994).

Results for Tricaprylin are as follows: Compared to untreated controls, a statistically significant dose-related increase in the incidence of pancreatic acinar cell hyperplasia and adenoma was reported for groups dosed with Tricaprylin. Tricaprylin did not induce any acinar cell carcinomas. Additionally, a dose-related decrease (not statistically significant) in the incidence of pancreatic islet cell hyperplasia and adenoma or carcinoma combined was noted in rats dosed with Tricaprylin. The incidence of squamous cell papilloma in the squamous portion of the stomach of rats of the highest dose group (10 ml/kg) was significantly greater when compared to the tumor incidence in untreated controls. Squamous cell papilloma was accompanied by focal to diffuse cell hyperplasia of the nonglandular stomach. The incidence of mononuclear cell leukemia in the 10 ml/kg dose group (9/53, 17% incidence) was much less than that noted for the untreated control group (23/50, 46% incidence). Additionally, compared to untreated controls, both the incidence and severity of nephropathy were diminished in the highest dose group (10 ml/kg) (NTP 1994).

The researchers noted that the results of this study demonstrated that Tricaprylin and safflower oil do not offer significant advantages over corn oil as a gavage vehicle in long-term rodent studies. This is based on results indicating that each of the three caused hyperplasia and adenoma of the exocrine pancreas, decreased incidences of mononuclear cell leukemia, and reduced incidences and severity of nephropathy in male F344/N rats (NTP 1994).

Tricaprylin served as the vehicle control in a study evaluating the neoplastic potential of monocrotaline pyrrole. Two control groups of SPF CFE rats (5 males, 5 females/group; average weight = 100 g) were used. In one of the groups, Tricaprylin (0.2 ml) was injected subcutaneously twice weekly for 30 weeks (60 injections). Dosing was followed by a 36-week nontreatment period. In the second group, injections were made twice weekly for a total of 75 weeks (150 injections). Animals with tumors were killed when their health deteriorated or when the neoplasm became ulcerated. Of the 20 rats treated, tumors were observed in 2 animals (at 50 and 61 weeks, respectively). Both tumors were sarcomas arising from the connective tissue at the injection site. According to the investigators, the occurrence of tumors in control rats was unexpected (Hooson and Grasso 1976).

Tricaprylin also served as a vehicle control in a study evaluating the carcinogenicity of the pesticide, maleic hydrazide. Tricaprylin was injected subcutaneously into 61 newborn mice (of 16 litters) in volumes of 0.1, 0.1, 0.2, and 0.2 ml, on days 1, 7, 14, and 21 after birth, respectively. The results were reported based on the number of survivors at the time that the first tumor was observed (23 males, 22 females injected with Tricaprylin). There were 47 male and 47 female survivors in the

untreated control group. Sixteen and 14 tumors were reported for the 23 male and 22 female survivors injected with Tricaprylin, respectively. In both males and females, most of the tumors were found in the lymphoid tissues. Tumors of the lung and liver were observed in male mice, but were not observed in females. Of the Tricaprylin-treated mice that survived (23 males, 22 females) the percentages of males and females with tumors were 60.9% and 59.1%, respectively. In untreated controls (47 male and 47 female survivors), the percentages of males and females with tumors were 51.1% and 42.6%, respectively (Cabral and Ponomarkov 1982).

In a study evaluating the carcinogenicity of di- and trifunctional α-chloro ethers and 1,4-Dichlorobutene-2 in ICR/Ha female Swiss mice (4 to 6 weeks old) , Tricaprylin (vehicle), and untreated controls were used. Tricaprylin was injected either subcutaneously or intraperitoneally weekly for 502 to 569 days (depending on level of survival). With the exception of the cranial region, all mice were necropsied either at the end of the experiment or at the time of interim death. Tissues were subjected to histopathological evaluation. In the subcutaneous injection experiment (left flank; 0.05 ml weekly), the vehicle control group consisted of 50 mice and the untreated control group consisted of 85 mice. No tumors were observed in untreated control mice or mice injected subcutaneously with Tricaprylin. In the intraperitoneal injection experiment (lower abdomen; 0.05 ml once weekly), the vehicle-control group consisted of 30 female mice and the untreated control group consisted of 85 female mice. No tumors were observed in untreated controls or mice injected intraperitoneally with Tricaprylin (Van Duuren, Goldschmidt, and Seidman 1975).

In addition to the preceding study, Tricaprylin has been used as a negative/solvent control in a number of carcinogenicity/cocarcinogenicity or tumorigenicity studies (Fugi and Epstein 1979; Prahalad et al. 1997; Nesnow et al. 1994). An untreated-control group was not used in either of these studies.

Trioctanoin (C8)

Trioctanoin was used as the vehicle control in a study evaluating the carcinogenic activity of polycyclic hydrocarbons. The control group consisted of 10 male hamsters (weights = 55 to 75 g). Trioctanoin (0.4 ml) was injected subcutaneously (single injection) into the nape of the neck of each animal. No tumors were reported over a period of 17 weeks (Wodinsky, Helinski, and Kensler 1964).

Trioctanoin also served as the vehicle control in another carcinogenicity study using Syrian golden hamsters. The focus of this study was the induction of respiratory tract tumors in Syrian golden hamsters by a single dose of 4-(methylnitrosamino)-1-(3-pyridyl)-1-(3-pyridyl)-1-butanone (NNK) and the effect of smoke inhalation. Respiratory tract tumors were not observed in two groups of hamsters (10 males, 10 females/group) injected subcutaneously with Trioctanoin (single injection at 8 weeks) and subjected to sham smoking in the 69 week study (Hecht et al. 1983).

In another study, differences in the incidence of mammary tumors between the offspring of pregnant COBS SD rats injected intraperitoneally with Trioctanoin (1 ml/kg on days 16 and 17 of gestation) and untreated control offspring were determined. The percentage of Trioctanoin-treated female offspring with mammary tumors was 43% (9 of 21 females), and the percentage of untreated female offspring with mammary tumors was 29% (10 of 35 females). In this study, Trioctanoin served as the vehicle control in a study evaluating the enhanced development of mammary tumors in rats following transplacental and neonatal exposure to ethylnitrosourea (Mandybur, Ormsby, and Buncher 1978).

No tumors were observed in the offspring of vehicle-control groups of pregnant Sendai virus-free Syrian Golden hamsters that received a single subcutaneous injection of Trioctanoin (40 males, 42 females, day 15 of gestation) or three subcutaneous injections of Trioctanoin (43 males, 40 females, last 3 days of gestation). This study was concerned with the transplacental carcinogenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (Correa et al. 1990).

The intraperitoneal administration of Trioctanoin to two strains of pregnant rabbits (IIIVO/J and WH/J) did not induce tumors in any of the offspring. Eighteen does of the WH/J strain and 10 does of the IIIVO/J strain were dosed over a 10-day period. In this study, the transplacental carcinogenic potential of *N*-Ethyl-*N*-nitrosourea (ENU) was evaluated, and Trioctanoin served as the vehicle control. Doses of ENU (10 mg/kg/day) were injected over a 10-day period, and equal volumes of Trioctanoin were administered to controls (Fox et al. 1980).

Triolein (C18)

Triolein served as a negative control in a carcinogenicity study involving rats. Ten control rats were injected subcutaneously with Triolein (0.2 to 0.5 cc, in groin). No tumors were observed at the injection site over a period of 540 days (Burrows, Hieger, and Kennaway 1936).

Cocarcinogenicity

Triolein (C18)

The cocarcinogenicity of Triolein was evaluated using groups of 33 castrated male Marsh mice and groups of 28 intact male BALB/c mice. Groups of Marsh mice were injected subcutaneously with 6β-hydroxyperoxy-4-cholesten-3-one (in Triolein or sesame oil) and with sesame oil (control). Groups of BALB/c mice were injected with 6β-hydroxyperoxy-4 - cholesten-3-one in either Triolein, sesame oil, or 2% Balb serum, or with either Triolein or sesame oil (controls) alone. Comparisons between groups were made up to age 19 months. In Marsh mice, 6β-hydroxyperoxy-4-cholesten-3-one (5 mg) in sesame oil and Triolein produced 9% and 18% sarcomas, respectively. In Balb/c mice, 6β-hydroxyperoxy-4-cholesten-3-one (10 mg) alone did not produce local sarcomas, but caused 7% local hemorrhagic cysts when tested in sesame oil. Tumors were not observed in any of the groups (both strains) injected with Triolein or

sesame oil alone. In another comparison, 6β-hydroxyperoxy-4-cholesten-3-one did not increase the incidence of lung adenomas in Marsh mice over that observed in Triolein and sesame oil control groups. However, in Balb/c mice, the incidence of 6β-hydroxyperoxy-4-cholesten-3-one (in saline)-induced lung adenomas (39%) was significantly greater when compared to Triolein and sesame oil controls (Bryson and Bischoff 1964).

The researchers in the preceding study added that, to date, 6β-hydroxyperoxy-4-cholesten-3-on e in sesame oil, cottonseed oil, and/or Triolein has produced sarcomas in Marsh and C57 mice and in Evans rats, but not in Swiss and Balb mice. Additionally, 6β-hydroxyperoxy-4-cholesten-3-one , administered as an isotonic aqueous suspension, did not produce neoplasms in Marsh, Balb, or Evans strains. The investigators also stated that, when effective, Triolein (a major constituent of the oils tested) apparently acts as the local cocarcinogenic factor (Bryson and Bischoff 1964).

Tumor Inhibition

Trilaurin (C12)

The effect of Trilaurin on the promotion stage of carcinogenesis was evaluated using groups of ten Swiss Webster mice. In the test group, the back of each mouse was shaved and dimethylbenzanthracene (DMBA) (250 μg/0.25 ml) was applied to the shaved area 3 days later. Croton oil (0.03% in acetone) was applied to the same site 3 days after DMBA application, and Trilaurin was brushed on three minutes later. Applications of each chemical were made three times per week for 20 weeks, after which the animals were killed by cervical dislocation. Four additional groups were treated with DMBA (alone), croton oil (alone), DMBA + croton oil, and DMBA + Trilaurin, respectively, for the same duration. Cutaneous neoplasms and neoplasms of the internal organs were recorded, and the incidence of neoplasms determined. After 20 weeks of exposure, no neoplasms were found in the groups treated with DMBA (alone) and croton oil (alone), respectively, and all mice in the group treated with DMBA + croton oil had neoplasms. Trilaurin completely inhibited the formation of neoplasms (0% incidence) initiated by DMBA and promoted by croton oil. No neoplasms were observed in the group treated with DMBA + Trilaurin (Nolasco et al. 1994).

Tricaprylin (C8)

Inbred Nb rats with implants of Nb2 lymphoma (liver implantation) were treated orally with two 150-mg doses of Tricaprylin. Extensive damage to tumor cells was evident microscopically 4 to11 hours after implantation; hepatocytes were unaffected. On day 17, nuclei were pyknotic and angular, and cells were not in close contact (Burton 1991).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Tricaprylin (C8)

Two groups of 20 female mice received oral doses of 2 ml/kg and 10 ml/kg Tricaprylin, respectively, during gestation. Of the 220 live fetuses from the 2-ml/kg dose group, the following six were malformed: cleft palate (1 fetus), club foot (3 fetuses), and assimilation of the ribs (2 fetuses). Of the 219 live fetuses from the 10-ml/kg group, the following 8 were malformed: cleft palate (3 fetuses), club foot (4 fetuses), and assimilation of the cervical vertebrae (1 fetus). Curled tail (1 fetus), cleft palate (1 fetus), and club foot (1 fetus) were the only malformations reported for 3 of 220 live control fetuses. The investigators concluded that Tricaprylin was not teratogenic in mice (Ohta et al. 1970).

In another experiment from the above study, eight female mice were dosed orally with Tricaprylin during gestation. No malformations were reported for any of the 61 live fetuses (Ohta et al. 1970).

Tricaprylin was effective in producing fusion of the endometrial epithelium (symplasma formation) and decidualization of the stroma in pseudopregnant New Zealand white rabbits. (With this in mind, the investigators noted that many of the oils used as vehicles for fat-soluble materials, such as the steroidal sex hormones, have significant estrogenic activity.) On day 0, Tricaprylin (0.1 ml) was injected into isolated segments of the uterus in which pseudopregnancy had been induced by IV injection of human chorionic gonadotropin (HCG). The animals were killed as groups of three on days 1, 2, 3, 4, and 6. Saline, simple ligation of the uterus or uterine trauma served as control treatments in other uterine segments in the same animals. Trauma and ligation with saline, but not ligation alone, induced formation of symplasma. Decidualization was observed after trauma but not after ligation or saline injection alone. Compared to control treatments, Tricaprylin was much more effective in inducing symplasma formation. Symplasma, most typically observed in the rabbit, has been specifically described as a fusion of originally columnar cells into large, multinucleated cells with intensely acidophilic cytoplasm. According to the researchers, it is usually found at the implantation site and covering areas of decidua at the margin of the placenta, as well as in areas of decidualization induced by trauma or other artificial means (Davies and Davenport 1979).

Tricaprylin was used as a vehicle control in a study evaluating the developmental toxicity of dichloroacetonitrile. Tricaprylin (dose not stated) was administered orally to pregnant female Long-Evans hooded rats (65 to 80 days old) on days 6 to 18 of gestation. Another control group was dosed with water according to the same procedure. Pregnant females were killed on day 20 of gestation. No statistically significant differences were found in reproductive parameters between Tricaprylin and water control groups (Smith et al. 1989).

Trioctanoin (C8)

The developmental toxicity of Trioctanoin was evaluated using time-mated, female specific-pathogen-free CD-1 mice (6 to 8 weeks old). Nonpregnant mice were dosed orally for 8 consecutive days in a dose-finding study; the LD_{10} was used in the reproductive phase. In this phase of the study, time-mated mice received oral doses of 4750 mg/kg/day (dose

volume = 5 ml/kg, corn oil vehicle) on gestation days 6 to 13 and were allowed to deliver litters. Litter size, birth weight, and neonatal growth and survival to postnatal day 3 were recorded as indices of potential developmental toxicity. The proportion of pregnant survivors that delivered a viable litter (at least one liveborn pup) was compared with the concurrent vehicle control (corn oil) using a one-tail Fisher's exact test. For mice that delivered a viable litter, the following were analyzed by pairwise multiple comparisons of control and treated groups using the two-tail Mann-Whitney *U* test: the number of liveborn pups per litter, percent neonatal survival to postnatal day 3, average pup weight at birth, and average pup weight gain by postnatal day 3. Study results indicated no significant differences in any of the parameters evaluated between pregnant mice dosed with Trioctanoin and corn oil–treated controls (Hardin et al. 1987).

Trioctanoin was used as a vehicle control in a study evaluating the teratogenicity of 1-ethyl-1-nitrosourea. The following strains of pregnant mice (mated at 10 weeks of age) were tested: AKR/J, SWR/J, DBA/2J, C57Bl/6J, and C57L/J. In test groups, 1-ethyl-1-nitrosourea was injected intraperitoneally (0.5 mmole/kg body weight) on days 8 and 12 of gestation. Control groups received an equivalent volume of Trioctanoin. Various kinds of eye abnormalities were observed in 6.2% of 291 control fetuses of the five strains studied; other malformations were also observed. However, no untreated-control group or historical control data were used for comparative purposes (Diwan 1974).

Trioctanoin was used as the vehicle control in a sperm abnormality test. Ten male control mice ($B6C3F_1$/Hap hybrid strain; 10- to 12-week-old) received an intraperitoneal injection of Trioctanoin (0.25 ml per injection) daily for 5 days. After day 35, the animals were killed, caudae epididymides removed, and slides of sperm preparations made. The percentage of abnormal sperm in 500 sperm per animal was determined to be $4.5\% \pm 1.7\%$. No untreated controls or historical control data were used for comparative purposes (Lockard et al. 1982).

CLINICAL ASSESSMENT OF SAFETY

Metabolism

Tricaprin (C10)

Following the oral administration of Tricaprin to three human subjects, a considerable amount of sebacic acid (C10) was isolated from the urine along with smaller quantities of suberic acid (C8) and adipic acid (C6) (Verkade and vander Lee 1934).

Trioctanoin (C8)

[^{13}C]-Trioctanoin was administered orally to five normal term neonates and five growing preterm infants. ^{13}C enrichment in carbon dioxide was analyzed using isotope ratio mass spectrometry; oxidation rates over 6 hours were calculated. The peak for ^{13}C appearance was between 120 and 240 minutes post administration in preterm infants and between 90 and 180 minutes post administration in full-term infants. Oxidation rates for [^{13}C]-Trioctanoin were $46.2\% \pm 3.6\%$ in preterm infants and $53.5\% \pm 13.8\%$ in normal neonates. The difference between these two values was not statistically significant. Study results indicated that Trioctanoin was utilized sufficiently, even in the neonatal period, during which energy intake is restricted by immaturity of digestive or excretory function (Hoshi et al. 1992).

Triolein (C18)

Eight adult subjects (ages 21 to 51) were fed 10 μCi [^{14}C]-Triolein in 5 g olive oil together with a standard breakfast. The collection and assay of expired air began 1 hour after dosing and continued until lunch time. The maximum rate of excretion of Glyceryl-Trioleate-1-^{14}C as $^{14}CO_2$ in expired air occurred 5 to 6 hours after the beginning of the experiment (Blomstrand and Kager 1973).

In another study, five adult subjects (ages 31 to 63) were fed a breakfast containing 20 μCi [^{14}C]-Triolein. The quantity of expired $^{14}CO_2$ was estimated from measurements of specific activity of $^{14}CO_2$ in duplicate samples of breath over a period of days, the last of which occurred at 28 days post ingestion. At day 1, 15% to 33% of the ingested dose was expired, and 25% to 40% of the dose was expired at 10 days post-ingestion. From day 10 on, $^{14}CO_2$ was expired at a slow, but constant rate (Pedersen and Marqversen 1981).

The fate of orally administered [1-^{13}C]- and [8-^{13}C]-Triolein was evaluated using four healthy human subjects (two males, two females). In the first experiment, 100 mg of [1-^{13}C]-Triolein was administered orally (postprandial). One week later in a second experiment, the four subjects received 100 mg of [8-^{13}C]-Triolein. The fate of both radioactive compounds was traced in serum lipids. A trend of an increase in absolute concentration of triglyceride (TG) oleic acid was noted. ^{13}C enrichment in palmitic, stearic, linoleic, and oleic acids of these fractions was determined using gas chromatography/isotope ratio mass spectrometry. At time points 1, 2, 4, 7, and 9 hours after dosing, a range of 2% to 24% of [1-^{13}C]-Triolein was recovered in the serum TG fraction, compared to 10% to 60% of the [8-^{13}C]-Triolein dose. Thus, after administration of [8-^{13}C]-Triolein , the TG oleic acid in serum was significantly more highly enriched (significantly higher enrichment peak) in ^{13}C than after [1-^{13}C]-Triolein administration. This difference could have been due to faster elimination of [1-^{13}C]-Triolein from the serum. ^{13}C enrichment in other fatty acids of the TG fraction as well as phospholipid and cholesterol ester fractions were in the range of natural ^{13}C abundance (Metges, Kempe, and Wolfram 1994).

Eight patients with chronic pancreatitis were fed a breakfast containing [^{14}C]-Triolein (10 μCi). These patients, regarded as having normal lipid assimilation (<7 g of fat per day excreted), excreted in the feces $\leq$10.4% of the dose of ^{14}C ingested (Pedersen and Halgreen 1985).

Tripalmitin (C16)

The metabolic fate of a triglyceride oral load labeled with [1,1,1-$^{13}C_3$]-Tripalmitin was investigated by noting the appearance of labeled palmitate in the circulating nonesterified

fatty acids (NEFA) and TG. Six healthy adult subjects (five females, one male; 21 to 29 years old) were used in this evaluation. The average body mass index (BMI) for these subjects was 21 kg/m^2. Sunflower oil (30 g) enriched with 300 mg of [1,1,1-$^{13}C_3$]-Tripalmitin was ingested by each subject, after which blood samples were collected. Blood was collected at 10, 20, and 30 minutes, and then every 30 minutes up to 480 minutes. [1-^{13}C]-palmitate appeared in the plasma TG at 90 minutes, and the mole percent excess (MPE) of [1-^{13}C]-palmitate in TG increased and reached a plateau after 240 minutes. At 240 minutes, the MPE of [1-^{13}C]-palmitate was significantly greater in NEFA than in TG. At the conclusion of the study, the MPE of [1-^{13}C]-palmitate in TG remained significantly greater than baseline values ($p < .0001$) (Binnert et al. 1995).

In the same study, four additional adult subjects (three males, one female; 22 to 30 years old), the reesterification of NEFA was determined during [1-^{13}C]-palmitate infusion. The average BMI for these subjects was 21 kg/m^3. Albumin-bound [1-^{13}C]-palmitate was infused intravenously for 150 minutes, and blood samples were collected at 120 and 150 minutes. Hepatic reesterification of intravenously infused [1-^{13}C]-palmitate was estimated from the appearance of labeled palmitate in TG. A constant ^{13}C-palmitate enrichment of 1.6 ± 0.2 MPE was noted in NEFA after 120 minutes of [1-^{13}C]-palmitate infusion. However, [^{13}C]-palmitate enrichment increased from 0.23 ± 0.07 to 0.37 ± 0.07 MPE ($p < .001$) in plasma TG between 120 and 150 minutes. The estimated contribution from NEFA to palmitate of total TG (estimated reesterification) increased from $13.9\% \pm 3.6\%$ to $22.6\% \pm 2.6\%$ over the same period (Binnert et al. 1995).

The metabolic fate of an oral long-chain TG load was determined using 10 healthy female subjects (mean age $= 23 \pm 2$ years BMI $= 20.3 \pm 1.6$). The women were studied during a 6-hour period after ingestion of 30 g of olive oil made radioactive with [1,1,1-$^{13}C_3$]-Triolein. Total lipid oxidation was determined using indirect calorimetry. After 90 minutes, radioactivity was greater in chylomicron triglycerides (CM-TG) than in NEFA of very-low-density lipoprotein (VLDL). CM-TG were radioactive first, followed by NEFA and then VLDL. Thus, in this study, a long-chain TG mainly followed the classical lymph pathway. A plateau of enrichment was noted for CM-TG and NEFA at 180 minutes, demonstrating the entry of exogenous lipids into the NEFA pool. For VLDL-TG, a plateau of enrichment was noted after 300 minutes. The extent of enrichment for VLDL-TG ($0.38\% \pm 0.04\%$) was similar to that noted for NEFA ($0.36\% \pm 0.03\%$). The investigators stated that these similar results were suggestive of a precursor-product relationship. The percentage of the TG load that was oxidized was $19\% \pm 2\%$. Exogenous TG accounted for 70% of the total lipid oxidation over the period of 300 to 360 minutes. The investigators concluded that after ingestion of a lipid load, a cycle of fatty acids TG from CM to NEFA and from NEFA to VLDL takes place. Additionally, this lipid load has a sparing effect on endogenous lipid stores (Binnert et al. 1996).

Ocular Irritation

Tribehenin (C22)

The ocular irritation potential of an eye enhancer (eye area cosmetic product) containing 0.32% Tribehenin was evaluated using a group of 20 subjects (males and females). The test substance (undiluted cream, 10 to 30 μl) was instilled into one eye of each subject. Eye examinations were performed by a board-certified ophthalmologist before and after instillation of the test substance. During the subjective evaluation, few subjects reported transient burning/stinging sensation. However, itching, dryness, pain, and foreign body sensation were minimal throughout the study. During the objective evaluation, increased lacrimation was noted in one subject within 120 minutes post instillation, whereas, none of the remaining subjects had increased lacrimation at 5 minutes post instillation. Eyelid inflammation was not observed during the study. Mild to moderate irritation of the upper and/or lower palpebral conjunctivae was noted in all subjects at 30 seconds post instillation. However, at the time of the final evaluation (120 minutes post instillation), the subjects either had no irritation or mild irritation. Mild bulbar conjunctival irritation was noted in the majority of the subjects within the first few minutes after instillation. These reactions subsided during the remainder of the study (CTFA No date a, 1998c).

The in-use safety of two eye enhancers (eye area cosmetic products) containing 0.32% Tribehenin was evaluated using 40 female subjects (between 18 and 65 years old) who were contact lens wearers and regular users of eye shadow products. The subjects were instructed to use the two products (20 subjects per cream) for four consecutive weeks. Pre- and post-test eye examinations were performed by a board-certified ophthalmologist to support the claims of "ophthalmologist-tested" and "safe for contact lens wearers." Clinically relevant alterations in visual acuity were not observed in any of the subjects during the course of the study. Eye examinations performed by the ophthalmologist did not reveal any signs of ocular irritation either before or after product use. Additionally, none of the subjects reported any problems that were related to product use. It was concluded that both products were safe for their intended use (CTFA No date a, 1998c)

In another in-use study conducted according to the preceding test procedure, the safety of another eye enhancer containing 0.32% Tribehenin was evaluated using 31 female subjects (contact lens wearers). Eye examinations performed by an ophthalmologist did not reveal any signs of ocular irritation after product use. Additionally, clinically relevant alterations in visual acuity were not observed in any of the subjects tested. It was concluded that the eye enhancer was safe for its intended use (CTFA No date a, 1998c).

Tristearin (C18)

The ocular irritation potential of an eye defining pencil containing 1.68% Tristearin was evaluated in an in-use study using a group of 31 female subjects who were contact lens wearers. The

test procedure is stated in the preceding section. Ocular irritation was not observed in either of the subjects prior to product use or after four consecutive weeks of daily use. It was concluded that the eye defining pencil was safe for its intended use (CTFA No date a, 1998c).

Skin Irritation

Trilaurin (C12)

The skin irritation potential of an eyeliner containing 36.3% Trilaurin was evaluated in a single-insult occlusive patch test using 17 subjects (ages not stated). A control group of 17 subjects was also used. 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). No skin irritation was observed in any of the subjects (CTFA 1984c).

Trioctanoin (C8)

The primary skin irritation potential of Trioctanoin was evaluated using a panel of 25 men and women. Results were negative at 24 and 72 hours. Details concerning the test protocol were not included (Unichema International 1996).

Skin Irritation and Sensitization

Trilaurin (C12)

The skin sensitization potential of an eyebrow pencil containing 40% Trilaurin was evaluated using 91 subjects (88 males, 3 females). The subjects ranged in age from 18 to >65 years old. A total of nine induction applications (24-hour occlusive patches, covered with test material) were made to the same test site on each subject. Alternate test sites were used for the challenge phase. The eyebrow pencil induced mostly mild skin irritation during induction in one subject and there was no evidence of delayed contact hypersensitivity in any of the 91 subjects (Hill Top Research, Inc. 1988).

Tristearin (C18)

The skin irritation and sensitization potential of an eye defining pencil containing 1.68% Tristearin was evaluated in a 6-week study using 160 subjects (mainly females). Seven subjects withdrew from the study for reasons unrelated to administration of the test substance, reducing the test population to 153 subjects. The repeated-insult patch test (RIPT) protocol used was a modification of the Draize-Shelanski RIPT procedure. The undiluted test substance was applied liberally (under occlusive patches) to the scapular back three times per week for a total of nine applications. After a 2-week nontreatment period, two consecutive occlusive challenge patches containing the test substance were applied to a different site on the scapular back. Reactions were not observed in any of the subjects during the induction or challenge phase. It was concluded that the eye defining pencil did not induce irritant contact dermatitis or allergic contact dermatitis (CTFA No date a, 1998c).

Tribehenin (C22)

The skin irritation and sensitization potential of an eye enhancer (eye area cosmetic product) containing 0.32% Tribehenin was evaluated in a 6-week study using 211 subjects. One hundred ninety-eight subjects completed the study because 13 withdrew for reasons unrelated to the conduct of the study. The RIPT protocol was a modification of the procedure by Draize. Occlusive patches (occlusive plastic chambers) moistened with approximately 0.02 g of the cream were applied to the test site (upper arm or back) and secured with an occlusive bandage. During the 3-week induction period, patches were applied (same site) three times per week for 48 to 72 hours. The challenge phase was initiated 2 weeks after the end of induction. Challenge patches were applied to new test sites for 72 hours. Reactions were scored at 96 hours post application according to the following scale: 1 (erythema) to 4 (erythema, induration, and bullae). G (minimal glazing, such as "peau d'orange"), O (negative), and + (equivocal reaction) designations were also used. An equivocal reaction was observed in three subjects during the induction phase. Challenge reactions were not observed in any of the subjects tested. It was concluded that the eye enhancer did not induce clinically significant irritant contact dermatitis or allergic contact dermatitis (CTFA No date a, 1998c).

Both a hand cream and a lip cream containing 0.38% Tribehenin were evaluated in human RIPTs according to a procedure similar to that stated in the preceding paragraph. The hand cream and lip cream were tested in one and two RIPTs, respectively. The modifications of the test procedure previously mentioned were as follows: In each test, enrollment was sufficient to ensure that 200 men and/or women completed the study. The application period for induction and challenge patches (occlusive) was 24 hours; the quantity of test substance per patch application was not stated. Induction reactions were scored at 48 and 72 hours post application and challenge reactions were scored at 48 and 96 hours. The nontreatment period between induction and challenge phases ranged from 10 to 15 days. It was concluded that the hand cream and the lip cream were neither skin irritants nor sensitizers. Details concerning the study results were not included (CTFA No date a, 1998c).

Skin Sensitization

Trioctanoin (C8)

Reportedly, Trioctanoin did not induce sensitization in a contact allergy test involving human subjects. Details concerning the test protocol were not provided (Unichema International 1996).

Comedogenicity

The comedogenicity of a lip enhancer (cream) containing 0.38% Tribehenin was evaluated using 18 subjects (18 to 45 years old). One hundred microliters of the cream were dosed onto the surface of each occlusive patch that was taped to the upper back. Test sites were situated at the right and left of the spinal column. The subjects were instructed to return to the clinic every

Monday, Wednesday, and Friday for a total of 4 consecutive weeks (28 days) of patch removal, scoring of irritation reactions, and application of fresh patches. Reactions were scored using the North American Contact Dermatitis grading scale: 0 (no reaction) to +3 (a bullous reaction or an ulcer). At the time of the final visit, reactions were scored and follicular biopsies (cyanoacrylate follicular biopsy technique) taken at test and control sites. Specimens were evaluated according to the following scale using a stereomicroscope: 0 (no microcomedones, 0%) to 3 (severe, 75% to 100% larger globoid microcomedones). Data from the global assessments of test and control sites were compared statistically for biological significance ($p \leq .05$). A recorded mean value of 1 or greater that is significantly different from the negative control was considered positive for comedogenicity. Study results are summarized below:

None of the subjects had clinically significant skin irritation during the study. However, occasionally, macular erythema (score = +0.5) was observed in one subject. Statistical comparisons of microcomedone formation between test and control sites yielded a p value of >05. Thus, it was concluded that there was no difference between test and control sites and that the lip enhancer was not comedogenic (CTFA No date a, 1998c).

Case Reports

Triarachidin (C20), Trilinolein (C18), Triolein, (C18), and Tripalmitin (C16)

Thirteen eczema patients (median age = 68) with contact allergy to olive oil were patch-tested with the following glyceryl triesters, constituents of olive oil: Triolein (~30% in petrolatum [pet]), Tripalmitin (~30% in pet), Trilinolein (~5% in pet), and Triarachidin (~30% in pet). Patch tests were applied to normal skin of the back using Finn chambers secured with Scanpor tape. The chambers were removed after 48 hours, and reactions were recorded according to International Contact Dermatitis Research Group recommendations. All patch tests with the glyceryl triesters were negative (Malmkvist, Pettersson, and Svensson 1990).

In another case report, a woman (71 years old) and a man (60 years old) with dermatitis were patch-tested with Triolein (30% in vaseline). The results were negative (van Joost, Smitt, and van Ketel 1981).

SUMMARY

The safety of the following glyceryl triesters in cosmetics is reviewed in this report: 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.

The ingredients mentioned above are used mostly as skin-conditioning agents—occlusive and/or viscosity-increasing agents—nonaqueous. Frequency of use data submitted to FDA in 1998 indicate that 12 of the 23 ingredients in this safety assessment (Trilaurin, Tricaprylin, Tribehenin, Triisononanoin, Triisostearin, Trilinolein, Trimyristin, Trioctanoin, Tripalmitin, Tristearin, Glyceryl Triacetyl Hydroxystearate, and Glyceryl Triacetyl Ricinoleate) are being used in cosmetics. Collectively, these data indicate use in a total of 443 cosmetic products. Concentration of use data received from the cosmetics industry in 1999 indicated use of Glyceryl Triesters in cosmetic products at concentrations up to 46.3%.

Metabolism data indicate that most triglycerides (or glyceryl triesters) are split into monoglycerides, free fatty acids, and glycerol in the small intestine and absorbed by the intestinal mucosa.

In mice and guinea pigs, little skin penetration was observed, althrough Tricaprylin did enhance the skin penetration of drugs in vivo (Wistar rats) and in vitro (hairless mice). The skin penetration enhancement of drugs in the presence of Triolein has also been reported.

Acute oral LD_{50} values range from 5 g/kg in mice (Tribehenin, C8 aliphatic acid chains) to >20 g/kg in rats (Tristearin, C18). In other acute oral toxicity studies, Trioctanoin (C8) was not toxic following oral administration to male mice at a dose of 50 ml/kg, and Triisostearin did not induce toxicity in rats at a dose of 2 g/kg. In an acute intravenous toxicity study, Tricaprylin (C8) induced very minimal acute effects following administration to rats and mice, although, in another acute study, spasms in the hindlegs and respiratory distress were observed in mice injected intravenously with a 25% Tricaprylin emulsion. In acute parenteral toxicity studies, Tricaprylin induced very minimal toxicity in groups of mice and rats dosed intraperitoneally/subcutaneously.

The short-term oral administration of Trilaurin (C12), Tristearin (C18), or Triolein (C18) to weanling rats did not result in gross or microscopic lesions; however, in another short-term study, significant differences in hematological and clinical chemistry parameters as well as organ weights were noted after administration of Tricaprylin (C8) to male and female Wistar rats.

No significant differences were found in growth rate or the incidence of lesions between groups of rats fed a mixture containing 0.0002% Trilaurin (C12) for 2 years and controls. In another chronic study, cardiac lipidosis and/or focal fibrosis was observed in rats fed a basal diet consisting of 30 cal % Trierucin (C22) for 24 weeks. Renal tubular dilatation, proteinaceous casts, or fibrosis were also reported.

When the chronic oral toxicity of Tricaprylin (C8) was evaluated using groups of male rats, significant reductions in hematological/clinical chemistry parameters and significant increases in organ weight were noted after 26 weeks of dosing. Few lesions in the kidneys, myocardium, and aorta were noted when Tricaprylin was tested in another chronic oral toxicity study.

An eyeliner containing 36.3% Trilaurin (C12) and a 20% solution of Tribehenin (C22) in liquid paraffin were, at most, mildly irritating to the eyes of rabbits. Trioctanoin (C8) and

Triisostearin (C18) did not induce ocular irritation in rabbits. An eye enhancer cream containing 0.32% Tribehenin and a hand cream containing 0.38% Tribehenin were classified as nonirritants in an in vitro chorioallantoic membrane vascular assay for determining the ocular irritation potential of chemicals.

Triisostearin (C18) and a 20% solution of Tribehenin (C22) in liquid paraffin were, at most, mildly irritating when applied to the skin of rabbits. However, Trioctanoin (C8) and an eyeliner containing 36.3% Trilaurin (C12) did not induce cutaneous irritation in rabbits. Neither Tribehenin (C22) nor Trioctanoin (C8) induced sensitization in the Magnusson-Kligman guinea pig maximization test. Triisostearin (C18) did not induce significant cutaneous reactions in a study evaluating the phototoxicity and photoallergenicity potential of this ingredient in guinea pigs.

Most of the mutagenicity data in this report are on the ingredient, Tricaprylin (C8). In the Ames test, Tricaprylin was mutagenic in one of four *S. typhimurium* strains tested. Negative test results were reported in the following assays: dominant lethal test, host-mediated mitotic gene conversion assay, chromosomal aberrations assay, micronucleus test, SCE assay, spot test for gene mutations, and cytogenetic assay for clastogenic activity.

In the Ames test, Trilaurin (C12), Trioctanoin (C8), Triolein (C18), Tristearin (C18), and Triisostearin (C18) were not mutagenic in *S. typhimurium* strains. However, Trioctanoin was mutagenic in the spot test for gene mutations. In other tests, no clastogenic activity was noted when Trioctanoin was tested in a cytogenetics assay and results were negative in a sister chromatid exchanges mutagenicity assay.

Following intraperitoneal injection of Tricaprylin (C8) into 30 female mice in a tumorigenicity study, lung tumors were observed in 37% of the animals. In the untreated-control group of 30 mice, the lung tumor incidence was 23%. The results of an oral carcinogenicity study by the National Toxicology Program (NTP) indicated that Tricaprylin (C8) caused a statistically significant dose-related increase in the incidence of pancreatic acinar cell hyperplasia and adenoma in rats. Tricaprylin did not induce acinar cell carcinomas. Additionally, the incidence of squamous cell papilloma in the squamous portion of the stomach of rats in the highest dose group (10 ml/kg Tricaprylin) was significantly greater when compared to controls.

Trilaurin (C12) completely inhibited the formation of neoplasms initiated by DMBA and promoted by croton oil. Additionally, extensive damage to tumor cells (lymphoma implants in the liver) was noted in rats after oral dosing with Tricaprylin (C8).

Tricaprylin (C8) was not teratogenic in mice or rats when administered orally. In another study on reproductive effects, Tricaprylin was effective in producing fusion of the endometrial epithelium (symplasma formation) and decidualization of the stroma in pseudopregnant New Zealand white rabbits.

The oral administration of Trioctanoin (C8) to mice did not result in any significant differences in indices of potential developmental toxicity (i.e., litter size, birth weight, and neonatal growth and survival to postnatal day 3) between test and control groups. Test results for 291 fetuses from various strains of mice injected intraperitoneally with Trioctanoin (vehicle control) in a teratogenicity study indicated various kinds of eye abnormalities in 6.2% of the fetuses.

An eye enhancer cream containing 0.32% Tribehenin induced reactions ranging from mild to moderate ocular irritation in a group of 20 subjects, which resolved to either mild irritation or no irritation reactions at 2 hours post exposure. In a clinical in-use safety test of two eye enhancer creams containing 0.32% Tribehenin, neither ocular irritation nor clinically relevant alterations in visual acuity were observed after 4 consecutive weeks of daily product use. Similar results were reported after testing of another eye enhancer cream containing 0.32% Tribehenin and an eye defining pencil containing 1.68% Tristearin in separate studies according to the same procedure. All of the subjects tested in these studies were contact lens wearers.

An eyeliner containing 36.3% Trilaurin (C12) did not induce skin irritation reactions in test subjects. Trioctanoin (C8) itself did not induce skin irritation. A lip enhancer cream containing 0.38% Tribehenin was not comedogenic and did not induce clinically significant skin irritation in any of the subjects evaluated in a 28-day test. RIPT results (occlusive patches) for the following products were negative: eye enhancer cream containing 0.32% Tribehenin (198 subjects), hand cream containing 0.38% Tribehenin (at least 200 subjects), lip cream containing 0.38% Tribehenin (at least 200 subjects), and an eye defining pencil containing 1.68% Tristearin. None of these products induced clinically significant irritant or allergic contact dermatitis.

In a skin sensitization test involving 91 subjects, there was no evidence of delayed contact hypersensitivity after repeated applications (occlusive patches) of an eyebrow pencil containing 40% Trilaurin (C12). Also, reportedly, Trioctanoin (C8) did not induce sensitization in a contact allergy test.

DISCUSSION

The Panel noted that, as part of an effort to evaluate vehicles used in carcinogenicity studies, the NTP conducted a 2-year carcinogenicity study in rats given Tricaprylin by gavage. This treatment was associated with a statistically significant dose-related increase in pancreatic acinar cell hyperplasia and adenoma, but there were no acinar carcinomas, the incidence of mononuclear leukemia was less, and nephropathy findings were reduced, compared to corn oil controls. The Panel agreed that, overall, the study concluded that Tricaprylin did not offer significant advantages over corn oil as vehicles in carcinogenicity studies. Trilaurin was also found to inhibit the formation of neoplasms initiated by DMBA and promoted by croton oil.

The available short- and long-term toxicity test results (NTP oral carcinogenicity study on Tricaprylin included) summarized above, do not warrant any restrictions on the use of any of the

Glyceryl Triesters included in this safety assessment in rinse-off or leave-on cosmetic products. The Expert Panel recognizes that some of the Glyceryl Triesters included in this review are not in use, but would be considered safe if used at concentrations similar to those of Glyceryl Triesters that are being used in cosmetic products.

Although minimal percutaneous absorption of Triolein has been demonstrated in vivo using guinea pigs (but not hairless mice) and in vitro using full-thickness skin from hairless mice, the Expert Panel recognizes that, reportedly, Triolein and Tricaprylin can enhance the skin penetration of other chemicals, and recommends that care should be exercised in using these and other Glyceryl Triesters in cosmetic products.

CONCLUSION

On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that the following ingredients are safe as used in cosmetics: 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.

REFERENCES

Abdellatif, A. M., and R. O. Vles. 1973. Short-term and long-term pathological effects of glyceryl trierucate and of increasing levels of dietary rapeseed oil in rats. *Nutr. Metab.* 15:219–231.

Abramovici, B., R. Sabatier, L. Maury, and J. Hoachim. 1991. Spectral and galenic study of glycerol tribehenate (Compritol): Statistical interpretation for the analysis of multidimensional variance. *Pharm. Acta Helv.* 66:348–352.

Altura, B. M., and S. G. Hershey. 1970. Effects of glyceryl trioleate on the reticuloendothelial system and survival after experimental shock. *J. Pharmacol. Exp. Ther.* 175:555–564.

Ames, B. N., J. McCann, and E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. *Mutat. Res.* 31:347–364.

Andersen, F. A., ed. 2000. Final report on the safety assessment of Trihydroxystearin. *IJT* 19:89–94.

Argilés, J., and E. Herrera. 1989. Appearance of circulating and tissue 14C-lipids after oral 14C-tripalmitate administration in the late pregnant rat. *Metabolism* 38:104–108.

Baker, P. L., M. C. Kuenzig, and L. F. Peltier. 1969. Experimental fat embolism in dogs. *J. Trauma* 9:577–586.

Bayer, R. 1978. In vivo induction of sister chromatid exchanges by three polyaromatic hydrocarbons. *Carcinog. Compr. Surv.* 3:423–428.

Becker, W., and A. Bruce. 1985. Autoradiographic studies with fatty acids and some other lipids: A review. *Prog. Lipid Res.* 24:325–346.

Bergstrom, S., R. Blomstrand, and B. Borgstrom. 1954. Route of absorption and distribution of oleic acid and triolein in the rat. *Biochem. J.* 58:600–604.

Binnert, C., M. Laville, C. Pachiaudi, V. Rigalleau, and M. Beylot. 1995. Use of gas chromatography/isotope ratio-mass spectrometry to study triglyceride metabolism in humans. *Lipids* 30:869–873.

Binnert, C., C. Pachiaudi, M. Beylot, M. Croset, R. Cohen, J. P. Riou, and M. Laville. 1996. Metabolic fate of an oral long-chain triglyceride load in humans. *Am. J. Physiol.* 270:E445–E450.

Biogir, S. A. Conseil Recherche. 1989. Assessment of cutaneous tolerance in rabbit. Index of primary cutaneous irritation. Unpublished data submitted by CTFA, April 26, 1999 (13 pages).[2]

Blomstrand, R., and L. Kager. 1973. The combustion of triolein-1-14C and its inhibition by alcohol in man. *Life Sci.* 13:113–123.

Bryson, G., and F. Bischoff. 1964. Triolein as a cocarcinogen. *Fed. Proc.* 23:106.

Bryson, G., and F. Bischoff. 1969. The limitations of safety testing. *Prog. Exp. Tumor. Res.* 11:100–133.

Budavari, S. 1989. *The Merck index. An encyclopedia of chemicals, drugs, and biologicals*, 983–984, 1530–1531, 1534. Rahway, NJ: Merck.

Bull, A. W., L. J. Marnett, E. J. Dawe, and N. D. Nigro. 1983. Stimulation of deoxythymidine incorporation in the colon of rats treated intrarectally with bile acids and fats. *Carcinogenesis* 4:207–210.

Burrows, H., I. Hieger, and E. L. Kennaway. 1936. Experiments in carcinogenesis: The effect of the subcutaneous and intraperitoneal injection of lard, olive oil, and other fatty materials in rats and mice. *J. Pathogens Bacteria* 43:419–426.

Burton, A. F. 1991. Oncolytic effects of fatty acids in mice and rats. 1991. *Am. J. Clin. Nutr.* 53:1082S–1086S.

Cabral, J. R., and V. Ponomarkov. 1982. Carcinogenic study of the pesticide maleic hydrazide in mice. *Toxicology* 24:169–173.

Carlson, T. L., and B. A. Kottke. 1991. Effect of coconut oil on plasma apo A-I levels in WHHL and NZW rabbits. *Biochim. Biophys. Acta* 1083:221–229.

Castilho, M. C., M. I. Silveira, and A. Pena. 1989. Contribution to the characterization of olive oil: Composition of triacylglycerols by reversed phase high performance liquid chromatography. *Rev. Port. Farm.* 39:95–100.

Chan, P., J. T. Cheng, C. W. Tsao, C. S. Niu, and C. Y. Hong. 1996. The *in vitro* antioxidant activity of trilinolein and other lipid-related natural substances as measured by enhanced chemiluminescence. *Life Sci.* 59:2067–2073.

Chan, P., S. K. Tsai, B. N. Chiang, and C. Y. Hong. 1995. Trilinolein reduces infarct size and suppresses ventricular arrhythmias in rats subjected to coronary ligation. *Pharmacology* 51:118–126.

Correa, E., P. A. Joshi, A. Castonguay, and H. M. Schüller. 1990. The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone is an active transplacental carcinogen in Syrian golden hamsters. *Cancer Res.* 50:3435–3438.

Cosmetic, Toiletry, and Fragrance Association (CTFA). 1984a. Eye irritation data on Trilaurin. (Test No. 56-250). Unpublished data submitted by CTFA, June 26, 1998 (1 page).[2]

CTFA. 1984b. Primary skin irritation data on Trilaurin (Test No. 15-059). Unpublished data submitted by CTFA, June 26, 1998 (1 page).[2]

CTFA. 1984c. Human patch test data on Trilaurin. Unpublished data submitted by CTFA, June 26, 1998 (1 page).[2]

CTFA. 1998a. Concentration of use data and specifications from Japanese references. Unpublished data submitted by CTFA, July 9, 1998 (4 pages).[2]

CTFA. 1998b. Information on use concentrations. Unpublished data submitted by CTFA, January 21, 1998 (2 pages).[2]

CTFA. 1998c. Unpublished data sheet for stearamide DIBA-stearate and tristearin. Unpublished data submitted by CTFA, June 12, 1998 (4 pages).[2]

CTFA. 1999. Ingredient use survey—Updated tables. Information from finished product manufacturers. Unpublished data submitted by CTFA, March 3, 1999 (26 pages).[2]

CTFA. No date a. Clinical studies conducted with formulations containing: glyceryl tribehenate, sorbitan isostearate, strearamide DIBA-stearate, and tristearin. Unpublished data submitted by CTFA, August 10, 1998 (54 pages).[2]

CTFA. No date b. Protocol No. 3. Primary skin irritation. Unpublished data submitted by CTFA, March 12, 1999 (2 pages).[2]

[2]Available for review from the Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC, 20036, USA.

Davidek, J., J. Velisek, V. Kubelka, G. Janicek, and Z. Simicova. 1980. Glyceryl chlorohydrins and their esters as products of the hydrolysis of tripalmitin, tristearin, and triolein with hydrochloric acid. *Z. Lebensm. Unters. Forsch.* 171:14–17.

Davies, J., and G. R. Davenport. 1979. Symplasma formation and decidualization in the pseudopregnant rabbit after intraluminal instillation of tricaprylin. *J. Reprod. Fertil.* 55:141–145.

Deman, L., and J. M. Deman. 1982. Trans-fatty acids in milkfat. *J. Am. Oil Chem. Soc.* 60:1095–1098.

Diwan, B. A. 1974. Strain-dependent teratogenic effects of 1-ethyl-1-nitrosourea in inbred strains of mice. *Cancer Res.* 34:151–157.

Elder, R. L. ed. 1980. Final report of the safety assessment for caprylic/capric triglyceride. *J. Environ. Pathol. Toxicol.* 4:105–120.

Erdahl, W. L., and O. S. Privett. 1977. A new system for lipid analysis by liquid chromatography-mass spectrometry. *Lipids* 12:797.

Environmental Safety Laboratory. 1990. Skin sensitization study on Panaceate 800B (Glycerol Tris(2-ethylhexanoate)) (Study No. SM890863). Unpublished data submitted by CTFA, April 26, 1999 (3 pages).[2]

Fabien, R., J. D. Craske, and M. Wootton. 1993. Quantitative analysis of synthetic mixtures of triacylglycerols with fatty acids from caprylic to stearic. *J. Am. Oil Chem. Soc.* 70:551–554.

Food and Drug Administration (FDA). 1992. Modification in voluntary filing of cosmetic product ingredient and cosmetic raw material composition statements. *Fed. Register* 57:3128–3130.

FDA. 1998. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.

Fox, R. R., H. Meier, R. Pottathil, and H. G. Bedigian. 1980. Transplacental teratogenic and carcinogenic effects in rabbits chronically treated with N-ethyl-N-nitrosourea. *J. Natl. Cancer Inst.* 65:607–614.

Frank, F., T. Roberts, J. Snell, and C. Yates. 1971. Trimyristin from nutmeg. *J. Chem. Educ.* 48:255–256.

Fujii, K., and S. S. Epstein, 1979. Effects of piperonyl butoxide on the toxicity and hepatocarcinogenicity of 2-acetylaminofluorene and 4-acetyl aminobiphenyl and their n-hydroxylated derivatives, following administration of newborn mice. *Oncology* 3:105–112.

Generoso, W. M., K. T. Cain, and L. A. Hughes. 1985. Tests for dominant-lethal effects of 1,2-dibromo-3-chloropropane (DBCP) in male and female mice. *Mutat. Res.* 156:103–108.

Gennaro, A. R. 1990. *Remington's pharmaceutical sciences*, 18th ed., 387–388. Easton, PA: Mack Publishing Company.

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.* 10:821–830.

Goto, S., T. Uchida, C. K. Lee, T. Yasutake, and J. B., Zhang. 1993. Effect of various vehicles on ketoprofen permeation across excised hairless mouse skin. *J. Pharm. Sci.* 82:959–963.

Grant, J., ed. 1972. *Hackh's chemical dictionary*. 4th ed. 691. New York: McGraw-Hill.

Greenberger, N. J., J. B. Rodgers, and K. F. Isselbacher. 1966. Absorption of medium and long chain triglycerides: Factors influencing their hydrolysis and transport. *J. Clin. Investi.* 45:217–227.

Hamilton, J. D., J. P. Webb, and A. M. Dawson. 1969. The absorption of tristearin and stearic acid and tripalmitin and palmitic acid. Studies on the rate-limiting steps in rats. *Biochim. Biophys. Acta* 176:27–36.

Hardin, B. D., R. L. Schuler, J. R. Burg et al. 1987. Evaluation of 60 chemicals in a preliminary developmental toxicity test. *Teratog. Carcinog. Mutag.* 7:29–48.

Hecht, S. S., J. D. Adams, S. Numoto, and D. Hoffmann. 1983. Induction of respiratory tract tumors in Syrian golden hamsters by a single dose of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and the effect of smoke inhalation. *Carcinogenesis* 4:1287–1290.

Hill Top Research, Inc. 1988. Repeated insult patch test. Eyebrow pencil (03A) 73861-01 containing 40% trilaurin. Unpublished data submitted by CTFA, June 26, 1998 (10 pages).[2]

Hodge, H. O. 1954. Pilot study of rats fed 5% tristearin. (Submitted by FDA in response to an FOI request—1998; 8 pages).[2]

Hooson, J., and P. Grasso. 1976. Cytotoxic carcinogenic response to monocrotaline pyrrole. *J. Pathol.* 118:121–128.

Hoshi, J., H. Nishida, M. Yasui, M. Ohishi, and M. Takahasji. 1992. [13C] breath test of medium-chain triglycerides and oligosaccharides in neonates. *Acta Paediatr. Jpn.* 34:674–677.

Huntingdon Research Centre. 1977. Irritant effects of Syncrowax HR (glyceryl tribehenate) on rabbit skin. Unpublished data submitted by CTFA, April 26, 1999 (4 pages).[2]

Informatics, Inc. 1973. *GRAS (generally recognized as safe) food ingredients—Glycerine and glycerides*. NTIS Report No. PB-221 227. Springfield, VA: National Technical Information Service (NTIS).

Ingebretsen, W. R., Jr., and S. R. Wagle. 1974. Studies on the effects of tricaprylin on gluconeogenesis and ketogenesis in isolated perfused liver. *Proc. Soc. Exp. Biol. Med.* 147:578–580.

Johnson, R. C., S. K. Young, R. Cotter, L. Lin, and W. B. Rowe. 1990. Medium-chain-triglyceride lipid emulsion: Metabolism and tissue distribution. *Am. J. Clin. Nutr.* 52:502–508.

Kabara, J. J. 1984. *Cosmetic and drug preservation. Principles and practice*, 305–322, 618, 740. New York: Marcel Dekker.

Karlshamns Sweden AB. 1997. Product documentation. Akoline MCM (Glyceryl Caprylate/Caprate) and Akomed C (Triundecanoin). Unpublished data submitted by CTFA, May 29, 1998 (7 pages).[2]

Knasmüller, S., W. Huber, and R. Schulte-Hermann. 1993. Prevention of genotoxic effects by dietary constituents in various organs of mice treated with nitrosamines. *Basic Life Sci.* 61:447–457.

Lee, C. K., T. Uchida, K. Kitagawa, A. Yagi, N. S. Kim, and S. Goto. 1993. Effect of hydrophilic and lipophilic vehicles on skin permeation of tegafur, alclofenac, and ibuprofen with or without permeation enhancers. *Biol. Pharm. Bull.* 16:1264–1269.

Lewis, R. J., ed. 1993. *Hawley's condensed chemical dictionary*, 12th ed., 568,1088,1180. New York: Van Nostrand Reinhold.

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., 3-260–3-261, 3-502–3-504. Boca Raton, FL:CRC Press.

Lockard, J. M., J. W. Prater, and C. J. Viau et al. 1982. Comparative study of the genotoxic potential of eastern and western U.S. shale oils, crude petroleum and coal-derived oil. *Mutat. Res.* 102:221–235.

Lubke, M., J. Le Quere, and D. Barron. 1996. Prefractionation of aroma extracts from fat-containing food by high-performance size-exclusion chromatography. *J. Chromatogr.* 729:371–379.

Magnusson, B., and A. M. Kligman. 1969. The identification of contact allergens by animal assay. The guinea pig maximization test. *J. Invest. Dermatol.* 52:268–276.

Malmkvist, P. S., A. Pettersson, and A. Svensson. 1990. Olive oil as a cause of contact allergy in patients with venous eczema, and occupationally. *Contact Dermatitis* 23:73–76.

Mandybur, T. I., I. Ormsby, and C. R. Buncher. 1978. Enhanced development of mammary tumors in rats following transplacental and neonatal exposure to ethylnitrosourea. *Cancer Res.* 38:3182–3185.

Masuno, H., and H. Okuda. 1986. Hepatic triacylglycerol lipase in circulating blood of normal and tumor-bearing mice and its hydrolysis of very-low-density lipoprotein and synthetic acylglycerols. *Biochim. Biophys. Acta* 879:339–344.

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

Mattson, F. H., and R. A. Volpenhein. 1972. Rate and extent of absorption of the fatty acids of fully esterified glycerol, erythritol, xylitol, and sucrose as measured in thoracic duct cannulated rats. *J. Nutr.* 102:1177–1180.

Mattson, F. J., F. J. Baur, and L. W. Beck. 1951. The comparative nutritive values of mono-, di-, and triglycerides. *J. Am. Oil Chem. Soc.* 28:386–390.

Metais, P., A. Bach, and J. Warter. 1967. Utilization of fats containing long-, medium, or short-chain fatty acids, compared by studying the level of labeled carbon dioxide in the expired air (English Translation). *C. R. Seances Soc. Biol. Ses. Fil.* 161:1372–1376.

Metges, C. C., K. Kempe, and G. Wolfram. 1994. Enrichment of selected serum fatty acids after a small oral dosage of (1-13C)- and (8-13C) triolein in human volunteers analyzed by gas chromatography/combustion isotope ratio mass spectrometry. *Biol. Mass. Spectrum* 23:295–301.

Mingrone, G., A. V. Greco, E. Capristo, G. Benedetti, M. Castagneto, and G. Gasbarrini. 1995. An improved GLC method for a rapid, simultaneous analysis of both medium chain fatty acids and medium chain triglycerides in plasma. *Clin. Chim. Acta* 240:195–207.

Moloney, S. J. 1988. The *in vitro* percutaneous absorption of glycerol trioleate through hairless mouse skin. *J. Pharm. Pharmacol.* 40:819–821.

Mouton, D., Y. Bouthillier, N. Feingold, and J. Feingold. 1975. Genetic control of macrophage functions. I. Polygenic regulation of phagocytosis stimulation produced by glyceryl trioleate. *J. Exp. Med.* 141:306–321.

Nakamura, M., and O. S. Privett. 1969. Metabolism of glyceryl 1-14C-trilinoleate in rat testis. *Lipids* 4:93–98.

National Toxicology Program (NTP). 1994. *NTP technical report on the comparative toxicology studies of Corn Oil, Safflower Oil, and Tricaprylin (CAS Nos. 8001-30-7, 8001-23-8, and 538-23-8) in male F344/N rats as vehicles for gavage*. NTIS Report No. PB95-103958.

Nesnow, S., J. A. Ross, and G. Nelson, et al. 1994. Cyclopenta[cd]pyrene-induced tumorigenicity, Ki-ras codon 12 mutations and DNA adducts in strain A/J mouse lung. *Carcinogenesis* 15:601–606.

Nestmann, E. R., E. G. H. Lee, T. I. Matula, G. R. Douglas, and J. C. Mueller. 1980. Mutagenicity of constituents identified in pulp and paper mill effluents using the *Salmonella*/mammalian-microsome assay. *Mutat. Res.* 79:203–212.

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. N.Y. Acad. Sci.* 768:227–230.

Nihati-Shirkhodaee, F., and T. Shibamoto. 1992. Analysis of reactive aldehydes formed from the irradiated skin lipid triolein (abstract AGFD). Presented at 203rd American Chemical Society National Meeting, San Francisco, California, USA.

Nikitakis, J. M., and G. N. McEwen, Jr., eds. 1990. *CTFA compendium of cosmetic ingredient composition-Descriptions I*. Washington, DC: CTFA.

Nolasco, N. A., J. G. Balboa, E. Serrame, and C. Y. Lim-Sylianco. 1994. Effect of coconut oil, trilaurin, and tripalmitin on the promotion stage of carcinogenesis. *Philipp. J. Sci.* 123:161–169.

Nolasco, N. A., and C. Y. Lim-Sylianco. 1993. Antigenotoxic effects of trilaurin, trilinolein, and α,β-dilauroyl, α-linoleoyl glycerol. *Philipp. J. Sci.* 122:403–411.

Ohta, K., Y. Matsuoka, Y. Ichikawa, and K. Yamamoto. 1970. Toxicity, teratogenicity, and pharmacology of tricaprylin. *Oyo Yakuri (Pharmacometrics)* 4:871–882.

Oro, L., and A. Wretlind. 1961. Pharmacological effects of fatty acids, triolein, and cottonseed oil. *Acta Pharmacol. Toxicol.* 18:141–152.

Padley, F. B. 1969. The use of a flame-ionization detector to detect components separated by thin-layer chromatography. *J. Chromatogr.* 39:37–46.

Paulose, M. M., and S. S. Chang. 1978. Chemical reactions involved in deep-frying of foods: VIII. Characterization of nonvolatile decomposition products of triolein. *J. Am. Oil Chem. Soc.* 55:375–380.

Pedersen, N. T., and H. Halgreen. 1985. Simultaneous assessment of fat maldigestion and fat malabsorption by a double-isotope method using fecal radioactivity. *Gastroenterology* 88:47–54.

Pedersen, N. T., and J. Marqversen. 1981. Metabolism of ingested 14C-triolein. Estimation of radiation dose in tests of lipid assimilation using 14C- and 3H-labelled fatty acids. *Eur. J. Nucl. Med.* 6:327–329.

Powell, M. 1932. The metabolism of tricaprin. *J. Biol. Chem.* 95:43.

Prahalad, A. K., J. A. Ross, G. B. Nelson, B. G. Roop, L. C. King, S. Nesnow, and M. J. Mass. 1997. Dibenzo(a,I)pyrene-induced DNA adduction, tumorigenicity, and Ki-ras oncogene mutations in strain A/J mouse lung. *Carcinogenesis* 18:1955–1963.

Procter & Gamble Company. 1950. The comparative nutritive value of certain mono-, di-, and triglycerides. (Submitted by FDA in response to an FOI request—1998; 41 pages).[2]

Procter & Gamble Company. 1973. The fate of intravenously administered sucrose octaoleate. (Submitted by FDA in response to an FOI request—1998; 17 pages).[2]

Rao, G. A., D. E. Riley, and E. C. Larkin. 1985. Comparison of the thin layer chromatography/flame ionization detection system with other methods for the quantitative analysis of liver lipid contents in alcohol-fed rats and controls. *Lipids* 20:531–535.

Registry of Toxic Effects of Chemical Substances (RTECS). 1998. Acute oral toxicity of tribehenin. *RTECS database*. Bethesda, MD: National Library of Medicine.

Rempe, J. M., and L. G. Santucci, eds. 1997. *CTFA list of Japanese cosmetic ingredients*, 3rd ed., 41–43. Washington, DC: CTFA.

Safepharm Limited. 1980. An assessment of the acute oral toxicity of Dynasan 118 in the rat. Experiment No. 126/8006. (Submitted by FDA in response to an FOI request—1998; 9 pages).[2]

Sanitary Laboratory Kanagawa Prefecture. 1975a. Acute oral toxicity test (mouse) on TIO (Glyceryl Tris(2-ethylhexanoate). Unpublished data submitted by CTFA, April 26, 1999 (3 pages).[2]

Sanitary Laboratory Kanagawa Prefecture. 1975b. Test on irritation for skin (rabbit) on TIO (Glyceryl Tris(2-ethylhexanoate). Unpublished data submitted by CTFA, April 26, 1999 (3 pages).[2]

Schirmer, B. D., W. J. Kortz, R. S. Jones, and S. H., Quarfordt. 1983. Metabolism of triglyceride by in vitro tandem-perfused rat liver and hind end. *Am. J. Physiol.* 245:G106–G112.

Schmid, W. 1976. The micronucleus test for cytogenetic analysis. *Chem. Mutag.* 4:37–43.

Schwabe, A. D., V. D. Valdivieso, S. Merrill, C. Ortega, L. R. Bennet, and J. C. Thompson. 1967. Studies on the intestinal absorption of a medium chain fat (trioctanoin) in normal and pancreatectomized dogs. *Am. J. Dig. Dis.* 12:1114–1121.

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

STN International. 1997b. Properties of esters of glycerin and lauric acid. *HODOC database file*. Columbus, OH: STN International.

STN International. 1997c. Properties of esters of glycerin and lauric acid. *Beilstein database file*. Columbus, OH: STN International.

Shaffer, J. W., W. C. Sealy, A. V. Seaber, and J. L. Goldner. 1976. Etiology of fat embolism syndrome: early morphologic lung changes of respiratory distress syndrome produced by triolein. *Surg. Forum* 27:516–518.

Siebert, D., U. Bayer, and H. Marquardt. 1979. Application of mitotic gene conversion in *Saccharomyces cerevisiae* in a pattern of four assays, *in vitro* and *in vivo*, for mutagenicity testing. *Mutat. Res.* 67:145–156.

Smith, M. K., J. L. Randall, J. A. Stober, and E. J. Reed. 1989. Developmental toxicity of dichloroacetonitrile: a by-product of drinking water disinfection. *Fundam. Appl. Toxicol.* 12:765–772.

Stuart, A. C., and II Smith. 1975. Histological effects of lipids on the liver and spleen of mice. *J. Pathol.* 115:63–72.

Styles, J. A., and M. G. Penman. 1985. The mouse spot test evaluation of its performance in identifying chemical mutagens and carcinogens. *Mutat. Res.* 154:183–204.

Suzuki, M., K. Asaba, H. Komatsu, and M. Mochizuka. 1978. Autoradiographic study on percutaneous absorption of several oils useful for cosmetics. *J. Soc. Cosmet. Chem.* 29:265–282.

Unichema International. 1992. Safety evaluation of glyceryl tribehenate. Unpublished data submitted by CTFA, May 4, 1998 (5 pages).[2]

Unichema International. 1996. Glycerol tris(2-ethylhexanoate) safety evaluation. Unpublished data submitted by CTFA, May 4, 1998 (7 pages).[2]

Unichema International. 1997a. Isostearate esters of glycerol and polyglycerol: Safety evaluation. Unpublished data submitted by CTFA, May 4, 1998 (8 pages).[2]

Unichema International. 1997b. Glyceryl monoesters of fatty acids: Safety evaluation. Unpublished data submitted by CTFA, May 4, 1998 (12 pages).[2]

Van Duuren B. L., B. M. Goldschmidt, and I. Seidman. 1975. Carcinogenic activity of di- and trifunctional alpha-chloro ethers and of 1,4-dichlorobutene-2 in ICR/HA Swiss mice. *Cancer Res*. 35:2553–2557.

van Joost, T., J. H. Smitt, and W. G. van Ketel. 1981. Sensitization to olive oil (olea europeae). *Contact Dermatitis* 7:309–310.

Verkade, P. C., and J. vander Lee. 1934. Fat metabolism. IV. Bilateral β-oxidation of the dicarboxylic acids formed by ω-oxidation of saturated fatty acids. *Z. Physiol. Chem*. 227:213.

Watts, R., and R. Dils. 1968. Separation of triglycerides by gas-liquid chromatography. *J. Lipid Res*. 9:40–51.

Wenninger, J. A., and G. N. McEwen, Jr., eds. 1997. *International cosmetic ingredient dictionary and handbook,* 7th ed., 569–570, 1417–1438. Washington, DC: CTFA.

Weyland, E. H., Y. C. Chen, Y. Wu, A. Koganti, H. A. Dunsford, and L. V. Rodriguez. 1995. Differences in the tumorigenic activity of a pure hydrocarbon and a complex mixture following ingestion: benzo[alpha]pyrene vs. Manufactured gas plant residue. *Chem. Res. Toxicol*. 8:949–954.

Wodinsky, I., A. Helinski, and C. J. Kensler. 1964. Susceptibility of Syrian hamsters to induction of fibrosarcomas with a single injection of 3,4,9,10-dibenzypyrene. *Nature* 203:308–309.

Wretlind, A. 1957. The toxicity of low-molecular triglycerides. *Acta Physiol. Scand*. 40:338–343.

Zeiger, E., J. Ashby, G. Bakale, K. Enslein, G. Klopman, and H. S. Rosenkranz. 1996. Prediction of *Salmonella* mutagenicity. *Mutagenesis* 11:471–484.