# Final Report on the Safety Assessment of Lecithin and Hydrogenated Lecithin[1]

**Lecithin is a naturally occurring mixture of the diglycerides of stearic, palmitic, and oleic acids, linked to the choline ester of phosphoric acid, commonly called phosphatidylcholine. Hydrogenated Lecithin is the product of controlled hydrogenation of Lecithin. Bilayers of these phospholipids in water may form liposomes, a spherical structure in which the acyl chains are inside and not exposed to the aqueous phase. Lecithin and Hydrogenated Lecithin are used in a large number of cosmetic formulations as skin conditioning agents-miscellaneous and as surfactant-emulsifying agents. Hydrogenated Lecithin is also used as a suspending agent-nonsurfactant. Historical data on concentration of use of Lecithin reveals that 0.1% to 1.0% is the concentration range most frequently seen, with concentrations up to 50% reported for two moisturizing products. A solution of 65% Lecithin is currently reported to be used at concentrations up to 3% in cosmetics. Nonocclusive application of Lecithin-containing liposomes to murine skin resulted in 30% penetration to the subdermis. In piglet skin, the same application resulted in 99% accumulating in the stratum corneum. In general, liposomes are considered effective in capturing other compounds inside their spherical structure and delivering any such captured compound through the skin barrier. As a result, caution should be exhibited in formulating cosmetic products that contain these ingredients in combination with other ingredients whose safety is based on their lack of absorption or where dermal absorption is a concern. Lecithin is virtually nontoxic in acute oral studies, short-term oral studies, and subchronic dermal studies in animals. Lecithin is not a reproductive toxicant, nor is it mutagenic in several assays. In an oral carcinogenicity study, brain neoplasms were found in mice exposed to Lecithin. In a subcutaneous carcinogenicity study, no neoplasms were found in mice and rats exposed to Lecithin. Adverse reactions to Lecithin in a metered-dose inhaler have been reported. Lecithin and Hydrogenated Lecithin were generally nonirritating and nonsensitizing in animal and human skin. Based on the available data, Lecithin and Hydrogenated Lecithin are safe as used in rinse-off cosmetic products; they may be safely used in leave-on products at concentrations up to 15%, the highest concentration tested in clinical irritation and sensitization studies; but the safety of use could not be substantiated in cosmetic products likely to be inhaled. Because of the possibility of formation of nitrosamines, these ingredients should not be used in cosmetic products in which *N*-nitroso compounds may be formed.**

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## INTRODUCTION

Lecithin refers to the phospholipid or phosphatide fraction of substances that occur in nature and can be isolated from both plants and animals (Federation of American Societies for Experimental Biology [FASEB] 1979). For cosmetic purposes, Lecithin functions as a skin conditioning agent—miscellaneous and as a surfactant—emulsifying agent in a variety of products (Wenninger, Canterbery, and McEwen 2000). Lecithin can also be hydrogenated, and Hydrogenated Lecithin has the same functions as Lecithin, and also functions as a suspending agent—nonsurfactant.

Studies on Lecithin-containing liposomes are included in this review.

## CHEMISTRY

### Definition and Structure

Lecithin (CAS Nos. 8002-43-5; 8030-76-0; 93685-90-6) is a naturally occurring mixture of the diglycerides of stearic, palmitic, and oleic acids, linked to the choline ester of phosphoric acid, and is found in living plants and animals (Wenninger, Canterbery, and McEwen 2000). In naturally occurring Lecithins, the phosphoric acid is attached to the glycerol at the $\alpha$-position; however, the phosphoric acid can also be attached in the $\beta$ position (Gennaro 1990). The structures of $\alpha$- and $\beta$-Lecithin are shown in Figure 1.

Lecithin is also known as Egg Yolk Lecithin; Lecithins, Egg Yolk; Lecithin, Soybean; Soybean Phospholipid (Wenninger, Canterbery, and McEwen 2000); Lecithol; Phosphatidylcholine (Budavari 1989); Choline Phosphoglyceride (Lehninger 1975); and Phospholutein (Hazardous Substances Database 1995). It has numerous trade names and composes part of various trade name mixtures.

Hydrogenated Lecithin (CAS No. 92128-87-5) is the end product of the controlled hydrogenation of Lecithin (q.v.) (Wenninger, Canterbery, and McEwen 2000). It is also known as Lecithin, Hydrogenated; Lecithins, Hydrogenated; and Hydrogenated Egg Yolk Phospholipids.

### Liposomes

A liposome is a structure in which the edges of phospholipid bilayers in lamellar dispersions in water seal readily with the

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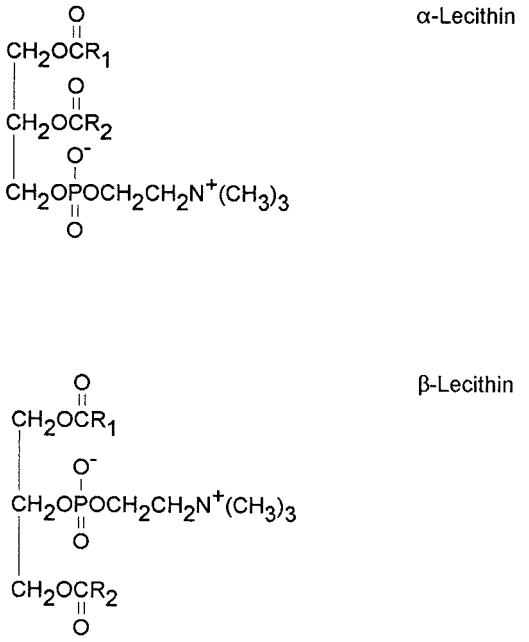


**FIGURE 1**
Chemical formulas for the $\alpha$ and $\beta$ forms of Lecithin.

formation of spherical structures whose acyl chains are also not exposed to the aqueous phase (Nattermann Phospholipid GmbH 1995). Liposomes can be formed by the addition of aqueous solution to a phospholipid film crystallized from an organic solvent. The liposomes that are formed are generally multilamellar vesicles (MLV) that are 20 to 5000 nm in diameter. Because of the ability of water to penetrate the crystal lattice of the phospholipid bilayer, MLV can be used to enclose water-soluble compounds. Upon sonification, MLV disperse into small unilamellar vesicles (SUV), 20 to 50 nm in diameter, which can be fused by freezing and thawing or evaporation to produce large unilamellar vesicles (LUV), <2500 nm in diameter, that have an enhanced encapsulation volume.

Liposome properties can be altered based upon composition. Liposomal permeability to water can be increased by shortening the length of the fatty acid chain. The inclusion of cholesterol in the liposome stabilizes the fatty acid chains, making them more rigid and decreasing liposomal permeability. Stability, clearance, and other pharmacokinetic properties can be altered by the inclusion of triglycerides, proteins, carbohydrates, lipoproteins, or charged particles. Different mixtures are used to target specific sites. The phospholipid composition of liposomes affects their actions on the skin after topical application. Altering the head groups of the liposome allows for the liposome to be neutral and lipophilic or negatively charged and hydrophilic. The surface charge of liposomes affect their interaction with membranes, their self-aggregation, and their rate of clearance from the body. Lecithin is the most widely used neutral phospholipid.

## Physical and Chemical Properties

The physical and chemical properties of Lecithin are summarized in Table 1 and the fatty acid composition of Lecithin is given in Table 2. The major unsaturated fatty acid in egg Lecithin is oleic acid, 32%, whereas soy Lecithin contains 61% linoleic acid (Allen et al. 1984).

## Manufacture and Production

In commercial practice, Lecithin refers to a mixture of acetone-insoluble phosphatides, including "true Lecithin" (phosphatidylcholines) and cephalin, with other substances such as carbohydrates, glyceride oils, and sterols that occur with the phosphatides (Sartoretto 1967). Refined grades of Lecithin can contain any of the following components in varying proportions and combinations depending on the type of fractionation used: phosphatidyl choline; phosphatidyl ethanolamine; phosphatidyl inositol; and substances such as triglycerides, fatty acids, and carbohydrates (National Academy of Sciences [NAS] 1996). Food-grade Lecithin is obtained from soybeans and other plant sources (NAS 1996). Lecithin is also derived from corn, vegetable seeds, egg yolk, and animal sources (Lewis 1993).

"Native Lecithin" is derived from soybean oil using the following four steps: hydration of the phosphatides contained in the oil; separation of the sludge; drying; and cooling (van Nieuwenhuyzen 1976). Egg Lecithin can be prepared by a temperature-solvent fractionation, giving an iodine number of 70 to 80, or by the Pangborn cadmium chloride method, giving an iodine number of 55 (Sinclair 1948).

## Natural Occurrence

Lecithin is a phosphatide found in all living organisms. It is a significant constituent of nervous tissue and brain substance (Budavari 1989). Lecithin is the predominant phospholipid (>50%) in most mammalian membranes (Zeisel 1993) and is usually localized in the outer surface of the plasma membrane (Taylor 1988). It is also a major phospholipid in lung surfactant and amniotic fluid (Diomede, Agosti, and Salmona 1993).

Crude soybean oil contains 2% to 3% Lecithin, and significant amounts are found in corn and wheat oil (FASEB 1979). Egg yolk contains approximately 10% Lecithin.

## Chemical Reactivity

Upon complete hydrolysis, each molecule of Lecithin yields two molecules of fatty acid and one molecule each of glycerol, phosphoric acid, and a basic nitrogenous compound (Gennaro 1990). The fatty acids obtained with hydrolysis are usually oleic, palmitic, and stearic acids and the basic nitrogenous compound obtained is usually choline. Lecithins oxidize readily on exposure to air.

The double bond of the unsaturated fatty acids present in the phosphoglyceride and triglyceride components of Lecithin is susceptible to oxidation with the possible formation of hydroperoxides and epoxides (FASEB 1979).

**TABLE 1**
Physical and chemical properties of Lecithin

| | | Reference |
|---|---|---|
| Approximate formula | $C_{43}H_{88}NO_9P$ | Nikitakis and McEwen 1990 |
| Molecular weight | 144.56 | Lide 1993 |
| Physical properties | Natural and refined grades vary from plastic to fluid, depending on free fatty acid and oil content and upon the presence or absence of other diluents. Color varies from light yellow to brown. | NAS 1996 |
| | Acid value ≈20, waxy mass; acid value ≈30, pourable, thick fluid | Budavari 1989 |
| | Amber, viscous liquid | Nikitakis and McEwen 1990 |
| | Light brown to brown, viscous semiliquid | Lewis 1993 |
| Grades | Technical, unbleached, bleached, fluid, plastic, edible, FCC, 96+% for biochemical or chromatographic standards | Lewis 1993 |
| Natural | Unbleached–plastic; unbleached–fluid; bleached–plastic; bleached–fluid; double-bleached–plastic; double-bleached–fluid | FASEB 1979 |
| Refined | Oil-free lecithins; alcohol-soluble phosphatide fractions; alcohol-insoluble phosphatide fractions | |
| Modified | Hydroxylated lecithin | |
| Odor and taste | Odorless or characteristic, slight nutlike odor; bland taste | NAS 1996 |
| | Faint fatty odor | Nikitakis and McEwen 1990 |
| Melting point | 236–237°C | Lide 1993 |
| pH | 6.6 | Sartoretto 1967 |
| Specific gravity | | |
| (24°/4°C) | 1.0305 | Budavari 1989 |
| (25°/25°C) | 1.02–1.06 | Nikitakis and McEwen 1990 |
| Specific rotation $[\alpha]_D^{24/}$ | +7.0 | Lide 1993 |
| Stability | Decomposes upon heating | Grant 1972 |
| | Soybean Lecithin does not darken or oxidize upon indefinite exposure to the atmosphere; egg Lecithin tends to darken and oxidize in air | Sartoretto 1967 |
| Acid value (maximum) | 30–plastic grade | Swern 1982 |
| | 32–fluid grade | |
| | <36–food grade | NAS 1996 |
| Saponification value | 196 | Budavari 1989 |
| | 170–178 | Nikitakis and McEwen 1990 |
| Chemical characterization | | |
| Benzene insolubles | 0.3% maximum | Nikitakis and McEwen 1990 |
| Acetone insolubles | 40.0% maximum | |
| Ash | 5.5% maximum | |
| Moisture | 2.0% maximum | |
| Chemical characterization of Soybean Lecithin | Three major phosphatides and phosphatidic acid, steryl glucosides, cerebrosides, and triglycerides, and trace amounts of riboflavin, biotin, tocopherol, and other vitamins; actual composition depends on the manufacturing process and variety of soybean | FASEB 1979 |

*(Continued on next page)*

**TABLE 1**
Physical and chemical properties of Lecithin *(Continued)*

| | | Reference |
|---|---|---|
| | Many phospholipids and triglycerides, with smaller amounts of sterols and carbohydrates | Ghyczy, Gareiss, and Kovats 1994 |
| | 2/3 phosphatides and 1/3 glyceride oil; 2.2% phosphorus and 0.9% nitrogen on the dry basis; moisture content is 1% | Sartoretto 1967 |
| | No glyceride oils; 4% phosphorus and 2% nitrogen on the dry basis; moisture content is 5% | Sartoretto 1967 |
| Solubility | "Commercial": soluble in aliphatic and aromatic hydrocarbons, partially soluble in aliphatic alcohols | Sartoretto 1967 |
| | "Soybean": soluble in mineral oils and fatty acids, practically insoluble in cold vegetable and animal oils | |
| | Insoluble in water | Grant 1972 |
| | Partially soluble in water—readily hydrates to form emulsions; partially soluble in alcohol and practically insoluble in acetone when all phosphatide fractions are present | NAS 1996 |
| | Soluble in cold absolute alcohol, chloroform, ether, petroleum ether, mineral oils, fatty acids; sparingly soluble in benzene; practically insoluble in cold vegetable and animal oils; insoluble in acetone; insoluble but swells in water and sodium chloride solution | Budavari 1989 |
| | Disperses in water to form a white emulsion | Nikitakis and McEwen 1990 |
| | Soluble in ether, chloroform, and petroleum ether | Lide 1993 |
| | Soluble in chloroform and benzene; partly soluble water and acetone | Lewis 1993 |
| Iodine value | 95 | Budavari 1989 |

**TABLE 2**
Fatty acid composition of some Lecithins

| | Natural Soybean Lecithin* | Oil-free Soybean Lecithin | Soybean Lecithin |
|---|---|---|---|
| Saturated fatty acids | | | |
| Palmitic acid | 16.4% | 19.8% | 11.7% |
| Stearic acid | 5.9% | 3.9% | 4.0% |
| Total Saturates | 22.3% | 23.7% | 24.7% |
| Unsaturated fatty acids | | | |
| Oleic acid | 16.9% | 9.2% | 9.8% |
| Linoleic acid | 54.1% | 60.2% | 55.0% |
| Linolenic acid | 6.8% | 6.8% | 4.0% |
| Palmitoleic acid | | | 8.6% |
| Total unsaturates | 77.8% | 76.2% | 77.4% |
| $C_{20}$–$C_{22}$ acids (including arachidonic acid) | | | 5.5% |
| Unsaturated/saturated ratio | 3.5:1 | 3.2:1 | 3.1:1 |
| Reference | FASEB 1979 | FASEB 1979 | Budavari 1989 |

*Natural Lecithin is used to describe separated, unrefined Lecithin.

In mixed micelles, degradation almost exclusively affects the Lecithin component through hydrolysis into free fatty acids and lysolecithin (Teelmann et al. 1984). Storage at room temperature for 4 years may result in degradation of 20% to 25% of the Lecithin.

### Analytical Methods

Lecithin has been resolved from extracted lipids in cultured tissue by high-performance thin-layer chromatography (Henault and Killian 1993), and has been assayed by high-performance liquid chromatography in preparations of human erythrocyte ghost membranes, lymphocytes, and thrombocytes (Rehman 1991). Lecithin has been determined using $^{31}P$ magnetic resonance spectroscopy (Merchant et al. 1991; Merchant and Glonek 1992). Lecithin in amniotic fluid had been assayed using thin-layer chromatography (Coch and Kessler 1972; Gilfillan et al. 1983) as well an electrochemical assay based on sequential enzymatic reactions causing the stoichiometric transformation of Lecithin to hydrogen peroxide (Diomede, Agosti, and Salmona 1993).

### Ultraviolet Absorbance

Published data on the ultraviolet absorbance of Lecithin and Hydrogenated Lecithin were not found.

### Impurities

Upon analysis of 17 Lecithin samples (both soy and egg) from various suppliers, all samples were contaminated with significant amounts of trimethylamine (0.76–14.62 $\mu$mol/g preparation) and some also contained dimethylamine (0.12–1.78 $\mu$mol/g preparation) (Zeisel, Wishnok, and Blusztajn 1983).

"Commercial lecithin" is a mixture of acetone-insoluble phosphatides for which the Food Chemical Codex (FCC) specifies that there be not less than 50% acetone-insoluble matter (phosphatides) (Lewis 1993). Soybean protein, 1.03 to 27.2 mg/g, was found in six of seven (soy) Lecithin samples (Smolinske 1992). In pure Lecithin (soya phosphatidylcholine) preparations, hydrolysis to the lyso product is slight (Nattermann Phospholipids GmbH 1995).

## USE

### Cosmetic

Lecithin and Hydrogenated Lecithin function as skin conditioning agents—miscellaneous and as surfactant—emulsifying agents: Hydrogenated Lecithin also functions as a suspending agent—nonsurfactant (Wenninger, Canterbery, and McEwen 2000). Lecithin can act as a dispersing agent for pigments and as an antioxidant (Wilkinson and Moore 1982). The product formulation data submitted to the Food and Drug Administration (FDA) in 1997 reported that Lecithin was used in 674 cosmetic formulations, two of which were specified as Lecithin, Soybean, and that it was part of a trade name mixture that had five uses (FDA 1997). Hydrogenated Lecithin was used in 29 cosmetic formulations (Table 3).

Concentration of use values are no longer reported to the FDA by the cosmetic industry (FDA 1992). However, product

**TABLE 3**
Product formulation data for Lecithin and Hydrogenated Lecithin (FDA 1997)

| Product category | Total no. of formulations in category | Total no. containing ingredient |
|---|---|---|
| | Lecithin | |
| Bath oils, tablets, and salts | 117 | 1 |
| Eyebrow pencil | 89 | 2 |
| Eyeliner | 499 | 9 |
| Eye shadow | 501 | 78 |
| Eye lotion | 18 | 4 |
| Mascara | 158 | 13 |
| Other eye makeup preparations | 116 | 14 |
| Other fragrance preparations | 137 | 1 |
| Hair conditioners | 596 | 39 |
| Hair sprays (aerosol fixatives) | 255 | 19 |
| Permanent waves | 297 | 2 |
| Rinses (noncoloring) | 42 | 1 |
| Shampoos (noncoloring) | 825 | 34 |
| Tonics, dressings, and other hair grooming aids | 512 | 21 |
| Wave sets | 55 | 1 |
| Other hair preparations | 311 | 10 |
| Blushers (all types) | 229 | 18 |
| Face powders | 245 | 23 |

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**TABLE 3**
Product formulation data for Lecithin and Hydrogenated Lecithin (FDA 1997) *(Continued)*

| Product category | Total no. of formulations in category | Total no. containing ingredient |
|---|---|---|
| Lecithin | | |
| Foundations | 283 | 107 |
| Lipstick | 758 | 32 |
| Makeup bases | 125 | 44 |
| Rouges | 12 | 1 |
| Makeup fixatives | 8 | 2 |
| Other makeup preparations | 122 | 19 |
| Cuticle softeners | 19 | 2 |
| Nail polish and enamel | 78 | 1 |
| Other manicuring preparations | 59 | 1 |
| Bath soaps and detergents | 341 | 1 |
| Other personal cleanliness products | 262 | 2 |
| Aftershave lotion | 212 | 2 |
| Shaving cream | 138 | 1 |
| Other shaving preparation products | 60 | 4 |
| Cleansing preparations | 630 | 13 |
| Face and neck preparations (excluding shaving preparations) | 251 | 13 |
| Body and hand preparations (excluding shaving preparations) | 776 | 26 |
| Foot powders and sprays | 32 | 1 |
| Moisturizing preparations | 743 | 47 |
| Night preparations | 185 | 16 |
| Paste masks (mud packs) | 247 | 15 |
| Skin fresheners | 181 | 2 |
| Other skin care preparations | 683 | 26 |
| Suntan gels, creams, and liquids | 134 | 1 |
| Indoor tanning preparations | 50 | 1 |
| Other suntan preparations | 43 | 2 |
| 1997 total | | **672** |
| Lecithin, Soybean | | |
| Foundations | 283 | 1 |
| Other skin care preparations | 683 | 1 |
| 1997 total | | **2** |
| Trade name | | |
| No. of uses as part of a trade name mixture | | **5** |
| Hydrogenated Lecithin | | |
| Eye shadow | 501 | 3 |
| Face powders | 245 | 4 |
| Foundations | 283 | 1 |
| Lipstick | 758 | 2 |
| Makeup bases | 125 | 1 |
| Rouges | 12 | 1 |
| Face and neck preparations (excluding shaving preparations) | 251 | 2 |
| Moisturizing preparations | 743 | 7 |
| Night preparations | 185 | 2 |
| Paste masks (mud packs) | 247 | 1 |
| Other skin care preparations | 683 | 3 |
| Suntan gels, creams, and liquids | 134 | 1 |
| Other suntan preparations | 43 | 1 |
| 1997 total | | **29** |

formulation data submitted to the FDA in 1984 stated that Lecithin was contained in 665 cosmetic formulations with the most frequently used concentration, reported for 325 formulations, being in the range of 0.1% to 1% and the maximum concentration of use, reported for two moisturizing products, being in the range of 25% to 50% (Table 4). Concentration of use data submitted by one company reported 65% Lecithin to be used at concentrations of 0.1% to 3.0% (CTFA 1996). Hydrogenated Lecithin was not reported as being used in 1984 (FDA 1984).

**TABLE 4**
Concentration of use data for Lecithin (FDA 1984)

| | Concentration of use (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Product category | 25–50 | 10–25 | 5–10 | 1–5 | 0.1–1 | 0–0.1 | Unknown | Total |
| Bath oils/tablets/salts | | | | | 1 | | 5 | 6 |
| Eyeliner | | | | 6 | 10 | | | 16 |
| Eye shadow | | | | 12 | 17 | 11 | | 40 |
| Eye lotion | | | | | | 1 | | 1 |
| Eye makeup remover | | | | 1 | | | | 1 |
| Mascara | | | | | 6 | 8 | | 14 |
| Other eye makeup preparations | | | | 1 | 6 | 2 | 3 | 12 |
| Other fragrance preparations | | | | | 1 | | | 1 |
| Hair conditioners | | | | 4 | 12 | 5 | 2 | 23 |
| Hair sprays (aerosol fixatives) | | | | 1 | | 5 | | 6 |
| Permanent waves | | | | | | 2 | 1 | 3 |
| Rinses (noncoloring) | | | | | 1 | | | 1 |
| Shampoos (noncoloring) | | | | | 7 | 17 | | 24 |
| Tonics/dressings/other hair-grooming aids | | | | 1 | 5 | 1 | 2 | 9 |
| Other hair preparations | | | | | 1 | | 1 | 2 |
| Blushers (all types) | | | | 4 | 23 | | 6 | 33 |
| Face powders | | | | | 1 | | | 1 |
| Foundations | | 1 | | 29 | 89 | 2 | 9 | 130 |
| Lipstick | | | | 12 | 13 | | | 25 |
| Makeup bases | | | | 27 | 54 | 1 | 44 | 126 |
| Rouges | | | | | 2 | | | 2 |
| Other makeup preparations | | | | 1 | 6 | | 2 | 9 |
| Nail creams/lotions | | | 1 | | | | 2 | 3 |
| Other manicuring preparations | | | | 1 | | | | 1 |
| Bath soaps/detergents | | | | | 1 | | | 1 |
| Other personal cleanliness products | | | | 2 | | | | 2 |
| Aftershave lotions | | | | 1 | 1 | | | 2 |
| Shaving cream (aerosol/brushless/lather) | | | | | 1 | | | 1 |
| Skin cleansing products (cold creams/lotions/liquids/pads) | | 1 | | 2 | 12 | 14 | 2 | 31 |
| Face/body hand (excluding shaving preparations) | | 3 | | 9 | 12 | 1 | 11 | 36 |
| Hormone products | | | 7 | | 1 | | | 8 |
| Moisturizing products | 2 | | | 10 | 24 | 2 | 14 | 52 |
| Night preparations | | | | 4 | 5 | 2 | 1 | 12 |
| Paste masks (mud packs) | | | | 2 | 1 | | | 3 |
| Skin lighteners | | | | | | | 3 | 3 |
| Skin fresheners | | | | | 1 | | 2 | 3 |
| Wrinkle smoothing products (removers) | | | | 1 | | | | 1 |
| Other skin care preparations | | | | 5 | 5 | 5 | 2 | 17 |
| Suntan gels/creams/liquids | | | | 1 | 3 | | | 4 |
| 1984 totals | **2** | **5** | **8** | **134** | **325** | **79** | **112** | **665** |

### International

Lecithin is listed in the *Japanese Comprehensive Licensing Standards of Cosmetic by Category* (*CLS*) as Lecithin and Egg Yolk Lecithin (Rempe and Santucci 1997). Lecithin, which conforms to the specifications of the *Japanese Standards of Food Additives*, and Egg Yolk Lecithin, which conforms to the specifications of the *Japanese Cosmetic Ingredients Codex* (*JCIC*), has precedent for use without restriction in all *CLS* categories except eyeliner preparations, for which there is no precedent for use. Hydrogenated Lecithin, as Hydrogenated Egg Yolk Phospholipids, is also listed in the *CLS*. Hydrogenated Egg Yolk Phospholipids, which conform to the specifications of the *JCIC*, have precedent for use without restriction in all *CLS* categories except eyeliner, lip, oral, and bath preparations, for which there is no precedent for use. Lecithin and Hydrogenated Lecithin are not listed in Annex II (list of substances which must not form part of the composition of cosmetic products) or Annex III (list of substances which cosmetic products must not contain except subject to the restrictions and conditions laid down) of the Cosmetics Directive of the European Union (European Economic Community 1995).

### Noncosmetic

FDA has published a Final Rule on the use of Lecithin as an anorectic for over-the-counter (OTC) drug use (CTFA 1991). The Final Rule has a category II conclusion (conditions under which OTC drug products are not generally recognized as safe) for reasons of safety and effectiveness. Lecithin is used in pharmaceuticals and has veterinary use as a lipotropic agent (Budavari 1989).

Lecithin is an edible and digestible surfactant of natural origin that is used in the food industry in general (Budavari 1989). Lecithin, meeting FCC specifications, has generally recognized as safe (GRAS) status in foods with no limitation other than current good manufacturing process (Rothschild 1986). It is also GRAS as a miscellaneous and/or general purpose feed additive. Lecithin is cleared by the Meat and Poultry Inspection Division as an antioxidant in lard and shortening in an amount sufficient for the purpose and as an emulsifier in oleomargarine, shortening, and various meat food products, with a limit of 0.5% in oleomargarine, and for use in other products in a sufficient amount for emulsification (Rothschild 1986). Lecithin migrates to food from packaging materials (Sax 1979).

Lecithin is used as an emulsifying, dispersing, wetting, and penetrating agent, as an antioxidant, a blending agent in oils and resins, and as a lubricant for textile fibers. It is also used in animal feeds, paints, the petroleum industry, printing inks, mold release for plastics, in rubber processing (Lewis 1993), and in treating leather (Budavari 1989).

## GENERAL BIOLOGY

### Absorption, Distribution, Metabolism, Excretion.

Five fasted human subjects, one male and four females, were given three capsules of radioactive Lecithin (1 g of Lecithin containing 50 $\mu$Ci di-[1′-$^{14}$C]linoleoyl-3-*sn*-glycerophosphocholine and 150 $\mu$Ci $^{3}$H-polyenephosphatidylcholine ) (Zierenberg and Grundy 1982). The subjects excreted 2 $\pm$ 0.7% and 4.5 $\pm$ 1.5% $^{3}$H and $^{14}$C, respectively, in the feces and 6 $\pm$ 0.8% and 1.2 $\pm$ 0.4% $^{3}$H and $^{14}$C, respectively, in the urine over 7 days. After a 2-hours lag time, radioactive lipids could be measured in the blood; the peak of $^{14}$C was reached between 4 and 12 hours and the peak of $^{3}$H was reached between 6 and 24 hours.

In rats and monkeys given Lecithin (soy phosphatidylcholine) orally, the major target organ was the liver, although significant amounts of radioactivity were detectable after 6 hours in striated muscle, depot fat, and the kidneys (Nattermann Phospholipid GmbH 1995). After repeated administration for 1 week, the organ distribution was similar, with additional small amounts of radioactivity in the lungs, testes, intestines, skin, thymus, and thyroid gland. Excretion via the feces was only 38%, and 15% of a single oral dose was exhaled. For both rats and monkeys, 17% to 18% of the dose was excreted in the urine in 5 days.

Fasted Wistar rats were given radioactive polyunsaturated Lecithin and absorption was monitored (LeKim and Betzing 1976). The absorption rate, as measured by disappearance from the gastrointestinal tract, was comparatively rapid in the first 6 to 8 hours and then became considerably slower. More than 90% of the radioactivity was absorbed from the intestinal tract within 24 hours of administration.

$^{14}$C-Dilinoleoylphosphatidylcholine, with the radioactivity attached at the 1- or 2-position in the acyl moiety or at the choline moiety, and dilinoleoylphosphatide, with $^{3}$H in the acyl moiety and $^{14}$C in the choline moiety, were given orally to fasted male and female Wistar rats at a dose of 70 mg/kg (Fox, Betzing, and LeKin 1979). A low percentage of radioactivity was excreted in the feces (1.1% $^{3}$H and $^{14}$C after 24 hours; 8.2% and 3.2%, respectively, after 120 hours), indicating that Lecithin was almost completely absorbed from the intestinal lumen. A considerable amount of radioactivity was found in the intestinal wall 3, 6, and 8 hours after dosing. The amount of $^{3}$H and $^{14}$C in the urine was 2.0% and 1.6%, respectively, 24 hours after dosing and 15.6% and 6.4%, respectively, 120 hours after dosing. The amount in expired air ($^{3}H_2O$ and $^{14}CO_2$) was 1.0% and 16.6% 24 hours after dosing and 6.6% and 32.0% 120 hours after dosing, respectively. The amount recovered in the carcass was 58.8% and 51.3%, respectively, 120 hours after dosing.

Human beings, dogs, and rats were given radioactive 1,2-dilinoleoyl-*sn*-glycero(3) phosphocholine (Nattermann Phospholipid GmbH 1995). More than 90% was absorbed from the intestinal lumen within 24 hours.

Normal human subjects consumed equimolar doses of (soy) Lecithin and urine was collected daily (Zeisel, Wishnok, and Blusztajn 1983). After ingestion of Lecithin, significantly greater amounts of dimethylamine and trimethylamine (TMA) were excreted as compared to control values obtained after consumption of a normal diet. If the Lecithin was "cleaned" prior to dosing (in which the Lecithin contained only 4% TMA as compared to the non-cleaned compound), methylamine excretion did not increase to as great an extent.

Methylamine excretion by rats was also examined by Zeisel, Wishnok, and Blusztajn (1983). Sprague-Dawley rats were fed a choline-free diet for 48 hours, and urine was collected during the second 24-hour period. During hour 49, the test animals were given Lecithin orally and a control group was given 2 ml 0.9% saline. Administration of 2.0 mmol Lecithin increased TMA excretion as compared to control values, whereas administration of "cleaned" Lecithin did not increase methylamine excretion.

In a study to determine the fate of dietary Lecithin, Hebrew University strain female rats were given of 1-palmitoyl-9,10-$^{3}$H-lecithin-$^{32}$P or 1-palmitoyl-9,10-$^{3}$H, 2-linoleoyl-1-$^{14}$C-lecithin-$^{32}$P or choline-(methyl)-$^{3}$H-lecithin-$^{32}$P or 1-acyl lysolecithin-$^{32}$P in corn oil (0.5 or 1.0 ml) by stomach tube (Scow, Stein, and Stein 1967). Lipids in chyle, chylomicrons, infranatant fraction, and corn oil were extracted. Radioactivity was counted on three channels using a liquid scintillation spectrometer (set to discriminate between the different particles emitted by $^{3}$H, $^{14}$C, and $^{32}$P). The authors determined the specific activity of each isotope in each fraction analyzed. Although the proportion of Lecithin in chylomicrons increased with the amount of Lecithin in the diet, most of the Lecithin in chylomicrons came from endogenous sources. The authors concluded that the major part of the Lecithin molecule is conserved during intestinal absorption and that, although Lecithin may be hydrolyzed and absorbed as lysolecithin, it is reacylated before being incorporated into chylomicrons.

Polyunsaturated Lecithin was not significantly absorbed in an intact state after oral administration to normal male New Zealand albino rabbits (Adams et al. 1969).

Lecithin (phosphatidylcholine) was labeled on the fatty acid or glycerol moieties or with $^{32}$P and injected into the intestine of rats (Parthasarathy, Subbaiah, and Ganguly 1974). Hydrolysis of the material was considerable 60 min after injection of 1-($^{14}$C-acyl) and 2-1-$^{14}$C-linoleoyl-Lecithin, resulting in the appearance of radioactive lysophosphatidylcholine and radioactive unesterified fatty acid in the lumen and the mucosa; the 2-($^{14}$C-acyl)–compound provided more radioactive unesterified fatty acids and less radioactive lysophosphatidylcholine. With both compounds, significant amounts of radioactive triglycerols appeared in the mucosa, with the 2-($^{14}$C-acyl)–compound providing a greater amount. After administration of $^{14}$C-glycerol-Lecithin, only a small portion of the 52% of the original radioactivity recovered 60 minutes after administration was incorporated into the triacylglycerols of the mucosa; the remainder was present as Lecithin and lysophosphatidylcholine in the lumen and the mucosa. After 180 minutes, total recovery decreased to 26% to 33% of the injected radioactivity, but the amount of $^{14}$C-glycerol incorporated into the triacylglycerol fraction of the mucosa was greater. Significant amounts of $^{32}$P radioactivity appeared in the intestine in the form of glycerophosphate and $P_i$ after administration of $^{32}$P-Lecithin.

Absorption by the gallbladder of uniformly labeled 0.1 to 1.0 mM $^{14}$C-Lecithin in bile containing bromosulphthalein was studied in guinea pigs in situ (Neiderhiser, Morningstar, and Roth 1973). After instillation of 1 ml of Lecithin-containing bile, 12% to 20% of the Lecithin was absorbed, with the amount absorbed appearing to be related to the amount of Lecithin instilled. At doses of 0.1 and 0.5 mM Lecithin, $7 \pm 2\%$ and $3 \pm 1\%$ of the radioactivity was located in the gallbladder wall, respectively, and the remainder of the radioactivity missing from the gallbladder lumen was recovered in the expired carbon dioxide, carcass, and the liver. (This information was not provided for the 1.0 mM dose.) Abnormalities were not found at microscopic examination.

Liposomes containing 50 mg (egg) Lecithin were labeled with hydrophilic and lipophilic fluorophores and 60 $\mu$l were applied to 2 cm$^2$ excised supravital human skin within 24 hours (Lasch, Laub, and Wohlrab 1991). Micrographs from various times after liposome application indicated that intact liposomes did not penetrate deeper than the stratum corneum.

Nonocclusive application of liposomes containing >80% Lecithin (soy phosphatidylcholine) resulted in the penetration of 30% of the phospholipid into murine skin subdermis (Cevc and Blume 1992). Penetration (measured by the uptake of $^{3}$H-dipalmitoyl phosphatidylcholine) was maximal when the liposomes dried on the skin surface. Uptake into the blood was detectable within 30 minutes and maximal after 6 hours.

(Soybean) Lecithin-based liposomes radiolabeled by incorporating $^{3}$H in the fatty acid chain were applied to shaved dorsal skin of three female piglets in an open manner at doses of 0.2, 0.4, 1.0, 1.9, and 3.6 mg phospholipid/cm$^2$ skin (Röding and Artmann 1992). The mean particle diameter of the liposomes was 250 nm. Twenty tape strippings were taken intermittently after 30, 60, and 180 minutes. The amount of phospholipid in the stratum corneum and underlying skin sections was determined after 30, 60, and 180 minutes using liquid scintillation counting on samples acquired by 20 tape strippings. The amount of phospholipid in the stratum corneum strips increased with time. The phospholipid concentration in the second strip 180 minutes after application of 0.2 and 3.6 mg phospholipid/cm$^2$ was 5.9 and 30.2 $\mu$g/cm$^2$, respectively. The phospholipid concentration in the stratum corneum averaged for the concentrations 1.0, 1.9, and 3.6 mg/cm$^2$ after 180 minutes was 100,000 $\mu$g phospholipid/g tissue; at 1 mm in the epidermis, the phospholipid concentration was 500 $\mu$g/g tissue. Greater than 99% of the applied phospholipid accumulated in the stratum corneum.

Small (30–60 nm) and large (400 nm diameter) liposomes composed of Lecithin (phosphatidylcholine) and equimolar cholesterol, containing carboxyfluorescein and $^{111}$In-bleomycin, were injected into the footpad of normal male Wistar rats; large liposomes were also injected into the footpad of Walker 256 tumor-bearing rats (Tümer et al. 1983). After injection, 1.3% per ml blood of carboxyfluorescein administered in the small liposomes entered the circulation in latent form in 1 hour and a peak concentration of 1.54% of injected dose per ml of blood, corresponding to 16.9% of the injected preparation, was achieved within 3.5 hours; latent carboxyfluorescein concentrations then declined slowly. These data indicated that the large liposomes

only marginally entered the circulation and were removed rapidly. At 48 hours after dosing, the uptake of radioactivity for normal rats was as follows with small and large liposomes (in percent of dose per g tissue or ml blood), respectively: liver, 3.7 and 0.04; spleen, 4.8 and 0.06; footpad, 35.1 and 43.9; blood, 0.08 and 0.10; popliteal lymph nodes, 463.0 and 195.1; lumbar lymph nodes, 182.6 and 47.6. For carcinoma-bearing rats injected with large liposomes, 218.4% and 252.7% per g of the radioactivity was in the popliteal and lumbar lymph nodes, respectively.

Liposomal Lecithin (soya phosphatidylcholine) was distributed more in the liver than in the spleen, and the reverse was true for Hydrogenated Lecithin (soya phosphatidylcholine) liposomes (Nattermann Phospholipid GmbH 1995).

## Biochemistry

The major route of Lecithin (phosphatidylcholine) biosynthesis in mammalian cells is the cytidine diphosphate (CDP) choline pathway (George et al. 1991). Other routes of Lecithin synthesis include methylation of phosphatidylethanolamine, $Ca^{2+}$-dependent base exchange, and reacylation of lysophospholipid. Other references regarding Lecithin synthesis include Bjørnstad and Bremer 1966; Price, Morris, and Hall 1989; Vance 1990; George et al. 1991; Tercé et al. 1991; Fisk and Kano-Sueoka 1992.

## Penetration Enhancement

The amount of vitamin A and vitamin E absorbed into the skin from liposomes and water-in-oil (w/o) and oil-in-water (o/w) emulsion ointments was examined using egg yolk and rape Lecithin (Szulc et al. 1994). Neutral liposomes were made using Lecithin and cholesterol in a 9:1 ratio. Liposomes made with egg yolk Lecithin contained 3.3% and 6.4% vitamin A and E, respectively, and liposomes made with rape Lecithin contained 11.3% and 24.4% vitamin A and E, respectively. The vitamins were placed into the emulsion ointment freely or bound to liposomes in quantities such that equal initial concentrations (approximately 0.1%) were present. Skin penetration was determined by applying a plastic frame containing the preparations to the left forearms of 30 subjects and after 6 hours, determining the vitamin quantity remaining in the frame using spectrophotometry. The authors suggested that greater amounts of vitamins A and E were absorbed using rape Lecithin in comparison to egg Lecithin. Actual penetration results are shown in Table 5.

**TABLE 5**
Penetration of vitamins A and E in the presence of Egg or Rape Lecithin (Szulc et al. 1994)

| | Penetration | |
|---|---|---|
| Lecithin source | Vitamin A | Vitamin E |
| Egg Lecithin—w/o | 50.4 ± 9.1% | 27.1 ± 8.8% |
| Rape Lecithin—w/o | 60.4 ± 14.5% | 42.7 ± 8.5% |
| Egg Lecithin—o/w | 64.4 ± 15.6% | 36.5 ± 7.3% |
| Rape Lecithin—o/w | 73.7 ± 6.8% | 39.7 ± 11.6% |

In a skin-blanching assay, pretreatment of the skin with 5 mg/ml (egg) Lecithin in a liposomal suspension prior to corticosteroid application altered the bioavailability of the corticosteroids (Jacobs, Martin, and Marriott 1988). This study is described in greater detail in the "Clinical Assessment of Safety—Skin Effects" section of the report.

## Drug Delivery

(Soybean) Lecithin organogels, which are readily obtained by adding a minimal amount of water to a solution of Lecithin in organic solvents, have been studied in vitro with human skin samples as matrices for the transdermal transport of drugs, that is, the transport of pharmacologically active compounds through the skin and into the blood vessels (Willimann et al. 1992). The presence of Lecithin in organic solution often increases solubility as compared to solvent alone; this was observed with broxaterol and nifedipine, and good solubility was obtained with $\beta$-estradiol, clonidine, and various other compounds. Using scopolamine, the transport rate with the Lecithin gel ($w_0$ [water/Lecithin] = 3) was approximately 10 times greater than that of aqueous solution. With broxaterol using a 200 mM Lecithin microemulsion solution ($w_0 = 0$) as donor, the transport rate was 47 $\mu$g/h/cm$^2$ at a drug concentration of 75 mg/ml; the transdermal delivery rate was proportional to concentration. In other preliminary studies, it was found that various other substances, such as amino acids and peptides, can be transported transdermally via Lecithin gels. The researchers offered the preliminary speculation that the Lecithin gels cause a slight disorganization of the skin structure, permitting the permeation of various substances.

Liposomal delivery of radioactive retinoic acid increased the dermal concentration of the drug two- to threefold with an equivalent reduction in systemic absorption and renal excretion (Nattermann Phospholipid GmbH 1995).

The use of (egg yolk) Lecithin and Hydrogenated (soya) Lecithin in polyvinyl alcohol hydrogels for rectal administration of propanol hydrochloride (Morimoto et al. 1990) and of Hydrogenated (soybean) Lecithin in indomethacin-containing suppositories (Nakajima et al. 1989) has been investigated. Prolonged release was generally observed.

Because of the use of liposomes as carriers of drugs, Hanschmann and Wolf (1986) investigated the cytoxicity of neutral and positively-charged (egg) Lecithin-containing empty MLV using the a mouse myeloma cell line. The neutral and positively charged MLV had a "considerable cytotoxic effect," leading the authors to suggest that the use of liposomes to protect an encapsulated drug against degradation and to enhance its uptake should be approached with care.

Also studying the effects of liposomes that may be used in drug delivery, Mayhew, Ito, and Lazo (1987) examined the toxicity of several liposomes on nine human tumor cell lines and one human diploid fibroblast cell line. Phosphatidylcholine

(Lecithin):cholesterol liposomes (1:1 molar ratio) prepared as multilamellar liposomes or sonicated mainly unilamellar liposomes were nontoxic ($ID_{50}$ > 4000 $\mu$M) to urinary bladder carcinoma RT-4, colon adenocarcinoma HT-29, lung carcinoma PC-1 and A549, rhabdomyosarcoma A204, and diploid fibroblast MLD cell lines. Dipalmitoylphosphatidylcholine liposomes, whether multilamellar or unilamellar, was similarly nontoxic ($ID_{50}$ > 3000 $\mu$M) across all cell lines tested. Liposomes containing stearylamine and cardiolipin were toxic at low concentrations ($ID_{50}$ = 200 $\mu$M). Phosphatidylserine and phosphatidylglycerol containing liposomes were toxic in the range 130 to 3000 $\mu$M. The authors urged care in selecting liposomes for use in human studies.

### Cytotoxicity

While studying the toxicity of hemoglobin solutions, Feola et al. (1989) found that phosphatidylethanolamine and phosphatidylserine from red blood cell membranes may be the active agents in the well recognized hemolyzed red blood cell toxicity. Phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine (Lecithin), and phosphatidylinositol were tested by stimulation of murine peritoneal macrophages. At doses of 5 $\mu$g/ml only phosphatidylethanolamine and phosphatidylserine were toxic. At doses of 50 and 100 $\mu$g/ml, phosphatidylcholine (Lecithin) and phosphatidylinositol were also toxic, but less so compared to phosphatidylethanolamine and phosphatidylserine. The authors postulate that phosphatidylethanolamine and phosphatidylserine are responsible for the toxicity of hemolysed red blood cells.

Reybrouck (1978) tested a number of materials that are potentially used as inactivators of disinfectant activity in studies of disinfectant effectiveness (i.e., to eliminate residual activity in postexposure recovery medium). The results demonstrated that Lecithin, 0.3% and 2%, had a germicidal effect toward *Staphalococus aureus* and *Pseudomonas aeroginosa* and so would not be suitable for the intended purpose.

Postulating that certain fatty acids may be responsible for the antimycobacterial activity of immunologically stimulated macrophages, Kondo and Kanai (1978) developed a model system in which tubercle bacilli were exposed to liposomes prepared from Lecithin and cholesterol treated with phospholipase $A_2$. The authors demonstrated that myristic acid and linoleic acid from the liposomes had the greatest antimycobacterial activity.

## TOXICOLOGY

### Acute Toxicity

*Oral*

Groups of five male and five female albino CD-1 outbred mice were dosed orally with 1, 2, 4, 8, or 16 g/kg Lecithin and observed for 14 days (Food and Drug Research Laboratories, Inc. [FDRL] 1973a). No animals died during the study; the oral $LD_{50}$ for albino CD-1 outbred mice was >16 g/kg.

The oral $LD_{50}$ for albino Wistar rats was determined according to the same procedure as above, using the same number of animals per group and the same dosages (FDRL 1973b). No animals died during the study and the oral $LD_{50}$ for albino Wistar rats was >16 mg/kg.

In a study using groups of five male and five female Dutch-Belted rabbits, following the same procedures as above, the animals were dosed with 3, 4, 8, 9, or 16 g/kg Lecithin (FDRL 1973c). The oral $LD_{50}$ for Dutch-Belted rabbits was estimated to be 4.75 $\pm$ 0.64 g/kg.

The maximal nontoxic oral dose of Lecithin (purified soya phospholipids containing 75% to 98% phosphatidylcholine) for the mouse, rat, rabbit, and dog was 20, 20, 5, and 10 g/kg, respectively (Nattermann Phospholipid GmbH 1995).

The maximum nontoxic oral dose of Hydrogenated Lecithin (a fully saturated phospholipid containing 80% to 90% stearic acid) for the mouse and rat was 10 g/kg (Nattermann Phospholipid GmbH 1995).

Five male and five female Sprague-Dawley rats were dosed orally with 5 g/kg 1:2 *w*:*v* Hydrogenated Lecithin in deionized water and observed for 14 days (Leberco-Celsis Testing 1997a). All animals appeared normal, and none died. The oral $LD_{50}$ of 1:2 *w*:*v* Hydrogenated Lecithin for Sprague-Dawley rats was >5 g/kg.

*Parenteral*

As another part of an effort to study the toxicity of hemoglobin solutions, Feola et al. (1989) found that phosphatidylethanolamine and phosphatidylserine from red blood cell membranes may be the active agents in the well recognized hemolyzed red blood cell toxicity. Phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine (Lecithin), and phosphatidylinositol were tested by intravenous administration of 0.05, 0.1, or 0.3 mg/kg in New Zealand rabbits. Surviving animals were killed after 24 hours (Feola et al. 1989). Lecithin did not produce changes in the circulatory or respiratory systems.

The maximal nontoxic intravenous (IV) dose of Lecithin (purified soya phospholipids containing 75% to 98% phos-phatidylcholine) for the mouse, rat, and rabbit was 4, 2, and 0.5 g/kg, respectively (Nattermann Phospholipid GmbH 1995). The maximal nontoxic subcutaneous dose for the mouse, rat, and rabbit was 10, 4, and 1 g/kg, respectively; the same respective doses were found for the maximal nontoxic intraperitoneal dose.

### Short-Term Toxicity

*Oral*

The no-effect daily oral dose of Lecithin (soya phosphatidylcholine) was determined using rats over periods of 4, 6, or 12 weeks (Nattermann Phospholipid GmbH 1995). In these studies, the animals were dosed with $\leq$0.8, $\leq$1.35, or $\leq$2.8 g/kg, respectively. The no-effect daily oral dose for rats was greater than the maximum dose used in each study. The no-effect daily oral dose for dogs dosed for 6 weeks was 1.9 g/kg (details not provided).

Four dogs were fed 5 g/day (soybean) Lecithin for 25 to 60 days (Davis 1944). After a latent period of ≥5 days, the erythrocyte count was gradually reduced, with a maximal decrease of 15% to 20% occurring 12 to 25 days after dose initiation. Erythrocyte numbers returned to normal 11 to 20 days after discontinuation of dosing.

Groups of four male chimpanzees were fed a diet containing 37.6 g/day unsaturated Lecithin or 26.6 g/day Hydrogenated (saturated) Lecithin and one group was given basal diet for 1 month; the diets were adjusted so that each contained the same amount of protein, carbohydrates, and fat and the same number of calories (Rosseneu et al. 1979). Total lipoprotein concentration was similar for the group fed the diet containing Lecithin as compared to controls. Plasma very-low-density lipoproteins and low-density lipoprotein concentrations were "strongly" increased for the group fed the diet containing Hydrogenated Lecithin.

*Parenteral*

The no-effect daily IV dose for rats dosed with Lecithin (soya phosphatidylcholine) for 4 or 12 weeks was 316 to 1000 and 100 to 1000 mg/kg, respectively (Nattermann Phospholipid GmbH 1995). The no-effect daily IV dose for dogs dosed for 4 weeks was >100 mg/kg (details not provided).

Teelman et al. (1984) dosed twelve SPF-bred Wistar-derived Fü-albino rats per sex intravenously for 4 weeks with 0.25, 0.75. or 2.25 ml/kg of a fresh mixed micelle solution containing 169.3 mg Lecithin or with 0.5 or 2.25 ml/kg of the mixed micelle solution, which was artificially decomposed, resulting in decomposition of approximately 25% Lecithin into fatty acids (~20 mg/ml) and lysolecithin (~40 mg/ml). A negative-control group was dosed with 0.9% physiological saline. After 4 weeks of dosing, six rats per sex of the control and high-dose groups and all rats of the low- and mid-dose groups were killed; the remaining six rats per sex of the control and high-dose groups were killed after a 2-week nontreatment period.

The low- and mid-doses of the fresh mixed micelles and the low dose of the artificially decomposed micelles were generally well tolerated throughout the 4 weeks of dosing. Administration of the high dose of the fresh and decomposed mixed micelles was also generally well tolerated, but seemingly slight transient dizziness and drowsiness were occasionally observed immediately upon injection, especially at the beginning of the study. In the high-dose group given the fresh mixed micelles, weight gain for males was slightly less (−11%) and for females slightly more (+5.5%) than that of control animals; weight gain of males given the decomposed mixed micelles was also less than control rats (−14%). The liver and intestines of the high-dose animals given the fresh micelles appeared normal, whereas cellularity in the red pulp of the spleen was slightly increased and hemosiderin content was moderately increased. Hematologic and clinical chemistry evaluations reported increased hemopoietic activity and increased bilirubin content in the plasma of male and female high-dose animals given fresh micelles. These changes were not observed in animals killed after the 2-week nontreatment period. For the high-dose animals given the decomposed mixed micelles, moderate to marked hemolysis with hematopoiesis was observed in males and females and moderate renal tubular basophilia and tubular dilation was observed in males. The animals killed after the 2-week nontreatment period were mostly normal; basophilia of the renal tubules remained but was markedly reduced.

A similar study was performed with the mixed micelle solutions using beagle-like dogs of the "Niederlauf" strain (Teelmann et al. 1984). Groups of two or three dogs per sex were given daily intravenous doses for 4 weeks of 0.25, 0.75, or 2.25 ml/kg of the fresh micelle solution or 0.5 or 2.25 ml/kg of the decomposed solution; three dogs per sex were given physiological saline and served as a control group. Administration of the high-dose decomposed micelle solution had to be suspended after 10 days due to side effects; after a 6-day nontreatment period, the animals were dosed with 1.50 ml/kg until study termination. After 4 weeks of dosing, all animals were killed except for one per sex of the control and high dose groups; these animals were killed after a 2-week nontreatment period.

The low- and mid-doses of the fresh mixed micelles and the low dose of the artificially decomposed micelles were generally well tolerated. However, retching and vomiting was observed in a few animals of the mid-dose group given the fresh mixture, especially during the first 2 weeks of dosing, and sporadic retching and vomiting occurring immediately after dosing and a slight to moderate elevation of the plasma cholesterol values occurred among animals of the low-dose group given the decomposed micelle solutions. All other parameters were normal for the animals of these groups.

Frequent retching and vomiting were observed in several animals given the high dose of the fresh mixed micelles. Salivation was often noted before and after dosing, and the animals appeared languid for about 1 hour after dosing. Body weight gain was not affected. Moderate to marked increases in the activity of alanine aminotransferase and increased concentrations of plasma cholesterol and plasma bilirubin were observed. The absolute and relative liver weights of these high-dose animals were moderately increased compared to those of control animals. All changes were reversible after cessation of dosing. For the animals of the high-dose group given the decomposed mixed micelles, retching, vomiting, salivation, marked intravascular hemolysis, increasing anemia, a reduction in numbers of erythrocytes and concentrations of hemoglobin, and a progressive deterioration of general condition were observed with administration of 2.25 ml/kg. During the 6-day nontreatment period, all parameters improved and remained almost normal after dosing with 1.5 ml/kg. At necropsy, a mild to moderate increase in absolute and relative liver weights and mild to moderate intracanalicular cholestasis of the liver were found at microscopic examination. Most test article–related changes were reversed during the 2-week nontreatment period, although some increase of liver weights and cholestasis were still present.

Groups of female C3H/HeJ or ICR mice were dosed intravenously with (egg) Lecithin-containing liposomes 3 days per week in a series of experiments (Allen et al. 1984). The day 20 phagocytic index was statistically significantly decreased in C3H/HeJ mice after eight 1-mg doses of Lecithin:cholesterol 2:1 MLV, Lecithin:cholesterol:stearylamine 9:5:1 MLV, and Lecithin:cholesterol:phosphatidylserine 9:5:1 MLV liposomes as compared to control values. The Lecithin:cholesterol 2:1 SUV liposome value was not significantly different from the control. Spleen weight in animals receiving nonextruded MLV were significantly greater than control values. In animals of a recovery group that had received 0.45 $\mu$ MLV or 0.2 $\mu$ REV Lecithin:cholesterol extruded liposomes, the phagocytic index continued to decrease for 3 weeks after the last dose and slowly recovered during the next 3 weeks. Liver and spleen weights of recovery animals were not significantly different from control values.

ICR mice were given 10 2-mg injections of liposomes containing $^{14}$C-sucrose and of 0.45 $\mu$ MLV Lecithin-cholesterol 2:1 containing 1% vitamin E, and the phagocytic index was determined 6 or 24 hours after each dose. The phagocytic index differed from control values only after doses 8 and 9 at the 6-hours determination, with the decreased values corresponding to a 30% increase in spleen weight. Mice were given $^{14}$C-sucrose liposomes 24 hours after the last injection, and tissues were sampled after 0.5, 2, 6, and 18 hours. The $^{14}$C-sucrose content in the livers of test animals was "consistently a few percent lower than in control animals and levels in spleen, blood and to some extent lung [were] higher in test animals." ICR mice that were given 2 mg of sphingomyelin-Lecithin 4:1 MLV containing 1% vitamin E had greater functional impairment than mice that were given Lecithin-cholesterol liposomes. Phagocytic index was immediately decreased, had a gradual increase to values above control, and then decreased to normal by week 7 after the last injection. Mice given the 4:1 liposomes were also given $^{14}$C-sucrose liposomes 24 hours after the last injection, and tissues were sampled. "Substantial alterations" in tissue distribution were observed, and a decrease in $^{14}$C content in the liver and an increase in the content in the spleen and blood were observed.

ICR mice were given 16 IV injections of 0.25 mg of Lecithin-cholesterol 2:1 MLV (0.45 $\mu$) at regular intervals over a 2-month period (Allen et al. 1984). No signs of toxicity were observed during dosing or during a 1-month recovery period following the last dose.

Female ICR mice were dosed by intravenously three times a week for a total of 10 or 11 doses with 2 mg of 0.45-$\mu$ MLV sphingomyelin:Lecithin and a control group was dosed with saline (Allen and Smuckler 1985). Granulomas were present in the liver after two injections, with the lesions randomly located in the parenchyma. A significant decrease in the phagocytic index was also observed at this time. Splenomegaly peaked after 8 to 10 doses and varied considerably. The liver normalized rapidly and the animals did not appear to have lesions. Phagocytic indices increased following dosing.

Mice dosed with (egg) Lecithin:cholesterol 2:1 liposomes had no significant structural alterations in the liver or spleen during dosing, and only a "small decrease" in phagocytic index. However, 2 weeks after the last injection, 75% of the mice had hepatic granulomas; these lesions persisted until 9 weeks after dosing (Allen et al. 1984).

## Subchronic Toxicity

*Dermal*

A group of 15 Crl:COBS CD(SD)BR female rats received dermal applications of 5130 mg/kg (5.1 ml/kg) of a commercial tanning oil containing 3.0% Lecithin 65% once daily, 5 days per week, for a total of 68 doses; the dose was estimated to be a 50× multiple of normal human use (assuming 102.6 mg/kg as average daily human use) (CTFA 1980a). The test material was applied by gentle inunction to a shaved dorsal area of the back. A negative-control group was untreated. Pharmacologic and toxicologic observations were made daily. Blood was drawn from 10 animals per group during weeks 7 and 13 for blood chemistry and hematology analysis, and pooled urine samples were also collected at this time. All animals were necropsied at study termination.

All test animals survived until study termination. Significant differences in body weight between test and control animals were not observed. With the exception of "sporadic minimal skin irritation," adverse reactions to dosing were not observed. Statistically significant decreased serum glucose and increased blood urea nitrogen (BUN) and activities of serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and serum alkaline phosphatase (SAP) determined at week 7 and decreased hemoglobin and serum glucose and increased white blood cell, BUN, SGPT and SGOT activities determined at week 13 were concluded to be toxicologically insignificant. No significant gross lesions were found at necropsy. Statistically significant increases in absolute liver weights, liver-to-body weight ratios, and absolute kidney weights were observed for the test animals as compared to the controls; these differences were also considered toxicologically insignificant. At microscopic examination, grade 1 hyperkeratosis was found in three animals. The researchers concluded that "except for skin irritation, no significant systemic toxic effects were noted" for a tanning oil containing 3.0% Lecithin 65% and that "based on the exaggerated dose levels used in this study . . . dermal application is not likely to produce adverse effects under conditions of consumer use."

A group of 15 female Sprague-Dawley rats were dosed with 450 mg/kg (0.45 ml/kg) of a liquid foundation containing 0.3% Lecithin 65% 5 days per week for 66 days following the procedure described above; the dose was estimated to be a 100× multiple of normal human use (assuming 4.5 mg/kg as average daily human use) (CTFA 1982). The negative control group was untreated. One animals died during week 9; the death was not considered treatment-related. Toxicological effects were not

observed. A statistically significant increase in serum glucose observed at week 13 was considered toxicologically insignificant. Dose-related lesions were not observed at necropsy. At microscopic examination, grade 1 hyperkeratosis was found in three test animals. The researchers concluded that a liquid foundation containing 0.3% Lecithin 65% "was well tolerated by the test animals" and that it was "considered to be safe from the viewpoint of cumulative, systemic toxicity."

*Oral*

A group of 15 male and 15 female rats was fed 6.0% (soya) Lecithin for 90 days and a control group was fed untreated feed (Gaunt, Grasso, and Gangolli 1967). The animals were housed five per cage. Body weights and feed consumption were determined weekly. Blood was collected from 10 animals/sex/group during week 6 and from all animals at study termination.

No animals died during the study, and all animals appeared normal. Body weights of test animals were not significantly different from control values. Feed consumption was slightly but statistically insignificantly decreased for males and females of the test groups as compared to that by controls. At week 6, hemoglobin and hematocrit values were statistically significantly decreased for female animals of the test group; this was not observed at study termination or for males of the test group at either time period. Serum chemistry and urinalysis did not differ significantly from control values. Absolute spleen and kidney weights were significantly increased for females of the test group; no significant differences were observed in relative organ weights between the test and control group.

The no-effect daily oral dose for rats dosed with Lecithin (soya phosphatidylcholine) for 24 weeks was >2.8 g/kg (Nattermann Phospholipid GmbH 1995) (details not provided).

## Chronic Toxicity

*Oral*

A group of 48 male and 48 female SPF Wistar rats was fed 4% (soya) Lecithin for 2 years while a control group was fed commercial diet only (Brantom et al. 1973). Feed consumption and body weights were determined prior to dosing, at intervals up to week 95, at week 102, and at study termination. The mean Lecithin intake was 1470 and 2280 mg/kg/day for males and females, respectively. No significant differences were observed for mortality, feed consumption, or body weight between the treated and control groups, but it was noted that feed consumption and body weight were sometimes greater in the treated group as compared to controls. Hematology values of animals of the treated group were similar to those of control animals, as were organ weights and gross and microscopic alterations. Parathyroid gland hyperplasia was increased, particularly in the males; this increase was attributed to an increased phosphate intake. The incidence of tumor formation was similar for the treated and control groups. A slightly increased incidence of myocardial fibrosis was associated with parathyroid gland hyperplasia.

The no-effect daily oral dose for rats dosed with Lecithin (soya phosphatidylcholine) for 48 weeks was >2800 mg/kg; for dogs dosed for 52 weeks, the no-effect daily oral dose was >750 mg/kg. In neither case were study details provided (Nattermann Phospholipid GmbH 1995).

## Dermal Irritation

The primary skin irritation of Lecithin or Lecithin-containing products was determined using rabbits in a number of single insult occlusive patch tests. All products were tested at 100% concentration. These studies are summarized in Table 6.

The skin irritation potential of a soap containing 0.83% Lecithin powder, tested at 0.5%, was determined in a guinea pig immersion study (CTFA 1983a). The immersion score was 9.5, indicating that "the product is practically nonirritating."

The skin irritation potential of Hydrogenated Lecithin was determined using six New Zealand white rabbits (Leberco-Celsis Testing 1997b). An occlusive patch containing 0.5 g Hydrogenated Lecithin was applied for 24 hours to an intact and an abraded site on the clipped skin on the mid-dorsal area of the trunk of each animal. The test sites were scored on a scale of 0 to 4 immediately and 48 hours after patch removal. Application of Hydrogenated Lecithin resulted in very slight erythema and no edema, and flaking skin was observed at one site. The primary irritation score was 0.21/8, and Hydrogenated Lecithin was not a primary dermal irritant.

## Ocular Irritation

The ocular irritation of Lecithin or Lecithin-containing products was determined using rabbits in a number of Draize tests.

**TABLE 6**
Primary skin irritation of Lecithin and Lecithin-containing formulations

| Product | Result | Reference |
|---|---|---|
| Lecithin 65% | Minimal irritation | CTFA 1976 |
| Lecithin 65% | Minimal irritation | CTFA 1978a |
| Tanning oil containing 3.0% Lecithin 65% | Practically nonirritating | CTFA 1979 |
| Eyeshadow containing 2.25% Lecithin 65% | Nonirritating | CTFA 1980b |
| Eyeliner containing 3.0% Lecithin 65% | Moderately irritating | CTFA 1980c |
| Tanning oil containing 3.0% Lecithin 65% (3 studies) | Practically nonirritating in all 3 studies | CTFA 1981a |

**TABLE 7**
Ocular irritation potential of Lecithin and Lecithin-containing formulations

| Product | Result | Reference |
|---|---|---|
| Lecithin 65% | Minimal irritation | CTFA 1976 |
| Lecithin 65% | Minimal irritation | CTFA 1978a |
| Tanning oil containing 3.0% Lecithin 65% | Minimal irritation | CTFA 1979 |
| Eyeshadow containing 2.25% Lecithin 65% | Mild irritation | CTFA 1980b |
| Eyeliner containing 3.0% Lecithin 65% | Nonirritating | CTFA 1980c |
| Tanning oil containing 3.0% Lecithin 65% | Minimal irritation | CTFA 1981a |
| Soap containing 0.83% Lecithin powder, 25% test concentration | Moderate irritation | CTFA 1983a |

All products were tested at 100% concentration, except where noted. The eyes were not rinsed after instillation. These studies are summarized in Table 7.

Fifty microliters of an (egg) Lecithin liposome preparation and a positively charged (egg) Lecithin liposome preparation containing stearylamine was placed in the left conjunctival sac of five male albino rabbits once every 15 minutes for 2 hours for a total of nine applications in a Draize test (Taniguchi et al. 1988). The control group of five rabbits had saline applied to the right conjunctival sac. The maximum mean total score did not exceed "practically nonirritating" and immediately decreased to "nonirritating." Slight hyperemia was observed in the conjunctiva of the right eye. No lesions were found at microscopic examination.

A rabbit blinking test was performed according to the methods of Tanaka et al. (1985) using groups of six male albino rabbits to determine the ocular irritation potential of neutral and positivelycharged (egg) Lecithin liposome preparations (Taniguchi et al. 1988). Fifty microliters of each test preparation was placed into the conjunctival sac of one eye of each rabbit and 50 $\mu$l of saline was placed in the conjunctival sac of the other eyes. The number of blinks of each eye was counted for 5 minutes following instillation. This procedure was repeated six times at 1-hours intervals, alternating the eye in which the test article was placed. Instillation of the neutral liposome preparation did not produce a statistically significant change in the number of blinks, but instillation of the positively charged liposome preparation significantly increased the number of blinks; the blinking count excluding that of both eyes together was also significantly increased.

The ocular potential of Hydrogenated Lecithin was determined by instilling 0.07 g of the test material into the conjunctival sac of one eye of six albino rabbits; the contralateral eyes were untreated and served as controls (Leberco-Celsis Testing 1997c). The eyes were scored 24, 48, and 72 hours postinstillation. Hydrogenated Lecithin produced minimal conjunctival irritation, and all signs of irritation were cleared by day 2. Hydrogenated Lecithin was not a primary ocular irritant.

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

### Teratogenic Effects

*Oral*

Groups of 21 to 23 gravid albino CD-1 mice (25-27 animals/group were mated) were dosed orally with 16.0, 74.3, 345.0, or 1600.0 mg/kg Lecithin in corn oil on days 6 to 15 of gestation (FDRL 1973d). A positive control group was given 150 mg/kg aspirin, and a sham-treated negative-control group was included. Body weights were determined on days 0, 6, 11, 15, and 17 of gestation, and the animals were killed on day 17 of gestation. All gravid animals survived until study termination. The researchers concluded "the administration of up to 1600 mg/kg (body weight) of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Groups of 22 to 24 gravid albino Wistar rats (25 animals/group were mated) were dosed orally with 16.0, 74.3, 345.0, or 1600.0 mg/kg Lecithin in corn oil on days 6 to 15 of gestation (FDRL 1973e). A positive control group was given 250 mg/kg aspirin and a negative-control group was given vehicle only. Body weights were determined on days 0, 6, 11, 15, and 20 of gestation, and the animals were killed on day 20 of gestation. All gravid animals survived until study termination. The researchers concluded "the administration of up to 1600 mg/kg (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Groups of 10 to 12 gravid Dutch-Belted rabbits were dosed orally with 4.75, 22.1, 100.3, or 475.0 mg/kg Lecithin in corn oil on days 6 to 18 of gestation (FDRL 1974). For the 4.75 mg/kg group, 23 animals were mated, and for the remaining test groups 15 to 16 animals/group were mated. A positive control group was

given 250 mg/kg aspirin and a negative-control group was given vehicle only. Body weights were determined on days 0, 6, 12, 18, and 29 of gestation, and the animals were killed on day 29 of gestation. One gravid animal of the 4.75-mg/kg dose group aborted on day 12 of gestation. Neonatal deaths were reported in all groups, including the negative control group. The researchers concluded "the administration of up to 475 mg/kg (body weight) of the test material to pregnant rabbits for 13 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

In studies examining maternal survival and teratogenicity, the lowest toxic daily oral dose of Lecithin (soya phosphatidylcholine) for rats dosed on days 6 to 15 of gestation, rabbits dosed on days 1 to 6 of gestation, and rabbits dosed on days 5 to 18 of gestation was >750, >1000, and >500 mg/kg, respectively. In another study examining peri- and postnatal toxicity and a study determining effects on fertility in which rats were dosed with Lecithin from day 16 of gestation to the end of the third week postpartum, the lowest toxic daily oral dose in both studies was >2800 mg/kg. In neither study were details provided (Nattermann Phospholipid GmbH 1995).

*Parenteral*

In a study examining peri-and postnatal toxicity, the lowest toxic daily IV dose of Lecithin (soya phosphatidylcholine) for rats dosed from day 16 of gestation to the end of the third week postpartum was >1000 mg/kg; study details were not provided (Nattermann Phospholipid GmbH 1995).

Groups of 30 mated SPF-bred Wistar-derived Fü-albino rats were dosed intravenously on days 6 to 15 of gestation with 1.0 or 2.0 ml/kg of an artificially decomposed mixed micelle solution that originally contained 169.3 mg Lecithin (described earlier in the "Short-Term Toxicity" section) (Teelmann et al. 1984). A group of control rats was given physiological saline. The animals were designated to a necropsy group, in which necropsy was performed on day 20 of gestation, or a rearing group, in which the offspring were reared until weaning. No dose-related maternal deaths or signs of maternal toxicity were observed. In the necropsy group, the resorption rate, litter size, and fetal body weights were similar for the treated and control groups. No functional abnormalities were reported for the litters that were delivered. No signs of teratogenicity or embryotoxicity were noted.

Groups of 20 mated albino rabbits were dosed intravenously on days 6 to 18 of gestation with 0.35 or 0.7 ml/kg artificially decomposed mixed micelle solution (Teelmann et al. 1984). A negative control group was given physiological saline. Two dams of the high dose group died during dosing, and body weight gains of treated dams was slightly reduced during dosing. No teratogenic or embryotoxic effects were observed in the low-dose group. In the high-dose group, no effect on resorption rate, body weight, or fetal survival was observed, but the number of abortions increased; this increase was considered unspecific and secondary to maternal toxicity. No test-article malformations were observed.

Groups of 20 mated Swiss Hare rabbits were dosed intravenously on days 6 to 18 of gestation with 0.7 or 2.0 ml/kg of a fresh mixed micelle solution containing 169.3 mg Lecithin described earlier (in the "Short-Term Toxicity" section), which was stored for 4 months, resulting in decomposition of approximately 5% Lecithin into fatty acids (~4 mg/ml) and lysolecithin (~8 mg/ml) (Teelmann et al. 1984). Administration of the low dose resulted in a slight but reversible reduction in body weight gain and moderate to marked diarrhea in several dams, resulting in the death of two dams of this group. In the high dose group, diarrhea was more marked after 1 to 3 days of treatment, and mortality was >50%; the remaining high dose animals were killed. The resorption rate and fetal body weights and survival were not affected by dosing, but abortions were increased; this was considered an unspecific, secondary effect of maternal toxicity and not a direct fetotoxic effect of the test article. No skeletal, soft tissue, or external malformations were observed for the low dose group.

### Effects on Sperm Motility

A transmembrane migration method (Hong, Chaput de Saintange, and Turned 1981) was used to determine the effect of 0.1, 0.3, 0.6, 1.0, and 3.0 mM Lecithin (phosphatidylcholine ) on the motility of human sperm (Hong et al. 1986). Lecithin had no significant effect on human sperm motility.

## GENOTOXICITY

The mutagenic potential of Lecithin was determined using *Salmonella typhimurium* strains TA1535, TA1537, and TA1538 and *Saccharomyces cerevisiae* D4 with and without metabolic activation (Litton Bionetics, Inc. 1975). Plate tests were performed using 0.02% Lecithin and *S. typhimurium* and suspension tests were performed using 0.01% to 0.04% and *S. typhimurium* strains and with 1.875% to 7.5% Lecithin and *S. cerevisiae*. The vehicle, dimethylsulfoxide, was used as the negative control. Positive controls were dimethylnitrosamine and 2-acetylaminofluorene with activation and ethylmethane sulfonate, 2-nitrofluorene, and quinacrine mustard without activation. Lecithin was not mutagenic in either the plate or suspension assays with or without metabolic activation.

Lecithin (highly purified soya phosphatidylcholine) was not mutagenic in an Ames test using five strains of *S. typhimurium*, in three yeast strains and human embryonic epithelial cell line (EUE) cells, or in the mouse host-mediated and urinary assays in vivo; study details were not provided (Nattermann Phospholipid GmbH 1995).

The effect of Lecithin (egg phosphatidylcholine):cholesterol (4:1) liposomes on $^{3}$H-thymidine incorporation into L1210 cell DNA was examined (Campbell 1983). The liposomes did not seem to inhibit $^{3}$H-thymidine incorporation.

An Ames test was performed using *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 with and without

metabolic activation on an artificially decomposed mixed micelle solution that originally contained 169.3 mg Lecithin (described earlier in the "Short-Term Toxicity"section) (Teelmann et al. 1984). The concentrations tested were 0.1, 1.0, 10.0, and 500.0 $\mu$l/plate. The artificially decomposed mixed micelle solution was not mutagenic with or without metabolic activation.

The addition of Lecithin (phosphatidylcholine) to incubations of TA98 and 1,8-dinitropyrene reduced mutagenicity (Shah, Combes, and Rowland 1991). However, the reduction in mutagenicity was less than that seen with uninduced S9.

### Nitrosamine Formation

Dimethylnitrosamine (DMNA) was reportedly formed in a model system in which 22.8 mmol sodium nitrite in 15 ml of water was added to a buffered solution, pH 5.6, containing 4.56 mmol of Lecithin and stirred at 78°C for 4 hours (Pensabene et al. 1975). The amount of DMNA formed (mg DMNA/kg of compound), confirmed by mass spectrometry, with various Lecithins was as follows: soy Lecithin (edible), 2.05; soy Lecithin (commercial), 0.70; vegetable Lecithin, 1.02; egg Lecithin, 5.40; bovine Lecithin (purified), 1.66; bovine Lecithin (60%), 30.76; and synthetic Lecithin, 319.7.

Lecithin is metabolized to choline by bacterial phospholipases and the released choline is dealkylated to dimethylamine, which is *N*-nitrosatable in the presence of nitrate (Hill 1979).

## CARCINOGENICITY

### Oral

TM strain mice were fed 5 to 10 mg Lecithin mixed with sugar (for palatability) and a second group was fed Lecithin and 4 to 5 mg cholesterol (Szepsenwol 1969). The mice were bred and their offspring dosed following the same procedures; dosing continued until all mice became moribund or had died. A control group was given laboratory feed ad libitum. The total number of mice fed Lecithin, Lecithin and cholesterol, or control feed was 166, 212, and 360, respectively. The brains of the animals that were killed upon the initiation of weight loss were examined; the brains of the animals that died at night were not examined because they could not be removed undamaged by the time the dead animal was found. No complete necropsy results were reported. Brain nerve cell tumors (2–5 mm) were found in 18 of 73 examined animals fed Lecithin and in 27 of 88 examined animals fed Lecithin and cholesterol, whereas no brain nerve cell tumors were found in 188 control animals.

### Parenteral

Groups of female dd mice were dosed subcutaneously as follows: 50 mice were given 0.1 ml of a 0.25% mixture of 4-nitroquinoline 1-oxide in 10% Lecithin in water in a single injection until the total dose was 2.5 mg, and the injections were repeated weekly to a different site on the back; 30 mice were dosed with a Lecithin-water mixture 10 times at the same total dose as in the previous group; 20 mice were not dosed and served as controls (Mori, Kondo, and Suzuki 1966). The mice were killed after 221 to 296 days. All of the animals dosed with 4-nitroquinoline 1-oxide/Lecithin surviving more than 221 days after dose initiation (36/50) had pulmonary neoplasms, with one case of skin neoplasia at the site of injection and one case of leukemia also were reported. No surviving mice dosed with Lecithin-water or untreated control mice had pulmonary or any other type of neoplasia. However, 3/28 animals of the Lecithin-water group and 3/18 control animals had adenomas; these were considered spontaneous.

Groups of female Buffalo rats were dosed subcutaneously as follows: 25 rats were given 0.2 ml of a 0.25% mixture of 4-nitroquinoline 1-oxide in 10% Lecithin in water in a single injection until the total dose reached 10 mg, and the injections were repeated weekly; 15 rats were dosed with a Lecithin-water mixture 20 times in the same total dose as in the previous group (Mori, Kondo, and Suzuki 1966). The rats were killed after 264 to 329 days. Nineteen of the 21 animals dosed with 4-nitroquinoline 1-oxide/Lecithin that survived more than 264 days after dose initiation had pulmonary neoplasms, with 11 subcutaneous sarcomas and two endometrial sarcomas also reported. No neoplasms were found in any of the 13/15 surviving rats dosed with Lecithin-water.

## CLINICAL ASSESSMENT OF SAFETY

### Dermal Irritation

Two single insult (24 hours) occlusive patch tests were performed using 20 subjects to determine the irritation potential of a tanning oil containing 3.0% Lecithin 65%; the lotion was applied undiluted (CTFA 1978b). A suntan oil and a "tanning blend" were used as reference controls in the studies, respectively. Significant differences in irritancy between the test material and the control were not observed, and the primary irritation index (PII) of the tanning oil was 0.00 in both studies.

A single-insult (24 hours) occlusive patch test was performed using 18 subjects to determine the irritancy potential of a soap containing 0.83% Lecithin powder; the soap was tested as a 0.5% aqueous solution (CTFA 1983b). A soap was used as a reference control. Significant differences in irritancy between the test material and the control were not observed, and the PII for the test soap was 0.25.

A 4-day cumulative irritancy assay of a foundation containing 0.3% Lecithin 65% was completed using 17 female subjects (CTFA 1981b). Occlusive patches containing 0.10 ml of undiluted test material were applied to the upper back of each subject for 24 hours on 4 consecutive days. An irritating positive control was used. The test sites were scored on a scale of 0 to 4 immediately after patch removal and 5 hours after removal of the fourth patch, and the PII was calculated after the 5-hour scoring. If a score of $\geq$2 was observed at any time during the study, patching was discontinued and that score was entered for

all subsequent scorings. Thirteen of the subjects reacted, with the greatest score being a score of 2 (moderate erythema) for one subject. The majority of the scores, seven subjects, was a ± reaction (barely perceptible erythema). The PII of a foundation containing 0.3% Lecithin 65% was 0.65.

A 4-day minicumulative assay of an eyeliner containing 3.0% Lecithin 65% was performed using 20 subjects according to the methods described previously, with the exception that the sites were only scored 5 hours after the fourth application (CTFA 1987). However, if a score ≥2 was noted at any time during the testing, patching was discontinued. Five of the subjects reacted with scores of ±, and the PII was 0.13.

A 21-day patch test was completed using 11 subjects, 2 males and 9 females, to determine the cumulative irritation potential of a tanning oil containing 3.0% Lecithin 65% (Hill Top Research, Inc. 1978). Vaseline Intensive Care baby oil and concentrate from a purchased deodorant were used as reference materials. Approximately 0.3 ml of each test material was applied for 23 hours to the backs of each subject under an occlusive patch, after which time the patches were removed and the sites washed. The sites were scored 1 hour after patch removal, and the same sites were used daily. A tanning oil containing 3.0% Lecithin 65% was nonirritating.

A double-blind 4-week clinical use study was performed using groups of approximately 38 female subjects to determine the irritation potential of a tanning oil containing 3.0% Lecithin 65% (CTFA 1978c). Control products were also used. Significant clinical or subjective irritation was not observed.

Application of Lecithin (soya phosphatidylcholine) as liposomes at a dose of 3 mg/cm$^2$ under an occlusive chamber for 48 hours was not irritating and 24- to 48-hour patch testing with Hydrogenated Lecithin (soy phosphatidylcholine) did not result in an irritant effect. In neither case were study details provided (Nattermann Phospholipid GmbH 1995).

### Sensitization

A Draize-Shelanski repeat-insult patch test (RIPT) was completed using 99 subjects to determine the sensitization potential of a tanning oil containing 3.0% Lecithin 65% (Research Testing Laboratories, Inc. 1978). The test material was applied under an occlusive patch three times weekly for a total of 10 applications using a "quadrant approach," that is, the first quadrant received patches 1, 4, 7, and 10 the second quadrant received patches 2, 5, and 8, and the third quadrant received patches 3, 6, and 9 (the length of patch duration was not stated). The test sites were scored according to the International Research Contact Dermatitis Group at 48 or 72 hours. Following a 10-day nontreatment period, a challenge patch was applied to a previously untested site on the fourth quadrant of the back; this site was evaluated after 48 and 96 hours.

One subject developed a "1+" reaction (a weak, nonvesicular, reaction) upon application of the seventh patch, and this reaction continued through the 96-hour challenge reading. This subject had 1+ reactions at 24 hours upon retesting with both undiluted test material and a 1:3 dilution of the test material; open tests with the product were negative when it was applied three times a day for 5 days. The researchers concluded that this subject had a "low level of sensitization," but it was "not of clinical significance" because it was a low level of reaction and the open tests were negative. Two subjects had 1+ reactions with the fourth patch only; these were not considered significant. The researchers conclude that a tanning oil containing 3.0% Lecithin 65% "did not demonstrate any irritation or sensitization."

A modified Draize assay was performed to determine the sensitization potential of 15% Hydrogenated Lecithin in petrolatum and was completed with 110 of 120 initial subjects (International Research Services, Inc. [IRSI] 1997a). During induction, 0.025 g of the test material was applied to the scapular area of the back under occlusive patches. A total of 10 applications were made. Forty-eight hours after patch application (72 hours on weekends), the patches were removed and the test sites were rinsed and evaluated. New patches were then applied. Twelve days after removal of the last patch, a challenge patch with the same dose used during induction was applied to a previously untested site. The challenge patch was removed 48 hours after application, and the site was evaluated 48 and 96 hours after application.

During the induction phase of the study, two 1+ reactions (erythema throughout the entire patch area) were observed in one subject. At the 48- and 96-hour challenge readings, one other subjects had 1+ reactions. The researchers concluded that "no evidence of sensitization" to 15% Hydrogenated Lecithin in petrolatum was observed.

A maximization study was completed using 25 subjects, 10 males and 15 females, to determine the contact-sensitization potential of a mascara containing 0.1% Lecithin 65% (Ivy Research Laboratories, Inc. 1982). Five 48-hour occlusive patches containing 0.3 g of the test material were applied to the volar aspect of the forearm following application of sodium lauryl sulfate (SLS). Following a 10-day nontreatment period, a 48-hour occlusive challenge patch was applied to a previously untreated site, and observations were made immediately and 24 hours after patch removal. Reactions were not observed at the sites of induction or challenge patches, and the researchers concluded that it was "unlikely that (a mascara containing 0.1% Lecithin 65%) would present a danger ocontact-sensitization during normal, intended use."

### Phototoxicity/Photosensitization

The phototoxic potential of 15% Lecithin and 15% Hydrogenated Lecithin in petrolatum was determined in a study completed using 10 subjects, 1 male and 9 females (IRSI 1997b). The solar minimal erythema dose (MED) of each subject was determined using a solar simulator (Solar Light Model 14S with a dichroic reflector, WG320, WG345, and UG5 filters). On day 1, duplicate patches of 0.017 to 0.025 mg of each test material were applied to contralateral sites on the back of each subject. The patches were removed and the test sites were scored on a

scale of 0 to 4 48 hours after patch application. One test site for each material was then exposed to a dose of UVA numerically equivalent to 10 times the UVB MED delivered through the solar simulator using a WG345 (2 mm) filter followed by a dose of UVB equivalent to 0.5 times the MED. An untreated site was irradiated in the same manner and served as an irradiated control. The irradiated sites were examined and scored 5 minutes and 24 hours after irradiation.

The "mean reaction scores" for Lecithin at the treated, treated and UV, and UV-only sites were 0, 0.1, and 0–48 hours after application; 0, 0.1, and 0 immediately after irradiation; and 0, 0.3, and 0–24 hours after irradiation, respectively. The mean reaction scores for Hydrogenated Lecithin at the treated, treated and UV, and UV-only sites were 0, 0.2, and 0–48 hours after application; 0, 0.2, and 0 immediately after irradiation; and 0.1, 0.4, and 0–48 hours after irradiation, respectively. Lecithin and Hydrogenated Lecithin, each 15% in petrolatum, were not phototoxic.

The sensitization and photosensitization potential of a foundation containing 0.3% Lecithin 65% was determined in a modified Draize-Shelanski RIPT completed using 198 subjects, half of whom were considered sensitive (Research Testing Laboratories, Inc. 1979). The test material was applied to the backs of the subjects for 48 or 72 hours under occlusive patches using the quadrant approach three times per week for a total of 10 applications. The sites were scored upon patch removal and 48 hours later, except for the last application, which was only scored upon removal. Following a 14-day nontreatment period, a challenge patch was applied to a previously untested site on the back and scored upon patch removal and 48 hours later. This procedure was generally followed for all subjects with the exception that half of the subjects were also subjected to UV light and their test sites were only scored upon patch removal. Rechallenges using an occlusive 48-hour patch test with undiluted and 1:3 diluted test material and open patches using undiluted test material were to be performed on subjects that had potential sensitization reactions.

The half of the subjects that were used in the photosensitization portion of the study were exposed to a UV light source (Hanovia Tanette Mark I lamp, 360 nm peak output), at a distance of 12 inches for 1 minute after removal of the 1st, 4th, 7th, 10th, and challenge patches. Photosensitization reactions were determined 48 hours after exposure. The researchers concluded that a foundation containing 0.3% Lecithin 65% "did not exhibit any potential for inducing sensitization."

The photoallergic potential of 15% Lecithin and 15% Hydrogenated Lecithin in petrolatum was determined in a modified Draize assay completed using 30 subjects, 5 males and 25 females (IRSI 1997c). The MED of each subject was determined using a solar simulator (Solar Light Model 14S with a dichroic reflector, WG320 and UG5 filters). Duplicate occlusive patches containing 0.017 to 0.025 mg of the test material were applied to contralateral sites on the back of each subject on induction days 1, 4, 7, and 9 and at challenge. On days 1, 4, and 7, the patches were removed after 48 hours and one set of test sites was irradiated (using WG320, UG5, and WG345 filters) with a dose of three MED of UVA. (The initial exposure inadvertently consisted of UVA + UVB.) These sites were scored on a scale of 0 to 4 48 hours after UV exposure. New patches were applied 48 hours prior to the next UV exposure, and this process was repeated for the three induction applications/exposures. The fourth set of induction patches were applied for 72 hours, and upon removal one set was irradiated and scored 24 hours later. The nonirradiated sites were patched in accordance with IRSI Standard Modified Draize RIPT. A challenge of duplicate occlusive patches were applied after a 13-day nontreatment period. One set of patched sites were exposed to a dose of 9.5 MED UVA and 0.5 MED of simulated solar light (UVA + UVB). The test sites were scored 48 hours after irradiation.

In the modified Draize assay portion of this study by IRSI (1997c), 0.017 to 0.025 mg of 15% Lecithin and 15% Hydrogenated Lecithin in petrolatum was applied three times weekly for a total of 10 applications. The patches were applied for 48 hours, with the exception that the 9th and 10th applications were 24 hours apart, and scored upon removal. Following a 13-day nontreatment period, challenge patches were applied to previously unpatched sites and scored 48 and 96 hours after application. Lecithin and Hydrogenated Lecithin, each 15% in petrolatum, were not photosensitizing.

## Skin Effects

An examination was made using light microscopy to determine the effect of Lecithin gels on the skin (Willimann et al. 1992). The skin was examined before and after treatment with 200 mM (soybean) Lecithin-isopropyl palmitate gel ($w_0$ [water/Lecithin] = 3) or isopropyl palmitate alone. No significant alterations of the skin were observed after 3 days of application of either substance, and no differences were observed when compared to skin samples treated with physiological saline solution. The only change seen was an increase in vacuole size of the cells in the spinous layer of the epidermis, but this was also seen in the skin of the saline-treated controls.

Freeze-fracture electron microscopy and small angle x-ray scattering were used to study the interaction between liposomes containing 10%, 28%, or 85% Lecithin (phosphatidylcholine) with hypophosphatidyl choline (phospholipids NAT 89, NAT 50, and NAT 106, respectively) and human stratum corneum (Bouwstra et al. 1992). The stratum corneum, which was obtained from abdominal skin, was soaked in a 10% $w/w$ liposome solution for 48 hours; control samples were soaked in phosphate buffered saline solution. The interactions were limited to the interface with the NAT 50 liposomes, but the changes in lipid structure extended into the deeper layers of the stratum corneum with the NAT 89 and NAT 106 liposomes. There was a "strong indication that the degree of interaction between vesicular dispersions and the skin mainly depends on the physicochemical properties of the compounds of which the liposomes are composed. The

interaction is most likely to be dependent on the size, mean number of bilayers, head groups, and alkyl chain length of the lipids."

Single applications of 3.2 mg phospholipid/$cm^2$ skin of three phospholipid mixtures were made to the hairless inner forearm of 10 dermatologically normal male and female subjects to determine their effect on skin humidity (Ghyczy, Gareiss, and Kovats 1994). The liposomes had a phospholipid fraction concentration of 10% and the pH was 6.5; the mixtures contained 10%, 29%, or 76% Lecithin. Saline solution, 0.9%, was used as a control. Skin moisture was monitored at all test areas in triplicate prior to dosing and at 30, 60, 120, and 180 minutes using a Corneometer; relative humidity was 45% and temperature was 26°C. An "acute and significant increase in skin humidity" was produced by the formulation containing 76% Lecithin. A weak effect was observed with the formulation containing 29% Lecithin, whereas a decrease in skin moisture was seen with the formulation containing 10% Lecithin. The researchers concluded that Lecithin provided a "moisturizing effect to the stratum corneum" because it was the only phospholipid in the formulations that changed greatly in content. A second multiple-application study also demonstrated a steady-state increase in skin moisture after repeated application of Lecithin-based liposomes.

The effect on skin roughness of liposomes that contained Lecithin as the only phospholipid was examined using 19 female subjects; the amount of Lecithin was 21% of the formulation and the pH was 6.0 (Ghyczy, Gareiss, and Kovats 1994). "DAC cream" base formula (Deutscher Arzneimettel Codex [German Pharmaceutic Codex]) was used as the control. A dose of 0.8 to 1.0 mg phospholipid/$cm^2$ and 2 to 2.5 mg DAC cream/$cm^2$ per day was applied to the skin of the volar forearm twice daily for 28 days. Skin roughness was measured using image analysis prior to study initiation, 2 hours after application on days 3, 7, 14, and 28, and 2 days after study termination. The researchers stated that "the decrease in skin roughness when using the soy [Lecithin] liposomes was significant." Application of DAC cream increased skin moisture throughout the study, but it generally did not cause a smoothing effect.

A skin-blanching assay using a nonocclusive multiple dosage regimen to examine the effect of pretreating skin with 5 mg/ml (egg) Lecithin in a liposomal suspension on the bioavailability of four corticosteroid formulations was performed using three male and seven female subjects (Jacobs, Martin, and Marriott 1988). The liposomal suspension, 0.5 ml, was applied to the flexor surface of one arm twice daily for 7 days prior to steroid application and during the 2 weeks of testing; Sørensen's phosphate buffer was applied similarly to the opposite arm, which served as a control. Five milligrams of hydrocortisone 0.1% cream, clobetasone butyrate 0.05% cream, betamethasone 0.1% cream, and clobetasol propionate 0.05% cream were applied to 7 × 7 = mm sites on the flexor surface of each forearm within 1 hour of lipid or buffer application. The corticosteroid preparations were applied twice daily on day 1 and once daily on days 2 to 5; the preparations were not applied on days 6 and 7, but application was repeated on days 8 to 12 as during days 1 to 5. Estimation of pallor was made four times daily on days 1 to 5 and 8 to 12 and once daily on days 6 to 7 and 13 to 15 using a scale with scores of 0 to 4.

The researchers stated that it appeared "that a repeated treatment of skin with [Lecithin], presented in a liposomal suspension, causes an alteration of the normal blanching profile." For a direct assessment of the effect of treatment with the Lecithin liposomal suspension on blanching activity, the results of the four corticosteroids were summed for all subjects at each reading and expressed as a percentage of the total possible score. Differences in daily peak responses obtained from Lecithin-treated and control arms were statistically significant on days 4, 5, and 9 to 12, and the scores observed for the treated arm were greater than those observed for the control arm. Also, the difference increased over time; the researchers attributed this increase to a reduction in the tachyphylactic response on the Lecithin-treated arms. The researchers concluded that "the repeated skin treatment with a [Lecithin] solution may increase the total lipid content of the skin and thereby alter the bioavailability of steroids. Alternatively, the application of [Lecithin] could create a relatively fluid lipid film in intimate association with the skin surface, into which the steroids partition and enter the lipid regions of the stratum corneum by diffusion or lipid exchange. . . . Such a lipid film, by possessing a degree of occlusion, is likely to promote the hydration of the stratum corneum and thereby facilitate corticosteroid absorption."

## Inhalation Toxicity

As reported by Smolinske (1992) paradoxical bronchospasm was reported in 23 of 1450 (1.6%) asthmatics treated with a metered-dose inhaler containing (soy) Lecithin. In a follow-up study, a 4.4% incidence of immediate bronchoconstriction was reported and was ascribed to one or more of the excipients; however, the researchers did not determine which of the excipients was responsible (Yarbrough, Mansfield, and Ting 1985). After reformulation of a metered-dose inhaler to contain (soy) Lecithin, escalating reports of adverse reactions within 1 month of reformulation resulted in withdrawal of the new formulation (Smolinske 1992).

## Oral Exposure

The Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives reported in 1972 that it estimated that the average diet provided about 1 to 5 g daily of Lecithin (FASEB 1979). Up to 100 g/day of commercial (soy) Lecithin has been administered without toxic effects, and 25 g/day was generally well tolerated (Nattermann Phospholipid GmbH 1995).

Long-term daily ingestion of up to 48 g of a commercial (soybean) Lecithin was well-tolerated by patients with serum lipoprotein abnormalities and was not associated with any

abnormalities of thyroid, hepatic, or renal function as measured by serum chemistry tests (Tompkins and King 1974). Phospholipid concentrations were increased in duodenal bile and cholesterol crystals, present before Lecithin administration, disappeared.

### Occupational Exposure

Two men involved with baking reported clinical signs in relation to an occupational exposure to (soy) Lecithin (Lavaud et al. 1994). In both cases, a standard prick test was done for common inhalant allergens in bakeries and $\alpha$-amylase, and the sites were examined after 20 minutes and 6 hours. For Lecithin, skin tests were performed intradermally. A bronchial challenge was also performed during asymptomatic periods with an initial Lecithin solution at concenteration of $10^{-6}$; this concentration was increased progressively with 30-minute intervals. Three nonatopic subjects and three asthmatic patients were used as controls. Both subjects had positive skin tests with Lecithin, with wheals of 20 and 30 $mm^2$ observed; no late reaction was noted. In the bronchial challenge, drops of 50% and 45% in $FEV_1$(not defined) were observed 5 minutes after inhalation of a $10^{-3}$ dilution for the two subjects, respectively; significant late reactions were not observed.

Intradermal testing was performed using 19 male soybean-processing workers and a control group of 31 transport workers not exposed to industrial dust or fumes (Zusk et al. 1991). Intradermal tests were performed using (soy) Lecithin as well as aqueous extracts of soybean and common allergens, with reactions being read after 20 minutes. Three of the 19 workers (15.8%) reacted to the Lecithin antigen, whereas none of the controls reacted to Lecithin.

## SUMMARY

Lecithin, a phospholipid commonly known as phosphatidylcholine, and Hydrogenated Lecithin functions in cosmetics as miscellaneous skin-conditioning agents and as surfactants. The composition of Lecithin is dependent upon the substance from which it is isolated. In 1997, it was reported to the FDA that Lecithin and Hydrogenated Lecithin were used in 679 and 29 cosmetic formulations, respectively. Data submitted to the FDA in 1984 reported that Lecithin was used at concentrations of $\leq$50%. Lecithin is often included in the composition of liposomes, structures in which the edges of phospholipid bilayers in lamellar dispersions in water seal readily with the formation of spherical structures whose acyl chains are also not exposed to the aqueous phase.

Lecithin that meets the FCC specifications had GRAS status in food.

Oral administration of radioactive Lecithin to humans, monkeys, and rats resulted in most of the radioactivity being absorbed from the intestinal tract. Ingestion of Lecithin by human subjects resulted in significant increases in the excretion of dimethylamine and trimethylamine as compared to control values from subjects that consumed a normal diet. Nonocclusive application of Lecithin-containing liposomes resulted in penetration of 30% of the phospholipid into murine skin subdermis. Open application of Lecithin-based liposomes to dorsal piglet skin resulted in >99% of the applied phospholipid accumulating in the stratum corneum. Injection of small and large Lecithin-containing liposomes into the footpad of rats demonstrated that small liposomes entered the circulation in latent form, with a peak concentration of 17% of the injected dose at 3.5 hours, whereas the large liposomes marginally entered the circulation and were rapidly removed.

In acute studies, the maximal nontoxic oral dose of Lecithin for the mouse, rat, rabbit, and dog was 20, 20, 5, and 10 g/kg, respectively, the maximal nontoxic IV dose for the mouse, rat, and rabbit was 4, 2, and 0.5 g/kg, respectively, and the maximal nontoxic subcutaneous and intraperitoneal doses for the mouse, rat, and rabbit were 10, 4, and 1 g/kg, respectively. The maximum nontoxic oral dose of Hydrogenated Lecithin for the mouse and rat was 10 g/kg. In short-term studies, the no-effect daily oral and IV dose of Lecithin was >2.8 and >1 g/kg for rats, respectively, and >1.9 and >0.1 mg/kg for dogs. Some decreases in the phagocytic index were observed in short-term studies in mice injected with Lecithin-containing liposomes. In subchronic studies, dermal application to rats of cosmetic formulations containing 3% Lecithin 65% did not produce systemic toxicity. In a subchronic and a chronic study, the no-effect daily oral dose of Lecithin for rats was >2.8 g/kg and in a chronic study, the no-effect daily oral dose for dogs was >0.75 g/kg.

In single-insult occlusive patch tests, Lecithin 65% was minimally irritating, products containing 3% Lecithin 65% were practically non- to mildly irritating, and a product containing 2.25% Lecithin 65% was nonirritating to the skin of rabbits and in a guinea pig immersion study, 0.5% of a soap containing 0.83% Lecithin powder was practically nonirritating. Using rabbits, Hydrogenated Lecithin was not a primary dermal irritant. Lecithin 65% and products containing 2.25% or 3.0% Lecithin 65% were non- to minimally irritating to unrinsed rabbit eyes, whereas a soap containing 0.83% Lecithin powder, tested at 25%, was moderately irritating. Lecithin-containing liposomes were practically nonirritating in a Draize test, whereas in a blinking test, positively charged Lecithin liposome preparations significantly increased the number of blinks compared to controls. Using rabbits, Hydrogenated Lecithin was not a primary eye irritant.

In oral studies, $\leq$1600 mg/kg Lecithin was not a reproductive toxicant in mice or rats and $\leq$475 mg/kg was not a reproductive toxicant in rabbits. In an IV reproductive study, the lowest toxic daily IV dose for rats was >1000 mg/kg. Lecithin, $\leq$3.0 mM, had no significant effect on human sperm motility.

Lecithin was not mutagenic in plate or suspension assays with or without metabolic activation, and it was not mutagenic in the Ames test. Lecithin-containing liposomes did not inhibit $^3$H-thymidine incorporation and an artificially decomposed mixed micelle solution that originally contained Lecithin was not

mutagenic in the Ames assay with or without metabolic activation. In a model system, dimethylnitrosamine (DMNA) was formed when sodium nitrite in water was added to a buffered solution containing Lecithin; the amount of DMNA formed depended on the type of Lecithin used and ranged from 0.7 to 320 mg DMNA/kg of compound.

In an oral carcinogenicity study, brain neoplasms were found in 18/73 mice fed Lecithin, 27/88 fed Lecithin and cholesterol, and 0/188 control mice. In subcutaneous studies no mice or rats dosed with Lecithin-water developed neoplasms, whereas most or all of the mice and rats, respectively, dosed with a 0.25% mixture of 4-nitroquinoline 1-oxide in 10% Lecithin in water developed pulmonary neoplasms.

In clinical irritation studies, cosmetic formulations containing 0.3 or 3% Lecithin 65%, a soap containing 0.83% Lecithin powder, tested at 0.5%, and Lecithin liposomes were generally nonirritating; barely perceptible erythema was the most severe reaction observed. Hydrogenated Lecithin was also not an irritant. A tanning oil containing 3% Lecithin 65% and a mascara containing 0.1% Lecithin 65% were non-sensitizing, and a foundation containing 0.3% Lecithin 65% was not a sensitizer or photosensitizer. Hydrogenated Lecithin, 15% in petrolatum, was not a sensitizer and Lecithin and Hydrogenated Lecithin, both 15% in petrolatum, were not phototoxic or photosensitizing. Lecithin appeared to have a moisturizing effect on the stratum corneum and Lecithin-containing liposomes decreased skin roughness. Results of one study indicated that the interaction between Lecithin-containing liposomes and the skin was dependent on the physicochemical properties of the liposomes. Pretreatment of skin with Lecithin in a liposomal suspension prior to corticosteroid application could increase total lipid content or, alternatively, create a fluid lipid film, and may alter the bioavailability of corticosteroids.

Upon reformulation of a metered-dose inhaler to contain Lecithin, escalating reports of adverse reactions were received within 1 month of reformulation resulted in its withdrawal.

## DISCUSSION

The Expert Panel found the data included in this review adequate to determine that Lecithin and Hydrogenated Lecithin are safe as used in rinse-off products and, based on the results of sensitization and photosensitization studies, safe for use at concentrations of $\leq$15% in leave-on products. However, the safety of use could not be substantiated in cosmetic products when Lecithin or Hydrogenated Lecithin is likely to be inhaled.

On September 20, 1996, the Expert Panel had issued a Notice of Insufficient Data Announcement that included, among other data needs, the need for inhalation toxicity data. These data were not provided. When the requested data are available, the Expert Panel will reconsider the Final Report in accordance with Section 46 of the Cosmetic Ingredient Review Procedures, Amendment of a Final Report.

The Expert Panel noted that Lecithin-containing liposomes may enhance the penetration of other ingredients through the skin. The Expert Panel cautioned that care should be taken in formulating cosmetic products that contain ingredients that the Expert Panel determined safe for use based on their lack of dermal absorption, or when dermal absorption is a concern. The Expert Panel also noted that Lecithin and Hydrogenated Lecithin should not be used in cosmetic products in which *N*-nitroso compounds may be formed.

## CONCLUSION

On the basis of the animal and clinical data presented in this report, the Expert Panel concluded that Lecithin and Hydrogenated Lecithin are safe as used in rinse-off products, safe for use in leave-on products at concentrations of $\leq$15%, and that the data are insufficient to determine the safety for use in cosmetic products where Lecithin or Hydrogenated Lecithin are likely to be inhaled. Lecithin and Hydrogenated Lecithin should not be used in cosmetic products in which *N*-nitroso compounds may be formed.

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