

GRAS Notice (GRN) No. 534

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<http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm>

ORIGINAL SUBMISSION

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GRN 000534

July 22, 2014

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

RE: Notifications of GRAS Determinations for Canola-derived Lecithin and Soybean-derived Hydrogenated Lecithin in Food

Dear Sir/Madam:

In accordance with proposed 21 CFR 170.36 (Notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18939-18964), I am submitting in triplicate, as the agent to the notifier, American Lecithin Company, two GRAS Notifications for canola-derived lecithin and for soybean-derived hydrogenated lecithin under the conditions of their intended uses in food.

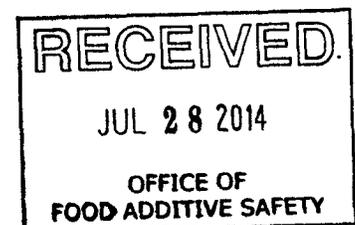
Please contact me if you have any questions.

Sincerely,

(b) (6)



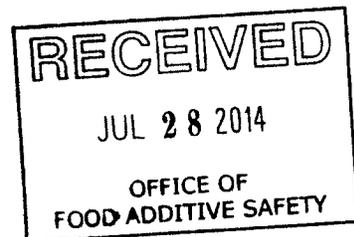
Charles Manley PhD, CFS
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GRN 000534

**GRAS Determination for Soybean-derived Hydrogenated
Lecithin in Food**



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I. GRAS Exemption Claim

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR § 170.36(c)(1) [62 FR 18938 (17 April 1997)]

American Lecithin Company has determined that soybean-derived hydrogenated lecithin is Generally Recognized As Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of their intended uses in food. Therefore, the uses of soybean-derived hydrogenated lecithin in food, as described below, is exempt from the requirement of premarket approval.

Signed,

(b) (6)

Charles Manley



7/22/14
Date

Agent for:

American Lecithin Company
115 Hurley Road Unit 2B
Oxford, CT 06478

Name and Address of Notifier:

Randall E. Zigmont
President
American Lecithin Company
115 Hurley Road Unit 2B
Oxford, CT 06478

B. Common Name of the Notified Substance

The substance is commonly known as lecithin. Commercial lecithin is a complex mixture of phosphatides obtained from various edible food sources. Because the composition of phosphatides in lecithin is dependent upon its source and due to differences in degree of saturation of the phosphatidyl fatty acids, we are describing lecithin whose constituent phosphatides contain fully saturated fatty acid chains as hydrogenated lecithin (HL) to distinguish it from (non-hydrogenated) lecithins whose constituent phosphatides contain fatty acids with varying degrees of saturation. Soybean-derived hydrogenated lecithin is recognized as HL in this GRAS notification.

C. Conditions of Intended Use in Food

HL is intended for addition to various conventional foods as an aid, such as an emulsifier, releasing agent, wetting agent, or instantizing agent, for typical technological manufacturing processes and for addition to some foods as a choline-enriching dietary ingredient.

Table 1 lists the proposed uses and use-levels of HL in food. American Lecithin anticipates that the uses of HL will replace, for some products, other lecithins, and thus the total lecithin intake is not anticipated to increase based on the use of HL.

According to 21 CFR 184.1400, lecithin from soy, safflower and corn oils is allowed to be used in food with no limitation other than current good manufacturing practices. That being said, Table 1 shows the typical usage levels for hydrogenated lecithins respectively in various applications. For economic reasons, it is highly unlikely that food manufacturers would exceed the recommended usage levels.

Infants are growing at such a fast rate that their demands for all nutrients is very high. Choline is no exception. Breast milk naturally contains choline; analysis shows that there are 9 +/- 2 mg of choline per deciliter of breast milk. The choline present in breast milk is in the form of free choline, glycerphosphocholine (GPC), phosphatidylcholine (PC) and sphingomyelin.

Since choline has been determined to be an essential nutrient and necessary to support optimal growth, development and health, the FDA requires that infant formulas be supplemented with a choline source. The FDA requires choline supplementation in infant formula at the rate of 7 mg/100kcal. The following choline sources are considered generally recognized as safe (GRAS): commercial lecithin, PC, choline bitartrate and choline chloride. But not all sources of choline are used as effectively by the body. When choline supplementation is done through choline salts such as choline bitartrate and choline chloride, as much as 60% of the choline from these sources is lost in the intestines by the intestinal bacteria converting it into an unusable form known as trimethylamine. Trimethylamine can give the body an offensive fishy odor. Whereas, when choline supplementation is done using lecithin or PC, it appears that it is a more bioavailable and timed release source of choline.

When using Lecithin/PC in an Infant Formula, it serves a dual role in the product. PC functions as an excellent oil in water emulsifier stabilizing a liquid formulation and preventing separation of the lipid and water phases. In addition, it can be utilized as a bio-available and sustained release source of choline supplementation. PC is 13% choline by weight.

Table 1: Average Use Levels, Maximum Use Levels, and Average Single Portion Intakes of HL in Foods, expressed in g / 100 g

Food	Use Hydrogenated Lecithin (Grade I) PHOSPHOLIPON® 80 H, LIPOID S 75-3 N and PHOSPHOLIPON® 90 H, LIPOID S 100-3 (Grade II)	
	average	max. levels
Baked Goods		
Breads and other Yeast Raised Products	0.2*	0.5*
Cake	0.4*	1.0*
Low Fat Cookies, Crackers Pretzels	0.4*	1.0*
Pizza Crust	0.2*	1.0*
Waffles, Pancakes	0.2*	1.0*
Dairy Products		
Milk Based Beverages	0.5	1.5
Milk Analog Beverages - Soy, Almond, Coconut, etc.		
Instantization		
Frostings	0.2	1.5
Peanut Butter/Spreads	0.1	0.5
Release	0.2	0.5
Sauces/Gravies	0.2	1.0
Extrusion		
Extruded Snacks	0.5	1.5
Confections		
Chewing Gum	0.3	1.5
Gummy Candy	0.3	1.0
Chocolate Fudge Topping	0.2	1.5
Ice Cream/Frozen Desserts	0.50	1.0
Margarine/Spreads	0.1	0.3
Ovenable Breadings and Coatings	0.3	1.5

*based on flour

HL is also a valuable nutritional ingredients for dietary purposes. PC is a bioavailable source of choline, an essential nutrient for humans as precursor of the neurotransmitter acetylcholine. Lecithin is the predominant source of PC, and thus choline, in the human diet. Choline and PC (lecithin) are generally regarded as safe and non-toxic for consumption. No maximum tolerated dose of lecithin was specified by the Joint FAO/WHO Expert Committee on Food Additives (WHO, 1974). The normal intake of lecithin and PC has been estimated to be approximately 6 g and 800 mg/day, respectively. The Institute of Medicine has determined that an adequate intake of choline is 550 mg/day for adult males, 450 mg/day for adult females and pregnant women, and 550 mg/day for nursing mothers (Institute of Medicine, 1998). The Food and Nutrition Board recommends an upper limit of dietary reference intake of 3.5 g choline/person/day for adults age

14 through 70 (Food and Nutrition Board, 2006). For adults, the usual recommended dose of PC for dietary choline supplementation is 1200 mg to 3450 mg (maximum) of PC per day. Based on these recommendations, ALC intends to market HL as a dietary ingredient added to food at use levels, listed in Table 2, corresponding to up to approximately 1.2 g PC per day.

Table 2: Average Use Levels[†] of HL as a Dietary Ingredient

Food Category	PHOSPHOLIPON [®] 80 H, LIPOID S 75-3 N (Grade I)	PHOSPHOLIPON [®] 90 H, LIPOID S 100-3 (Grade II)
Breakfast bars 40 g	4%	3.2%
Cereals and other Grain Products 40 – 55 g	4 – 2.9%	3.2 – 2.3%
Shakes or Shakes Substitutes 240 ml	0.67%	0.53%

[†] Percentages correspond to a daily intake of approximately 1.2 g phosphatidylcholine

Commercial plant-derived lecithin is derived from plant oils that contain not less than 50% of acetone-insoluble matter. This is the raw material used for deoiled lecithin fraction containing >97% acetone insolubles, the most important of which are the phospholipids PC, phosphatidylinositol and phosphatidylethanolamine. HL is at minimum 70% phosphatidylcholine (PC), which contains approximately 13% choline. Therefore, the recommended adequate intake of choline would be provided by approximately 5.5 g of HL for adults and the upper limit of intake would be approximately 38 g for adults (Food and Nutrition Board, 2006). Based on the food category portion sizes and recommended use levels of HL in various foods, the possible average daily intake of HL is calculated to be approximately 4 g/person/day. For adults, this is equivalent to a daily intake of roughly 67 mg/kg bw/day, whereas for children this is equivalent to a daily intake of 135 mg/kg bw/day (based on a bodyweight of 30 kg). This upper limit of intake is well within the recommended daily intake (Food and Nutrition Board, 2006).

In accordance with The Code of Federal Regulations (21 CFR 184.1b), lecithin can be used in food with no limitation other than good manufacturing practice (GMP).

D. Basis for the GRAS Determination

Pursuant to 21 CFR 170.30, HL has been determined to be GRAS on the basis of scientific procedures. This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of HL as a component of food, on previous FDA GRAS Notifications of related substances, and on the primary components of HL with comparable use levels. Lecithin is considered to be GRAS by US Food and Drug Administration (21 CFR 184.1400) (FDA, 2006).

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Lecithin from different specific sources and of slightly different chemical composition are considered GRAS. In 2006, FDA GRAS Notification 000186, in accordance with 21 CFR 170.30, Soy Lecithin Phosphatidylserine Complex was determined to be GRAS on the basis of scientific procedures and information provided by Lipogen Products Ltd (Israel) (Lipogen, 2006). In 2008, FDA GRAS Notification 000226, pursuant to 21 CFR 170.30, of Krill-based Lecithin was determined to be GRAS on the basis of scientific procedures and information provided by Enzymotec (Israel) (Enzymotec, 2008). Hydrolyzed lecithin has been the subject of GRAS Notifications to FDA also. In 2004, FDA GRAS Notification 000134, pursuant to 21 CFR 170.30, C-Fraction Soy Protein Hydrolyzate with Bound Phospholipids (CSPHP) was determined to be GRAS by scientific procedures based on information provided by Kyowa Hakko (USA) (Kyowa Hakko, 2004).

Hydrogenated lecithin was reviewed by the LSRO Select Committee on GRAS substances and determined to be non-harmful in SCOGS Report No. 106 (Life Sciences Research Office, 1979).

E. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Randall Zigmont
American Lecithin Company
115 Hurley Road Unit 2B
Oxford, CT 06478

Should the U.S. Food and Drug Administration (FDA) have any questions or additional information requests regarding this notification, American Lecithin Company will supply these data and information.

II. Detailed Information About the Identity of the Substance

A. Identity

Chemical or common names

Phosphatidylcholine
1,2-diacyl-sn-glycero-3-phosphocholine
Lecithin
Hydrogenated lecithin
Hydrogenated phosphatidylcholine

Trade Names

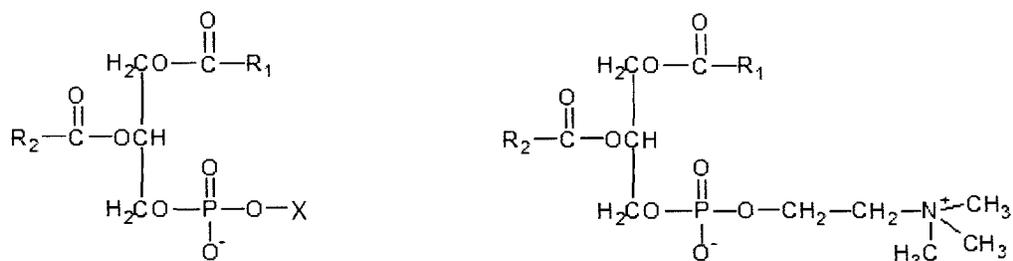
PHOSPHOLIPON® 80 H

LIPOID S 75-3N
PHOSPHOLIPON® 90 H
LIPOID S 100-3

Structure of Lecithin

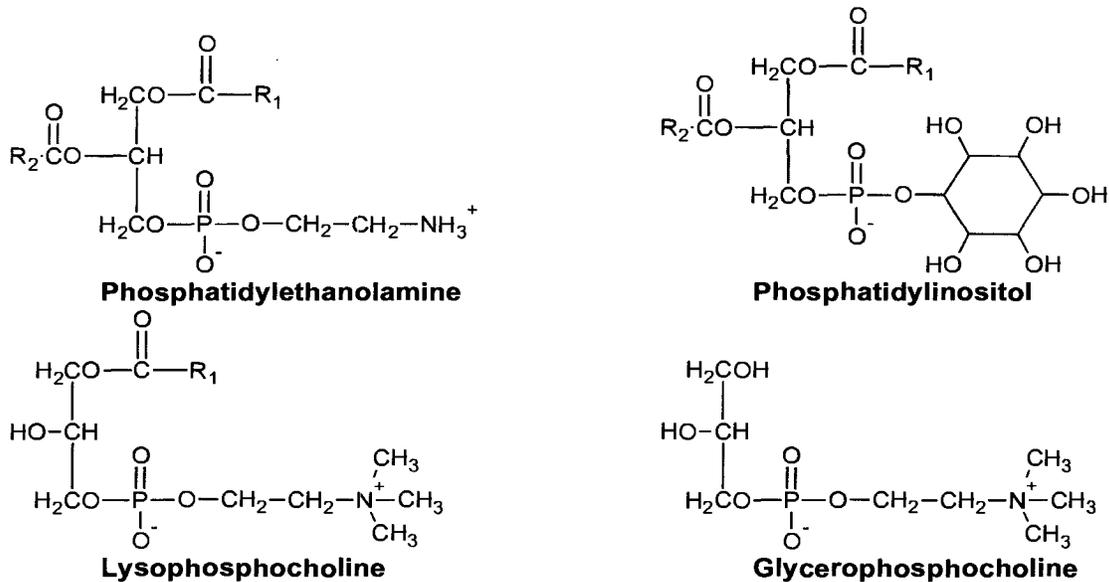
Phosphoglycerides have the structure shown below (Fig. 1), where R₁ and R₂ are unspecified fatty acids and X, the head group, is a polar or charged molecule. The fatty acids are typical of those found in the source from which the lecithin is extracted. Hydrogenated soybean-derived lecithin is composed of a higher proportion of saturated fatty acids. Soybean-derived hydrogenated lecithin meets the specifications for low iodine value (n.m.t. 3), a reflection of its low degree of unsaturation.

Figure 1: Structure of a phosphoglyceride and phosphatidylcholine



Commercially, lecithin has a much less specific structure. Lecithin is a generic term that designates a natural complex mixture of acetone-insoluble phosphatides and various amounts of other substances, such as triglycerides, fatty acids and carbohydrates. In addition to “true” lecithin, PC, commercial lecithin contains other phosphoglycerides: phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, glycerophosphocholine (GPC), and lysophosphocholine (LPC). Refined grades of lecithin may contain any of these substances in varying proportions and combinations depending on the type of fractionation used. Different fractions can be obtained by de-oiling and further purification, such as one enriched with the main component phosphatidylcholine. PC is the main phosphatidyl compound. It is a naturally occurring mixture of the diglycerides of stearic, palmitic, oleic and other unsaturated acids, linked to the choline ester of a phosphoric acid. Fractionation is the process by which the less abundant naturally related components, such as phosphatidylethanolamine, phosphatidylinositol, triglycerides, fatty acids, and carbohydrates, are removed to certain levels. Lecithin fractions are obtained by controlled hydrogenation in different fractions of hydrogenated lecithin or hydrogenated phosphatidylcholine (Figure 2).

Figure 2: Structures of other phosphoglycerides in lecithins



Lecithin is naturally consumed through a diet that includes lecithin rich foods such as egg yolk, soybeans, grains, fish, legumes, yeast, and peanuts, to name a few. Soybean, sunflower, and rapeseed are the primary plant sources of commercial lecithin, with soybean being the most common source.

Product Specifications

Hydrogenated lecithin is derived from natural lecithins that can be obtained from canola, soybean, sunflower or other plant sources. It is a complex mixture of acetone-insoluble phosphatides and various amounts of other substances, such as triglycerides, fatty acids and carbohydrates, that chiefly consists of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. Refined grades of lecithin may contain any of these substances in varying proportions and combinations depending on the type of fractionation used. Different fractions can be obtained by de-oiling and further purification, such as one enriched with the main component phosphatidylcholine. PC is the main phosphatidyl compound. It is a naturally occurring mixture of the diglycerides of stearic, palmitic, oleic and other unsaturated acids, linked to the choline ester of a phosphoric acid. Fractionation is the process by which the less abundant naturally related components, such as phosphatidylethanolamine, phosphatidylinositol, triglycerides, fatty acids, and carbohydrates, are removed to certain levels. Hydrogenated lecithin and hydrogenated phosphatidylcholine are obtained by controlled hydrogenation of the respective purified lecithin fractions.

ALC's Grade I hydrogenated lecithin from soybean (PHOSPHOLIPON® 80 H, LIPOID S 75-3 N) typically contains about 90% phospholipids with the phosphatidylcholine (PC) content being greater than 70%. Further concomitant components such as lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), phosphatidic acid (PA) and phosphatidylinositol (PI) are present

in low levels (below 4%). ALC's Grade II hydrogenated phosphatidylcholine (PHOSPHOLIPON® 90 H, LIPOID S 100-3) is a further purified fraction of Grade I, and contains about 95% phosphatidylcholine as the main phospholipid. It only contains small amounts of lysophosphatidylcholine (2%-3%) and the other concomitant phospholipids (<0.1%).

Table 3a lists the phospholipid composition of all grades of ALC soybean-derived HL and Table 3b lists the fatty acid composition of all grades of ALC soybean-derived HL. The phosphoglyceride content among the various grades of ALC HL and that of lecithins derived from other common sources can be found in Table 3a below and Table A-1 of Appendix A, respectively. A comparison among the phosphoglyceride-bound fatty acid compositions among the various grades of ALC HL and those of lecithins derived from other common sources can be found in Table 3b below and Table A-2 of Appendix A, respectively. These tables show that HL is nearly identical to other commercially available non-hydrogenated lecithins except that the most prevalent fatty acid in soybean-derived HL is stearic acid (which is a direct result of hydrogenation). The specifications for American Lecithin Company HL are summarized below in Table 3c.

Table 3a: Phospholipid Composition of ALC Soybean-derived Hydrogenated Lecithin

Grade	Hydrogenated Lecithin, Grade I LIPOID S 75-3 N, PHOSPHOLIPON® 80 H	Hydrogenated Phosphatidylcholine, Grade II LIPOID S 100-3, PHOSPHOLIPON® 90 H
Parameter (w/w%)		
Total Phospholipids*	87	97
PC	78	95
LPC	4	2-3
PE	4	<0.1
PA	1-2	<0.1
PI	<1	<0.1

*Total Phospholipids: Sum of PC, LPC, PE, PI, PA

Table 3b: Fatty Acid Composition of ALC Soybean-derived Hydrogenated Lecithin (as % w/w of fatty acids)

	Hydrogenated Lecithin, Grade I LIPOID S 75-3 N, PHOSPHOLIPON® 80 H Hydrogenated Phosphatidylcholine, Grade II LIPOID S 100-3, PHOSPHOLIPON® 90 H
14:0 Myristic Acid	<0.1
16:0 Palmitic acid	11.8
18:0 Stearic acid	87.4
18:1 Oleic acid	<0.1
18:2 Linoleic acid	<0.1
18:3 Linolenic acid	<0.1
20:0 Arachic acid	0.3
22:0 Behenic acid	<0.1

Table 3c: Soybean-derived Hydrogenated Lecithin Specifications

Parameter	Soybean-derived Hydrogenated Lecithin, Grade I
Hydrogenated phosphatidylcholine (%)	≥ 70
Hydrogenated Lysophosphatidylcholine (%)	< 6.0
Water (KF) (%)	< 2.0
Ethanol (%)	< 0.5
Iodine Value	< 3.0
Heavy Metals (ppm)	≤ 10

The two grades of HL that American Lecithin Company (ALC) intends to bring on the market are listed and described below.

Deoiled Soybean-derived Hydrogenated Lecithin, Grade I:

ALC Products (Trade Names): PHOSPHOLIPON® 80 H, LIPOID S 75-3 N
Description: Hydrogenated phospholipids from soybean constituted by 70% hydrogenated phosphatidylcholine (main component)
CAS No.: 92128-87-5

Purified Hydrogenated Soybean-derived Phosphatidylcholine, Grade II:

ALC Products (Trade Names): PHOSPHOLIPON® 90 H, LIPOID S 100-3
Description: Hydrogenated phosphatidylcholine (non-hydrogenated phosphatidylcholine starting material isolated from soybean) (approximately 95% hydrogenated phosphatidylcholine)
CAS No.: 97281-48-6

B. Method of Manufacture and Compositional Analysis

Lecithin is an oil component resulting from hexane extraction of crushed rapeseeds. Since lecithin is more hydrophilic than the oils, it is possible to make them absorb added water. When lecithins absorb water, they swell to form characteristic liquid crystal mesophases. This process is called degumming. This viscous lecithin gum, or slurry, must be dried quickly under vacuum and controlled temperature, otherwise the lecithins will either spoil microbially or brown as a result of overheating.

De-oiling

A number of additional processing steps are used to further refine crude lecithin. The phospholipid fraction is insoluble in acetone and, thus, addition of acetone allows removal of the inactive, vegetable oil fraction of the crude lecithin. The de-oiled lecithin is obtained after

evaporation of the acetone. The extraction can either be a batch- or a continuous process. De-oiled lecithin is >97% acetone insoluble matter and has several advantages: the powder has convenient handling characteristics and has a more neutral taste since the unsaturated fatty acids of vegetable oil has been removed; de-oiled lecithin is more hydrophilic than standard lecithin. The valuable phosphatidylcholine fraction can be enriched by fractionation, but fractionated forms have limited commercial applications.

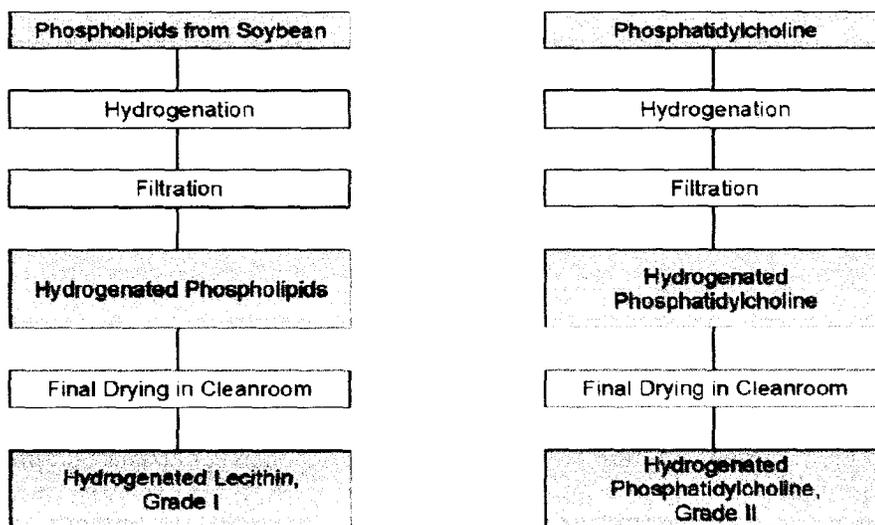
Fractionation

Phosphatidylcholine (PC) is more soluble in ethanol than all other phospholipids. This allows a semi-quantitative fractionation of PC, which can be separated from a "bottom fraction" with relatively less PC, the so called PC-fraction. It is possible to obtain PC contents in the range of 30-50 % by this process. Further fractionation requires, more expensive, chromatographic separation techniques that allow commercial production of 70% phosphatidylcholine and to pure phosphatidylcholine which, contrary to common belief, is a solid material at ambient temperatures and very difficult to handle in food unless sprayed on a carrier. However, fractionated lecithin has several advantages: the PC-fraction is choline enriched for dietary purposes; it has its own characteristic hydrophilicity and emulsification properties; it is less sensitive to calcium in hard water and it is less sensitive to browning on extended heating.

Hydrogenation

Hydrogenated lecithin and hydrogenated phosphatidylcholine are obtained by controlled hydrogenation of the respective purified lecithin fractions. This is followed by filtration to remove the reduction catalyst (Figure 4).

Figure 4. Manufacturing Process Diagram for Hydrogenated Lecithin, Grade I and Hydrogenated Phosphatidylcholine, Grade II



C. Intended Use

HL is intended for addition to a limited number of conventional foods as a nutritional ingredient. To be used as an emulsifier, wetting or instantizing agent, viscosity modifier, releasing agent, extrusion aid, low flavor binding material, and high quality dietary fat source. It is intended for the general population at the levels identified previously in Tables 1 and 2 to dairy products (milk drinks and yogurt types), soy products, spreads, breakfast bars, and soft candy. No specific subpopulations are anticipated to consume the product in higher amounts than the general population. It is also recognized that there are Standard of Identity requirements for some of the proposed foods, and there is therefore no intention to refer to them by the commonly recognized names such as milk, chocolate, or yogurt.

D. Safety Assessment of HL

Previous Considerations

The safety of Lecithin has been considered in several previous reviews. Lecithin is considered Generally Recognized as Safe (GRAS) by the US Food and Drug Administration under 21 CFR 184.1400. Enzyme-modified lecithin is affirmed as GRAS under 21 CFR 184.1063 Lecithin was reviewed by the Select Committee on GRAS Substances (SCOGS) in 1979 (Life Sciences Research Office, 1979). Hydrolyzed lecithin has been the subject of GRAS Notifications to FDA. In 2004, GRAS Notification 000134, pursuant to 21 CFR 170.30, the C-Fraction Soy Protein Hydrolyzate with Bound Phospholipids (CSPHP) was determined to be GRAS by

scientific procedures based on information provided by Kyowa Hakko (USA) (CFSAN/FDA, 2004). In 2006, GRAS Notification 000186, in accordance with 21 CFR 170.30, the Soy Lecithin Phosphatidylserine Complex was determined to be GRAS on the basis of scientific procedures and information provided by Lipogen (Israel) (Lipogen Products Ltd., 2006). In 2008, GRAS Notification 000226, pursuant to 21 CFR 170.30, Krill-based Lecithin was determined to be GRAS on the basis of scientific procedures and information provided by Enzymotec (Israel) (CFSAN/FDA, 2008). The available toxicity data for lecithin and hydrogenated lecithin have been previously comprehensively reviewed (Cosmetic Ingredients Review, 2001).

In a critical long-term study, groups of male and female SPF Wistar rats (48/sex) were fed soy lecithin for 2 years at 4% of the diet, resulting in mean intake of 1470 and 2280 mg/kg bw/day for males and females, respectively (Brantom et al. 1973). Feed consumption and body weights were determined at time 0 (prior to dosing), at regular timepoints through week 95, at week 102, and then at study termination. No significant differences were observed in mortality, feed consumption, or body weight between the treated and control groups, but minor differences in feed consumption and body weight were noted for the treated group as compared to controls, presumably due to increased lipid intake. Hematological parameters, organ weights, and gross and microscopic alterations for treated animals were similar to those of control animals. Parathyroid gland hyperplasia was increased, particularly in the treated males, which also contributed to a slightly increased incidence of myocardial fibrosis. This increase was attributed to an increased phosphate intake. The incidence of tumor formation was similar for the treated and control groups.

Natural Occurrence and Function of Lecithin

Phosphatidylcholine (once given the trivial name 'lecithin') is usually the most abundant phospholipid in animal and plants, often amounting to almost 50% of the total, and as such it is obviously the key building block of membrane bilayers. In particular, it makes up a very high proportion of the outer leaflet of the plasma membrane. It is a neutral or zwitterionic phospholipid over a pH range from strongly acid to strongly alkaline. It is well established that PC (lecithin) from either plant or animal sources is handled the same metabolically. In animal tissues, PC tends to exist mainly in the diacyl form, but small proportions (in comparison to phosphatidylethanolamine and phosphatidylserine) of alkylacyl and alkenylacyl forms may also be present. As a generalization, animal phosphatidylcholine tends to contain more of the C₁₈ unsaturated fatty acids than the other zwitterionic phospholipid, phosphatidylethanolamine. The saturated fatty acids are most abundant in position *sn*-1, while the polyunsaturated components are concentrated in position *sn*-2. Indeed, C₂₀ and C₂₂ polyenoic acids are exclusively in position *sn*-2. In plants, the positional distributions of fatty acids in phosphatidylcholine is that saturated fatty acids are concentrated at position *sn*-1, monounsaturated fatty acids are distributed approximately equally between the two positions, and there is a preponderance of di- and tri-unsaturated fatty acids in position *sn*-2. The principal compositional difference between canola-derived lecithin and soy or sunflower lecithin is that the former has a high percentage (56%) of mono-unsaturated fatty acid (oleic) distributed more or less equally between *sn*-1 and *sn*-2 while soy or sunflower lecithin has a high percentage (66-70%) of di- and tri-unsaturated fatty acids (linoleic and linolenic, respectively) distributed primarily to the *sn*-2 position. Given their natural presence in the body as chief components of cell membranes, and since the major

phosphoglycerides can be catabolized and synthesized de novo in mammalian systems, lecithins are assigned a low concern level (concern level 1).

Choline

Choline is an important nutritional ingredient that when consumed in necessary amount prevents liver damage and the development of fatty liver syndrome (Zeisel et al., 1991). Based on a review of all available data recommended adequate intakes of 550 mg/day (adult males), 425 mg/day (adult females), 450 mg/day (pregnant women), and 550 mg/day (nursing mothers) were established by the Institute of Medicine (Institute of Medicine, 1998). At extremely high levels, excessive intake of choline results in hypotension, sweating, diarrhea, and fishy body odor (Institute of Medicine, 1998).

Overview of Biochemistry

Lecithin is absorbed into the mucosal cells of the small intestine, mainly in the duodenum and upper jejunum. Prior to absorption, the pancreatic enzyme phospholipase A₂ (Arnesjo et al., 1969; Belleville and Clement, 1969a,b) hydrolyzes the fatty acids at the position *sn*-2 to yield lysophosphatidylcholine, which is then absorbed by the enterocytes (Nieuwenhuizen et al., 1974). Following absorption, reacylation of lysolecithin takes place in these intestinal mucosal cells, reforming phosphatidylcholine, while the previously released fatty acids can be further used for triglyceride synthesis (Tso and Fujimoto, 1994). Phosphatidylcholine is then transported by the lymphatic system in the form of chylomicrons to the blood and metabolized by peripheral tissues. Phosphatidylcholine is also produced endogenously; for example, the liver secretes between 15-20 grams of phospholipids into the intestinal lumen and, of that, over 90% is phosphatidylcholine (Tso et al., 1977; Tso and Simmonds, 1977). Endogenous phosphatidylcholine is a major source for a multitude of phospholipids serving a multitude of body functions. The principal structural difference among these phospholipids is the composition and positional distributions of fatty acids on the glycerol moiety.

The extensive re-modeling of phosphatidylcholine *in vivo* involves hydrolysis of the *sn*-2 ester bond by phospholipase A₂, hydrolysis of the *sn*-1 ester bond by phospholipase A₁, and subsequent re-acylation. There are at least fifteen different isozymes in the phospholipase A₂ super-family, which differ in calcium dependence, cellular location, and structure, that hydrolyze phosphatidylcholine specifically, generating a fatty acid and lysophospholipid. Complete hydrolysis of phosphatidylcholine yields two fatty acids and α -glycerylphosphorylcholine (AGPC). AGPC is the unacylated backbone skeleton from which phosphatidylcholine is synthesized *in vivo*. It can be converted to phosphatidylcholine (Amentia et al., 2001) via a pathway involving choline and phosphorylcholine intermediates.

Recent Data Related to the Safety of Lecithin

Acute Toxicity

AGPC exhibits a low order of acute toxicity both by oral and parenteral routes in rodents and dogs (Brownawell et al., 2010). The oral LD₅₀ in rodents was $\geq 10,000$ mg/kg. Toxic symptoms

at the lethal dose after administration consisted of motor and respiratory depression. At doses up to 3,000 mg/kg in beagle dogs the only symptoms were reduced animal activity.

Subchronic Toxicity

Groups of male and female Sprague–Dawley (SD) rats (10/sex/group) were randomly divided and administered 0, 100, 300, or 1000 mg AGPC/kg bw/day by gavage for 28 consecutive days. Daily clinical observation and weekly body weights failed to reveal any differences between test and control animals. Urinalysis and blood chemical determinations performed prior to necropsy show no statistically significant difference between test and control groups. At necropsy, organ weights also showed no significant changes between test and control animals and histopathological examination did not show any alterations that could be related to administration of the test material.

Groups of males and females SD rats (18/sex/group) were administered 0, 100, 300, or 1000 mg AGPC/kg bw/day by gavage (5 mL/kg) for 26 weeks. Individual daily clinical observations were performed during both the pre-test and dosing phases of the study. The ten deaths recorded over the 26 weeks were due mainly to gavage errors or pulmonary infection. No death could be related to treatment. A reduction in motor activity and of reactivity to stimulation was observed in animals receiving the 1000 mg AGPC/kg bw/day group (1000 mg/kg) starting after 3–4 weeks. Body weights and food consumption measured weekly during the first 3 months of treatment, and every 2 weeks thereafter showed reduced food consumption and body weight gain only in the high dose group beginning at week 4. Hematology and blood chemical determinations performed at week 13 and 26 and urinalysis performed at week 26 revealed a slight increase in creatinine in the high dose group at week 13 and a decrease in plasma triglycerides in males and females, reduction in plasma bilirubin, ALT, and creatinine in females in the high dose group at week 26. Triglycerides were reduced in mid-dose males at week 26. Measurement of organ weights after 26 weeks revealed a reduction in heart weight in mid- and high-dose females but the relative weight (to bodyweight) was unchanged. Necropsy (data not shown) and histopathological evaluations did not reveal any treatment related effects. The Expert Panel has concluded that the 100 mg/kg bw/day level of AGPC was a “no observable adverse effect” (NOAEL) level in rats.

A 28-day (1M & 1F) and 26 week studies (3M & 3F) were also performed with Beagle dogs using the study protocol designed for the SD rat studies and dose levels of 0, 75, 150, or 300 mg/kg bw/day. The only effect in the 28-day study was mild reduced activity in males at the highest dose level. During the 13th week of treatment, blood samples were drawn from the retro-orbital plexus under fasting conditions for limited hematology and clinical chemistry evaluations. Blood and urine was collected from 10/sex/group after 26 week of treatment. In the 26-week study mild reduced activity lasting 2 to 5 h after dosing began the second week of the study in the high-dose animal group (300 mg/kg daily). Body weight gain was reduced at 13 weeks but not at 26 weeks. No hematological changes were observed during the 26-week treatment period. Clinical chemistry evaluations performed at week 13 showed a significant increase in plasma cholesterol and decrease in alkaline phosphatase levels in the mid-dose group, but these changes were absent at week 26. In the high-dose group, significant changes were observed in plasma bilirubin, plasma triglycerides, and alkaline phosphatase, which were reduced 34%, 56%, and

9%, respectively, relative to controls. The weights of the liver and of the heart showed a dose-related decrease that did not achieve statistical significance. Histopathological evaluation of tissues did not reveal any treatment related effects. There were no changes associated with the decrease in liver enzymes and liver and heart weight. Based on the reduced activity after dosing lasting up to 5 h, clinical chemistry evaluations suggesting reduced liver function, and reduced liver and heart weights of the 300 mg AGPC/kg bw/day group, a NOAEL of 150 mg/kg bw/day was determined for AGPC by the study authors.

Genetic Toxicity

In vitro

Incubation of AGPC with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 or TA1538 produced no significant increases in the number of revertants or with or without metabolic activation, up to 10,000 µg/plate. AGPC was nonmutagenic in mutations of *S. pombe* P1 at concentrations up to 3000 µg/mL with or without microsomal activation. AGPC did not alter the mitotic gene conversion frequency of *S. cerevisiae* at concentrations up to 10,000 µg/mL. Also, no concentration of AGPC with or without microsomal activation increased the frequency of Ade2 or Trp5 gene conversion above controls.

In vivo

In *in vivo* assays, AGPC administered subcutaneously to rats at doses up to 300 mg/kg bw showed no evidence of mitotic gene conversion of *S. cerevisiae* at the Ade2 or Trp5 genes. In mouse micronucleus test, AGPC did not induce any significant increases in the incidence of micronucleated immature erythrocytes and was not cytotoxic in Swiss mice administered AGPC at doses of 3, 30 or 300 mg/kg bw, compared to the vehicle control. Based on this, the Expert Panel concluded that the *in vivo* precursor of lecithin, AGPC, is not genotoxic in the micronucleus assay or any other of the assays performed.

Based on the dose of 150 mg/kg bw/day resulting in no adverse effects in dogs exposed to AGPC and the fact that AGPC is a metabolic precursor of PC (Abbiati et al., 1993), the approximate equivalent dose levels of PC (AGPC plus two C18 fatty acids) and choline would be approximately 450 mg/kg bw/day and 60 mg/kg bw/day, respectively. Based on the fact that HL is approximately 70% phosphatidylcholine (PC) at minimum, the intake of HL equivalent to the 150 mg AGPC is approximately 650 mg/kg bw/day or 39 g/person/day. This intake of lecithin is similar to the NOAEL determined for lecithin in a 2-year feeding study with rats given 1400 mg lecithin/kg bw/day (equivalent to a human dose of about 84 g daily) except for an increased incidence of tertiary parathyroid hyperplasia (Brantom et al., 1973). The parathyroid hyperplasia seen in the rats likely resulted from the increased phosphate load in the diet (Block and Port, 2000). No adverse effects have been noted in volunteers taking 20 g or more of lecithin daily for several months.

In summary, there is no reason to believe that HL would pose any significant adverse health effects at levels consumed in a normal diet, given the metabolic sequences of lecithin in the body and recent data showing a lack of any toxicity on a precursor of lecithin or the lack of toxicity for lecithin in a 2-year study at levels of lecithin (>1400 mg/kg bw/day) exceeding dietary intake by at least a factor of 10.

III. Conclusions

In conclusion, based on the information provided above and the fact that the constituents of HL are commonly found in food, and because these lipids and phospholipids are essentially the same, and will be handled metabolically the same as those produced endogenously or consumed in the diet, we conclude that scientific experts, generally, would recognize HL to be as safe and as acceptable as other commercially available lecithins. Further, we believe that there are no significant questions regarding the safety of HL that would appear to require additional safety studies, due to the prior consideration and acceptability by the Agency for other plant-derived lecithins and phospholipids, the recent multi-species toxicity study (Brownawell et al., 2011) of the basic building block, AGPC, of lecithin, and the 2-year feeding study with plant-derived lecithin (Brantom et al. 1973).

IV. Conclusion of Expert Panel

We, the Expert Panel, have independently and collectively critically evaluated the data and information summarized above and conclude that, based on scientific procedures, HL, meeting appropriate food grade specifications and produced in compliance with current good manufacturing practice, is Generally Recognized As Safe (GRAS) for use in foods under the conditions of intended use described herein.

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Appendix A:

Table A-1: Phospholipid Composition of Lecithin from Various Sources (% w/w of product)

Phospholipid	Fluid Soy	Fluid Sunflower	Fluid Canola	Deoiled Soy	Deoiled Sunflower	Deoiled Canola	Deoiled Egg
Total*	39	32	35	58	51	49	24
PC	16	15	15	24	24	24	19
PE	13	7	8	20	9	12	5
PI	10	10	9	14	18	13	0

*Sum of PC, PE, and PI

Table A-2: Fatty Acid Composition of Lecithins from Various Sources (as %w/w of fatty acids)

Fatty Acid	Deoiled Soy	Deoiled Sunflower	Deoiled Canola	Deoiled Egg
Monounsaturated Total	10	12	56	27
Oleic (18:1)	10	12	56	27
Erucic (22:1)	<1	<1	<1	<1
Polyunsaturated Total	66	61	33	23
Linoleic (18:2)	60	55	29	17
Linolenic (18:3)	6	<1	4	0
Arachidonic (20:4)	0	0	0	4
Docosahexaenoic (22:6)	0	0	0	2
Saturated Total	24	21	8	47
Palmitic (16:0)	20	17	7	33
Stearic (18:0)	4	4	1	14
Arachidic (20:0)	0	0	0	0
Trans Fatty Acid	<0.5	<0.5	<0.5	<0.5

Appendix B: Batch Data of Bacterial Counts for PHOSPHOLIPON® 80 H (Soybean-derived Hydrogenated Lecithin)

Bacterial Organism (/g)	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
C.F.U.	< 10	< 10	< 10	< 10	< 10
Yeasts & Moulds	< 10	< 10	< 10	< 10	< 10
E. coli	0	0	0	0	0
Staphyloc. aureus	0	0	0	0	0
Pseudomon. aerug.	0	0	0	0	0

Appendix C: Curriculum Vitae of Expert Panel Members

Pages 000027-000238 of Curriculum Vitae removed in accordance with the Privacy Act of 1974.

Submission End

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