ORIGINAL SUBMISSION

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March 8, 2016

GRN 000636

Dr. Antonia Mattia
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

Subject: GRAS Notice for Phosphatidylserine derived from Sunflower Lecithin

Dear Dr. Antonia Mattia:

On behalf of ECA Healthcare, Inc., we are submitting for FDA review a GRAS notification for phosphatidylserine (PS) derived from sunflower lecithin. The attached documents contain the specific information that addresses the safe human food uses for the notified substance. We believe that this determination and notification are in compliance with proposed Sec. 170.36 of Part 21 of the Code of Federal Regulations (21 CFR section170.36) as published in the Federal Register, Vol. 62, No. 74, FR 18937, April 17, 1997.

We enclose an original and two copies of this notification for your review. Please feel free to contact me if additional information or clarification is needed as you proceed with the review. We would appreciate your kind attention to this matter.

Sincerely,

(b) (6)

3/8/2016

Susan Cho, Ph.D.
Susanscho1@yahoo.com
Agent for ECA Healthcare, Inc.

enclosure

GRAS EXEMPTION CLAIM for SunPS™ Manufactured by ECA Healthcare, Inc.

Prepared by: NutraSource, Inc.
6309 Morning Dew Court
Clarksville, MD 21029
Tel: 410-531-3336;
Susanscho1@yahoo.com

A. GRAS EXEMPTION CLAIM: phosphatidylserine (PS) from sunflower lecithin (SunPS®) - **Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36**

ECA Healthcare, Inc. (hereinafter referred to as ECA) has determined that its phosphatidylserine (PS) from sunflower lecithin (SunPS™) 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 described in the following sections. Since these procedures specify the conditions of its intended use in food, the use of ECA's SunPS™ is exempt from the requirement of premarket approval.

Signed

Susan Cho Date:
Agent for ECA Healthcare, Inc.

B. Notifier's Name and Address

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C. Name of GRAS Substance

Common name is Phosphatidylserine (PS). Trade name is SunPS^{TM}, manufactured by ECA Healthcare, Inc.

D. Product Description

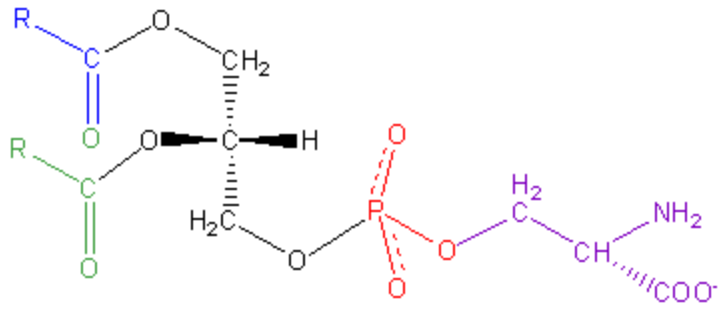
D.1. Identity

Chemical name: Phosphatidylserine (PS).
Per IUPAC-CBN nomenclature, it is a 1,2-diacyl-sn-glycero-3-phospho-L-serine.

Chemical Abstract Registry Number:
There is no CAS Reg. Number assigned specifically to PS derived from sunflower lecithin. The generic CAS Reg. Number assigned to PS is: 84776-79-4.

Chemical Formula: The empirical formula of the most abundant molecule (comprising two linoleic acids) is $C_{42}H_{73}O_{10}PNCa$.

Structure: PS consists of a glycerophosphate skeleton conjugated with two fatty acids and L-serine via a phosphodiester linkage. The structural diagram below shows the general representation of the glycerophosphate backbone with R as fatty acids. The counter ion for the phosphate moiety is Ca^{2+} .



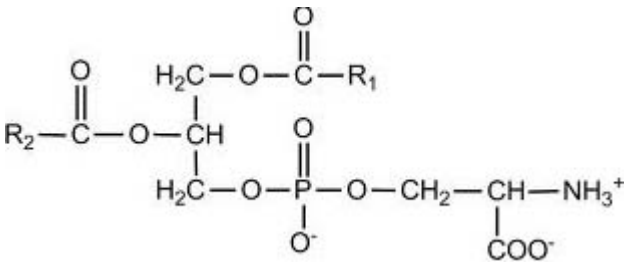


Figure 1. General structure of PS, where R= alkyl group; the counter ion for the phosphate moiety is Ca^{2+} in the most abundant form.

Fatty Acid Profile:

The mean percentages of the fatty acids (FA) in PS from various sources are presented in Table 1. Table 2 presents the FA profile of SunPS™. The bovine source is mainly composed of stearic and oleic acids; the main fatty acids in plant sources have linoleic acid and oleic acid; and fish sources have docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and palmitic acid as the main fatty acids. Different sources do not significantly impact safety profiles of PS.

Table 1. Fatty acid (FA) profiles of soy-, sunflower-, fish-, krill-, and bovine-derived PS

Fatty Acid	Typical FA composition (as % of total FA)				
	Soy-derived PS[1]	Sunflower-derived PS	Fish-derived PS[2]	Krill-derived PS[3]	Bovine-derived PS[4]
Caprylic acid (C8:0)			1		
Myristic acid (C14:0)			2	2	
Palmitic acid (C16:0)	14	11	23	23.5	3
Palmitoleic acid (C16:1)			2	1.8	
Stearic acid (C18:0)	4	2.9	2	1	40
Oleic acid (C18:1 n-9)	15	15.8	13	13	35
Vaccenic acid (C18:1n-11)					
Linoleic acid (C18:2n-6)	62	70.11	2	1.2	
alpha-Linolenic acid (C18:3 n-3)	5	0.2	1	1	
Octadecatetraenoic acid (C18:4n-3)				2	
Eicosenoic (C20:1n-9)			2	0.6	6
Arachidonic acid (C20:4n-6)			1	0.7	
Eicosapentaenoic acid (C20:5n-3; EPA)			12	31	
Erucic acid (C22:1)				1.3	6
Docosapentaenoic acid (C22:5)			1	0.7	
Docosahexaenoic acid (C22:6n-3; DHA)			33	14	7
Nervonic acid (C24:1n-9)				0.3	3
Others			5	5	

[1] GRN No. 000223; [2] GRN No. 000279;[3] GRN No. 000311.

[4] Adopted from Claro et al. (1999) and GRN 545. DHA=Docosahexaenoic acid; EPA=Eicosapentaenoic acid; FA= fatty acid.

Table 2. Typical fatty acid (FA) composition of ECA's SunPS™

Fatty acid	Percentage (as % of total FA)
Palmitic acid	14.58
Stearic acid	3.88
Oleic acid	15.90
Linoleic acid	60.32
Linolenic acid	5.32
Total	100

D.2. Manufacturing Process

The phosphatidylcholine-enriched lecithin is enzymatically trans-phosphatidylated with L-serine using phospholipase D, which catalyzes the substitution of the choline head-group with serine to form PS. Following the enzymatic reaction, PS is separated from the reaction mixture, purified and dried.

ECA uses a Hazard Analysis and Critical Control Point (HACCP)-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications described below. Processing aids, such as ethanol and other ingredients including excipients used in the manufacturing process, are food-grade as specified in the Food Chemical Codex (FCC). The ECA's manufacturing facility and process are certified with the NSF International, based in Ann Arbor, Michigan, USA.

D.3. Typical Composition and Specifications

SunPS™ is produced as an off-white to brown-colored powder. Table 3 presents the typical composition of SunPS™ in comparison to those described in other GRNs for PS. Table 4 shows specifications of SunPS™. Analysis of pesticides (less than 0.05 ppm) and dioxin congeners (less than 0.5 ppm) showed that the levels for the pesticides and other contaminants are minimal in this product. Specifications are comparable to those established in the previous GRAS notices (GRNs 186, 197, 223, and 545; PS content: GRN 186, ≥19%; GRN 197, ≥90%; GRN 223, ≥70%; GRN 545, ≥60%).

ECA's product is specified to contain ≥50% PS. The product also contains other phospholipids and glycerides naturally occurring in sunflower lecithin. These other phospholipids include lysoPS, phosphatidic acid, lyso phosphatidic acid, and associated phospholipids. Compared to the sunflower PS described in GRN 545, the specifications of SunPS™ are approximately 10% lower in the PS content and 10% higher in other phospholipids. These phospholipid profiles are not expected to impact the safety profile of PS products since other phospholipids follow similar metabolic pathways to that of PS. Phospholipids including phosphatidic acid, lyso PS, and lyso phosphatidic acid, are a class of lipids that are a major component of all cell membranes. Due to their safety and functionality, phospholipids are widely used as an emulsifier in food formulations.

Table 3. Typical composition of SunPS™ and other PS

Parameter	SunPS	GRN 545	GRN 186	GRN 197	GRN 223	GRN 279	GRN 311
PS, %	52.3	66.8	19*	90	72.0	48.0	55.0
Phosphatidyl acid, %	7.3	9.2	≤81	3.2	10.6	6.6	5.6
Phosphatidylcholine, %	3.6	0.4		NA	NA	1.6	0.8
Lyso PS, %	0.8	0.9		0.3	0.5	0.8	0.8
Lyso phosphatidyl acid, %	0.6	0.7		0.3	0.3	NA	NA
Phosphatidyl inositol, %	3.5	1.3		NA	NA	1.6	1.6
Other phospholipids, %	17.2	8.0		0.5	4.3	19.8	12.8
Glycerides (Tri-, di- and mono-), %	3.1	4.9		0.1	2.8		
DHA+EPA, %	NM	NM	NM	NM	NM	22	23
Calcium, %	2.8	2.5	NA	0.2	2.5	NA	NA
Sodium, %		NA	NA	3.1	≤0.1	NA	NA
Silicon dioxide should be less than 1.5%	1-1.5		≤1				
Free L-serine, %	0.5	0.4	NA	0.3	≤0.1	NA	NA
Loss on drying, %	1.1	NA	≤5.0	1.0	≤0.2	NA	NA
Ash, %	14.2	14.6	NA	NA	12.7	NA	NA
Volatiles, %	NM	NM	NM	NM	NM	2.0	1.5

NA=not available; NM=not measured; PS= Phosphatidylserine; *This value represents the sum of PS and lysoPS.

Table 4. Specifications of SunPS™

Parameter	Specifications, %	Assay method
Color	Off-white, light yellow to brown	Visual
Phosphatidylserine	≥50.0%	^{31}P-NMR
Loss on drying	≤2.0%	Karl Fisher
Peroxide value	≤ 5 meq/Kg	AOCS official Cd 8-53
Microbiological assays		
Total plate count	≤1000 cfu/g	USP 61
Yeast and mold	≤100 cfu/g	USP 61
E. coli	Negative (cfu/g)	USP 61
Salmonella	Negative (cfu/20g)	USP 61
Heavy metals		
Lead	≤1 ppm	USP 251
Arsenic	≤1 ppm	USP 211
Cadmium	≤1 ppm	AAS
Mercury	≤0.1 ppm	USP 261
Aflatoxins (B1, B2, G1, G2)	≤0.2 ppb	HPLC-FLD
Ethanol	≤1,000 ppm	GC

E. Applicable Conditions for Use of the Notified Substance

E.1. Current Regulatory Status

In 2003, the ability of dietary supplementation with PS (both PS derived from soy lecithin and bovine cortex) to support cognition and interrupt cognitive deterioration was recognized by the FDA in its approval of the qualified health claim, 'Consumption of PS may reduce the risk of dementia in the elderly', with a disclaimer, 'Very limited and preliminary scientific research suggests that phosphatidylserine may reduce the risk of dementia in the elderly' (FDA, 2003). In the FDA's response to this health claim petition, the FDA concluded that the use of PS as a dietary supplement is safe and lawful under 21 C.F.R. § 101.14 provided that bovine-derived sources, if used, are not derived from bovine tissues from cattle born, raised, or slaughtered in any country where bovine spongiform encephalopathy exists.

In addition, the FDA has issued no question letters on six GRAS notices related to food uses of PS derived from sunflower lecithin (GRN 545; FDA, 2015), soy lecithin (GRNs 186, 197, and 223; FDA 2006a, 2006b, 2007), and marine oil (GRNs 279 and 311; FDA, 2009, 2010). In these GRAS notices, toxicity-related studies on PS from the literature were presented to support the safety of PS. The FDA did not question the acceptability and suitability of these studies to establish the safety of PS for the proposed food uses. The safety and related information in the above mentioned GRAS notices are hereby incorporated by reference into this independent GRAS determination.

E.2. Intended Use Levels and Food Categories

SunPS^{TM} will be used in the same food categories and at the same use levels as those described in GRN 223 and 545. As shown in Table 5, ECA proposes to use SunPS^{TM} as a nutrient [21 CFR_ §170.3(o)(20)], and as an alternative to other sources of PS, at levels up to 100 mg PS per serving in dairy product analogs (imitation milk and soy milk), grain products (nutritional bars: breakfast, granola, and protein), milk products (flavored milk and milk drinks, excluding fluid milk, milk-based meal replacements, and yogurt), and processed fruits and fruit juices (fruit flavored drink), at use levels of 50 mg PS per serving in breakfast cereals and milk (fluid- regular, filled, buttermilk, and dry reconstituted), and up to 300 mg in medical foods. Medical foods are defined as foods that are specially formulated and intended for the dietary management of a disease that has distinctive nutritional needs that cannot be met by the normal diet alone.

ECA does not intend to use PS as a component of infant formula or in foods under the USDA's jurisdiction such as meat, poultry, and egg products.

Table 5. Intended use and maximum use levels of PS

Food category	Proposed food use	PS max. use level (mg/RACC)	RACC, g or ml	Use level, %
Breakfast cereals	Instant and regular hot cereals	50	240	0.0208
	Ready-to-eat cereals	50	15-55	0.0909-0.333
Dairy product analogs	Imitation milk	100	240	0.042
	Soy milk	100	240	0.042
Grain products	Nutritional bars (breakfast, granola, protein)	100	240	0.625
Milk products	Flavored milk and milk drinks, fluid	100	240	0.042
	Milk, fluid (regular, filled, buttermilk, and dry reconstituted)	50	240	0.0208
	Milk-based meal replacements	100	240	0.042
	Yogurt	100	225	0.044
Processed fruits and fruit juices	Fruit flavored drinks	100	240	0.042

Adopted from GRNs 223 and 545. RACC= Reference Amount Customarily Consumed; PS= Phosphatidylserine.

E.3. Estimated Dietary Intakes (EDIs) of PS Under the Intended Food Uses

Currently, dietary intakes of PS, from its natural presence in the diet, is estimated to be in the range of 75 - 184 mg/person/day.

Since SunPS™ will be used in the same food categories and at the same use levels as those described in GRN 223 and 545, these exposure calculations presented in those GRNs are valid for SunPS as well. As noted in GRN 223 and 545, consumption of types of food categories intended for addition of PS by the total U.S. population resulted in estimated mean all-user intakes of PS of 44.8 mg/person/day (0.95 mg/kg body weight [bw]/day. When heavy consumers (90^{th} percentile) were assessed, the 90^{th} percentile all-user intakes of PS from all intended food uses by the total population were 98.7 mg/person/day (2.51 mg/kg bw/day). A summary of the estimated daily intakes of PS from the intended food categories is presented in Table 6.

These estimates are highly optimistic since all foods under the intended uses will not be used at the maximum use levels. Based on the totality of the science and as discussed below, these intake levels are considered safe.

Table 6. EDIs of PS under the intended use in all users.

Age group, years	% users	N of total users	mg/day		mg/kg bw/day	
			Mean	90th percentile	Mean	90th percentile
0-2	52.5	1,880	27.4	60.5	2.21	4.86
3-11	79.8	5,030	41.9	91.1	1.72	3.64
12-19, females	54.1	380	45.8	89.1	0.83	1.67
12-19, males	55.0	383	60.7	117.8	1.01	2.18
20+ females	53.3	2,438	42.2	96.4	0.65	1.47
20+ males	46.0	2,230	49.6	105.0	0.61	1.31
Total population	59.9	12,341	44.8	98.7	0.95	2.15

Adopted from GRN 545. EDI = estimated dietary intake; PS= Phosphatidylserine; BW = body weight; N= number.

E.4. Basis for the GRAS Determination

The subject of the present GRAS assessment is SunPS™, PS derived from sunflower lecithin. PS, a structural component of cells, is found in all biological membranes of plants, animals, and other life forms. As the specifications in this GRAS determination are similar to the specifications in the previous FDA GRAS Notice (GRN 545), it is recognized that the information and data in the GRAS Notice received and reviewed by FDA are pertinent to the safety of the PS product derived from sunflower lecithin in this GRAS determination. Therefore, this notice incorporates by reference the safety and metabolism studies and other pertinent information of PS discussed in GRN 545. In addition, as soy PS and PS derived from sunflower and marine sources follow the similar metabolic pathway, this notice also incorporates by reference the safety and metabolism studies and other pertinent information discussed in GRNs 183, 197, and 223 (FDA, 2001, 2010a; PS from soy lecithin) and GRNs 279 and 311 (PS from marine source; FDA, 2009, 2010).

The intended use of sunflower PS (SunPS™) has been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the Generally Recognized as Safe (GRAS) determination. In addition, because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

Technical Element (Safety) of the GRAS Determination

Numerous human and animal studies have reported benefits of SunPS™ with no major adverse effects. ECA uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications. There is broad-based and widely disseminated knowledge concerning the chemistry of PS. This GRAS determination is based on the data and information generally available and consented opinion about the safety of PS from a sunflower source. The literature indicates that PS offers consumers benefits without adverse effects.

The following safety evaluation fully considers the composition, intake, nutritional, microbiological, and toxicological properties of SunPS™ as well as appropriate corroborative data.

1. ECA's SunPS™ is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes. Analytical data from multiple lots indicate that SunPS™ complies reliably with the established food-grade product specifications and meets all applicable purity standards. ECA's SunPS™ meets the specifications of the Food Chemical Codex, 8th edition.

2. PS is an endogenous substance found in the human body. The bioavailability of the ingested PS is limited due to extensive hydrolysis in the intestine prior to absorption, and that absorbed PS is transported and rapidly converted into other endogenous constituents. Orally ingested PS is hydrolyzed in the intestine prior to its absorption. The absorbed PS is transported and rapidly converted into other endogenous constituents. Although no animal toxicity studies and human studies were done on PS from this sunflower source, we recognize that metabolic fates of PS are expected to be similar regardless of its source.

3. Historical consumption of PS supports the safety of PS. PS is commonly found in common foods such as meat, fish, legumes, etc. PS has been marketed as a dietary supplement for the past two decades without any adverse effects (except gastrointestinal side effects such as nausea and indigestion). A typical recommended dose of PS as a dietary supplement is 100 mg three times a day (300 mg/day).

4. In numerous human clinical studies, the safety of PS has been confirmed at daily doses of up to 300 mg for up to 6 months (Hellhammer et al., 2004, 2012, 2014; Jorissen et al., 2001, 2002). The safety of PS has been proven in human clinical studies including susceptible groups (elderly and children) and healthy individuals. Appendix A summarizes human clinical studies conducted on PS derived from various sources.

5. The 90th percentile EDI under the intended use is estimated to be 98.7 mg PS/person (2.51 mg/kg bw/day) for all-users. The 90th percentile intake of PS is approximately 3-fold lower than the safe levels (300 mg/day) determined on the basis of available human safety studies. The EDI estimates are based on the assumption that SunPS™ will replace currently marketed PS derived from various sources. Thus, no increase in exposure is expected.

6. A variety of animal toxicity studies and *in vitro* mutagenicity/genotoxicity studies corroborate the human clinical safety data. The animal studies did not show any significant toxicity at doses up to approximately 1,000 mg/kg/day (Heywood, 1987).

7. In the previous six GRAS notices (GRNs 186, 197, 223, 279, 311, and 545) to the FDA, the safety of PS derived from marine, soy, and sunflower sources has been established in toxicological studies in animals and mutagenicity studies and is further supported by

clinical studies in humans. In particular, the FDA had no question on the safety of PS derived from sunflower (GRN 545).

8. Additional studies published subsequent to the FDA GRAS notices continue to support the safety of PS as a food ingredient (Hellhammer et al., 2014; Lifshitz et al., 2015; Vakhapova et al., 2014).

9. The FDA has agreed to exercise enforcement discretion with a Qualified Health Claim Petition on PS. The petitioner in this submission demonstrated that soy-derived PS is safe at levels up to 500 mg/day (FDA, 2003).

10. The compositional data and product properties are consistent with carefully controlled cGMP production and purification. ECA's SunPS™ preparation contains no impurities or contaminants of concern.

Based on the above-described data and information, we conclude that SunPS™, when used as a nutrient, is reasonably expected to be safe.

Common Knowledge Element of a GRAS Determination

FDA notes that general recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food. The two following components meet a common knowledge element of a GRAS determination:

1. Data and information related to safety are generally available, and this has been established by utilizing published, peer-reviewed scientific journals, and
2. Several reviews by experts in the field also have documented the safety of PS. In addition, PS has been the subject of six GRAS notices submitted to the FDA for use as a nutrient. In each case, the FDA responded that they had no questions on the proposed use and did not object to the respective GRAS determination. In addition, there is consensus among qualified scientists about the safety of the substance for its intended use.

Because this safety evaluation was based on generally available and widely accepted data and information and there was consensus among qualified scientists about the safety of PS for its intended use, it also satisfies the "common knowledge" element of a GRAS determination.

F. Availability of Information

The detailed data and information that serve as a basis for this GRAS determination will be provided to the U. S. FDA upon request, or are available for the FDA's review and copying during reasonable business hours at the offices of NutraSource, Inc. located at 6309 Morning Dew Ct., Clarksville, MD 21029, USA.

G. Basis of GRAS determination: Through scientific procedures.

References

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Appendix A. Human Clinical Studies of PS

Table A-1. Human Clinical Studies of PS from marine source

No. of subjects (PS-treated)	Daily dose, mg	Duration	Design	Adverse effects reported	Reference
A recent study published since the last FDA review of 2014-2015					
157 non-demented participants with memory complaints, 50-90 y	100 mg PS+ 26 mg DHA+ EPA	15 wk OLE from Vakhap-ova et al., 2011	OL	PS-DHA was found to be safe and well tolerated, with no significant side effects	Vakhap-ova et al., 2014
Studies references in previous GRNs					
Children with ADHD					
200 ADHD children, 6-13 y	300	15 wk DB+ 15 wk OL	DB-PC+ OL	Well tolerated. No major adverse events. Adverse events reported included – gastrointestinal discomfort, atopic dermatitis, hyperactivity, tics, nausea, elevated SGOT, tantrum episodes, insomnia, high triacylglyceride level, and soft stool.	Manor et al., 2013
60 children with putative ADHD, 8-13 y	300	90 d	DB-PC	No side effects. Well tolerated.	Vaisman et al., 2008
Elderly with memory complaints					
26 patients with subjective memory complaints, 60-90 y	100[a]	12 wk	OL	No significant changes were found in resting BP, pulse and weight during the study period. In addition, no major adverse events were reported.	Richter et al., 2011
157 non-demented participants with memory complaints, 50-90 y	300 mg PS+ 79 mg DHA+ EPA	15 wk	DB-PC	No serious adverse events were classified. No clinically meaningful differences between treatment groups on the tested blood parameters.	Vakhap-ova et al., 2011

Modified from GRN 545. [a] In combination with 600 mg GPC, 20 mg vinpocetine, 50 mg uridine-5-monophosphate (disodium), 550 mg plant extracts (150 mg wild blueberry, 125 mg ashwagandha, 150 mg grape seed, 125 hops, ginger and rosemary. ADHD = attention deficit hyperactivity disorder;

BP = blood pressure; d=days; DB=double blind; EEG=electro encephalogram; mo=months; OL=open label; OLE=open label extension; PC=placebo controlled; PS= Phosphatidylserine; wk=weeks; y=years.

Table A-2. Human Clinical Studies of Soy PS

No. of subjects (PS-treated)	Daily Dose, mg	Dura-tion	Design	Adverse effects reported	Reference
A recent study published since the last FDA review of 2014-2015					
75 healthy male volunteers, mean 26 y	400 mg PS + 400 mg PA	6 wk	DB-PC	No significant adverse events reported	Hellhammer et al., 2014
Studies references in previous GRNs					
Healthy subjects					
16 healthy subjects	200 in bars	42 d	DB-PC	None reported. No effect on heart rate values	Baumeister et al., 2008
48 healthy males, mean 20.8 y	300	l mo	DB-PC	No side effects in treatment group (2 in placebo)	Benton et al., 2001
80 healthy subjects, 20-45 y	400, 600, 800[a]	4wk	DB-PC	None reported	Hellhammer et al., 2004
60 healthy nonsmoking men, 30 - 60 y	300	12 wk	DB-PC	No significant adverse events reported; Weight gain, high blood pressure and uneasiness were reported by two subjects from the treatment group	Hellhammer et al., 2012
20 healthy young golfers, 20-55	200	6 wk	DB-PC	None reported. No influence on mean heart rate.	Jager et al., 2007
18 physically active males , mean 22.5 y	400	14 d	OL	Not effected on cortisol, total testosterone, or mood.	Parker et al., 2011
10 healthy males	600	10 d	DB-PC	None reported	Starks et al., 2008
Children with ADHD					
36 ADHD children, 4–14 y	200	2 mo	DB-PC	The treatment was well-tolerated and no adverse effects were observed.	Hirayama et al., 2014
Elderly with cognitive decline or impairment					
120 elderly with memory	300 or 600	12 wk	DB-PC	No adverse events and no significant differences were found in standard biochemical and	Jorissen et al., 2002

impairment				hematological safety parameters, blood pressure, and heart rate.	
73 elderly with mild cognitive impairment, 50–69 y	100 or 300	6 mo	DB-PC	No adverse events were observed. No clinically significant change in vital signs, hematological, and biological blood or urine parameters. Differences in blood glucose levels were considered clinically insignificant.	Kato-Kataoka et al., 2010
8 elderly with subjective memory complaints, >60 years	300	6 wk	OL	Not reported	Richter et al., 2010
30 elderly with memory complaints	300	12 wk	OL	S-PS significantly reduces BP. S-PS consumption was well tolerated and no serious adverse events were reported	Richter et al., 2013
15 with mild cognitive decline, 65-78 y	300	12 wk	OL	No changes noted in serum electrolytes, glucose, thyroid function, and differential blood counts; no adverse effects noted.	Schreiber et al., 2000

Expanded from GRN 545. [a]Each 100 mg PS contains additional: 125 mg phosphatic acid, 270 mg of other PL, 5 mg of silicon dioxide. ADHD = attention deficit hyperactivity disorder; BP = blood pressure; d=days; DB=double blind; EEG=electro encephalogram; mo=months; OL=open label; OLE=open label extension; PC=placebo controlled; PS= Phosphatidylserine; wk=weeks; y=years.

Table A-3. Human Clinical Studies of PS from Bovine Cortex

No. of subjects	Daily Dose, mg	Duration	Design	Adverse effects	Reference
Studies references in previous GRNs					
30 hospitalized patients with moderate mental impairment, mean 72.4 y	300	60 d	OL	No symptoms of adverse reactions were observed	Allegro et al., 1987
142 subjects with gradual decline of intelligence, 40-80 y	200	90 d	DB	No change noted in pre- and post-dose clinical exams, clinical chemistries, and blood counts; no adverse events	Amaducci et al., 1988
30 patients with mild cognitive decline, mean 69.2 y	300	60 d	OL	No adverse effects were reported	Caffarra et al., 1987
130 patients, uncharacterized	300	60 d	DB	No treatment related clinically significant adverse effects.	Cenacchi et al., 1987
425 elderly with moderate to severe cognitive decline	300	180 d	DB	Dizziness, vomiting and dyspepsia reported in a few patients, mainly in the placebo group. No pharmacological interactions	Cenacchi et al., 1993
149 elderly with AAMI, 50-75 y	300	12 wk	DB	Well tolerated; no adverse events	Crook et al., 1991
51 patients with probable AD, 55-85 y	300	12 wk	DB	Well tolerated; no adverse events	Crook et al., 1992
35 patients with senile dementia of AD type, 65-91 y	300	6 wk	DB	No significant side effects noted	Delwaide et al., 1986
33 patients with mild primary degenerative dementia	300	8 wk	DB	No adverse effects were reported	Engel et al., 1992
331 patients with senile dementia	300	2 mo	DB	No adverse effects were reported	Funfgeld et al., 1989
35 patients with moderate cognitive decline, 61-80 y	300	60 d	OL	No adverse effects were reported	Granata & Michele, 1987

80 elderly with mild to moderate dementia, 48-79 y	400	6 mo	OL	No adverse effects were reported	Heiss et al., 1993
70 patients with probable AD, 48-79 y	400	6 mo	OL	No adverse effects were reported	Heiss et al., 1994
10 elderly women with depressive disorders, 70-81 y	300	30 d	PC-CO	No adverse effects were reported	Maggioni et al., 1990
9 healthy men, 18-40 y	800	10 d	DB	None reported; BP unchanged	Monteleone et al., 1992
87 patients with severe cognitive impairment, mean 71.2 y	300	60 d	DB	No change noted in pre- and post-dose clinical and neurological exams, clinical chemistries, and EEG.	Palmieri et al., 1987
27 with senile cognitive decline, 55-80 y	300	60 d	OL	No change in pre- and post-dose blood biochemistry parameters.	Puca et al., 1987
30 (10 MID, 10 SDAT, 10 depression, mean 67-71 y	400	60 d	OL	No reported changes in liver and kidney function blood biochemistry or blood counts.	Rabboni et al., 1990
39 (20) patients with cerebro-vascular disease	300	2 mo	DB-PC	No differences in side effects between the groups. Transient epigastric discomforts were reported, which disappeared by the end of the study	Ransmayr et al., 1987
34 patients with mild cognitive decline, 60-80 y	300	60 d	OL	No remarkable side effects	Sinforiani et al., 1987
170 patients with cognitive deterioration, 55-80 y	300	90 d	DB	No adverse effects were reported	Villardita et al., 1987

Expanded from GRN 545. Expanded from GRN 545. AD=Alzheimer disease; AAMI=age-associated memory impairment; BP = blood pressure; d=days; DB=double blind; EEG=electro encephalogram; MID= Multi-infarct dementia; mo=months; OL=open label; PC=placebo controlled; PS= Phosphatidylserine; SDAT= Senile dementia–Alzheimer type; wk=weeks; y=years.

APPENDIX B. Summary of Animal Toxicity Studies of PS

Dose	Daily dose	Duration	Results	Reference
A Recent Study Published since the FDA's Last Review of 2014-2015				
Rat	0, 1,050, 2,100, and 3,250 mg/kg bw PS-DHA (Marine source)	13 wk subchronic toxicity study with an *in-utero* exposure phase	NOAEL for F1 =2,100 mg/kg bw/d for PS-DHA or 850 mg/kg bw for PS (98% purity)	Lifshitz et al., 2015
Studies Referenced in Previous GRNs*				
Rat, Sprague Dawley	5 g/kg bw	Single dose	LD_{50} >5 g/kg bw	Heywood et al., 1987
Rat, Sprague Dawley	0, 10, 100, and 1,000 mg/kg bw	26 wk	NOAEL=close to 1,000 mg/kg bw	Heywood et al., 1987
Dog, beagle	0, 10, 100, and 1,000 mg/kg bw	26 wk	NOAEL=1,000 mg/kg bw	Heywood et al., 1987
Rat, Sprague Dawley	0, 10, 100, and 200 mg/kg bw	Days 6 to 15 of gestation; teratogenicity	NOAEL=200 mg/kg bw	Heywood et al., 1987
Rabbit	0, 10, 100, and 450 mg/kg bw	Days 6 to 18 of pregnancy; teratogenicity	NOAEL=450 mg/kg bw	Heywood et al., 1987

PS= Phosphatidylserine; BW = body weight; NOAEL= no observed adverse effect; PS source-bovine.

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EXPERT PANEL STATEMENT

GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR THE ADDITION OF PHOSPHATIDYLSERINE (PS) DERIVED FROM SUNFLOWER LECITHIN TO FOODS

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GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR THE ADDITION OF PHOSPHATIDYLSERINE (PS) DERIVED FROM SUNFLOWER TO FOODS

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GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR THE ADDITION OF PHOSPHATIDYLSERINE (PS) DERIVED FROM SUNFLOWER TO FOODS

I. INTRODUCTION

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened by NutraSource, Inc., at the request of ECA Healthcare, Inc. (hereinafter referred to as ECA), to determine the Generally Recognized As Safe (GRAS) status of its phosphatidylserine (PS) from sunflower lecithin (SunPS™) as a nutritional food ingredient as defined in 21 CFR§170.3(o)(20) in foods. A comprehensive search of the scientific literature for safety and toxicity information on PS was conducted through NutraSouce, Inc. and made available to the Expert Panel members. The Expert Panel members independently and critically evaluated materials submitted by ECA and other information deemed appropriate or necessary. ECA assures that all published and unpublished safety-related information in its possession and relevant to the subject of this safety assessment has been provided to NutraSource, Inc. and has been accurately summarized in this GRAS monograph. Following an independent, critical evaluation, the Expert Panel unanimously agreed to the decision described herein.

The purpose of this dossier is to (1) Outline the identity and composition of SunPS™, (2) Estimate exposure under the intended use, (3) Document the literature pertaining to the safety, toxicity, and food uses of PS, and (4) Assemble an independent Expert Panel of recognized experts to evaluate the data and information in this document to determine if the document is sufficient to establish GRAS status. The data and information summarized in this dossier demonstrate that the intended use of SunPS™, produced using current Good Manufacturing Practices (cGMP) and meeting food-grade specifications, is GRAS, based on scientific procedures, as described herein.

II. INFORMATION ABOUT THE IDENTITY OF THE GRAS SUBSTANCE

II.A. Background

Phosphatidylserine (PS) is the major acidic phospholipid class that accounts for 13–15% of the phospholipids in the human cerebral cortex (Glade and Smith, 2015; Kim et al., 2014). The human body contains about 30 g of PS, about half (approximately 13 g) of which is found in the brain. PS plays a vital role in several metabolic processes such as activation of cell-membrane bound enzymes and is involved in neuronal signaling. In the plasma membrane, PS is localized exclusively in the cytoplasmic leaflet where it forms part of the protein docking sites necessary for the activation of several key signaling pathways (Kim et al., 2014).

Dietary PS supplements are known to improve cognitive function, mood, and stress management in humans and experimental animals, and the intake of PS has been associated with an improvement in psychiatric disorders, such as bipolar and major depressive disorders, as well as the prevention of inflammatory neurodegenerative events (Glade and Smith, 2015). Aging of the

human brain is associated with biochemical alterations and structural deterioration that impair neurotransmission. Exogenous PS (300-800 mg/day) safely slows, halts, or reverses biochemical alterations and structural deterioration in nerve cells (Glade and Smith, 2015). It supports human cognitive functions, including the formation of short-term memory, the consolidation of long-term memory, the ability to create new memories, the ability to retrieve memories, the ability to learn and recall information, the ability to focus attention and concentrate, the ability to reason and solve problems, language skills, and the ability to communicate. It also supports locomotor functions, especially rapid reactions and reflexes (Glade and Smith, 2015). Moreover, in combination with phosphatidic acid (PA), PS has been shown to reduce cortisol levels and enhance well-being under acute social stress (Hellhammer et al., 2004, 2014). PA has different roles in the cell: it is a precursor for other lipids such as PS or phosphatidylcholine via the conversion of PA to diacylglycerol (Sheng et al., 2015) where PA influences membrane curvature and acts as a signaling lipid for diacylglycerol (Sheng et al., 2015).

In this GRAS assessment, PS is intended to be used in a dry powder form as an alternative for the currently marketed PS from sunflower, fish, soy, and other sources that are used as nutritional ingredients for foods and medical foods for the general population. Thus, the overall exposure to PS is not expected to increase as a result of the introduction of SunPS™ onto the market.

The FDA has issued no question letters on six GRAS notices related to food uses of PS derived from soy lecithin (GRNs 186, 197, and 223), sunflower lecithin (GRN 545), and marine oil (GRNs 279 and 311). In these GRAS notices, toxicity-related studies on PS from the literature were presented to support the safety of utilizing PS. The FDA did not question the acceptability and suitability of these studies to establish the safety of PS derived from various sources. The safety and related information in the above-mentioned GRAS notices is hereby incorporated by reference to this independent GRAS determination.

III. CLAIM OF GRAS STATUS

III.A. Claim of Exemption from the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR § 170.36(c)(1)

Phosphatidylserine, derived from sunflower, for use as a nutrient, has been determined to be Generally Recognized As Safe (GRAS) and, therefore, is exempt from the requirement of premarket approval under the conditions of its intended use as described below. The basis for this finding is described in the following sections.

III.B. Common or Trade Name:

The common name of the subject of this GRAS document is phosphatidylserine derived from sunflower. The product will be marketed under the trade name SunPS™.

III.C. Name and Address of Responsible Individual:

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ECA Healthcare Inc. accepts responsibility for the GRAS determination that has been made for phosphatidylserine derived from sunflower (herein after referred to as SunPS™) as described in this GRAS document; consequently, phosphatidylserine derived from sunflower (SunPS™) meeting the conditions described herein is exempt from premarket approval requirements for food ingredients.

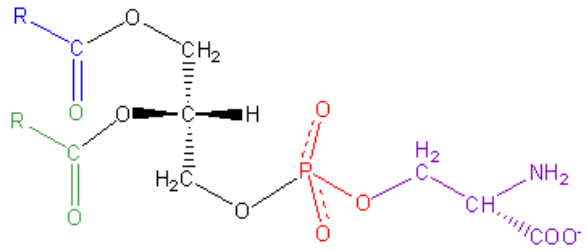
III.D. Chemistry and Physico-chemical Properties

Chemical name: Phosphatidylserine (PS).
Per IUPAC-CBN nomenclature, it is a 1,2-diacyl-sn-glycero-3-phospho-L-serine.

Chemical Abstract Registry Number:
There is no CAS Reg. Number assigned specifically to PS derived from sunflower. The generic CAS Reg. Number assigned to PS is: 84776-79-4.

Chemical Formula: The empirical formula of the most abundant molecule (comprising two linoleic acids) is $C_{42}H_{73}O_{10}PNCa$.

Structure: PS consists of a glycerophosphate skeleton conjugated with two fatty acids and L-serine via a phosphodiester linkage. The structural diagram below shows the general representation of the glycerophosphate backbone with R as fatty acids. The counter ion for the phosphate moiety is Ca^{2+} .



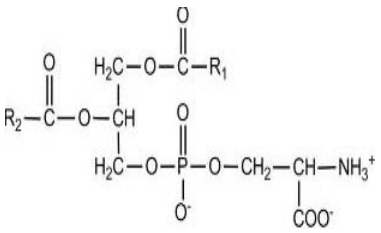


Figure 1. General structure of PS where R= alkyl group; the counter ion for the phosphate moiety is Ca^{2+} in most abundant form.

Fatty Acid Profile:

The mean percentages of the fatty acids (FA) in PS from other sources are presented in Table 1. Table 2 presents the FA profile of SunPS™. Bovine source is mainly composed of stearic and oleic acids as the main fatty acids: plant sources have linoleic acid and oleic acid; and fish sources have docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and palmitic acid as the predominant fatty acids. Different sources do not significantly impact safety profiles of PS. Additional studies also have confirmed this in the treatment level as well (Sakai et al., 1996; Suzuki et al., 2001).

Table 1. Fatty Acid (FA) profiles of sunflower-, soy-, fish-, krill-, and bovine-derived PS

Fatty Acid	Typical FA composition (as % of total FAs)				
	Sunflower-derived PS	Soy-derived PS[1]	Fish-derived PS[2]	Krill-derived PS[3]	Bovine-derived PS[4]
Caprylic acid (C8:0)			1		
Myristic acid (C14:0)			2	2	
Palmitic acid (C16:0)	11	14	23	23.5	3
Palmitoleic acid (C16:1)			2	1.8	
Stearic acid (C18:0)	2.9	4	2	1	40
Oleic acid (C18:1 n-9)	15.8	15	13	13	35
Vaccenic acid (C18:1n-11)					
Linoleic acid (C18:2n-6)	70.11	62	2	1.2	
alpha-Linolenic acid (C18:3 n-3)	0.2	5	1	1	
Octadecatetraenoic acid (C18:4n-3)				2	
Eicosenoic (C20:1n-9)			2	0.6	6
Arachidonic acid (C20:4n-6)			1	0.7	
Eicosapentaenoic acid (C20:5n-3; EPA)			12	31	
Erucic acid (C22:1)				1.3	6
Docosapentaenoic acid (C22:5)			1	0.7	
Docosahexaenoic acid (C22:6n-3; DHA)			33	14	7
Nervonic acid (C24:1n-9)				0.3	3
Others			5	5	

[1] GRN No. 223; [2] GRN No. 279;[3] GRN No. 311.

[4] Adopted from Claro et al. (1999) and GRN 545. DHA=Docosahexaenoic acid; EPA=Eicosapentaenoic acid; FA= Fatty Acid; PS= Phosphatidylserine.

Table 2. Typical FA composition of SunPS™, % of total FA

Fatty acid	% of total FA
Palmitic acid	11.0
Stearic acid	2.9
Oleic acid	15.8
Linoleic acid	70.1
Linolenic acid	0.2
Total	100

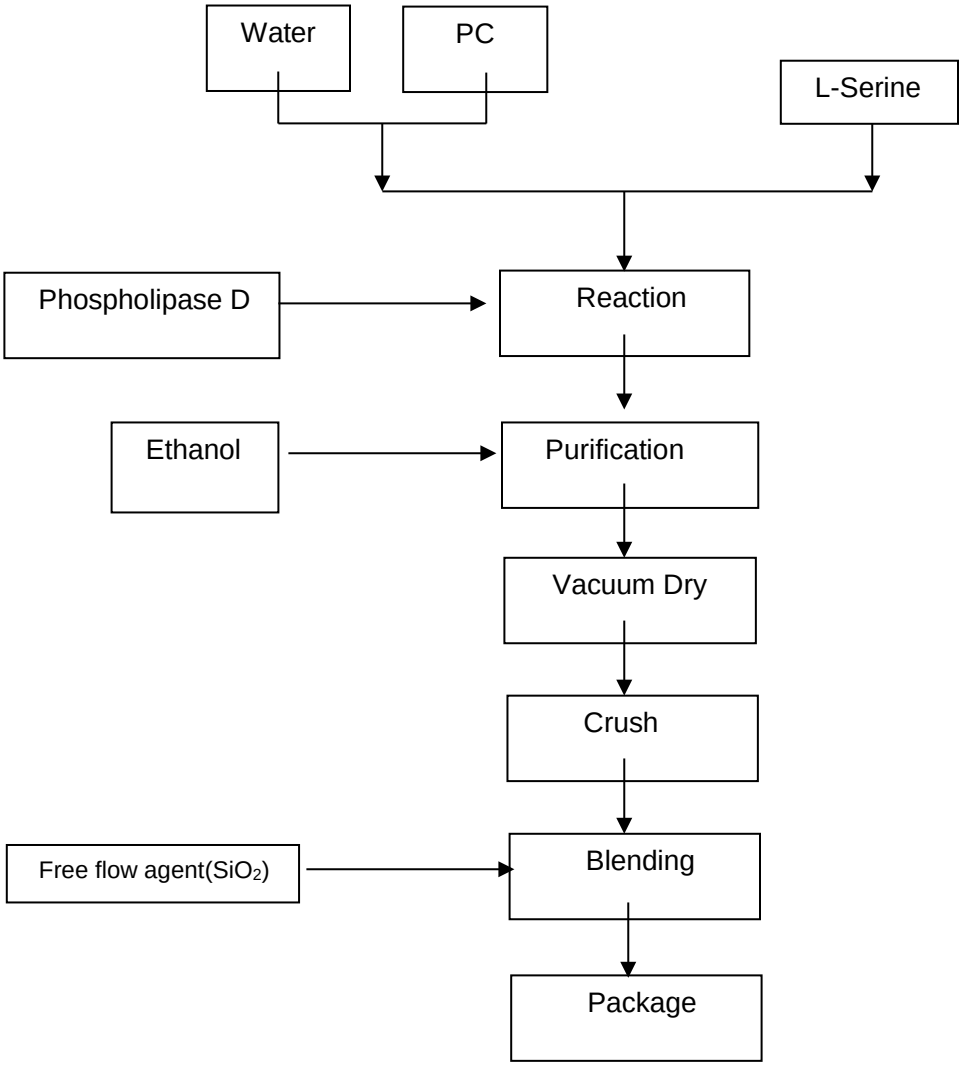
FA=Fatty Acid; PS=Phosphatidylserine; SunPS™ = PS derived from sunflower.

III.E. Manufacturing Process of SunPS™

The phosphatidylcholine-enriched lecithin from sunflower is enzymatically trans-phosphatidylated with L-serine using a phospholipase enzyme. The enzyme used for trans-phosphatidylation is derived from a microorganism that is non-pathogenic and non-toxicogenic. This enzymatic process catalyzes the substitution of the choline head-group with serine to form PS. The enzyme treatment does not alter the fatty acids attached to the molecule or its stereochemistry . Following the enzymatic reaction, the solid product is separated from the reaction mixture, purified, and dried. A final blending with approved food-grade excipients, including silicon dioxide, is carried out in order to produce a free-flowing powder.

ECA's SunPS™ is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes. ECA uses a Hazard Analysis and Critical Control Point (HACCP)-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications. Processing aids, such as ethanol and other ingredients including excipients used in the manufacturing process, are food-grade as specified in the Food Chemical Codex (FCC). The ECA's manufacturing facility and process are certified with the NSF International, based in Ann Arbor, Michigan, USA.

Figure 2. Flow diagram of SunPS™ manufacturing process



III.F. Typical Composition and Specifications

SunPS™ is produced as a grayish brown powder. The typical composition and specifications are shown in Tables 3 and 4, respectively. Analytical data from five different non-consecutive manufacturing lots are presented in Appendix A. Analysis of pesticides (less than 0.05 ppm) and dioxin congeners (less than 0.5 ppm) showed that the levels for pesticides and other contaminants are minimal in this product. Compared to sunflower PS described in GRN 545, the specifications of SunPS™ are approximately 10% lower in PS content and 10% higher in the other phospholipids. However, specifications are comparable to those established in the other GRAS notices (PS content: GRN 186, ≥19%; GRN 197, ≥90%; GRN 223, ≥70%). The PS product also contains other phospholipids (33%) and ash (14%) naturally occurring in sunflower lecithin. These other phospholipids include lysoPS, phosphatidic acid, lyso phosphatidic acid, and associated phospholipids. These phospholipids profiles are not expected to impact the safety profile of PS preparations.

Table 3. Typical composition of SunPS™

Parameter	SunPS™	GRN 545	Assay method
PS, %	52.3	66.8	^{31}P-NMR
Phosphatidyl acid, %	7.3	9.2	^{31}P-NMR
Phosphacholine, %	3.6	0.4	^{31}P-NMR
Lyso PS, %	0.8	0.9	^{31}P-NMR
Lyso phosphatidyl acid, %	0.6	0.7	^{31}P-NMR
Phosphatidyl inositol, %	3.5	1.3	^{31}P-NMR
Other phospholipids, %	17.2	8.0	^{31}P-NMR
Glyceride (Tri-, di- and mono-), %	3.1	4.9	GC-FID
Sum of other Phospholipids and glycerides, %	36.1	25.4	
Calcium, %	2.8	2.5	ICP-OES
Silicon dioxide, %	1 - 1.5		
Free L-serine, %	0.5	0.4	Ninhydrin reaction
Loss on drying, %	1.1		Karl Fisher
Ash, %	14.2	14.6	
Heavy metals			
Lead, ppm	≤1		USP≤251>
Arsenic, ppm	≤1		USP≤211>
Cadmium, ppm	≤1		AAS
Mercury, ppm	≤0.1		USP≤261>

PS= Phosphatidylserine.

Table 4. Specifications of SunPS™

Parameter	Specifications, %	Assay method
Color	Grayish brown	Visual
PS	≥50.0%	^{31}P-NMR
Loss on drying	≤2.0%	Karl Fisher
Peroxide value	≤5 meq/Kg	AOCS official Cd 8-53
Microbiological assays		
Total plate count	≤1000 cfu/g	USP 61
Yeast and mold	≤100 cfu/g	USP 61
E. coli	Negative (cfu/g)	USP 61
Salmonella	Negative (cfu/20g)	USP 61
Heavy metals		
Lead	≤1 ppm	USP 251
Arsenic	≤1 ppm	USP 211
Cadmium	≤1ppm	AAS
Mercury	≤0.1ppm	USP 261
Aflatoxins (B1, B2, G1, G2)	≤0.2 ppb	HPLC-FLD
Ethanol	≤1,000 ppm	USP 467

PS=Phosphatidylserine

IV. INTENDED USES AND EXPOSURE ESTIMATES

IV.A. Intended Technical Effects

SunPS™ is intended for use in powder form as a nutritional ingredient to provide a supplementary source of PS in consumer diets. While there is no specified Dietary Reference Intake (DRI) level for PS, intake of PS via food sources has been shown to be beneficial for brain function. Although PS is naturally present in the diet from foods such as certain fish, poultry, and meats (especially organ meats), its supplementation to food is gaining attention due to its potential health benefits.

IV.B. Intended Use

As shown in Table 5, ECA proposes to use SunPS™ as a nutrient [21 CFR §170.3(o)(20)], and as an alternative to soy-, marine-, or bovine PS, at levels up to 100 mg PS per serving in dairy product analogs (imitation milk and soy milk), grain products (nutritional bars: breakfast, granola, and protein), milk products (flavored milk and milk drinks, excluding fluid milk, milk-based meal replacements, and yogurt), and processed fruits and fruit juices (fruit flavored drink), at use levels of 50 mg PS per serving in breakfast cereals and milk (fluid - regular, filled, buttermilk, and dry reconstituted), and up to 300 mg in medical foods. Medical foods are defined as foods that are specially formulated and intended for the dietary management of a disease that has distinctive nutritional needs that cannot be met by the normal diet alone.

ECA does not intend to use PS as a component of infant formula or in foods under the USDA's jurisdiction such as meat, poultry, and egg products.

Table 5. Intended use and use levels of SunPS™

Food category	Proposed food use	Use level (mg/RACC)	RACC, g or ml	Use level, %
Breakfast cereals	Instant and regular hot cereals	50	240	0.0208
	Ready-to-eat cereals	50	15-55	0.0909-0.333
Dairy product analogs	Imitation milk	100	240	0.042
	Soy milk	100	240	0.042
Grain products	Nutritional bars (breakfast, granola, protein)	100	240	0.250
Milk products	Flavored milk and milk drinks, fluid	100	240	0.042
	Milk, fluid (regular, filled, buttermilk, and dry reconstituted)	50	240	0.0208
	Milk-based meal replacements	100	240	0.042
	Yogurt	100	225	0.044
Processed fruits and fruit juices	Fruit flavored drinks	100	240	0.042

Adopted from GRN 223 and 545. RACC=Reference Amount Customarily Consumed; PS= Phosphatidylserine.

IV.C. Estimated Daily Intakes (EDI) under the Intended Use

IV.C.1. Intake from Natural Presence in Food

PS is found in small amounts in foods such as meats, eggs, soy products, certain legumes, and milk. Dietary intake of PS, from its natural presence in the diet, is estimated to be in the range of 75 - 184 mg/person/day (Bruni et al.,1989; FDA, 2006b; Hamm, 2004). Common sources of industrially produced phospholipids are soya, rapeseed, sunflower, chicken eggs, bovine milk, fish, eggs, etc. Each source has a unique profile of individual phospholipid species and, consequently, differing applications in food, nutrition, and pharmaceuticals. In food formulations, phospholipids can act as an emulsifier, enabling oils to form a colloid with water.

Although some foods with standards of identity are included in the list of foods, at present the use of SunPS™ is intended for foods without a standard of identity.

IV.C.2. EDIs from the Intended Uses

Since SunPS™ will be used in the same food categories and at the same use levels as those described in GRN 223 and 545, the exposure calculations presented in those GRNs are valid for SunPS™ as well (Table 6). In these GRNs, the EDIs of PS from soy or sunflower sources under the intended use was determined using the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-96 database (USDA, 1998).

As noted in GRN 223 and 545 , approximately 60% of the total U.S. population was identified as potential consumers of PS from the proposed food uses. Although infants are included in the intake determinations, PS is not intended to be used in products such as baby foods or infant formula that are specifically marketed for use by infants. Consumption of types of food categories intended for addition of PS by the total U.S. population resulted in estimated mean all-user intakes of PS of 44.8 mg/person/day (0.95 mg/kg body weight [bw]/day. When heavy consumers (90th percentile) were assessed, the 90th percentile all-user intakes of PS from all intended food-uses by the total population was 98.7 mg/person/day (2.51 mg/kg bw/day). A summary of the estimated daily intakes of PS from the intended food categories is presented in Table 6. The EDI of high consumers under the intended use (98.7 mg/day) is comparable to the dietary intake of PS from its natural presence in dietary sources (75-184 mg/day).
These estimates are highly optimistic since all foods under the intended uses will not be used at the maximum use levels. Based on totality of science, and the information discussed below, these intake levels are considered safe.

Table 6. EDIs of PS under the intended use in all-users

Age group, years	% users	N of total users	mg/day		mg/kg bw/day	
			Mean	90th percentile	Mean	90th percentile
0-2	52.5	1,880	27.4	60.5	2.21	4.86
3-11	79.8	5,030	41.9	91.1	1.72	3.64
12-19, females	54.1	380	45.8	89.1	0.83	1.67
12-19, males	55.0	383	60.7	117.8	1.01	2.18
20+ females	53.3	2,438	42.2	96.4	0.65	1.47
20+ males	46.0	2,230	49.6	105.0	0.61	1.31
Total population	59.9	12,341	44.8	98.7	0.95	2.15

Adopted from GRN 545. EDI = estimated dietary intake; PS= Phosphatidylserine; BW = body weight; N= number.

V. BASIS FOR GRAS DETERMINATION

V.A. Current Regulatory Status

In 2003, the ability of dietary supplementation with PS (both PS derived from bovine cortex and soybean lecithin) to support cognition and interrupt cognitive deterioration was recognized by the FDA in its approval of the qualified health claim, 'Consumption of PS may reduce the risk of dementia in the elderly', with a disclaimer, 'Very limited and preliminary scientific research suggests that phosphatidylserine may reduce the risk of dementia in the elderly' (FDA, 2003). In the FDA's response to this health claim petition, the FDA concluded that the use of PS as a dietary supplement is safe and lawful under 21 C.F.R. § 101.14 provided that bovine-derived sources, if used, are not derived from bovine tissues from cattle born, raised, or slaughtered in any country where bovine spongiform encephalopathy exists.

In addition, the FDA has issued no question letters on six GRAS notices related to food uses of PS derived from soy lecithin (GRNs 186, 197 and 223), sunflower lecithin (GRN 545), and fish lecithin (GRN 279 and 311). In these GRAS notices, toxicity-related studies on PS from the literature were presented to support the safety of utilizing PS. The FDA did not question the acceptability and suitability of these studies to establish the safety of PS for the revised, proposed food uses. The safety and related information in the above mentioned GRAS notices is hereby incorporated by reference to this independent GRAS determination.

The pertinent information is summarized below:
GRN 186: Soy lecithin enzymatically modified to have increased phosphatidylserine. Intended use - Ingredient in food in general, except meat and poultry. Lipogen Products (9000) Ltd., Israel. Date of closure - July 20, 2006.
http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=186.

GRN 197: Phosphatidylserine; Intended use - Ingredient in yogurt (excluding fat-free yogurts), powdered milk, ready to drink soymilk, meal replacements, cereal bars, powdered beverage mixes, chewing gum, and breakfast cereals at 20 mg per serving. Degussa Food Ingredients GmbH, Germany. Date of closure - September 20, 2006.
http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=197.

GRN223: Phosphatidylserine; Intended use - Ingredient in milk, flavored milk, milk drinks (excluding milk, fluid), milk imitation (soy milk), milk-based meal replacement, yogurt, breakfast bars, and fruit flavored drink at levels of 100 milligrams (mg) phosphatidylserine per serving and in breakfast cereals and milk, fluid at 50 mg/serving. Enzymotec Ltd., Israel. Date of closure - December 20, 2007.
http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=223.

GRN 279: Phosphatidylserine derived from fish; Intended use - Ingredient in breakfast cereals, dairy product analogs, grain products and pastas, milk products, and processed fruits and fruit juices at levels intended to provide 30 mg of phosphatidylserine per serving; and, as an ingredient in medical foods at levels that would not exceed 300 mg of phosphatidylserine per day. Enzymotec Ltd., Israel. Date of closure - July 29, 2009.
http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=279.

GRN 311: Krill-based phosphatidylserine; Intended use - Ingredient in breakfast cereals, dairy product analogs, grain products and pastas, milk products, and processed fruits and fruit juices, at a use level intended to provide 30 mg of phosphatidylserine per serving; and, as an ingredient in medical foods at levels that would not exceed 300 mg of phosphatidylserine per day. Enzymotec Ltd., Israel. Date of closure – June 15, 2010.
http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=311.

GRN 545: Phosphatidylserine derived from sunflower; Intended use – same as GRN 223 except medical foods. Intended for use in milk, flavored milk, milk drinks (excluding milk, fluid), milk imitation (soy milk), milk-based meal replacement, yogurt, breakfast bars, and fruit flavored drink at levels of 100 mg PS per serving; in breakfast cereals and milk, fluid at 50 mg/serving; and in medical foods at levels not to exceed 300 mg/serving. Enzymotec

Ltd., Israel. Date of closure – June 5, 2015.
http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=545.

Present GRAS assessment by ECA-Healthcare, China: PS derived from sunflower; Intended use –same as GRNs 223 and 545.

V.B. Review of Safety Data

As noted above, the FDA has issued 'no question' letters on six GRAS notices of PS regardless of its source (GRNs 186, 197, 223, 279, 311, and 545; FDA, 2006a, 2006b, 2007, 2009, 2010, and 2015). As the specifications in this GRAS determination are similar to those noted in those FDA GRAS notices (although the concentration of PS in SunPS™ is lower than that of the previous notice) and FA profiles do not impact the safety of PS, it is recognized that the information and data in the GRAS notices are pertinent to the safety of the SunPS™ in this GRAS determination. Therefore, this notice incorporates by reference the safety and metabolism studies discussed in GRN 545 and the other five GRAS notices. The subject of the present GRAS assessment is PS from sunflower lecithin (powder form). Additionally, this GRAS determination discusses additional mutagenicity and genotoxicity study ((Lifshitz et al., 2015), an animal toxicity study (Lifshitz et al., 2015) and human clinical studies (Hellhammer et al., 2014; Vakhapova et al., 2014) that have been published since the FDA's last GRAS notice review of 2014-2015.

V.B.1. Absorption, Metabolism, and Excretion of PS

GRN 545 and the other GRNs discuss the metabolic fate of PS as follows: Following dietary ingestion of PS, pancreatic digestive enzymes cleave specific FAs. The lysophospholipids thus formed are absorbed by the mucosal cells of the intestine and could be reacylated into PS. The FAs released can be further used for triglyceride (TG) synthesis (Tso, 1994). Because of the high activity of decarboxylases in the mucosal cells, the majority of the PS is converted into other phospholipids. PS is decarboxylated mainly to phosphatidylethanolamine (Wise et al., 1965). The reacylated PS, phosphatidylethanolamine and other phospholipids enter the lymph and circulation and are redistributed. The available evidence indicates that only part of the ingested PS reaches systemic circulation as part of the phospholipid pool. For all routes of administration, approximately 60% of the ingested PS is excreted in feces, while 10% is eliminated in urine (Toffano et al., 1987). The major metabolite recovered in the feces was lysoPS (about 50%) after oral administration (Toffano et al., 1987).

Although the FA composition between bovine cortex-, soy-, marine-, or sunflower-derived PS differs, these differences are unlikely to affect the safety profile. Compared to saturated FA present in bovine sources, unsaturated FA present in plant sources are not expected to have more adverse effects. Bovine cortex PS (BCPS) primarily contains saturated and monounsaturated fatty acids, as well as some DHA (Hendler and Rorvik, 2001), and marine-derived PS mainly contain omega-3 polyunsaturated fatty acids (PUFA) and saturated FA. Sunflower- and soy-derived PS mainly contain PUFA. Thus, the human studies of PS derived from bovine cortex- and marine sources can be used for the safety evaluation of PS derived from sunflower lecithin.

Compared to sunflower PS described in GRN 545, the specifications of SunPS™ are approximately 10% lower in the PS content and 10% higher for other phospholipids content.

These phospholipids profiles are not expected to impact the safety profile of PS preparations since other phospholipids follow similar metabolic pathways to that of PS (Murru et al., 2013). Phophoslipids including phosphatidic acid, lyso PS, and lyso phosphatidic acid, are a class of lipids that are a major component of all cell membranes.

V.B.2. Mutagenicity and Genotoxicity Studies

Historically, PS is derived from animal sources such as bovine cortex. In recent years, because of potential contamination concerns from bovine spongiform encephalopathy (BSE) prions, other sources of PS such as plant and marine sources have been explored. As noted above, these differences in FA profiles are unlikely to affect the safety profile of PS regardless of its origin.

The mutagenic potential of PS from the BCPS was investigated in human lymphocytes, a chromosomal damage assay, mouse-lymphoma cell mutation tests, cultured human epithelial cell DNA repair assays, and in an *in vivo* mouse micronucleus assay (Heywood et al., 1987). It is concluded that BCPC is not genotoxic or clastogenic under the conditions described in the 1987 Heywood paper. A recent study by Lifshitz et al. (2015) confirmed the findings from previous research as follows:

Bacterial reverse mutation assay of PS-DHA

PS-DHA was shown not to be mutagenic in either the bacterial reverse mutation assay or the human lymphocyte micronucleus assay (Lifshitz et al., 2015). In the bacterial reverse mutation assay, PS-DHA was tested for mutagenic activity in the Ames test using the histidine-requiring *S. typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100 and in the tryptophan-requiring *E. coli* strain WP2 uvrA in both the absence and presence of S9-mix. In these tests, PS-DHA did not induce any dose-related increases in the mean number of revertant colonies.

Cultured human lymphocyte micronucleus assay

PS-DHA was examined for its potential to induce micronuclei in cultured binucleated human lymphocytes, in both the absence and presence of a metabolic activation system (S9-mix) (Lifshitz et al., 2015). In this *in vitro* mammalian cell micronucleus test, blood obtained from two different donors was used and ethanol was used as a solvent for the test substance. The final concentrations of the test substance in the culture medium ranged from 0.39 to 200 μg/ml. Cytotoxicity was determined from Cytokinesis-Block Proliferation Index (CBPI). The results obtained from the two *in vitro* micronucleus tests at doses up to 200 μg/ml demonstrated that PS-DHA was not clastogenic and/or aneugenic to cultured human lymphocytes.

V.B.3. Animal Toxicity Studies

V.B.3.1. A Recent Animal Toxicity Study

Recently, the safety of fish PS conjugated to DHA (PS-DHA) was examined in a subchronic toxicity study with an in-utero exposure phase (Lifshitz et al., 2015; Table 7). Rats were exposed to diets containing 1.5, 3, or 4.5% PS-DHA or two control diets. The test ingredient used in the current study comprised 81% phospholipids, of which PS comprised 49%. The FA profile reflects the fish lecithin source, comprising approximately 15% DHA and 7.5% EPA. Parental (F0) animals were fed throughout mating, gestation and lactation. Subsequently, a subchronic,

13-week study was conducted on the F1 animals followed by 4 weeks of recovery. The genotoxicity tests showed no mutagenicity potential. No significant toxicological findings were found in the F0 rats or the F1 pups. In a 13-week study, an increase in the presence of renal minimal-mild multifocal corticomedullary mineralization was noted in nine females of the high-dose group. This change was not associated with any inflammatory or degenerative changes in the kidneys. The no-observed-adverse-effect level (NOAEL) in the present study was placed at 3% in the diet (mid-dose group), equivalent to an overall intake of at least 2,100 mg/kg body weight (bw)/day for PS-DHA or 850 mg/kg bw/day for 98% purity PS in the F1 generation.

Table 7. Summary of a recent animal toxicity study of PS

Dose	Daily dose	Duration	Results	Reference
Rat	0, 1,050, 2,100, and 3,250 mg/kg bw PS-DHA	13 wk subchronic toxicity study with an in-utero exposure phase	NOAEL for F1 =2,100 mg/kg bw/d for PS-DHA or 850 mg/kg bw for 98% purity PS	Lifshitz et al., 2015

PS= Phosphatidylserine; SD = Sprague Dawley; BW = body weight; NOAEL= no observed adverse effect level.

V.B.3.2. Animal Toxicity Studies Referenced in Previous GRNs

Previous GRNs summarized traditional toxicity studies done on BCPS and marine PS containing DHA. The acute oral LD_{50} of PS was determined to be greater than 5 g/kg bw and subchronic studies found the NOAEL of PS as 1,000 mg/kg bw/day in rats and dogs (Heywood et al., 1987). In addition, PS was not teratogenic at daily doses of up to 200-450 mg/kg bw for 12 days during pregnancy in rats and rabbits (Heywood et al., 1987).

V.B.3. Human Clinical Studies

V.B.3.1. Recent Human Clinical Studies

Recent studies published since FDA's 2015 review (Hellhammer et al., 2014; Vakhapova et al., 2014) reported the data consistent with the agency's prior decision (Table 8). In healthy subjects, daily supplementation with 300 mg PS or 400-800 mg of PS-phosphatic acid (PA) complex (PAS) safely attenuated the increase in cortisol secretion induced by acute stressors, including moderate- to high intensity exercise (Hellhammer et al., 2014). Hellhammer et al. (2014) reported that in chronically stressed subjects, soy PS 400 can be expected to buffer a hyper-responsivity of the hypothalamic-pituitary-adrenal axis to acute stressors by normalizing cortisol responses. In contrast, PAS 400 did not affect endocrine stress response in high chronically stressed subjects who do not have elevated cortisol levels.

Marine PS also improved cognitive function (Table 8; Vakhapova et al., 2014). The improvements observed after 15 weeks of daily supplementation with 300 mg of PS from a study by Vakhapova et al. (2011) were sustained for another 15 weeks by continued dietary supplementation with 100 mg/day of PS (Vakhapova et al., 2014).

Table 8. Recent human clinical studies of PS

No. of subjects (PS-treated)	Daily dose, mg	Duration	Design	Adverse effects reported	Reference
PS from a marine source					
157 non-demented participants with memory complaints, 50-90 y	100 mg PS+ 26 mg DHA+ EPA	15 wk OLE from Vakhap-ova et al., 2011	OL	PS-DHA was found to be safe and well tolerated, with no significant side effects	Vakhap-ova et al., 2014
Soy PS					
75 healthy male volunteers, mean 26 y	400 mg PS + 400 mg PA	6 wk	DB-PC	No significant adverse events reported	Hell-hammer et al., 2014

[a] In combination with 600 mg GPC, 20 mg vinpocetine, 50 mg uridine-5-monophosphate (disodium), 550 mg plant extracts (150 mg wild blueberry, 125 mg ashwagandha, 150 mg grape seed, 125 hops, ginger and rosemary. d=days; DB=double blind; OL=open label; PC=placebo controlled; PS= Phosphatidylserine; wk=weeks.

V.B.4.2. Human Studies Referenced in Previous GRNs

Of the 38 human clinical trials, 21 studies evaluated BCPS, 13 trials (reported in 14 papers) tested soy-based PS (soy PS), and 4 trials employed marine-based PS (marine PS). Although these investigations were designed to study the efficacy of PS, clinical observations also included any adverse effects. None of these studies reported adverse effects of PS, regardless of its source.

PS derived from sunflower

Although fatty acid composition between bovine cortex-, soy-, marine-, or sunflower-derived PS differs, these differences are unlikely to affect the safety profile. Most toxicity/safety studies were done on BCPS (bovine source) and soy PS, which were found to be safe. Compared to saturated FA present in bovine source, unsaturated FA present in plant sources are not expected to have more adverse effects. BCPS primarily contains saturated and monounsaturated fatty acids, as well as some DHA (Hendler and Rorvik, 2001) and marine-derived PS mainly contain omega-3 polyunsaturated fatty acids (PUFA) and saturated FA. Sunflower- and soy-derived PS mainly contain PUFA. Thus, the human studies of PS derived from bovine cortex-, marine-, and soy-sources can be used for the safety evaluation of PS derived from sunflower.

PS from bovine source (BCPS)

Human clinical studies on oral BCPS have employed daily doses of 100 to 800 mg, with the duration of 10 days to 6 months in elderly patients with various age-related cognitive problems (Table 8-1; Allegro et al., 1987; Amaducci et al., 1988; Caffarra et al., 1987; Cenacchi et al., 1987, 1993; Crook et al., 1991, 1992; Delwaide et al., 1986; Engel et al., 1992; Funfgeld et al., 1989; Granata and Michele, 1987; Heiss et al., 1993, 1994; Maggioni et al., 1990; Monteleone et al., 1992; Palmieri et al., 1987; Puca et al., 1987; Rabboni et al., 1990; Sinforiani et al., 1987; Villardita et al., 1987). None of the studies listed above reported adverse effects of PS on measured outcomes. In addition, no adverse events were associated with PS supplementation.

PS from a marine source (Marine PS)

Improved verbal immediate recall also was observed in a double-blind, placebo controlled clinical trial in a large group of elderly subjects with memory complaints when treated with a daily dose of 300 mg PS containing DHA and EPA in a 3:1 ratio (Vayacog™ Enzymotec Ltd., Israel) (Vakhapova et al., 2011). A subset with relatively good cognitive performance at baseline showed the greatest improvement (Vakhapova et al., 2011).

PS derived from soybean (Soy PS)

The findings from the BCPS studies were confirmed and extended in studies evaluating soy PS (Baumeister et al., 2008; Benton et al., 2001; Hellhammer et al., 2004, 2012, 2014; Hirayama et al., 2014; Jager et al., 2007; Jorissen et al., 2001, 2002; Kato-Kataoka et al., 2010; Parker et al., 2011; Richter et al., 2010, 2013; Schreiber et al., 2000; Starks et al., 2008).

All of the studies described above reported no adverse effects of PS in healthy subjects and patients with cognitive decline or memory impairment.

VI. SAFETY DETERMINATION

Numerous human and animal studies have reported benefits of PS with no major adverse effects. ECA uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications. There is broad-based and widely disseminated knowledge concerning the chemistry of PS. This GRAS determination is based on the data and information generally available and consented opinion about the safety of PS from a sunflower source. The literature indicates that SunPS™ offers consumers benefits without adverse effects.

PS, a structural component of cells, is found in all biological membranes of plants, animals, and other life forms. FDA has received several GRAS Notices for PS derived from different sources (GRNs 186, 197, 223, 279, 311, and 545). In each case, the FDA responded that they had no questions on the proposed use and did not object to the respective GRAS determination. In particular, the FDA had no question on the safety of PS derived from sunflower (GRN 545).

The following safety evaluation fully considers the composition, intake, nutritional, microbiological, and toxicological properties of SunPS™, as well as appropriate corroborative data.

1. ECA's SunPS™ is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes. Analytical data from multiple lots indicate that SunPS™ complies reliably with the established food-grade product specifications and meet all applicable purity standards.

2. PS is an endogenous substance found in the human body. The bioavailability of the ingested PS is limited due to extensive hydrolysis in the intestine prior to absorption, and that absorbed PS is transported and rapidly converted into other endogenous constituents. Orally ingested PS is hydrolyzed in the intestine prior to its absorption. The absorbed PS is transported and rapidly converted into other endogenous constituents. Although no animal toxicity studies and human studies were done on PS from this sunflower source, we recognize that metabolic fates of PS are expected to be similar regardless of its source.

3. Historical consumption of PS supports the safety of PS. PS is found in common foods such as meat, fish, legumes, etc. PS has been marketed as a dietary supplement for the past two decades without any adverse effects (except gastrointestinal side effects such as nausea and indigestion). A typical recommended dose of PS as a dietary supplement is 100 mg three times a day (300 mg/day).

4. The 90th percentile EDI under the intended use is estimated to be 98.7 mg PS/person (2.51 mg/kg bw/day) for all-users. The 90th percentile intake of PS is approximately 3-fold lower than the safe levels (300 mg/day) determined on the basis of available human safety studies. The EDI estimates are based on the assumption that SunPS™ will replace currently marketed PS derived from various sources. Thus, cumulative exposures are not expected.

5. A variety of animal toxicity studies and *in vitro* mutagenicity/genotoxicity studies corroborate the human clinical safety data. The animal studies did not show any significant toxicity at doses up to 1,000 mg/kg/day.

6. In numerous human clinical studies, safety of PS has been confirmed at daily doses of up to 300 mg for up to 6 months. The safety of PS has been proven in human clinical studies including susceptible groups (elderly and children) and healthy individuals.

7. Additional studies published subsequent to the FDA GRAS notices continue to support the safety of PS as a food ingredient.

8. The FDA has agreed to exercise enforcement discretion with a Qualified Health Claim Petition on PS. The petitioner in this submission demonstrated that soy-derived PS is safe at levels up to 500 mg/day.

9. The compositional data and product properties are consistent with carefully controlled cGMP production and purification. ECA's SunPS™ preparation contains no impurities or contaminants of concern.

10. Several reviews by experts in the field also have documented the safety of PS.

Based on the above-described data and information, we conclude that SunPS™, when used as a nutrient, is reasonably expected to be safe.

VII. CONCLUSION OF THE EXPERT PANEL: GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR THE ADDITION OF PHOSPHATIDYLSERINE (PS) DERIVED FROM SUNFLOWER LECITHIN TO FOODS

Prepared for ECA Healthcare Inc.

We, the undersigned expert panel members, have critically evaluated the materials summarized as follows: We conclude that phosphatidylserine (PS) from a sunflower source is safe and Generally Recognized As Safe (GRAS) for its intended use in foods. The U.S. Food and Drug Administration (FDA) has either listed or affirmed PS as GRAS according to the Title 21 Code of Federal Regulations (21 CFR 170.3(o)(20)). Our conclusion is based on published animal toxicology and human clinical studies of PS from various sources. We recognize that animal toxicity studies and human clinical studies of PS do not present risks associated with the intended use and use levels of SunPS™. Although no animal toxicity studies and human studies were done on PS from this sunflower source, we recognize that metabolic fates of PS are expected to be similar regardless of its source.

Our conclusion is based on published animal toxicology and human clinical studies of PS from various sources including PS derived from soy, fish, and bovine cortex. We recognize that animal toxicity studies and human clinical studies of PS do not present risks associated with the intended use and use levels of SunPS™. The exact chemical structures and compositions of PS have been established and fall into the non-toxic classification. Considering that PS derived from sunflower lecithin is of biological origin, that it exists naturally in many foods, and that it does not represent a known health hazard, SunPS™ is considered as a GRAS substance.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have concluded that SunPS™, when used as described in this dossier, is GRAS based on scientific procedures.

Susan Cho, Ph.D.
President, NutraSource, Inc. susanscho1@yahoo.com

Signature: _____ Date: _____

Robert L. Martin, Ph.D., Waldorf, MD 20601

Signature: _____ Date: _____

George C. Fahey, Ph.D.
Professor Emeritus, The University of Illinois-Urbana, Urbana, IL 61801

Signature: _____ Date: _____

VII. CONCLUSION OF THE EXPERT PANEL: GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR THE ADDITION OF PHOSPHATIDYLSERINE (PS) DERIVED FROM SUNFLOWER LECITHIN TO FOODS

Prepared for ECA Healthcare Inc.

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Our conclusion is based on published animal toxicology and human clinical studies of PS from various sources including PS derived from soy, fish, and bovine cortex. We recognize that animal toxicity studies and human clinical studies of PS do not present risks associated with the intended use and use levels of SunPS™. The exact chemical structures and compositions of PS have been established and fall into the non-toxic classification. Considering that PS derived from sunflower lecithin is of biological origin, that it exists naturally in many foods, and that it does not represent a known health hazard, SunPS™ is considered as a GRAS substance.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have concluded that SunPS™, when used as described in this dossier, is GRAS based on scientific procedures.

Susan Cho, Ph.D.
President, NutraSource, Inc. susanscho1@yahoo.com

Signature: (b) (6) Date: March 8, 2016

Robert L. Martin, Ph.D., Waldorf, MD 20601

Signatur (b) (6) Date: March 8, 2016

George C. Fahey, Ph.D.
Professor Emeritus, The University of Illinois-Urbana, Urbana, IL 61801

Signature: (b) (6) Date: March 7, 2016

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APPENDIX A. CERTIFICATE OF ANALYSIS - SunPS™

Parameter	Typical level/speci-fications	Lot 2015 0901	Lot 2015 0903	Lot 2015 0906	Lot 2015 1001	Lot 2015 1003
PS, %	52.3	53.1	52.6	52.1	52.2	52.4
Phosphatidyl acid, %	7.3	7.1	7.3	7.4	7.2	7.3
Phosphacholine, %	3.57	3.51	3.32	3.63	3.41	3.45
Lyso PS, %	0.8	0.8	0.7	0.7	0.8	0.8
Lyso phosphatidyl acid, %	0.59	0.58	0.56	0.6	0.58	0.57
Phosphatidyl inositol, %	3.5	3.4	3.5	3.3	3.4	3.5
Other phospholipids, %	17.24	17.41	17.30	17.31	17.32	17.33
Glyceride (Tri-, di- and mono-), %	3.1	3.0	3.2	3.1	3.0	3.2
Calcium, %	2.8	2.5	2.6	2.8	2.6	2.8
Free L-serine, %	0.5	0.4	0.5	0.4	0.5	0.5
Loss on drying, %	1.1	1.2	1.4	1.2	1.2	1.3
Ash, %	14.2	14.1	14.2	14.3	14.2	14.3
Peroxide value	≤ 5 meq/Kg	≤ 5 meq/Kg	≤ 5 meq/Kg	≤ 5 meq/Kg	≤ 5 meq/Kg	≤ 5 meq/Kg
Lead	≤1ppm	≤1ppm	≤1ppm	≤1ppm	≤1ppm	≤1ppm
Arsenic	≤1ppm	≤1ppm	≤1ppm	≤1ppm	≤1ppm	≤1ppm
Cadmium	≤1ppm	≤1ppm	≤1ppm	≤1ppm	≤1ppm	≤1ppm
Mercury	≤0.1ppm	≤0.1ppm	≤0.1ppm	≤0.1ppm	≤0.1ppm	≤0.1ppm
Aflatoxins (B1, B2, G1, G2)	≤0.2 ppb	≤0.2 ppb	≤0.2 ppb	≤0.2 ppb	≤0.2 ppb	≤0.2 ppb
Ethanol	≤1,000 ppm	ND	ND	ND	ND	ND
Organochlor Pesticides	≤0.05ppm	≤0.05ppm	≤0.05ppm	≤0.05ppm	≤0.05ppm	≤0.05ppm
Organophosphor Pesticides	≤0.05ppm	≤0.05ppm	≤0.05ppm	≤0.05ppm	≤0.05ppm	≤0.05ppm
Dioxins and Furans	≤0.5ppm	≤0.5ppm	≤0.5ppm	≤0.5ppm	≤0.5ppm	≤0.5ppm
Total plate count	≤1000 cfu/g	≤1000 cfu/g	≤1000 cfu/g	≤1000 cfu/g	≤1000 cfu/g	≤1000 cfu/g
Yeasts and Molds	≤100 cfu/g	≤100 cfu/g	≤100 cfu/g	≤100 cfu/g	≤100 cfu/g	≤100 cfu/g
E. coli	Negative (cfu/g)	Negative (cfu/g)	Negative (cfu/g)	Negative (cfu/g)	Negative (cfu/g)	Negative (cfu/g)
Salmonella	Negative (cfu/20g)	Negative (cfu/20g)	Negative (cfu/20g)	Negative (cfu/20g)	Negative (cfu/20g)	Negative (cfu/20g)

ECA Healthcare Inc.

Http://www.eca-healthcare.com

Certificate of Analysis

Product Name: SunPS™ Phosphatidylserine

Product Code: 1812613 Batch No.: (b) (6) P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

MFG.DATE OCT. 9, 2015 EXP. DATE: OCT. 8, 2017

Product Characteristics:

Shelf Life	24 months
Description	Grayish brown powder
Source	Sunflower
Solvents Used	Ethanol & water
Country of Origin	China

Test Items	Specification	Test	Method
Identification	Positive	Conform	NMR
Phosphatidylserine	≥50.0%	52.20%	NMR/HPLC
Phosphatidyl acid		7.20%	31P-NMR
Phosphatidyl Choline		3.41%	31P-NMR
Lyso Phosphatidylserine		0.80%	31P-NMR
Lyso phosphatidyl acid		0.58%	31P-NMR
Phosphatidyl inositol		3.40%	31P-NMR
Other phospholipids		17.32%	31P-NMR
Glyceride (Tri-, di- and mono-)		3.00%	GC-FID
Calcium		2.60%	ICP-OES
Free L-serine	≤1.0%	0.50%	Ninhydrin
Loss on drying	≤2.0%	1.20%	Karl Fischer
Ash		14.20%	Gravimetric
Microbiological Profile			
Total Plate Count	≤1,000cfu/g	<1,000cfu/	USP<61>
Yeast & Mold	≤100cfu/g	<100cfu/g	USP<61>
E.coli	Negative	Conform	USP<61>
Salmonella	Negative	Conform	USP<61>
Additional Testing			
Heavy Metals			
Lead*	≤1ppm	<1ppm	USP<251>
Arsenic*	≤1ppm	<1ppm	USP<211>
Cadmium*	≤1ppm	<1ppm	AAS
Mercury*	≤0.1ppm	<0.1ppm	USP<261>
Aflatoxins (B1,B2,G1,G2)*	≤0.2ppb	Conform	HPLC-FLD
Organochlor Pesticides	≤0.05ppm	Conform	GC/MS
Organophosphor Pesticides	≤0.05ppm	Conform	GC/MS
Dioxins and Furans	≤0.5ppm	Conform	HRMS
Ethanol	≤0.5%	Conform	GC

Conclusion: Complies With Specifications.

Storage & Packaging:

Store in tightly closed containers at room temperature. Protected from moisture and pest infestation.

◆ The items with * are tested periodically.

Stamp and Signature

ECA HealthCare Inc.,
Add.: A-1002 Minggu Technology Park, 7001 Zhongchun Rd., Shanghai 201101, China
Tel.: +86 21 3358 0655 Fax: +86 21 3358 0611 Email: info@eca-healthcare.com

ECA Healthcare Inc.

Http://www.eca-healthcare.com

Certificate of Analysis

Product Name: SunPS™ Phosphatidylserine

Product Code: 1812613 Batch No.: (b) (6) P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

MFG.DATE SEP. 2, 2015 EXP. DATE: SEP. 1, 2017

Product Characteristics:

Shelf Life	24 months
Description	Grayish brown powder
Source	Sunflower
Solvents Used	Ethanol & water
Country of Origin	China

Test Items	Specification	Test	Method
Identification	Positive	Conform	NMR
Phosphatidylserine	≥50.0%	53.10%	NMR/HPLC
Phosphatidyl acid		7.10%	31P-NMR
Phosphatidyl Choline		3.51%	31P-NMR
Lyso Phosphatidylserine		0.80%	31P-NMR
Lyso phosphatidyl acid		0.58%	31P-NMR
Phosphatidyl inositol		3.40%	31P-NMR
Other phospholipids		17.41%	31P-NMR
Glyceride (Tri-, di- and mono-)		3.00%	GC-FID
Calcium		2.50%	ICP-OES
Free L-serine	≤1.0%	0.40%	Ninhydrin
Loss on drying	≤2.0%	1.20%	Karl Fischer
Ash		14.10%	Gravimetric
Microbiological Profile			
Total Plate Count	≤1,000cfu/g	<1,000cfu/	USP<61>
Yeast & Mold	≤100cfu/g	<100cfu/g	USP<61>
E.coli	Negative	Conform	USP<61>
Salmonella	Negative	Conform	USP<61>
Additional Testing			
Heavy Metals			
Lead*	≤1ppm	<1ppm	USP<251>
Arsenic*	≤1ppm	<1ppm	USP<211>
Cadmium*	≤1ppm	<1ppm	AAS
Mercury*	≤0.1ppm	<0.1ppm	USP<261>
Aflatoxins (B1,B2,G1,G2)*	≤0.2ppb	Conform	HPLC-FLD
Organochlor Pesticides	≤0.05ppm	Conform	GC/MS
Organophosphor Pesticides	≤0.05ppm	Conform	GC/MS
Dioxins and Furans	≤0.5ppm	Conform	HRMS
Ethanol	≤0.5%	Conform	GC

Conclusion: Complies With Specifications.

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Store in tightly closed containers at room temperature. Protected from moisture and pest infestation.

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Tel.: +86 21 3358 0655 Fax: +86 21 3358 0611 Email: info@eca-healthcare.com

ECA Healthcare Inc.

Http://www.eca-healthcare.com

Certificate of Analysis

Product Name: SunPS™ Phosphatidylserine

Product Code: 1812613 Batch No.: (b) (6) P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

MFG.DATE SEP. 5, 2015 EXP. DATE: SEP. 4, 2017

Product Characteristics:

Shelf Life	24 months
Description	Grayish brown powder
Source	Sunflower
Solvents Used	Ethanol & water
Country of Origin	China

Test Items	Specification	Test	Method
Identification	Positive	Conform	NMR
Phosphatidylserine	≥50.0%	52.60%	NMR/HPLC
Phosphatidyl acid		7.30%	31P-NMR
Phosphatidyl Choline		3.32%	31P-NMR
Lyso Phosphatidylserine		0.70%	31P-NMR
Lyso phosphatidyl acid		0.56%	31P-NMR
Phosphatidyl inositol		3.50%	31P-NMR
Other phospholipids		17.30%	31P-NMR
Glyceride (Tri-, di- and mono-)		3.20%	GC-FID
Calcium		2.60%	ICP-OES
Free L-serine	≤1.0%	0.50%	Ninhydrin
Loss on drying	≤2.0%	1.40%	Karl Fischer
Ash		14.20%	Gravimetric
Microbiological Profile			
Total Plate Count	≤1,000cfu/g	<1,000cfu/	USP<61>
Yeast & Mold	≤100cfu/g	<100cfu/g	USP<61>
E.coli	Negative	Conform	USP<61>
Salmonella	Negative	Conform	USP<61>
Additional Testing			
Heavy Metals			
Lead*	≤1ppm	<1ppm	USP<251>
Arsenic*	≤1ppm	<1ppm	USP<211>
Cadmium*	≤1ppm	<1ppm	AAS
Mercury*	≤0.1ppm	<0.1ppm	USP<261>
Aflatoxins (B1,B2,G1,G2)*	≤0.2ppb	Conform	HPLC-FLD
Organochlor Pesticides	≤0.05ppm	Conform	GC/MS
Organophosphor Pesticides	≤0.05ppm	Conform	GC/MS
Dioxins and Furans	≤0.5ppm	Conform	HRMS
Ethanol	≤0.5%	Conform	GC

Conclusion: Complies With Specifications.

Storage & Packaging:

Store in tightly closed containers at room temperature. Protected from moisture and pest infestation.

◆ The items with * are tested periodically.

Stamp and Signature

ECA HealthCare Inc.,
Add.: A-1002 Minggu Technology Park, 7001 Zhongchun Rd., Shanghai 201101, China
Tel.: +86 21 3358 0655 Fax: +86 21 3358 0611 Email: info@eca-healthcare.com

ECA Healthcare Inc.

Http://www.eca-healthcare.com

Certificate of Analysis

Product Name: SunPS™ Phosphatidylserine

Product Code: 1812613 Batch No.: (b) (6) P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

MFG.DATE SEP. 5, 2015 EXP. DATE: SEP. 4, 2017

Product Characteristics:

Shelf Life	24 months
Description	Grayish brown powder
Source	Sunflower
Solvents Used	Ethanol & water
Country of Origin	China

Test Items	Specification	Test	Method
Identification	Positive	Conform	NMR
Phosphatidylserine	≥50.0%	52.10%	NMR/HPLC
Phosphatidyl acid		7.40%	31P-NMR
Phosphatidyl Choline		3.63%	31P-NMR
Lyso Phosphatidylserine		0.70%	31P-NMR
Lyso phosphatidyl acid		0.60%	31P-NMR
Phosphatidyl inositol		3.30%	31P-NMR
Other phospholipids		17.31%	31P-NMR
Glyceride (Tri-, di- and mono-)		3.10%	GC-FID
Calcium		2.80%	ICP-OES
Free L-serine	≤1.0%	0.40%	Ninhydrin
Loss on drying	≤2.0%	1.20%	Karl Fischer
Ash		14.30%	Gravimetric
Microbiological Profile			
Total Plate Count	≤1,000cfu/g	<1,000cfu/	USP<61>
Yeast & Mold	≤100cfu/g	<100cfu/g	USP<61>
E.coli	Negative	Conform	USP<61>
Salmonella	Negative	Conform	USP<61>
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Aflatoxins (B1,B2,G1,G2)*	≤0.2ppb	Conform	HPLC-FLD
Organochlor Pesticides	≤0.05ppm	Conform	GC/MS
Organophosphor Pesticides	≤0.05ppm	Conform	GC/MS
Dioxins and Furans	≤0.5ppm	Conform	HRMS
Ethanol	≤0.5%	Conform	GC

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Certificate of Analysis

Product Name: SunPS™ Phosphatidylserine

Product Code: 1812613 Batch No.: (b) (6) P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

MFG.DATE OCT. 15, 2015 EXP. DATE: OCT. 14, 2017

Product Characteristics:

Shelf Life	24 months
Description	Grayish brown powder
Source	Sunflower
Solvents Used	Ethanol & water
Country of Origin	China

Test Items	Specification	Test	Method
Identification	Positive	Conform	NMR
Phosphatidylserine	≥50.0%	52.40%	NMR/HPLC
Phosphatidyl acid		7.30%	31P-NMR
Phosphatidyl Choline		3.45%	31P-NMR
Lyso Phosphatidylserine		0.80%	31P-NMR
Lyso phosphatidyl acid		0.57%	31P-NMR
Phosphatidyl inositol		3.50%	31P-NMR
Other phospholipids		17.33%	31P-NMR
Glyceride (Tri-, di- and mono-)		3.20%	GC-FID
Calcium		2.80%	ICP-OES
Free L-serine	≤1.0%	0.50%	Ninhydrin
Loss on drying	≤2.0%	1.30%	Karl Fischer
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SUBMISSION END