ORIGINAL SUBMISSION

#637

NutraSource, Inc.
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GRN 000637

March 4, 2016

Dr. Antonia Mattia
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5 100 Paint Branch Parkway
College Park, MD 20740

Subject: GRAS Notice for Phosphatidylserine derived from Soy Lecithin

Dear Dr. Antonia Mattia:

On behalf of ECA Healthcare, Inc., we are submitting for FDA review a GRAS notification for phosphatidylserine derived from soy 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)

3/4/2016

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

enclosure

GRAS EXEMPTION CLAIM for BioPS® Manufactured by

ECA Healthcare
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 soy lecithin (BioPS®) - **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 soy lecithin (BioPS®) 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 BioPS® is exempt from the requirement of premarket approval.

Signed

Susan Cho, Ph.D. Date:
Agent for ECA Healthcare, Inc.

B. Notifier's Name and Address

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E mail: jiang.su@ecahealthcare.com

C. Name of GRAS Substance

Common name is Phosphatidylserine (PS). Trade name is BioPS®, manufactured by ECA Healthcare, Inc.

D. Product Description

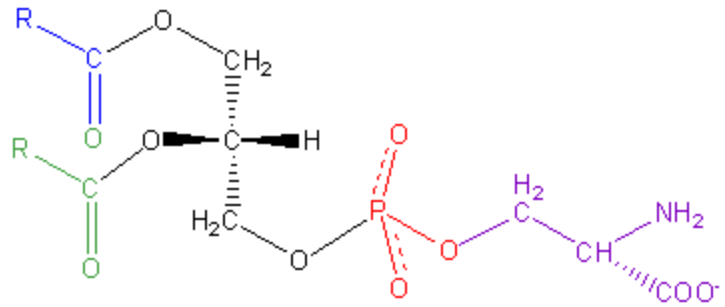
D.1. Identity

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 soybean. 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+} .



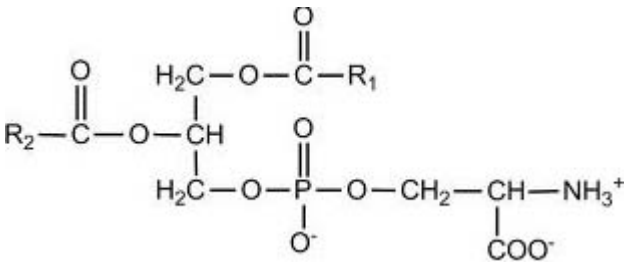


Figure 1. General structure of PS, where R= alkyl group; The counter ion for the phosphate moiety is Ca^{2+} in most abundant form.

Fatty Acid Profile:

The mean percentages of the fatty acids (FA) in PS from various sources are presented in Table 1. Table 2 presents the FA profile of BioPS®. The bovine source is mainly composed of stearic and oleic acids; the main fatty acids in plant sources are 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. 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 223; [2] GRN 279;[3] GRN 311;

[4] Adopted from GRN 545. DHA=Docosahexaenoic acid; EPA=Eicosapentaenoic acid; FA= fatty acid.

Table 2. Typical FA composition of ECA's BioPS®

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 transphosphatidylated 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's BioPS® is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes. All processing aids are food-grade. 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.

D.3. Typical Composition and Specifications

BioPS® is produced as an off white to brown-colored powder. Table 3 presents the typical composition of BioPS® in comparison with those described in other GRNs for PS of soy origin. Table 4 shows specifications of BioPS®. 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 (PS content: GRN 186, ≥19%; GRN 197, ≥90%; GRN 223, 72%).

ECA's product is specified to contain approximately 50% PS. The product also contains other phospholipids and glycerides naturally occurring in soy lecithin. These other phospholipids include lysoPS, phosphatidic acid, LS phosphatidic acid, and associated phospholipids. Compared to other soy PS described in GRNs 197 and 223, BioPS® is 20-40% lower in PS content, but higher in other phospholipids. Compared to soy PS described in GRN 186, BioPS® is 30% higher in PS content and lower in other phospholipids. These phospholipid profiles are not expected to impact the safety profile of PS preparations.

Table 3. Typical composition (%) of BioPS® and other Soy PS

Parameter	BioPS	GRN 186	GRN 197	GRN 223
PS, %	57.38	≥19*	≥90	72
Phosphatidyl acid, %	6.94	≤81	3.2	10.6
Phosphacholine, %	0.05		NA	NA
Lyso PS, %	0.44		0.3	0.5
Lyso phosphatidyl acid, %	0.42		0.3	0.3
Phosphatidyl inositol, %	0.51		NA	NA
Other phospholipids, %	11.87		0.4	4.3
Glyceride (Tri-, di- and mono-), %	5.0		0.1	2.8
Calcium, %	2.5	NA	0.2	2.5
Sodium, %		NA	3.1	≤0.1
Silicon dioxide, %	1.0-1.5	≤1		
Free L-serine, %	≤0.4	NA	0.3	≤0.1
Loss on drying, %	≤2.0	≤5.0	1.0	≤0.2
Ash, %	14.3	NA	NA	12.7

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

Table 4. Specifications of BioPS®

Parameter	Specifications, %	Assay method
Color	Off-white, light yellow to 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	GC

PS=Phosphatidylserine

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 soy lecithin (GRNs 186, 197, and 223; FDA 2006a, 2006b, 2007), sunflower lecithin (GRN 545; FDA, 2015), and fish lecithin (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 utilizing 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 is hereby incorporated by reference into this independent GRAS determination.

E.2. Intended Use Levels and Food Categories

As shown in Table 5, ECA proposes to use BioPS® 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, and 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 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 BioPS® 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 BioPS® as well. 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). The FDA commonly uses the estimated daily intake for the 90th percentile consumer of a food additive as a measure of high chronic dietary intake. Hence, for the safety determinations, the resulting 90th percentile intakes of PS under the intended uses are considered.

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 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 BioPS®, PS derived from soy lecithin. 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. As the specifications in this GRAS determination are similar to the specifications in the previous FDA GRAS Notices, it is recognized that the information and data in the GRAS Notices received and reviewed by FDA are pertinent to the safety of the soy PS product in this GRAS determination. Therefore, this notice incorporates by reference the safety and metabolism studies and other pertinent information of PS discussed in GRN 183, 197, and 223 (FDA, 2001, 2010a). In addition, as soy PS and PS derived from sunflower and fish sources follow a similar metabolic pathway, this notice also incorporates by reference the safety and metabolism studies and other pertinent information discussed in GRN 545 (sunflower PS; FDA, 2015) and GRNs 279 and 311 (PS from fish source; FDA, 2009, 2010).

The intended use of soy PS (BioPS®) has been determined to be safe through 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 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 soy source. The literature indicates that PS 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 six GRAS Notices for PS derived from different sources (GRN 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 soy when PS content ranged from 19 to 90% (GRNs 186, 197, and 223).

The following safety evaluation fully considers the composition, intake, nutritional, microbiological, and toxicological properties of PS as well as appropriate corroborative data. ECA's BioPS® is manufactured under current cGMP using common food industry materials and processes. Analytical data from multiple lots indicate that BioPS® complies reliably with the established food-grade product specifications and meets all applicable purity standards.

1. 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 absorbed PS is transported and rapidly converted into other endogenous constituents.

2. 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).

3. Multiple human clinical studies with various subjects reported that oral administration of PS at doses of 100 to 800 mg/day did not result in any adverse effect regardless of its origin. In particular, 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; Appendix A). The safety of PS has been proven in human clinical studies including susceptible groups (elderly and children) and healthy individuals.

4. These studies employed PS derived from bovine cortex, soy, or marine sources. The available scientific evidence indicates that PS derived from soy lecithin is toxicologically equivalent to PS naturally found in the diet or derived from bovine cortex, sunflower, or fish.

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

6. ECA proposes to use a standardized PS derived from soy lecithin (BioPS®) as a nutrient 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. The intended use levels and food categories are the same as those for soy- and sunflower derived PS which were the subjects of GRAS Notice Numbers 223 and 545. Thus, the exposure to PS from BioPS® will be the same as that described for GRNs 223 and 545, i.e., 98.7 mg/person/day which is well below the safe levels of intake for humans at 300 mg PS per person per day. The EDI estimates are based on the assumption that BioPS® will replace currently marketed PS derived from various sources. Thus, no increase in exposures is expected.

7. 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).

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 BioPS® preparation contains no impurities or contaminants of concern.

Based on the above-described data and information, we conclude that BioPS®, when used as a nutrient, is reasonably expected to be safe. Additionally, ECA has conducted an updated literature search since 2014. ECA did not uncover any additional information that is relevant to the use of PS.

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. PS has been evaluated by FDA and several expert groups and found to be safe for use in food. 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.

Additionally, ECAQ has conducted an updated literature search since 2014. ECA did not uncover an additional information that is relevant to the use of PS.

F. Allergen Labeling

ECA is aware that the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) amends the Federal Food, Drug, and Cosmetic Act to require that the label of a food that is or contains an ingredient that bears or contains a "major food allergen" declare the presence of the allergen (section 403(w)). FALCPA defines a "major food allergen" as one of eight foods or food groups (milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans) or a food ingredient that contains protein derived from one of those foods. ECA will ensure that its PS will be appropriately labeled. Appendix C shows that ECS's BioPS® is free of allergens.

G. 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.

H. Basis of GRAS determination: Through scientific procedures.

References

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Appendix A. Summary of Human Clinical Studies of Soy PS

No. of subjects (PS-treated)	Dose, mg/d	Dura-tion	Design	Adverse effects reported	Reference
A 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 referenced 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 effect 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., 2013
Elderly with cognitive decline or impairment					
120 elderly with memory impairment	300 or 600	12 wk	DB-PC	No adverse events and no significant differences were found in standard biochemical and hematological safety parameters, blood pressure, or heart rate.	Jorissen et al., 2002
73 elderly with mild	100 or 300	6 mo	DB-PC	No adverse event was observed. No clinically significant change in	Kato Kataoka et

cognitive Impairment, 50–69 y				vital signs, hematological and biological blood or urine parameters. Differences in blood glucose levels were considered clinically insignificant.	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. d=days; DB=double blind; EEG=electro encephalogram; mo=months; OL=open label; OLE=open label extension; PC=placebo controlled; wk=weeks; y=years.

APPENDIX B. Summary of Animal Toxicity Studies of PS

Dose	Daily dose	Duration	Results	Reference
A Recent Study				
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 SOY LECITHIN TO FOODS

Coordinated by: NutraSource, Inc.
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GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR THE ADDITION OF PHOSPHATIDYLSERINE (PS) DERIVED FROM SOY LECITHIN TO FOODS

I. INTRODUCTION

The undersigned, an independent panel of recognized experts (herein after 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 soy lecithin (BioPS®) 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 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 BioPS®, (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 BioPS®, 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.

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).

In this GRAS assessment, PS is intended to be used in dry powder form as an alternative for the currently marketed PS from soy, marine, sunflower, 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 BioPS® 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; FDA 2006a, 2006b, 2007), sunflower lecithin (GRN 545; FDA 2015), and marine lecithin (GRN 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 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 notice 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 soy lecithin, 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:

Common name is Phosphatidylserine (PS). Trade name is BioPS®, manufactured by ECA Healthcare, Inc.

III.C. Name and Address of Responsible Individual:

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III.D. Chemistry and Physicochemical 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 soybean. 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+} .

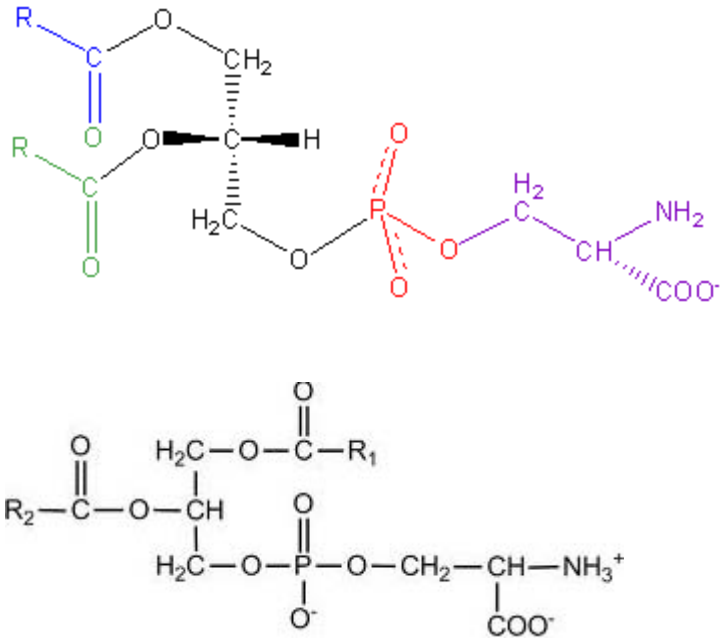


Figure 1. General structure of PS where R= alkyl group; The counter ion for the phosphate moiety is Ca^{2+} in the most abundant form.

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 BioPS®. The bovine source is mainly composed of stearic and oleic acids as the main fatty acids; the main fatty acids in plant sources are 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. 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 223; [2] GRN 279;[3] GRN 311;
[4] Adopted from GRN 545. DHA=Docosahexaenoic acid; EPA=Eicosapentaenoic acid; FA= fatty acid.

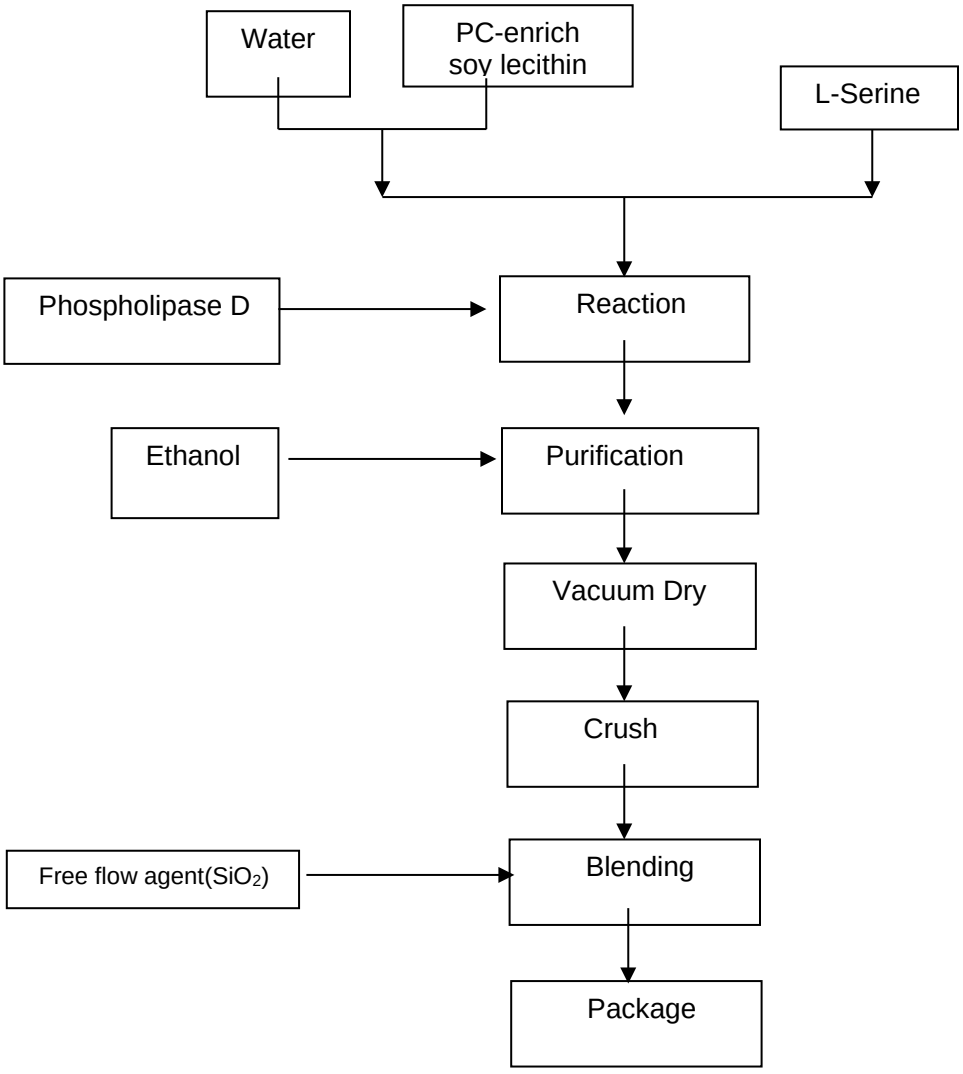
Table 2. Typical FA composition of ECA's BioPS®

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

III.E. Manufacturing Process

PS is manufactured from high phosphatidylcholine (PC)-enriched soybean lecithin (non-GMO). The phosphatidylcholine-enriched lecithin is enzymatically trans-phosphatidylated with L-serine using a phospholipase D enzyme. The enzyme used for trans-phosphatidylation is derived from a microorganism that is nonpathogenic and nontoxicogenic (similar to GRN 186). This enzymatic process catalyzes the substitution of the choline head-group with serine to form PS. The enzyme treatment does not alter the FAs 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 food-grade silicon dioxide is carried out in order to produce a free-flowing powder. A final blending with approved food-grade excipients, including silicon dioxide, is carried out in order to produce a free-flowing powder. ECA's BioPS® is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes. All processing aids are food-grade. 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.

Figure 2. Flow diagram of the BioPS® manufacturing process



III.F. Typical Composition and Specifications

BioPS® is produced as an off white to brown-colored powder. Table 3 presents the typical composition of BioPS® in comparison to those of other GRNs for PS from soy origin. Table 4 shows specifications of BioPS®. Analytical data from five different 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 the pesticides and other contaminants are minimal in this product. Specifications are comparable to those established in the previous GRAS notices (GRNs 186, 197, and 223; PS content: GRN 186, >19%; GRN 197, 90%; GRN 223, 72%).

ECA's product is specified to contain approximately 50% PS. The product also contains other phospholipids and glycerides naturally occurring in soy lecithin. These other phospholipids include lysoPS, phosphatidic acid, lyso phosphatidic acid, and associated phospholipids.

Compared to other soy PS described in GRNs 197 and 223, BioPS® is 20-40% lower in PS content, but higher in other phospholipids. Compared to soy PS described in GRN 186, BioPS® is 30% higher in PS content and lower in other phospholipids. These phospholipids profiles are not expected to impact the safety profile of PS preparations.

Table 3. Typical composition (%) of BioPS® and other Soy PS

Parameter	BioPS	GRN 186 powder	GRN 197	GRN 223
PS	57.38	≥19*	90	72
Phosphatidyl acid	6.94	≤81	3.2	10.6
Phosphacholine	0.05		NA	NA
Lyso PS	0.44		0.3	0.5
Lyso phosphatidyl acid	0.42		0.3	0.3
Phosphatidyl inositol	0.51		NA	NA
Other phospholipids	11.87		0.4	4.3
Glyceride (Tri-, di- and mono-)	5.0		0.1	2.8
Calcium	2.5	NA	0.2	2.5
Sodium		NA	3.1	≤0.1
Silicon dioxide	1.0-1.5	≤1		
Free L-serine	≤0.4	NA	0.3	≤0.1
Loss on drying	≤2.0	≤5.0	1.0	≤0.2
Ash	14.3	NA	NA	12.7

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

Table 4. Specifications of BioPS®

Parameter	Specifications, %	Assay method
Color	Off-white, light yellow to 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	≤1,000 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

PS=Phosphatidylserine

IV. INTENDED USES AND EXPOSURE ESTIMATES

IV.A. Intended Technical Effects

BioPS® is intended for use in powder form as a nutritional ingredient to provide a supplementary source of PS in consumers' 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 such as certain fish, poultry, and meats (especially organ meats), using it to supplement food is gaining attention due to its potential health benefits.

IV.B. Intended Use

BioPS® 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 BioPS® 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, and 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 GRNs 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. Bruni et al. (1989) reported an estimated daily intake of PS of about 75 mg/day. On the basis of a scientific analysis of PS exposure, Hamm (2004) determined an average intake of PS as 130 mg/day, with light eaters of meat and fish consuming about 100 mg and vegans consuming less than 50 mg/day. Consumers of meat are estimated to ingest approximately 80 mg of naturally occurring PS per day (GRN 186; FDA, 2006a). In another notification (GRN 197; FDA, 2006b), the estimated average and 90th percentile intakes of PS for an adult from natural sources were reported as 98 and 184 mg/person/day, respectively. Taken together, dietary intakes of PS, from its natural presence in the diet, is estimated to be in the range of 75 - 184 mg/person/day. Although some foods with standards of identity are included in the list of foods, at present the use of BioPS® is intended for foods without a standard of identity.

IV.C.2. EDIs from the Intended Use

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

daily intake for the 90th percentile consumer of a food additive as a measure of high chronic dietary intake. Hence, for the safety determinations, the resulting 90th percentile intakes of PS under the intended uses are considered.

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 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.

V. BASIS FOR GRAS DETERMINATION

V.A. 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 soy lecithin (GRNs 186, 197, and 223; FDA 2006a, 2006b, 2007), sunflower lecithin (GRN 545; FDA, 2015), and marine oil (GRN 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 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 into this independent GRAS determination.

The pertinent information is available as indicated below:

GRN 186: Soy lecithin enzymatically modified to have increased phosphatidylserine. Lipogen Products (9000) Ltd., Israel. Date of closure - July 20, 2006a. http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=186.

GRN 197: Phosphatidylserine; Degussa Food Ingredients GmbH, Germany. Date of closure - September 20, 2006b. http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=197.

GRN 223: Phosphatidylserine; 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; 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; 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; 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: BioPS®, PS derived from soy lecithin; Intended use –same as GRNs 223 and 545. Submitted by ECA-Healthcare, China.

Soy lecithin is GRAS according to 21 CFR 184.1400.
Silicon dioxide is GRAS according to 21 CFR 172.480.

V.B. Review of Safety Data

FDA has received several GRAS Notices for PS derived from different sources. As noted above, the FDA has issued 'no question' letters on six GRAS notices of PS regardless of its origin. As the specifications in this GRAS determination are similar to the specifications in the previous FDA GRAS Notices, it is recognized that the information and data in the GRAS Notices received and reviewed by FDA are pertinent to the safety of the soy PS product in this GRAS determination. Therefore, this notice incorporates by reference the safety and metabolism studies and other pertinent information of PS discussed in GRN 186, 197, 223 (FDA, 2006a, 2006b, 2007). 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 GRN 545 (sunflower PS; FDA, 2015) and GRNs 279 and 311 (PS from marine source; FDA, 2009, 2010). Additionally, this GRAS determination discusses additional human studies that have been published since the FDA's last GRAS notice review of 2014-2015.

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

Previous 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. Available evidence indicates that only part of the ingested PS reaches systemic circulation as part of the phospholipid pool. Approximately 60% of dietary PS is excreted in the feces with 10% eliminated in urine (Toffano et al., 1987). Approximately 40% of PS is metabolized with lysophosphatidylcholine and lysoPS as the main metabolites.

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 from soy lecithin.

Compared to other soy PS described in GRNs 197 and 223, BioPS® is 20-40% lower in PS content, but higher in other phospholipids. Compared to soy PS described in GRN 186, BioPS® is 30% higher in PS content and lower 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 (Murru et al., 2013). Phosphoslipids including phosphatidic acid, lyso PS, and lyso phosphatidic acid, are a class of lipids that are a major component of all cell membranes. After oral administration, phospholipids are rapidly absorbed by the intestinal mucosa via conversion to lysophosphoplids because phospholipids are hydrolyzed by pancreatic

phospholipase A2 which releases the FA from the sn-2 position. After absorption by the enterocytes, this lysophospholipids can be reacylated into phospholipids, while the previously released FA can be used for TG synthesis.

V.B.2. Mutagenicity and Genotoxicity Studies

The mutagenic potential of PS from the bovine cortex source (BCPS) was investigated in human lymphocytes, 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: 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).

Bacterial Reverse Mutation Assay of PS-DHA

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

A Recent Subchronic Toxicity Study with *In Utero* Exposure (Lifshitz et al., 2015)

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). The study design consisted of two phases: (1) an *in utero* exposure phase in which the F0 parents were fed the test or control diets starting four weeks prior to mating and continued throughout mating, gestation, and lactation until the weaning of the F1 rats and (2) a traditional 90-day feeding study in which selected F1 offspring consumed the same diets as did the F0 generation rats. 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 of 81% phospholipids of which PS comprised 49%. The FA profile reflects the fish lecithin source, comprising approxmately15% 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.

F0 rats (Lifshitz et al., 2015): No significant toxicological findings were found in the F0 rats or the F1 pups. The overall clinical condition and behavior were not adversely affected by the test substance, and none of the parent rats died as a result of the test material. Gross examination of the females did not reveal any effect of the test substance on the maternal organs or tissues. There were no treatment-related clinical signs during the pre-mating, mating, post-mating, gestation, or lactation periods. There were no noticeable differences between the treatment groups and the controls in pre-coital time, male or female fertility index, or the number of pregnant females. There were no statistically significant differences between the treatment groups and the controls in duration of gestation, and the gestation index was 100% in all groups. There were no statistically significant differences in body weights and food intake during the pre-mating, post-mating, gestation, or lactation periods between the groups. Macroscopic examination of the F0 rats did not reveal any treatment-related abnormalities. All pregnant females bore live pups, and the number of females with stillborn pups were comparable among the groups. There were no noticeable differences in live birth index, viability index, pup mortality, sex ratio, prenatal loss, perinatal loss, or lactation index.

F1 rats (Lifshitz et al., 2015): In this subchronic study, the PS-DHA-fed F1 rats did not show any treatment-related changes in neurobehavioral observations, ophthalmoscopy, growth, or food or water intake. In addition, there were no treatment-related abnormalities in clinical signs or hematology, or clinical chemistry. At the end of the treatment period, cholesterol, phospholipids and TG concentrations were decreased in males and females of the mid-dose, high-dose, and reference control groups (fed 3% soy lecithin + 1.7% DHA). These decreases in plasma lipids were expected and are ascribed to the lipid-lowering effects of PUFA. No significant changes were observed in urinary volume or density, either at the end of the treatment period or the recovery period. At the end of the treatment period, the absolute weights of the spleens were significantly increased in the females of the mid- and high-dose groups, while the relative spleen weight was statistically significantly increased in the females of the high-dose group. Many authors have reported increased spleen weights without corroborating histopathological findings in mice and rats administered polyunsaturated fatty acids. In the absence of histopathological changes, increased spleen weights are generally considered to represent physiological or metabolic responses to the PUFAs rather than adverse responses.

In the 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. One female of the reference group showed multifocal corticomedullary mineralization. This finding was still observed at end of the recovery period in all four surviving females of the high-dose recovery group. Focal mineralization was noted in five reference control females, one control, and one mid-dose female and three low-dose females. The animals' multifocal mineralization was not accompanied by renal degeneration, cellular necrosis, or any other morphological, biochemical, or physiological changes. In addition, mineralization of the kidneys at the corticomedullary junction (nephrocalcinosis) is a frequent finding in rats. Female rats appear to be more susceptible to this phenomenon than males. The findings in the current study are consistent with an exacerbation of a physiological process that occurs in the female Wistar rat (Rao, 2002) and based on the historical control data. Another possible explanation for this finding was diet-induced nephrocalcinosis. A low molar ratio of dietary calcium to phosphorus (Ca:P molar ratio

of less than 1) is the most likely cause of nephrocalcinosis associated with semi-purified or commercial diets (Rao, 2002). PS-DHA contains a relatively high amount of phosphorus. Based on the above, there is no clear explanation for the nephrocalcinosis, but it cannot be ruled out as either strain susceptibility or diet-induced. Despite the lack of any other urinary effect, or renal degenerative and/or inflammatory changes associated with this minimal mineral deposition, the study's NOAEL is based on this finding. The NOAEL in this study was determined at 3% in the diet (mid-dose group), equivalent to an overall intake of at least 2,100 mg/kg bw/day for PS-DHA or 850 mg/kg bw/day for PS (98% purity) in the F1 generation (Lifshitz et al., 2015).

Studies Referenced in Previous GRNs

Previous GRNs reported traditional toxicity studies that were done on BCPS and fish PS containing DHA (Table 7). The acute oral Lethal Dose $(LD)_{50}$ of PS was determined to be greater than 5 g/kg bw and subchronic studies found the No-Observed-Adverse-Effect-Level (NOAEL) of PS as close to 1,000 mg/kg bw/day in rats and dogs (Heywood et al., 1987). In addition, PS was found to be non-teratogenic in rats and rabbits (Heywood et al., 1987).

Table 7. Summary of animal toxicity studies of PS

Dose	Daily dose	Duration	Results	Reference
A Recent Study				
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 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-Level.

V.B.4. Human Clinical Studies

As discussed in section IV.B.2., this document also incorporates by reference the information and data included in the previous GRN reports. Additionally, this GRAS determination discusses additional human studies (Hellhammer et al., 2014; Vakhapova et al., 2014) that have been published since the FDA's last GRAS notice review of 2014-2015 (Table 8).

Recently Published Studies

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 that is induced by acute stressors, including moderate- to high intensity exercise (Hellhammer et al., 2004, 2012, 2014).

In a study by Hellhammer et al. (2014), PS, in combination with phosphatidic acid (PA), has been shown to reduce cortisol levels and enhance well-being under acute social stress in young healthy males. Hellhammer et al. (2014) reported that in chronically stressed subjects, daily consumption of 200 mg soy PS (plus 200 mg PA) can be expected to buffer a hyper-responsivity of the hypothalamic-pituitary-adrenal axis to acute stressors by normalizing cortisol responses. In contrast, 200 mg soy PS plus 200 mg PA did not affect endocrine stress response in high chronically stressed subjects who did not have elevated cortisol levels.

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 (from marine source) containing 79 mg DHA and EPA in a 3:1 ratio (Vakhapova et al., 2011). A subset with relatively good cognitive performance at baseline showed the greatest improvement (Vakhapova et al., 2011). 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 daily supplementation with 100 mg PS (Vakhapova et al., 2014).

The Studies Reviewed in Previous GRNs

Of the 38 human clinical trials previously reviewed, 13 trials (reported in 14 papers) tested soy-based PS (soy PS), 21 studies evaluated BCPS, 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. Thus, these studies can be used for the safety evaluation of PS. None of these studies reported adverse effects of PS, regardless of its origin. None of the studies listed below reported adverse effects of PS on measured outcomes. In addition, no adverse events or adverse effects were associated with PS supplementation.

Human clinical studies conducted on oral BCPS in the 1980s and 1990s employed daily doses of 100 - 800 mg, with the duration of 10 days to 6 months in elderly patients with various age-related cognitive problems (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; Ransmayr et al., 1987; Sinforiani et al., 1987; Villardita et al., 1987). None of the studies listed here reported adverse effects of PS on measured outcomes. In addition, no adverse events or adverse effects were associated with PS supplementation.

Due to safety concerns of the risk for prion contamination in BCPS, soybean-derived PS (Soy PS) was established as a safe alternative. In addition, soy PS may be more preferable to BCPS from the perspective of FA composition; soy PS consists of mostly long chain polyunsaturated fatty acids (LC-PUFAs) such as linoleic acid and linolenic acid while BCPS is composed of oleic acid and stearic acid, saturated FAs.

The findings from the BCPS studies were confirmed and extended in studies evaluating soy PS in the past two decades (Baumeister et al., 2008; Benton et al., 2001; Hellhammer et al., 2004, 2012; 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). None of the studies listed below reported adverse effects of PS on measured outcomes when PS was administered at daily doses up to 600 mg for 3-6 months (Jorissen et al., 2001, 2002). In addition, no adverse events were associated with PS supplementation.

As demonstrated in studies of BCPS, marine PS also improved cognitive function (Manor et al., 2013; Richter et al., 2011; Vaisman et al., 2008; Vakhapova et al., 2011). None of the studies listed below reported adverse effects of PS on measured outcomes when PS was administered at daily doses up to 300 mg for 3-7 months (Manor et al., 2003; Vaisman et al., 2008).

Table 8. Recent human clinical studies of PS

No. of subjects (PS-treated)	Daily dose, mg	Duration	Design	Measured outcomes	Adverse effects reported	Reference
75 (50) healthy male volunteers, mean 26 y	400 mg PS + 400 mg PA from soy	6 wk	DB-PC	Endocrine stress response such as adrenocorticotropic hormone (ACTH), saliva, and serum cortisol), and biological (heart rate, pulse rate) and psychological stress responses to acute stress induced by the Trier Social Stress Test	No significant adverse events reported	Hellhammer et al., 2014
157 non-demented participants with memory Complaints, 50-90 years	300 mg PS+ 79 mg DHA+EPA (DHA/ EPA ratio of 3: 1), fish source	15 wk	DB-PC	Cognition (Rey Auditory Verbal Learning Test, Rey Complex Figure Test, and a computerized cognitive battery. Clinicians' Global Impression of Change) and blood parameters	No serious adverse events were classified. No clinically meaningful differences between treatment groups on the tested blood parameters	Vakhapova et al., 2011, 2014

PAS capsule consists of 100 mg PS and 125 mg phosphatic-acid (PA), plus 270 mg of other inert phospholipids (Phosphatidylcholine, Phosphatidylinositol, Phosphatidylethanolamine, and lysophospholipids) and 5 mg silicon dioxide (anti-caking material). PS=Phosphatidylserine; ACTH= adrenocorticotropic hormone; ADHD=attention deficit hyperactivity disorder; DB=double blind; OL=open label; PC=placebo controlled; X=crossover; d=day; mo=months; wk=weeks; y=years.

VI. SAFETY ASSESSMENT

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 soy source. The literature indicates that PS 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 six GRAS Notices for PS derived from different sources (GRN 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 soy when PS content ranged from 19 to 90% (GRNs 186, 197, and 223).

The following safety evaluation fully considers the composition, intake, nutritional, microbiological, and toxicological properties of PS, as well as appropriate corroborative data. ECA's BioPS® is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes. Analytical data from multiple lots indicate that BioPS® complies reliably with the established food-grade product specifications and meet all applicable purity standards.

1. 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.

2. 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).

3. Multiple human clinical studies with various subjects reported that oral administration of PS at doses of 100 to 800 mg/day did not result in any adverse effect regardless of its origin. In particular, the 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.

4. These studies employed PS derived from bovine cortex, soy or marine sources. The available scientific evidence indicates that PS derived from soy lecithin is toxicologically equivalent to PS naturally found in diet or derived from bovine cortex, sunflower, or fish.

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. ECA proposes to use a standardized PS derived from soy lecithin (BioPS®) as a nutrient 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. The intended use levels and food categories are the same as those for soy- and sunflower derived PS which was the subject of GRAS Notice Numbers 223 and 545. Thus, the exposure to PS from BioPS® will be the same as that described for GRNs 223 and 545, i.e., 98.7 mg/person/day which is well below the safe levels of intake for humans at 300 mg PS per person per day. The EDI estimates are based on the assumption that BioPS® will replace currently marketed PS derived from various sources. Thus, no increase in exposures is expected.

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 BioPS® preparation contains no impurities or contaminants of concern.

10. Several reviews by experts in the field also have documented the safety of PS. Additionally, ECA has conducted an updated literature search since 2013. ECA did not uncover any additional information that is relevant to the use of PS.

Based on the above-described data and information, we conclude that BioPS®, 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 SOY 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) derived from soy lecithin 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)). Intended use of PS described in this GRAS determination has been adopted from the previous GRAS notifications for PS from various sources including soy lecithin, which already have received FDA no question letters.

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 BioPS®. The 90th percentile estimated daily intake of PS is approximately 3-fold lower than the safe intake levels (300 mg/day) determined on the basis of available safety studies. Considering that PS derived from soy lecithin is of biological origin, that it exists naturally in many foods, and that it does not represent a known health hazard, BioPS® 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 BioPS®, when used as described in this dossier, is GRAS based on scientific procedures.

Susan Cho, Ph.D.
President, NutraSource, Inc. Clarksville, MD 21029; susanscho1@yahoo.com

Signature: _____ Date: _____

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

Signature: _____ Date: _____

George C. Fahey, Ph.D.
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Signature: _____ Date: _____

VII. CONCLUSION OF THE EXPERT PANEL:

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APPENDIX A. CERTIFICATE OF ANALYSIS - BIOPS®

Parameter	Typical level/specifications	Lot 20150610	Lot 20150804	Lot 20150908	Lot 20150912	Lot 20151011
PS	57.38	57.65	58.01	57.33	57.79	57.71
Phosphatidyl acid, %	6.94	6.85	6.55	7.01	6.53	6.62
Phosphatidyl choline, %	0.05	0.04	0.05	0.04	0.05	0.05
Lyso PS, %	0.44	0.42	0.40	0.43	0.42	0.43
Lyso phosphatidyl acid, %	0.42	0.46	0.39	0.43	0.41	0.42
Phosphatidyl inositol, %	0.51	0.52	0.57	0.56	0.55	0.52
Other phospholipids, %	11.87	11.77	11.80	11.60	11.79	11.62
Glyceride (Tri-, di- and mono-), %	5.0	5.1	5.2	5.0	4.9	5.0
Calcium, %	2.5	2.3	2.5	2.4	2.5	2.4
Free L-serine, %	0.4	0.5	0.4	0.5	0.5	0.4
Loss on drying, %	2.0	1.7	1.5	1.5	1.3	1.4
Ash, %	14.3	14.2	14.3	14.4	14.2	14.1
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
Yeast and Mold	≤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)

Appendix C. Allergencity Test Results

eurofins

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SU0002114

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 10 B1, Longshan Road, SND
Suzhou, 215163
Jiangsu Province, P.R.China
Tel: +86 400 828 5088
Fax: +86 512 68785966

Report date: 04-Jan-2016

Print By:Kelly Ji

CERTIFICATE OF ANALYSIS

Certificate No.: AR-16-SU-000449-01

Sample

Client Sample Description BioPS Phosphatidylserine	Sample Code	50215S058320
	Date of order	18-Dec-2015
	Sample received	18-Dec-2015
	Start of Analysis	18-Dec-2015
	End of Analysis	04-Jan-2016
	Reception temperature	16℃
	Quantity of Sample	1*110g
	Sample packaging	Sealed plastic bag
	Sample appearance	Powder
Client Sample Code 20150912		

Results and comments are shown on the following page(s)

The result(s) relate(s) only to the item (s) tested.
Eurofins General Terms and Conditions apply.

For and on behalf of
Eurofins Technology Service (Suzhou) Co., Ltd

(b) (6)

Pathik Vyas
Technical Director

Results of Analysis

	Results	LOQ	LOD	Unit	Comments
SU590	Allergen – Soya, ELISA				
Soya protein	<2.5	2.5		mg/kg	

☆ means the test is subcontracted within Eurofins group
◎ means the test is subcontracted outside Eurofins group

END OF REPORT

ECA Healthcare Inc.

Http://www.eca-healthcare.com

Certificate of Analysis

Product Name: BioPS® Phosphatidylserine 50% Powder, Eurofins IP Certified Non-GM

Product Code: 1808835 Batch No.: (b) (6) P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

MFG.DATE JUN. 10, 2015 EXP. DATE: JUN. 09, 2017

Product Characteristics:

Molecular Weight	750~850
CAS NO.	84776-79-4
Shelf Life	24 months
Description	Light Yellow to Brown Powder
Source	Non-GMO Soybeans
Solvents Used	Ethanol & water
Country of Origin	China

Test Items	Specification	Test	Method
Identification	Positive	Conform	NMR
Phosphatidylserine	≥50.0%	57.65%	NMR/HPLC
Phosphatidyl acid		6.85%	31P-NMR
Phosphatidyl Choline		0.04%	31P-NMR
Lyso Phosphatidylserine		0.42%	31P-NMR
Lyso phosphatidyl acid		0.46%	31P-NMR
Phosphatidyl inositol		0.52%	31P-NMR
Other phospholipids		11.77%	31P-NMR
Glyceride (Tri-, di- and mono-)		5.10%	GC-FID
Calcium		2.30%	ICP-OES
Free L-serine	≤1.0%	0.50%	Ninhydrin
Loss on drying	≤2.0%	1.70%	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: BioPS® Phosphatidylserine 50% Powder, Eurofins IP Certified Non-GM

Product Code: 1808835 Batch No.: (b) (6) P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

MFG.DATE APR. 4, 2015 EXP. DATE: APR. 03, 2017

Product Characteristics:

Molecular Weight	750~850
CAS NO.	84776-79-4
Shelf Life	24 months
Description	Light Yellow to Brown Powder
Source	Non-GMO Soybeans
Solvents Used	Ethanol & water
Country of Origin	China

Test Items	Specification	Test	Method
Identification	Positive	Conform	NMR
Phosphatidylserine	≥50.0%	58.01%	NMR/HPLC
Phosphatidyl acid		6.55%	31P-NMR
Phosphatidyl Choline		0.05%	31P-NMR
Lyso Phosphatidylserine		0.40%	31P-NMR
Lyso phosphatidyl acid		0.39%	31P-NMR
Phosphatidyl inositol		0.57%	31P-NMR
Other phospholipids		11.80%	31P-NMR
Glyceride (Tri-, di- and mono-)		5.20%	GC-FID
Calcium		2.50%	ICP-OES
Free L-serine	≤1.0%	0.40%	Ninhydrin
Loss on drying	≤2.0%	1.50%	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>
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

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ECA Healthcare Inc.

Http://www.eca-healthcare.com

Certificate of Analysis

Product Name: BioPS® Phosphatidylserine 50% Powder, Eurofins IP Certified Non-GM

Product Code: 1808835 Batch No.: (b) (6) P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

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

Product Characteristics:

Molecular Weight	750~850
CAS NO.	84776-79-4
Shelf Life	24 months
Description	Light Yellow to Brown Powder
Source	Non-GMO Soybeans
Solvents Used	Ethanol & water
Country of Origin	China

Test Items	Specification	Test	Method
Identification	Positive	Conform	NMR
Phosphatidylserine	≥50.0%	57.33%	NMR/HPLC
Phosphatidyl acid		7.01%	31P-NMR
Phosphatidyl Choline		0.04%	31P-NMR
Lyso Phosphatidylserine		0.43%	31P-NMR
Lyso phosphatidyl acid		0.43%	31P-NMR
Phosphatidyl inositol		0.56%	31P-NMR
Other phospholipids		11.60%	31P-NMR
Glyceride (Tri-, di- and mono-)		5.00%	GC-FID
Calcium		2.40%	ICP-OES
Free L-serine	≤1.0%	0.50%	Ninhydrin
Loss on drying	≤2.0%	1.50%	Karl Fischer
Ash		14.40%	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

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Certificate of Analysis

Product Name: BioPS® Phosphatidylserine 50% Powder, Eurofins IP Certified Non-GM

Product Code: 1808835 Batch No.: (b) (6) P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

MFG.DATE SEP. 22, 2015 EXP. DATE: SEP. 21, 2017

Product Characteristics:

Molecular Weight	750~850
CAS NO.	84776-79-4
Shelf Life	24 months
Description	Light Yellow to Brown Powder
Source	Non-GMO Soybeans
Solvents Used	Ethanol & water
Country of Origin	China

Test Items	Specification	Test	Method
Identification	Positive	Conform	NMR
Phosphatidylserine	≥50.0%	57.79%	NMR/HPLC
Phosphatidyl acid		6.53%	31P-NMR
Phosphatidyl Choline		0.05%	31P-NMR
Lyso Phosphatidylserine		0.42%	31P-NMR
Lyso phosphatidyl acid		0.41%	31P-NMR
Phosphatidyl inositol		0.55%	31P-NMR
Other phospholipids		11.79%	31P-NMR
Glyceride (Tri-, di- and mono-)		4.90%	GC-FID
Calcium		2.50%	ICP-OES
Free L-serine	≤1.0%	0.50%	Ninhydrin
Loss on drying	≤2.0%	1.30%	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.,
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Certificate of Analysis

Product Name: BioPS® Phosphatidylserine 50% Powder, Eurofins IP Certified Non-GM

Product Code: 1808835 Batch No.: (b) (6) P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

MFG.DATE OCT. 20, 2015 EXP. DATE: OCT. 20, 2017

Product Characteristics:

Molecular Weight	750~850
CAS NO.	84776-79-4
Shelf Life	24 months
Description	Light Yellow to Brown Powder
Source	Non-GMO Soybeans
Solvents Used	Ethanol & water
Country of Origin	China

Test Items	Specification	Test	Method
Identification	Positive	Conform	NMR
Phosphatidylserine	≥50.0%	57.71%	NMR/HPLC
Phosphatidyl acid		6.62%	31P-NMR
Phosphatidyl Choline		0.05%	31P-NMR
Lyso Phosphatidylserine		0.43%	31P-NMR
Lyso phosphatidyl acid		0.42%	31P-NMR
Phosphatidyl inositol		0.52%	31P-NMR
Other phospholipids		11.62%	31P-NMR
Glyceride (Tri-, di- and mono-)		5.00%	GC-FID
Calcium		2.40%	ICP-OES
Free L-serine	≤1.0%	0.40%	Ninhydrin
Loss on drying	≤2.0%	1.40%	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.

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

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SUBMISSION END