**ORIGINAL SUBMISSION**

NutraSource, Inc.
6309 Morning Dew Ct, Clarksville, MD 21029
(410)-531-3336 or (301) 875-6454

GRN 000643

March 23, 2016

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 Fish 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 fish 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)

3/23/2016

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

enclosure

# GRAS EXEMPTION CLAIM for DHAPS™ Manufactured by ECA Healthcare

GRN 000643

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 Fish Lecithin (DHAPS™) - 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 fish lecithin (DHAPS™) 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 DHAPS™ is exempt from the requirement of premarket approval.

Signed

(b) (6)

3/23/2013

Susan Cho Date:
Agent for ECA Healthcare, Inc.

**B. Notifier's Name and Address**

Jiang Su, Managing Director
ECA Healthcare, Inc.
1017 North Building, 1839 Qixin Rd
Shanghai, China 201101
Tel: +86+139-1704-0601 or 909-859-4956 (Cell phone in California)
Fax: +86-21-3358-0611
E mail: jiang.su@ecahealthcare.com

**C. Name of GRAS Substance**

Common name is Phosphatidylserine (PS). Trade name is DHAPS™, manufactured by ECA Healthcare, Inc.

# GRAS EXEMPTION CLAIM for DHAPS™ 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 Fish Lecithin (DHAPS™) - 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 fish lecithin (DHAPS™) 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 DHAPS™ is exempt from the requirement of premarket approval.

Signed

Susan Cho Date:
Agent for ECA Healthcare, Inc.

## B. Notifier's Name and Address

Jiang Su, Managing Director
ECA Healthcare, Inc.
1017 North Building, 1839 Qixin Rd
Shanghai, China 201101
Tel: +86+139-1704-0601 or 909-859-4956 (Cell phone in California)
Fax: +86-21-3358-0611
E mail: jiang.su@ecahealthcare.com

## C. Name of GRAS Substance

Common name is Phosphatidylserine (PS). Trade name is DHAPS™, 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 fish 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 $Ca^{2+}$.

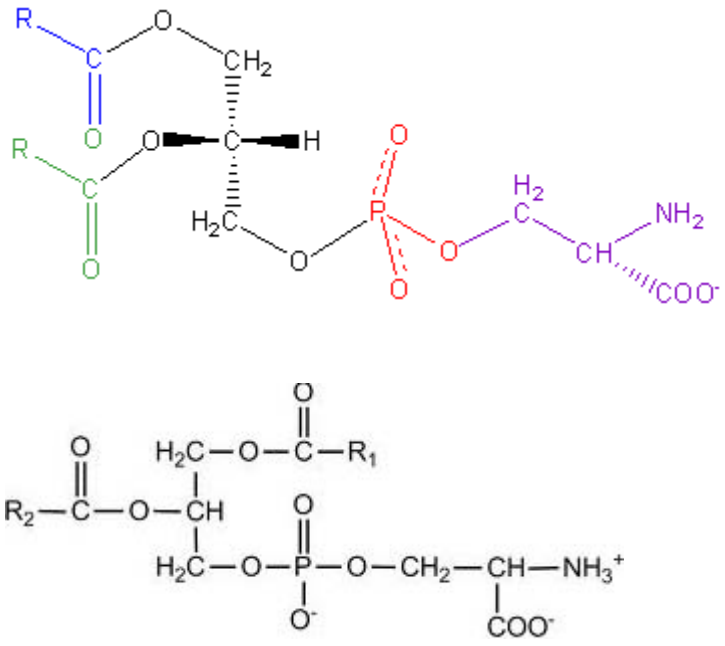


Figure 1. General structure of PS, where R= alkyl group; The counter ion for the phosphate moiety is $Ca^{2+}$ in most abundant form.

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 DHAPS™. 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 predominant fatty acids. Different sources do not significantly impact safety profiles of PS.

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 Claro et al. (1999) and GRN 545. DHA=Docosahexaenoic acid; EPA=Eicosapentaenoic acid; FA= fatty acid.

Table 2. Typical FA composition of ECA's DHAPS™

| Fatty Acid | Percentage (as % of total fatty acids) |
|---|---|
| C8:0 | 1 |
| C14:0 | 2 |
| C16:0 (palmitic acid) | 23 |
| C16:1 | 2 |
| C18:0 | 2 |
| C18:1(oleic acid) | 13 |
| C18:2 | 2 |
| C18:3 | 1 |
| C20:1 | 2 |
| C20:4 | 1 |
| C20:5(EPA) | 12 |
| C22:5 | 1 |
| C22:6 (DHA) | 33 |
| Others | 5 |
| Total | 100 |

FA = fatty acid; DHA=Docosahexaenoic acid; EPA=Eicosapentaenoic acid.

**D.2. Manufacturing Process**

DHAPS™ is manufactured from high phosphatidylcholine-enriched herring fish lecithin (DHA-lecithin is extracted with ethanol from herring roe). 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 DHAPS™ 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 described below.

**D.3. Typical Composition and Specifications**

DHAPS™ is produced as an off-white to brown-colored powder. Table 3 presents the typical composition of DHAPS™ in comparison with those described in other GRNs for PS of soy origin. Table 4 shows specifications of DHAPS™. 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, lysophosphatidic acid, and associated phospholipids. Compared to other soy PS described in GRNs 197 and 223, specifications of DHAPS™ are 20-40%

lower in PS content, but higher in other phospholipids. Compared to soy PS described in GRN 186, DHAPS™ 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 DHAPS™ and other PS

| Parameter | DHAPS | GRN 186 Soy PS | GRN 279 Krill PS | GRN 311 Fish PS |
|---|---|---|---|---|
| PS, % | 30.6 | 19* | 48 | 55 |
| Phosphatidyl acid, % | 6.9 | ≤81 | 29 | 35 |
| Phosphatidylcholine, % | 0.05 | | | |
| Lyso PS, % | 0.44 | | | |
| Lyso phosphatidyl acid, % | 0.42 | | | |
| Phosphatidyl inositol, % | 0.51 | | | |
| Other phospholipids, % | 11.9 | | | |
| Glyceride (Tri-, di- and mono-), % | 5.0 | | | |
| DHA, % | 20 | | DHA+ EPA=22 | DHA+ EPA=23 |
| EPA, % | 6 | | | |
| Calcium, % | 2.5 | NA | | |
| Sodium, % | | NA | | |
| Silicon dioxide, % | 1.0-1.5 | ≤1 | | |
| Free L-serine, % | ≤0.4 | NA | | |
| Loss on drying, % | ≤2.0 | ≤5.0 | ≤2.0 | ≤1.5 |
| Ash, % | 14.3 | NA | | |

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

Table 4. Specifications of DHAPS™

| 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 |
| Yeasts and molds | ≤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 | ≤0.1ppm | USP 261 |

| | | |
|---|---|---|
| Aflatoxins (B1, B2, G1, G2) | ≤0.2 ppb | HPLC-FLD |
| Ethanol | ≤5,000 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 issued no question letters on six GRAS notices (GRN 294) 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 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.

### E.2. Intended Use Levels and Food Categories

As shown in Table 5, ECA proposes to use DHAPS™ 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 | 0.250 |
| 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 to 184 mg/person/day.

Since DHAPS® will be used in the same food categories and at same use levels as those described in GRN 223 and 186, these exposure calculations presented in those GRNs are valid for DHAPS™ as well. In these GRNs, the 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 90$^{th}$ percentile consumer of a food additive as a measure of high chronic dietary intake. Hence, for the safety determinations, the resulting 90$^{th}$ percentile intakes of PS under the intended uses are considered.

As noted in GRN 223 and 186, 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 (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 | 90$^{th}$ percentile | Mean | 90$^{th}$ 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 DHAPS™, PS derived from fish 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 fish 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 GRNs 279 and 311 (fish PS; FDA, 2009, 2010). In addition, as fish PS and PS derived from sunflower and soy 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 (soy PS, GRN 183, 197, and 223, FDA, 2006a, 2006b, 2007; sunflower PS, FDA, 2015).

The intended use of fish PS (DHAPS™) 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 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 fish 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 questions 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 DHAPS™ is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes. Analytical data from multiple lots indicate that DHAPS™ 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 (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. These studies employed PS derived from bovine cortex, soy, or marine sources. The available scientific evidence indicates that PS derived from fish lecithin is toxicologically equivalent to PS naturally found in the diet or derived from bovine cortex or soy.

4. 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).

5. ECA proposes to use a standardized PS derived from fish lecithin (DHAPS™) 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 GRNs 223 and 545. Thus, the exposure to PS from DHAPS™ 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 DHAPS™ will replace currently marketed PS derived from various sources. Thus, no increase in exposures is expected.

6. 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).

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

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

Based on the above-described data and information, we conclude that DHAPS™, 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, ECA 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 in full compliance with the Food Allergen Labeling and Consumer Protection Act of 2004 (Title I1 of Public Law 108-282). Appendix C shows that ECS's DHAPS$^{TM}$ 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**

Claro FT, Silva RH, Frussa-Filho R. Bovine brain phosphatidylserine attenuates scopolamine-induced amnesia. Physiol Behav. 1999;67:551-54.

FDA. 2003. Qualified Health Claim: Final Decision Letter - Phosphatidylserine and Cognitive Dysfunction and Dementia. http://www.fda.gov/Food/IngredientsPackagingLabeling/LabelingNutrition/ucm072999.htm.

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

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

FDA. 2007. GRN223: Phosphatidylserine. Enzymotec Ltd., Israel. Date of closure - December 20, 2007. http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=223.

FDA. 2009. 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.

FDA. 2010. 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.

FDA. 2015. 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.

Hellhammer J, Vogt D, Franz N, Freitas U, Rutenberg D. A soy-based phosphatidylserine/ phosphatidic acid complex (PAS) normalizes the stress reactivity of hypothalamus-pituitary-adrenal-axis in chronically stressed male subjects: a randomized, placebo-controlled study. Lipids Health Dis. 2014;13:121. 11 pages.

Hellhammer J, Hero T, Franz N, Contreras C, Schubert M. Omega-3 fatty acids administered in phosphatidylserine improved certain aspects of high chronic stress in men. Nutr Res. 2012;32:241-50.

Hellhammer J, Fries E, Buss C, Engert V, Tuch A, Rutenberg D, Hellhammer D. Effects of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) on the endocrine and psychological responses to mental stress. Stress. 2004;7:119-26.

Jorissen BL, Brouns F, Van Boxtel MP, Riedel WJ. Safety of soy-derived phosphatidylserine in elderly people. Nutr Neurosci. 2002;5:337-43.

Jorissen BL, Brouns F, Van Boxtel MP, Ponds RW, Verhey FR, Jolles J, Riedel WJ. The influence of soy-derived phosphatidylserine on cognition in age-associated memory impairment. Nutr Neurosci. 2001;4:121-34.

Lifshitz Y, Levi L, Eyal I, Cohen T, Tessler S. Sub-chronic (13-week) oral toxicity study, preceded by an in utero exposure phase and genotoxicity studies with fish source phosphatidylserine in rats. Food Chem Toxicol. 2015;86:234-44.

Vakhapova V, Cohen T, Richter Y, Herzog Y, Kam Y, Korczyn AD. Phosphatidylserine containing omega-3 fatty acids may improve memory abilities in nondemented elderly individuals with memory complaints: results from an open-label extension study. Dement Geriatr Cogn Disord. 2014;38:39-45.

**Appendix A-1. Human Clinical Studies of PS from Fish 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.

**Appendix A-2. Summary of Human Clinical Studies of Soy PS**

| No. of subjects (PS-treated) | Daily dose, mg | 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] | 4 wk | 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 | No effects 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 parameter, blood pressure, or heart rate. | Jorissen et al., 2002 |
| 73 elderly with mild cognitive | 100 or 300 | 6 mo | DB-PC | No adverse event was observed. No clinically significant change in vital signs, hematological and | Kato-Kataoka et al., 2010 |

| | | | | | |
|---|---|---|---|---|---|
| Impairment, 50–69 y | | | | biological blood or urine parameters. Differences in blood glucose levels were considered clinically insignificant. | |
| 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 A-3. Human Clinical Studies of PS from Bovine Cortex**

| No. of subjects | Daily dose, mg | Duration | Design | Adverse effects | Reference |
|---|---|---|---|---|---|
| Studies referenced 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 and Di, 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 | | | | |
| 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.

References Cited in the Appendix Tables

Allegro L, Favaretto V, Ziliotto G. Oral phosphatidylserine in elderly patients with cognitive deterioration. An open study. Clin Trials J. 1987;24:104-08.

Amaducci L. Phosphatidylserine in the treatment of Alzheimer's disease: results of a multicenter study. Psychopharmacol Bull. 1988;24:130-34.

Baumeister J, Barthel T, Geiss KR, Weiss M Influence of phosphatidylserine on cognitive performance and cortical activity afterinduced stress. Nutr Neurosci.2008;11:103-10.

Benton D, Donohoe RT, Sillance B, Nabb S. The influence of phosphatidylserine supplementation on mood and heart rate when faced with an acute stressor. Nutr Neurosci. 2001;4:169-78.

Caffarra P, Santamaria V. The effects of phosphatidylserine in patients with mild cognitive decline. Clin Trials J. 1987;24:109-14.

Cenacchi T, Baggio C, Palin E. Human tolerability of oral V assessed through laboratory examinations. Clin Trials J. 1987;24:125-30.

Cenacchi T, Bertoldin T, Farina C, Fiori MG, Crepaldi G. Cognitive decline in the elderly: A double-blind, placebo-controlled multicenter study on the efficacy of phosphatidylserine administration. Aging Clin Exp Res. 1993;5:123-33.

Crook TH, Tinklenberg J, Yesavage J, Petrie W, Nunzi MG, Massari DC. Effects of phosphatidylserine in age-associated memory impairment. Neurology. 1991;41:644-49.

Crook T, Petrie W, Wells C, Massari DC. Effects of phosphatidylserine in Alzheimer's disease. Psychopharmacol Bull. 1992;28:61-6.

Delwaide PJ, Gyselynck-Mambourg AM, Hurlet A, Ylieff M. Double-blind randomized controlled study of phosphatidylserine in senile demented patients. Acta Neurol Scand. 1986;73:136-40.

Engel RR, Satzger W, Günther W, Kathmann N, Bove D, Gerke S, Münch U, Hippius H. Double-blind cross-over study of phosphatidylserine vs. placebo in patients with early dementia of the Alzheimer type. Eur Neuropsychopharmacol. 1992;2:149-55.

Funfgeld EW, Baggen M, Nedwidek P, Richstein B, Mistlberger G. Double-blind study with phosphatidylserine (PS) in Parkinsonian patients with senile dementia of Alzheimer's type (SDAT). Prog Clin Biol Res. 1989;317:1235-46.

Granata Q, Di Michele J. Phosphatidylserine in elderly patients. Clin Trials J. 1987 24:99-103.

Heiss WD, Kessler J, Slansky I, Mielke R, Szelies B, Herholz K. Activation PET as an instrument to determine therapeutic efficacy in Alzheimer's disease. Annals NY Acad Sci. 1993; 695:327-31.

Heiss WD, Kessler J, Mielke R, Szelies B, Herholz K. Long-term effects of phosphatidylserine, pyritinol, and cognitive training in Alzheimer's disease. A neuropsychological, EEG, and PET investigation. Dementia. 1994;5:88-98.

Hellhammer J, Vogt D, Franz N, Freitas U, Rutenberg D. A soy-based phosphatidylserine/ phosphatidic acid complex (PAS) normalizes the stress reactivity of hypothalamus-pituitary-adrenal-axis in chronically stressed male subjects: a randomized, placebo-controlled study. Lipids Health Dis. 2014;13:121. 11 pages.

Hellhammer J, Hero T, Franz N, Contreras C, Schubert M. Omega-3 fatty acids administered in phosphatidylserine improved certain aspects of high chronic stress in men. Nutr Res. 2012;32:241-50.

Hellhammer J, Fries E, Buss C, Engert V, Tuch A, Rutenberg D, Hellhammer D. Effects of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) on the endocrine and psychological responses to mental stress. Stress. 2004;7:119-26.

Heywood R, Cozens DD, Richold M. Toxicology of a phosphatidylserine preparation from bovine brain (BC-PS). Clin Trials J. 1987;24:25-32.

Hirayama S, Terasawa K, Rabeler R, Hirayama T, Inoue T, Tatsumi Y, Purpura M, Jäger R. The effect of phosphatidylserine administration on memory and symptoms of attention-deficit hyperactivity disorder: a randomised, double-blind, placebo-controlled clinical trial. J Hum Nutr Diet. 2014;27 Suppl 2:284-91.

Jager R, Purpura M, Geiss KR, Wei1 M, Baumeister J, Amatulli F, Schröder L, Herwegen H. The effect of phosphatidylserine on golf performance. J Int Soc Sports Nutr. 2007; 4:23. 4 pages.

Jorissen BL, Brouns F, Van Boxtel MP, Riedel WJ. Safety of soy-derived phosphatidylserine in elderly people. Nutr Neurosci. 2002;5:337-43.

Kato-Kataoka A, Sakai M, Ebina R, Nonaka C, Asano T, Miyamori T. Soybean derived phosphatidylserine improves memory function of the elderly Japanese subjects with memory complaints. J Clin Biochem Nutr. 2010;47:246-55.

Lifshitz Y, Levi L, Eyal I, Cohen T, Tessler S. Sub-chronic (13-week) oral toxicity study, preceded by an in utero exposure phase and genotoxicity studies with fish source phosphatidylserine in rats. Food Chem Toxicol. 2015;86:234-44.

Maggioni M, Picotti GB, Bondiolotti GP, Panerai A, Cenacchi T, Nobile P, Brambilla F. Effects of phosphatidylserine therapy in geriatric patients with depressive disorders. Acta Psychiatr Scand. 1990;81:265-70.

Manor I, Magen A, Keidar D, Rosen S, Tasker H, Cohen T, Richter Y, Zaaroor-Regev D, Manor Y, Weizman A. Safety of phosphatidylserine containing omega3 fatty acids in ADHD children: a double-blind placebo-controlled trial followed by an open label extension. Eur Psychiatr. 2013;28:386-91.

Monteleone P, Maj M, Beinat L, Natale M, Kemali D. Blunting by chronic phosphatidylserine administration of the stress-induced activation of the hypothalamo-pituitary-adrenal axis in healthy men. Eur J Clin Pharmacol. 1992;42:385-88.

Monteleone P, Beinat L, Tanzillo C, Maj M, Kemali D. Effects of phosphatidylserine on the neuroendocrine response to physical stress in humans. Neuroendocrinol. 1990;52:243-48.

Palatini P, Viola G, Bigon E, Menegus AM, Bruni A. Pharmacokinetic characterization of phosphatidylserine liposomes in the rat. Br J Pharmacol. 1991;102:345-50.

Palmieri G, Palmieri R, Inzoll MR, Agrati AM, Vargiu A. Doubleblind controlled trial of phosphatidylserine in patients with senile mental deterioration. Clin Trials J. 1987;24:73-83.

Parker AG, Gordon J, Thornton A, Byars A, Lubker J, Bartlett M, Byrd M, Oliver J, Simbo S, Rasmussen C, Greenwood M, Kreide RB. The effects of IQPLUS Focus on cognitive function, mood and endocrine response before and following acute exercise. J Intl Soc Sports Nutr. 2011;8:16. 6 pages.

Puca FM, Bavarese MA, Minervini MG. Exploratory trial of phosphatidylserine efficacy in mildly demented patients. Clin Trials J. 1987;24:94-8.

Rabboni M, Maggioni FS, Giannelli A, Beinat L. Neuroendocrine and behavioral effects of phosphatidylserine in elderly patients with abiotrophic or vascular dementia or mild depression. Clin Trials J. 1990;27:230-40.

Ransmayr G, Ploerer S, Gerstenbrand F, Bauer G. Double-blind placebo-controlled trial of phosphatidylserine in elderly patients with arteriosclerotic encephalopaty. Clin Trials J. 1987; 24:62-72.

Richter Y, Herzog Y, Eyal I, Cohen T. Cognitex supplementation in elderly adults with memory complaints: an uncontrolled open label trial. J Dietary Suppl. 2011;8:158-68.

Richter Y, Herzog Y, Cohen T, Steinhart Y. The effect of phosphatidylserine containing omega-3 fatty acids on memory abilities in subjects with subjective memory complaints: a pilot study. Clin Interv Aging. 2010;5:313-36.

Richter Y, Herzog Y, Lifshitz Y, Hayun R, Zchut S. The effect of soybean derived phosphatidylserine on cognitive performance in elderly with subjective memory complaints: a pilot study. Clin Interv Aging. 2013;8:557–63.

Schreiber S, Kampf-Sherf O, Gorfine M, Kelly D, Oppenheim Y, Lerer B. An open trial of plant-source derived phosphatydilserine for treatment of age-related cognitive decline. Isr J Psychiatry Relat Sci. 2000;37:302-07.

Sinforiani E, Agostinis C, Merlo P, Gualtieri S, Mauri M, Mancuso A. Cognitive decline in ageing brain. Therapeutic approach with phosphatidylserine. Clin Trials J. 1987 24:115-24.

Starks MA, Starks S, Kingsley M, Purpura M and Jager R. The effects of phosphatidylserine on endocrine response to moderate intensity exercise. J Intl Soc Sports Nutr. 2008;5:11.6 pages.

Villardita JC, Grioli S, Salmeri G, Nicoletti F, Pennisi G. Multicenter clinical trial of brain phosphatidylserine in elderly patients with intellectual deterioration. Clin Trials J. 1987 24:84-93.

Vaisman N, Kaysar N, Zaruk-Adasha Y, Pelled D, Brichon G, Zwingelstein G, Bodennec J. Correlation between changes in blood fatty acid composition and visual sustained attention

performance in children with inattention: effect of dietary n-3 fatty acids containing phospholipids. Am J Clin Nutr. 2008; 87:1170-80.

Vakhapova V, Richter Y, Cohen T, Herzog Y, Korczyn AD. Safety of phosphatidylserine containing omega-3 fatty acids in non-demented elderly: a doubleblind placebo-controlled trial followed by an open-label extension. BMC Neurol. 2011;11:79. 7 pages.

Vakhapova V, Cohen T, Richter Y, Herzog Y, Kam Y, Korczyn AD. Phosphatidylserine containing omega-3 fatty acids may improve memory abilities in nondemented elderly individuals with memory complaints: results from an open-label extension study. Dement Geriatr Cogn Disord. 2014;38:39-45.

## EXPERT PANEL STATEMENT

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

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

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

**Table of Contents**

**Tables**



**Figures**

# GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR THE ADDITION OF PHOSPHATIDYLSERINE (PS) DERIVED FROM FISH LECITHIN 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 fish lecithin (DHAPS™) 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 Healthcare 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 DHAPS™, (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 DHAPS™, 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 of 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 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 as an alternative to the currently marketed PS from soy, 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 DHAPS™ 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 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 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 a marine source (fish), 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 DHAPS™, manufactured by ECA Healthcare, Inc.

### III.C. Name and Address of Responsible Individual:

Jiang Su, Managing Director
ECA Healthcare, Inc.
1017 North Building 1839 Qixin Rd
Shanghai, China 201101
Tel: +86+139-1704-0601 or 909-859-4956 (Cell phone in California)
Fax: +86-21-3358-0611
E mail: jiang.su@ecahealthcare.com

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

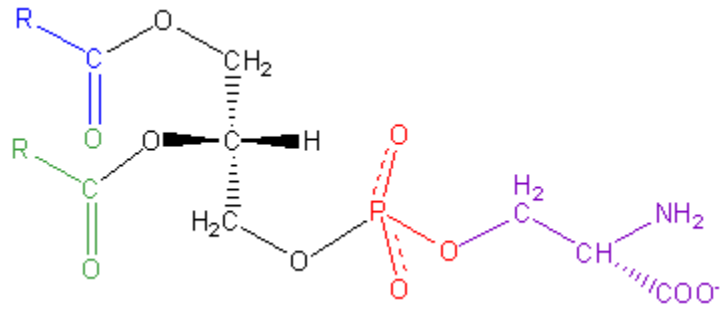
**III.D. Chemistry and Physicochemical Properties**

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 fish. 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 2 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 $Ca^{2+}$.



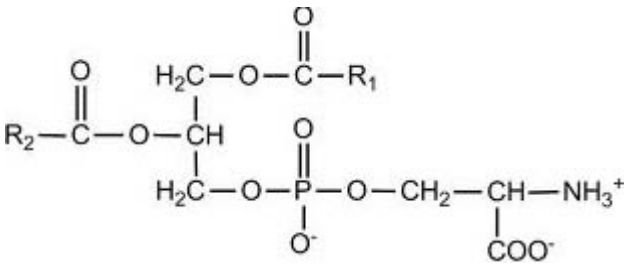


Figure 1. General structure of PS where R= alkyl group; the counter ion for the phosphate moiety is $Ca^{2+}$ in most abundant form.

Fatty acid profile:

The mean percentage of the fatty acids (FA) in PS from other sources is presented in Table 1. Table 2 presents the FA profile of DHAPS™.

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

| Fatty Acid | Typical fatty acid composition (as % of total fatty acids) | | | | |
|---|---|---|---|---|---|
| | 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 (DHA; 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 Claro et al. (1999) and GRN 545. DHA=Docosahexaenoic acid; EPA=Eicosapentaenoic acid.

Table 2. Typical FA composition of ECA's DHAPS™

| Fatty Acid | Percentage (as % of total fatty acids) |
|---|---|
| C8:0 | 1 |
| C14:0 | 2 |
| C16:0 (palmitic acid) | 23 |
| C16:1 | 2 |
| C18:0 | 2 |
| C18:1(oleic acid) | 13 |
| C18:2 | 2 |
| C18:3 | 1 |
| C20:1 | 2 |
| C20:4 | 1 |

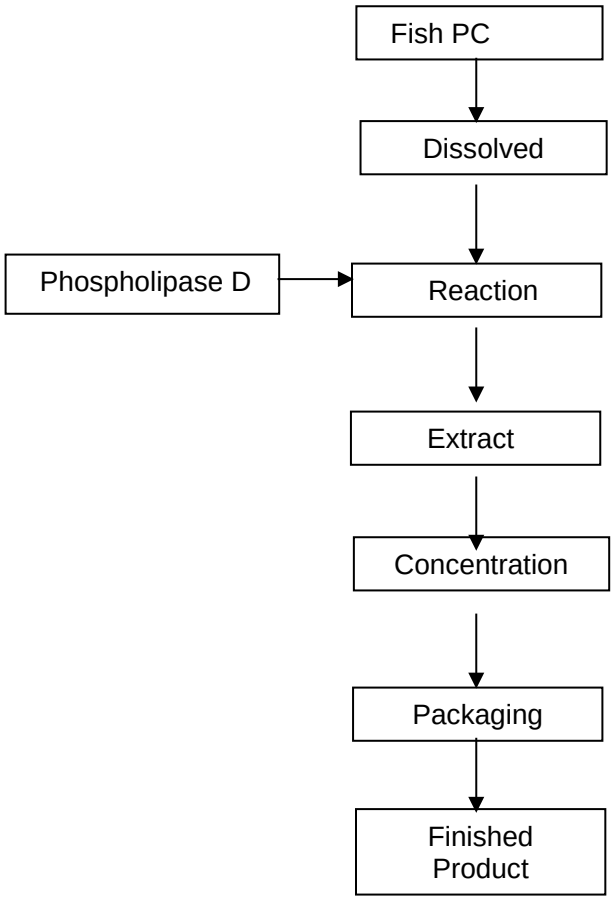
| | |
|---|---|
| C20:5(EPA) | 12 |
| C22:5 | 1 |
| C22:6 (DHA) | 33 |
| Others | 5 |
| Total | 100 |

FA = fatty acid; DHA=Docosahexaenoic acid; EPA=Eicosapentaenoic acid.

**III.E. Manufacturing Process**

DHAPS™ is manufactured from high phosphatidylcholine-enriched herring fish lecithin (DHA-lecithin is extracted with ethanol from herring roe). The phosphatidylcholine-enriched lecithin is enzymatically transphosphatidylated with L-serine using a phospholipase enzyme. The enzyme used for transphosphatidylation is derived from a microorganism that is nonpathogenic and nontoxicogenic. This enzymatic process catalyzes the substitution of the choline head-group with serine to form phosphatidylserine. 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. Processing aids used, such as ethyl alcohol, are food grade quality as specified in Food Chemical Codex (FCC). ECA's DHAPS™ is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes.

Figure 2. Flow diagram of DHAPS™ manufacturing process



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 Food Chemical Codex. The ECA manufacturing facility and process are certified with National Science Foundation International, based in Ann Arbor, Michigan, USA.

**III.F. Typical Composition and Specifications**

DHAPS™ is produced as a yellow to brown cream. The typical composition and specifications are shown in Tables 3 and 4, respectively. 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 pesticides and other contaminants are minimal in this product. Specifications are comparable to those established in the previous GRAS notices for impurities although the PS concentration is lower in DHAPS™ (PS content: GRN 279, 48%; GRN 311, >55%; ECA, >30%). Analytical data from multiple lots indicate that DHAPS™ complies reliably with the established food-grade product specifications and meet all applicable purity standards.

The product also contains other phospholipids naturally occurring in fish oil. These other phospholipids include lysoPS, phosphatidic acid, lyso phosphatidic acid, and associated phospholipids. FA profiles include docosahexaenoic acid (DHA; C22:6n-3; 20%), and eicosapentaenoic acid (EPA; C20:5n-3; 6%).

Table 3. Typical composition (%) of DHAPS™

<table>
<tr><td rowspan="2">Parameter</td><td colspan="3">Typical Composition (%)</td></tr>
<tr><td>ECA; PS from Herring</td><td>GRN 279 Krill PS</td><td>GRN 311 Herring PS</td></tr>
<tr><td>PS, %</td><td>30.62</td><td>48</td><td>55</td></tr>
<tr><td>Phosphatidyl acid, %</td><td>8.5</td><td rowspan="6">29</td><td rowspan="6">35</td></tr>
<tr><td>Phosphatidylcholine, %</td><td>1.5</td></tr>
<tr><td>Lyso PS, %</td><td>0.51</td></tr>
<tr><td>Lyso phosphatidyl acid, %</td><td>1.09</td></tr>
<tr><td>Phosphatidyl inositol, %</td><td>14.5</td></tr>
<tr><td>Other phospholipids, %</td><td>19.1</td></tr>
<tr><td>Glyceride (Tri-, di- and mono-), %</td><td>12.1</td><td></td><td></td></tr>
<tr><td>DHA, %</td><td>20.0</td><td rowspan="2">DHA=EPA=22%</td><td rowspan="2">DHA+EPA=23%</td></tr>
<tr><td>EPA, %</td><td>6</td></tr>
<tr><td>Calcium</td><td>2.9</td><td>NA</td><td>NA</td></tr>
<tr><td>Silicon dioxide, %</td><td>1.0-1.5</td><td></td><td></td></tr>
<tr><td>Free L-serine, %</td><td>0.4</td><td></td><td></td></tr>
<tr><td>Loss on drying, %</td><td>2.0</td><td><2.0 volatiles</td><td><2.0 volatiles</td></tr>
<tr><td>Ash, %</td><td>14.6</td><td>NA</td><td>NA</td></tr>
</table>

PS= Phosphatidylserine.

Table 4. Specifications of DHAPS™

| Parameter | Specifications, % | Assay method |
|---|---|---|
| Color | yellow to brown | Visual |
| PS | ≥30.0% | HPLC/$^{31}$P-NMR |
| Other Phospholipids | ≥44.0% | |
| Loss on drying | ≤2.0% | Karl Fisher |
| Peroxide value | ≤5 meq/Kg | Iodide titration |
| Microbiological assays | | |
| Total plate count | ≤1000 cfu/g | USP 61 |
| Yeasts and molds | ≤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 | ≤5,000 ppm | USP 467 |

PS= Phosphatidylserine.

## IV. INTENDED USES AND EXPOSURE ESTIMATES

### IV.A. Intended Technical Effects

DHAPS™ 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 in 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

As shown in Table 5, ECA proposes to use DHAPS™ as a nutrient [21 CFR §170.3(o)(20)], and as an alternative to soy or sunflower 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 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 |

Modified 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 to 184 mg/person/day (Bruni et al., 1989; FDA, 2006b; Hamm, 2004). Although some foods with standards of identity are included in the list of foods, at present the use of DHAPS™ is intended for foods without a standard of identity.

### IV.C.2. EDIs under the Intended Uses

Since DHAPS™ will be used in the same food categories and at same use levels as those described in GRN 223 and 545, the exposure calculations presented in those GRNs are valid for DHAPS™ 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). 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 ($90^{th}$ 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 intake 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 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 | $90^{th}$ percentile | Mean | $90^{th}$ 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 marine oil (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 into this independent GRAS determination.

The pertinent information is available as indicated 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, 2006a.
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, 2006b.
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 of 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: PS derived from fish lecithin; Intended use –same as GRNs 223 and 545. ECA-Healthcare, China.

**V.B. Review of Safety Data**

As noted above, the FDA has issued 'no question' letters on 6 GRAS notices of PS regardless of its origin. As the specifications in this GRAS determination are similar to those noted in the FDA GRAS notices (although the concentration of PS in DHAPS™ is lower than those of the previous notices) and fatty acid (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 PS product in this GRAS determination. Therefore, this notice incorporates by reference the safety and metabolism studies discussed in GRN 545 and the other 5 GRAS notices. The subject of the present GRAS assessment is PS from fish (a marine source) (powder form). 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**

Following dietary ingestion of PS, pancreatic digestive enzymes cleave specific FAs. The fatty acids on phospholipids (PLs) are absorbed and transported differently than FA on triglycerides (TGs). FAs esterified in the sn-2 position are hydrolyzed by pancreatic digestive enzymes, especially pancreatic phospholipase A2 (Arnesjo et al., 1969). The lysophosphatidylserine (lyso-PS) and fatty acids thus formed are absorbed by the mucosal cells of the intestine. The absorbed lysophospholipids could be reacylated into PS. The fatty acids 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 and Elwyn, 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.

Pharmacokinetic studies of PS in rats and mice show good bioavailability. After oral administration to rats, most of the isotope label recovered from blood samples remained identifiable as PS for up to 60 min after administration. After 24 h, metabolites were recovered from blood almost exclusively, mainly as lysophosphatidylethanolamine and lysophosphatidylcholine (Toffano et al., 1987). For all routes of administration, approximately 60% of the ingested PS is excreted in feces, while 10% is eliminated in urine. When radiolabeled PS is administered (orally or intraperitoneally) in animals, the radioactivity recovered in the urine was mainly metabolized (water soluble), whereas the 60-65% of fecal radioactivity was mainly associated with lipids. The major metabolite recovered in the feces was lysoPS (about 50%) after oral administration (Toffano et al., 1987).

Although FA composition between bovine cortex-, soy-, marine-, or sunflower-derived PS differs, these differences are unlikely to affect the safety profile. Most of toxicity/safety studies were done on fish PS, 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 and fish-derived PS mainly contains 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 from fish lecithin.

**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 derived from marine or bovine cortex (BCPS) was investigated in human lymphocytes, chromosomal damage assays, mouse-lymphoma cell mutation tests, cultured human epithelial cell DNA repair assays, and/or in an *in vivo* mouse micronucleus assay (Heywood et al., 1987). It is concluded that marine PS or BCPC is not genotoxic or clastogenic under the conditions described below. A recent study by Lifshitz et al. (2015) confirmed that PS from fish source was not mutagenic or genotoxic.

Bacterial reverse mutation assay of PS-DHA

A mutagenic potential of fish PS conjugated to DHA (PS-DHA) was tested (Lifshitz et al., 2015). PS-DHA contained 81% phospholipids of which PS comprised 49%. The FA profile reflects the fish lecithin source, comprising approximately15% DHA and 7.5% EPA. PS-DHA was shown not to be mutagenic in either the bacterial reverse mutation assay or the human lymphocyte micronucleus assay. 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**

Traditional toxicity studies were done on BCPS and marine PS containing DHA (Table 7).

**A Study Published Since the FDA's Last Review of 2014-2015**

Rat (fish PS)

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. Test ingredients used in this study comprised 81% phospholipids of which PS comprised 49%. The FA profile reflects the fish lecithin source, comprising approximately15% 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, 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 among 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 among the groups. Macroscopic examination of the F0 rats did not reveal any treatment-related abnormalities.

All pregnant females bore live pups, and the numbers of females with stillborn pups were comparable among 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 and hematology, or clinical chemistry. At the end of the treatment period, cholesterol, phospholipid, and triglyceride concentrations were decreased in males and females of the mid-dose, high-dose, and reference control group (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 PUFA's rather than adverse responses.

In this 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 the 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.

The NOAEL in this study was determined at 3% of 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 References in Previous GRNs**

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 in rats and rabbits (Heywood et al., 1987): results of teratogenicity studies at doses up to 200 mg/kg bw/day in rats and at doses up to 450 mg/kg/day in rabbits show that oral administration of PS did not affect embryonic and fetal development or reproductive performances.

Table 7. Summary of animal toxicity studies of PS

| Dose | Dose | Duration | Results | Reference |
|---|---|---|---|---|
| A recent Study Published since FDA's Last Review of 2014-2015 | | | | |
| Rat | 0, 1,050, 2,100, and 3,250 mg/kg bw PS-DHA from 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, SD | 5 g/kg bw | Single dose | $LD_{50}$ >5 g/kg bw | Heywood et al., 1987 |
| Rat, SD | 0, 10, 100, and | 26 wk | NOAEL=close | Heywood et al., |

| | | | | |
|---|---|---|---|---|
| | 1,000 mg/kg bw | | to 1,000 mg/kg bw | 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, SD | 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; SD = Sprague Dawley; 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.

**Recent Studies Published Since FDA's Last Review of 2014-2015**

Vakhapova et al. (2011) reported improvements in cognitive performance observed in nondemented elderly individuals with memory complaints after 15 weeks of daily supplementation with 300 mg of fish PS enriched with 76 mg DHA (PS-DHA). A follow-up study by Vakhapova et al. (2014) reported that continued dietary supplementation with 100 mg/day of fish PS enriched with 26 mg DHA/day for another 15 weeks sustained improved cognitive performance in nondemented elderly individuals with memory complaints.

In a study by Hellhammer et al. (2014), in combination with phosphatidic acid (PA, 200 mg), soy PS (200 mg/day) has been shown to reduce cortisol levels and enhance well-being under acute social stress in young healthy males who consumed PAS (200 mg PS+200 mg PA) for 42 days. No significant adverse effects of soy PS were reported.

**Studies Referenced in Previous GRNs**

In addition to the absence of reports in the published scientific literature of adverse reactions concerning oral supplementation with PS, the safety of dietary supplementation with PS has been demonstrated in many human clinical trials. The objective of the majority of these studies was to examine the effect of PS in reducing the symptoms of dementia and cognitive dysfunction in geriatric individuals, as well as improvement of cognitive functions in elderly and attention deficit hyperactivity disorder (ADHD) symptoms in children.

<u>PS derived from fish lecithin (fish PS or PS-DHA)</u>

Due to safety concerns of the risk for prion contamination in BCPS, fish-derived PS has been established as a safe alternative in the past two decades. Marine PS is known to improve cognitive functions in the elderly with various age-related cognitive problems (Table 8-1; Manor et al., 2013; Richter et al., 2011; Vaisman et al., 2008; Vakhapova et al., 2011, 2014). Children with ADHD also benefited from marine PS supplementation (Manor et al., 2013; Vaisman et al., 2008). No adverse effects of PS derived from fish were reported on measured outcomes. Daily doses of 300 mg PS enriched with DHA were well tolerated when tested for up to 15 weeks in both children with ADHD and elderly with memory complaints (Vakhapova et al., 2011; Vaisman et al., 2008). None of the studies listed above reported adverse effects of PS-DHA on measured outcomes.

PS derived from soy lecithin (soy PS)

Due to safety concerns of the risk for prion contamination in BCPS, soybean-derived PS (soy PS) was established as a safe alternative 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). All of the studies described above reported no adverse effects of PS in healthy subjects and patients with cognitive decline, or memory impairment. None of the studies 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.

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 (Allegro et al., 1987; Amaducci et al., 1988; Caffarra et al., 1987; Cenacchi et al., 1987, 1993; Crook et al., 1991, 1992, 1996; Delwaide et al., 1986; Engel et al., 1992; Funfgeld et al., 1989; Granata and Di, 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). Most studies of BCPS were conducted in 1980s and 1990s. None of the studies listed above reported adverse effects of PS on measured outcomes.

Other Safety Concern

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 in full compliance with the Food Allergen Labeling and Consumer Protection Act of 2004 (Title I1 of Public Law 108-282). Appendix C shows that ECS's DHAPS™ is free of allergens.

Table 8-1. Human clinical studies of fish PS

| No. of subjects (PS-treated) | Daily dose, mg | Duration | Design | Measured parameters | Adverse effects reported | Reference |
|---|---|---|---|---|---|---|
| A recent study published since the last FDA review in 2014-2015 | | | | | | |
| 122 non-demented participants with memory complaints, 50-90 y | 100 mg PS+ 26 mg DHA+EPA (DHA/ EPA ratio of 3: 1). | 30 wk (15 wk open-label extension + 15 wk 300 mg PS+79 mg DHA) | OL | Cognition (Rey Auditory Verbal Learning Test, Rey Complex Figure Test, etc.), safety evaluation (physical exam, blood pressure, heart rate, and weight) and adverse events | PS-DHA was found to be safe and well tolerated, with no significant side effects | Vakhapova et al., 2014 |
| Studies referenced in previous GRNs | | | | | | |
| 157 non-demented participants with memory complaints, 50-90 y | 300 mg PS+ 79 mg DHA+EPA (DHA/ EPA ratio of 3: 1). | 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 |
| 200 (137) ADHD children, 6-13 y | 150 mg PS (in 300 mg PS-Omega 3 complex) | 30 wk (15 wk blinded + 15 wk OL extension) | DB-PC+OL | Adverse events and the Side Effect Rating Scale, body weight, and growth | Well tolerated; no major adverse events; adverse events reported included – gastrointestinal discomfort, atopic dermatitis, hyperactivity, tics, nausea, elevated SGOT, tantrum episodes, insomnia, high TG level and soft stool; no significant differences between the two groups | Manor et al., 2013 |
| 26 (26) | 100 mg PS- | 12 wk | OL | Safety measures and | No significant changes were | Richter et |

| | | | | | | |
|---|---|---|---|---|---|---|
| patients with subjective memory complaints, 60-90 y | Omega in 600 mg complex | | | cognitive function | found in resting BP, pulse or weight during study period; no major adverse events reported | al., 2011 |
| 60 (18) children with putative ADHD, 8-13 y | 300 mg PS (+250 mg EPA+DHA esterified to PS; +168 mg other PL) | 90 d | DB-PC | Plasma and erythrocyte FA and lipid profiles and continuous performance test results (Test of Variables of Attention; TOVA) | No side effects; well tolerated | Vaisman et al., 2008 |

Expanded from GRN 545. Richer et al. (2011) study used 100 mg PS-omega 3, 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 mg hops, ginger and rosemary); Manor et al. (2013) used PS-Omega3 (Vayarin) that provided a daily dose of 300 mg of PS and 120 mg of EPA + docosahexaenoic acid (DHA) at a ratio of 2:1 EPA/DHA; ADHD = attention deficit hyperactivity disorder; BP = blood pressure; DB=double blind; OL=open label; PC=placebo controlled; PS= Phosphatidylserine; SGOT= serum glutamic oxaloacetic transaminase; TG = triglyceride; d=day; mo=months; wk=weeks; y=years.

Table 8-2. A recent human clinical study of soy PS

| No. of subjects (PS-treated) | Daily dose | Duration | Design | Measured parameters | Adverse effects reported | Reference |
|---|---|---|---|---|---|---|
| 75 healthy male volunteers, mean 26 y | 400 mg PAS (200 mg PS + 200 mg PA) | 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 |

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); DB=double blind; PC=placebo controlled; PS= Phosphatidylserine; BP = blood pressure; ADHD = attention deficit hyperactivity disorder; d=day; mo=months; wk=weeks; y=years.

## VI. SUMMARY

PS, a structural component of cells, is found in all biological membranes of plants, animals, and other life forms. 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.

ECA proposes to use a standardized marine derived PS (DHAPS™) 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-PS which were the subject of GRAS Notice Numbers 223 and 545. Thus, the exposure to PS from DHAPS™ 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 DHAPS™ will replace currently marketed PS derived from various sources. Thus, cumulative exposures are not expected to change.

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 effects regardless of its origin. These studies employed PS derived from fish, bovine cortex, or soy sources. The available scientific evidence indicates that PS derived from fish lecithin is toxicologically equivalent to PS naturally found in the diet or derived from bovine cortex or soy. Once inside the body, orally ingested PS is hydrolyzed in the intestine prior to its absorption. The absorbed PS is transported and rapidly converted into other endogenous constituents.

The acute oral $LD_{50}$ of PS in rats was reported as greater than 5 g/kg bw. In subchronic toxicity studies in rats and dogs, NOAEL was determined to be approximately 1,000 mg/kg bw/day when PS was administered for 6 months. In teratogenicity studies in rats and rabbits, PS did not affect embryonic and fetal development. Multiple genotoxicity studies showed that PS did not reveal any genotoxic or clastogenic activity.

Based on the above-described data and information, DHAPS™, when used as a nutrient, is expected to be safe.

## VII. SAFETY ASSESSMENT

Numerous human and animal studies have reported benefits of DHAPS™ 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 marine source. The literature indicates that DHAPS™ offers consumers benefits without adverse effects.

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

1. ECA's DHAPS™ is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes. Analytical data from multiple lots indicate that DHAPS™ complies reliably with the established food-grade product specifications and meets 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 the absorbed PS is transported and rapidly converted into other endogenous constituents.

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 90$^{th}$ 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 safety studies. The EDI estimates are based on the assumption that DHAPS™ 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. In subchronic toxicity studies in rats and dogs, NOAEL was determined to be close to 1,000 mg/kg bw/day when PS was administered for 6 months.

6. In 40 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 derived from various sources has

been proven in human clinical studies including susceptible groups (elderly and children) and healthy individuals.

7. In the previous six GRAS notices (GRN 186, 197, 223, 279, 311, and 545) to the FDA, the safety of PS derived from fish, 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 fish lecithin (GRN 279 and 311).

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

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

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

11. 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 DHAPS$^{TM}$, when used as a nutrient, is reasonably expected to be safe.

## VIII. CONCLUSION OF THE EXPERT PANEL: GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR THE ADDITION OF PHOSPHATIDYLSERINE FROM MARINE SOURCE (DHAPS™) 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 fish 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 fish, soy, and sunflower 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 including PS derived from fish. 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 DHAPS™. The exact chemical structures and compositions of PS have been established and fall into the non-toxic classification. Considering that PS derived from fish is of biological origin, that it exists naturally in many foods, and that it does not represent a known health hazard, DHAPS™ 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 DHAPS™ , 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.

Signature: _____ Date: _____

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

Signature: _____ Date: _____

## VIII. CONCLUSION OF THE EXPERT PANEL: GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR THE ADDITION OF PHOSPHATIDYLSERINE FROM MARINE SOURCE (DHAPS™) 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 fish 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 fish, soy, and sunflower 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 including PS derived from fish. 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 DHAPS™. The exact chemical structures and compositions of PS have been established and fall into the non-toxic classification. Considering that PS derived from fish is of biological origin, that it exists naturally in many foods, and that it does not represent a known health hazard, DHAPS™ 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 DHAPS™ , 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 12, 2016

Robert L. Martin, Ph.D.

Signature (b) (6) Date: March 10, 2016

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

Signature: (b) (6) Date: March 9 2016

## IX. REFERENCES

Allegro L, Favaretto V, Ziliotto G. Oral phosphatidylserine in elderly patients with cognitive deterioration. An open study. Clin Trials J. 1987;24:104-08.

Amaducci L. Phosphatidylserine in the treatment of Alzheimer's disease: results of a multicenter study. Psychopharmacol Bull. 1988;24:130-34.

Arnesjö B, Nilsson A, Barrowman J, Borgström B. Intestinal digestion and absorption of cholesterol and lecithin in the human. Intubation studies with a fat-soluble reference substance. Scand J Gastroenterol. 1969;4:653-65.

Baumeister J, Barthel T, Geiss KR, Weiss M Influence of phosphatidylserine on cognitive performance and cortical activity afterinduced stress. Nutritional Neurosci. 2008;11:103-10.

Benton D, Donohoe RT, Sillance B, Nabb S. The influence of phosphatidylserine supplementation on mood and heart rate when faced with an acute stressor. Nutr Neurosci. 2001;4:169-78.

Bruni A, Mietto L, Bellini F, Boarato E, Toffano G. 1989. Pharmacological and autopharmacological action of phosphatidylserine. In Phospholipids in the Nervous System: Biochemical and Molecular Biology. Eds: Bazan, N.G., Horrocks, L.A. and Toffano, G. Fidia Research Series, Vol. 17 Livana Press, Padova: pp. 217-24.

Caffarra P, Santamaria V. The effects of phosphatidylserine in patients with mild cognitive decline. Clin Trials J. 1987;24:109-14.

Cenacchi T, Baggio C, Palin E. Human tolerability of oral V assessed through laboratory examinations. Clin Trials J. 1987;24:125-30.

Cenacchi T, Bertoldin T, Farina C, Fiori MG, Crepaldi G. Cognitive decline in the elderly: A double-blind, placebo-controlled multicenter study on the efficacy of phosphatidylserine administration. Aging Clin Expl Res. 1993;5:123-33.

Claro FT, Silva RH, Frussa-Filho R. Bovine brain phosphatidylserine attenuates scopolamine-induced amnesia. Physiol Behav. 1999;67:551-54.

Crook TH, Tinklenberg J, Yesavage J, Petrie W, Nunzi MG, Massari DC. Effects of phosphatidylserine in age-associated memory impairment. Neurology. 1991;41:644-49.

Crook T, Petrie W, Wells C, Massari DC. Effects of phosphatidylserine in Alzheimer's disease. Psychopharmacol Bull. 1992;28:61-6.

Delwaide PJ, Gyselynck-Mambourg AM, Hurlet A, Ylieff M. Double-blind randomized controlled study of phosphatidylserine in senile demented patients. Acta Neurol Scand. 1986;73:136-40.

Engel RR, Satzger W, Günther W, Kathmann N, Bove D, Gerke S, Münch U, Hippius H. Double-blind cross-over study of phosphatidylserine vs. placebo in patients with early dementia of the Alzheimer type. Eur Neuropsychopharmacol. 1992;2:149-55.

FDA. 2003. Qualified Health Claim: Final Decision Letter - Phosphatidylserine and Cognitive Dysfunction and Dementia. http://www.fda.gov/Food/IngredientsPackagingLabeling/LabelingNutrition/ucm072999.htm.

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

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

FDA. 2007. GRN223: Phosphatidylserine. Enzymotec Ltd., Israel. Date of closure - December 20, 2007. http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=223.

FDA. 2009. 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.

FDA. 2010. 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.

FDA. 2015. 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.

Funfgeld EW, Baggen M, Nedwidek P, Richstein B, Mistlberger G. Double-blind study with phosphatidylserine (PS) in Parkinsonian patients with senile dementia of Alzheimer's type (SDAT). Prog Clin Biol Res. 1989;317:1235-46.

Glade MJ, Smith K. Phosphatidylserine and the human brain. Nutrition. 2015;31:781-86.

Granata Q, Di MJ. Phosphatidylserine in elderly patients. Clin Trials J. 1987 24:99-103.

Hamm M. 2004. Nutritional- scientific statement on the change of nutritive provision with phosphatidylserine (PS). Article provided by Dr. Hamm. Cited in GRN 545.

Heiss WD, Kessler J, Slansky I, Mielke R, Szelies B, Herholz K. Activation PET as an instrument to determine therapeutic efficacy in Alzheimer's disease. Annals NY Acad Sci. 1993; 695:327-31.

Heiss WD, Kessler J, Mielke R, Szelies B, Herholz K. Long-term effects of phosphatidylserine, pyritinol, and cognitive training in Alzheimer's disease. A neuropsychological, EEG, and PET investigation. Dementia. 1994;5:88-98.

Hellhammer J, Vogt D, Franz N, Freitas U, Rutenberg D. A soy-based phosphatidylserine/ phosphatidic acid complex (PAS) normalizes the stress reactivity of hypothalamus-pituitary-adrenal-axis in chronically stressed male subjects: a randomized, placebo-controlled study. Lipids Health Dis. 2014;13:121. 11 pages.

Hellhammer J, Hero T, Franz N, Contreras C, Schubert M. Omega-3 fatty acids administered in phosphatidylserine improved certain aspects of high chronic stress in men. Nutr Res. 2012;32:241-50.

Hellhammer J, Fries E, Buss C, Engert V, Tuch A, Rutenberg D, Hellhammer D. Effects of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) on the endocrine and psychological responses to mental stress. Stress. 2004;7:119-26.

Heywood R, Cozens DD, Richold M. Toxicology of a phosphatidylserine preparation from bovine brain (BC-PS). Clin Trials J. 1987;24:25-32.

Hirayama S, Terasawa K, Rabeler R, Hirayama T, Inoue T, Tatsumi Y, Purpura M, Jäger R. The effect of phosphatidylserine administration on memory and symptoms of attention-deficit hyperactivity disorder: a randomised, double-blind, placebo-controlled clinical trial. J Hum Nutr Diet. 2014;27 Suppl 2:284-91.

Jager R, Purpura M, Geiss KR, Wei1 M, Baumeister J, Amatulli F, Schröder L, Herwegen H. The effect of phosphatidylserine on golf performance. J Int Soc Sports Nutr. 2007; 4:23. 4 pages.

Jorissen BL, Brouns F, Van Boxtel MP, Riedel WJ. Safety of soy-derived phosphatidylserine in elderly people. Nutr Neurosci. 2002;5:337-43.

Jorissen BL, Brouns F, Van Boxtel MP, Ponds RW, Verhey FR, Jolles J, Riedel WJ. The influence of soy-derived phosphatidylserine on cognition in age-associated memory impairment. Nutr Neurosci. 2001;4:121-34.

Kato-Kataoka A, Sakai M, Ebina R, Nonaka C, Asano T, Miyamori T. Soybean derived phosphatidylserine improves memory function of the elderly Japanese subjects with memory complaints. J Clin Biochem Nutr. 2010;47:246-55.

Kim HY, Huang BX, Spector AA. Phosphatidylserine in the brain: metabolism and function. Prog Lipid Res. 2014;56:1-18.

Lifshitz Y, Levi L, Eyal I, Cohen T, Tessler S. Sub-chronic (13-week) oral toxicity study, preceded by an in utero exposure phase and genotoxicity studies with fish source phosphatidylserine in rats. Food Chem Toxicol. 2015;86:234-44.

Maggioni M, Picotti GB, Bondiolotti GP, Panerai A, Cenacchi T, Nobile P, Brambilla F. Effects of phosphatidylserine therapy in geriatric patients with depressive disorders. Acta Psychiatr Scand. 1990;81:265-70.

Manor I, Magen A, Keidar D, Rosen S, Tasker H, Cohen T, Richter Y, Zaaroor-Regev D, Manor Y, Weizman A. Safety of phosphatidylserine containing omega3 fatty acids in ADHD children: a double-blind placebo-controlled trial followed by an open label extension. Eur Psychiatr. 2013;28:386-91.

Monteleone P, Maj M, Beinat L, Natale M, Kemali D. Blunting by chronic phosphatidylserine administration of the stress-induced activation of the hypothalamo-pituitary-adrenal axis in healthy men. Eur J Clin Pharmacol. 1992;42:385-8.

Palmieri G, Palmieri R, Inzoll MR, Agrati AM, Vargiu A. Doubleblind controlled trial of phosphatidylserine in patients with senile mental deterioration. Clin Trials J. 1987;24:73-83.

Parker AG, Gordon J, Thornton A, Byars A, Lubker J, Bartlett M, Byrd M, Oliver J, Simbo S, Rasmussen C, Greenwood M, Kreide RB. The effects of IQPLUS Focus on cognitive function, mood and endocrine response before and following acute exercise. J Intl Soc Sports Nutr. 2011;8:16. 6 pages.

Puca FM, Bavarese MA, Minervini MG. Exploratory trial of phosphatidylserine efficacy in mildly demented patients. Clin Trials J. 1987;24:94-98.

Rabboni M, Maggioni FS, Giannelli A, Beinat L. Neuroendocrine and behavioral effects of phosphatidylserine in elderly patients with abiotrophic or vascular dementia or mild depression. Clin Trials J. 1990;27:230-40.

Ransmayr G, Ploerer S, Gerstenbrand F, Bauer G. Double-blind placebo-controlled trial of phosphatidylserine in elderly patients with arteriosclerotic encephalopaty. Clin Trials J. 1987; 24:62-72.

Rao GN. Diet and kidney diseases in rats. Toxicol Pathol. 2002;30:651-56.

Richter Y, Herzog Y, Eyal I, Cohen T. Cognitex supplementation in elderly adults with memory complaints: an uncontrolled open label trial. J Dietary Suppl. 2011;8:158-68.

Richter Y, Herzog Y, Cohen T, Steinhart Y. The effect of phosphatidylserine containing omega-3 fatty acids on memory abilities in subjects with subjective memory complaints: a pilot study. Clin Interv Aging. 2010;5:313-16.

Richter Y, Herzog Y, Lifshitz Y, Hayun R, Zchut S. The effect of soybean derived

phosphatidylserine on cognitive performance in elderly with subjective memory complaints: a pilot study. Clin Interv Aging. 2013;8:557-63.

Schreiber S, Kampf-Sherf O, Gorfine M, Kelly D, Oppenheim Y, Lerer B. An open trial of plant-source derived phosphatidylserine for treatment of age-related cognitive decline. Isr J Psychiatry Relat Sci. 2000;37:302-07.

Sinforiani E, Agostinis C, Merlo P, Gualtieri S, Mauri M, Mancuso A. Cognitive decline in ageing brain. Therapeutic approach with phosphatidylserine. Clin Trials J. 1987;24:115-24.

Starks MA, Starks S, Kingsley M, Purpura M, Jager R. The effects of phosphatidylserine on endocrine response to moderate intensity exercise. J Intl Soc Sports Nutr. 2008;5:11.6 pages.

Toffano G, Battistella A, Orlando P. Pharmacokinetics of radiolabeled brain phosphatidylserine. Clin Trials J. 1987;24:18-24.

Tso P. 1994. Intestinal lipid absorption. In L. R. Johnson (ed), Physiology of the Gastrointestinal Tract. Vol. 56 (L. R. Johnson, ed), Raven Press, New York, pp. 1867-907.

USDA. 1998.CSFII 1994-1996.
http://www.ars.usda.gov/Services/docs.htm?docid=14531.

Villardita JC, Grioli S, Salmeri G, Nicoletti F, Pennisi G. Multicenter clinical trial of brain phosphatidylserine in elderly patients with intellectual deterioration. Clin Trials J. 1987;24:84-93.

Vaisman N, Kaysar N, Zaruk-Adasha Y, Pelled D, Brichon G, Zwingelstein G, Bodennec J. Correlation between changes in blood fatty acid composition and visual sustained attention performance in children with inattention: effect of dietary n-3 fatty acids containing phospholipids. Am J Clin Nutr. 2008; 87:1170-80.

Vakhapova V, Richter Y, Cohen T, Herzog Y, Korczyn AD. Safety of phosphatidylserine containing omega-3 fatty acids in non-demented elderly: a doubleblind placebo-controlled trial followed by an open-label extension. BMC Neurol. 2011;11:79. 7 pages.

Vakhapova V, Cohen T, Richter Y, Herzog Y, Kam Y, Korczyn AD. Phosphatidylserine containing omega-3 fatty acids may improve memory abilities in nondemented elderly individuals with memory complaints: results from an open-label extension study. Dement Geriatr Cogn Disord. 2014;38:39-45.

Wise EM, Jr. Elwyn D. Rates of reactions involved in phosphatide synthesis in liver and small intestine of intact rats. J Biol Chem. 1965;240:1537-48.

## Appendix A. CERTIFICATE OF ANALYSIS - DHAPS™

| Parameter | Typical level/ Speci-fications | Lot 2015 0301 | Lot 2015 0304 | Lot 2015 0401 | Lot 2015 0403 | Lot 2015 0406 |
|---|---|---|---|---|---|---|
| PS, % | 30.62 | 31.21 | 30.56 | 30.61 | 31.11 | 30.80 |
| Phosphatidyl acid, % | 8.5 | 8.2 | 8.7 | 8.2 | 8.3 | 8.4 |
| Phosphatidylcholine | 1.5 | 1.5 | 1.3 | 1.3 | 1.2 | 1.1 |
| Lyso PS, % | 0.51 | 0.41 | 0.52 | 0.53 | 0.5 | 0.48 |
| Lyso phosphatidyl acid, % | 1.09 | 1.11 | 1.06 | 1.10 | 1.01 | 1.05 |
| Phosphatidyl inositol, % | 14.5 | 14.8 | 14.5 | 14.2 | 14.1 | 14.3 |
| Other phospholipids, % | 19.1 | 19.4 | 19.5 | 18.9 | 18.7 | 19.1 |
| Glyceride (Tri-, di- and mono-), % | 12.1 | 12.1 | 12.4 | 12.0 | 12.0 | 12.2 |
| Calcium, % | 2.9 | 2.8 | 2.7 | 2.9 | 2.8 | 2.7 |
| Free L-serine, % | 0.4 | 0.3 | 0.4 | 0.4 | 0.3 | 0.3 |
| Loss on drying, % | 2.0 | 1.5 | 1.4 | 1.4 | 1.2 | 1.2 |
| Ash, % | 14.6 | 14.3 | 14.5 | 14.8 | 14.6 | 14.5 |
| Peroxide value, meq/Kg | < 5 | < 5 | < 5 | < 5 | < 5 | < 5 |
| Lead, ppm | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 |
| Arsenic, ppm | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 |
| Cadmium, ppm | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 | ≤1 |
| Mercury, ppm | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| Aflatoxins (B1, B2, G1, G2), ppb | ≤0.2 | ≤0.2 | ≤0.2 | ≤0.2 | ≤0.2 | ≤0.2 |
| Ethanol, ppm | ≤1,000 | | | | | |
| Organochlor Pesticides, ppm | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| Organophosphor Pesticides, ppm | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| Dioxins and Furans, ppm | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 |
| Total plate count, cfu/g | <1,000 | <1,000 | <1,000 | <1,000 | <1,000 | <1,000 |
| Yeasts and molds, cfu/g | <100 | <100 | <100 | <100 | <100 | <100 |
| *E. coli*, cfu/g | Negative | Negative | Negative | Negative (cfu/g) | Negative (cfu/g) | Negative (cfu/g) |
| *Salmonella*, cfu/20g | Negative | Negative | Negative | Negative | Negative | Negative |

Pages 000060-000064 of Curriculum Vitae removed in accordance with the Privacy Act of 1974.

# Analytical Report

| | | | |
|---|---|---|---|
| Sample Code | 502-2016-00009382 | Report date | 03-Mar-2016 |
| Certificate No. | AR-16-SU-008986-03 | | |

*This report invalidates all previous versions.

05507646000002691

ECA HealthCare Inc.
Zhang Fan
1017 North Bldg.1839 Qixin Rd., Shanghai
201101 Shanghai
Fax +86-21-3358 0611

| | |
|---|---|
| Our reference: | 502-2016-00009382/ AR-16-SU-008986-03 |
| Client Sample Code: | 20141201 |
| Sample described as: | DHAPS™ (Marine Phosphatidylserine) |
| Sample Packaging: | Sealed plastic bag |
| Sample reception date: | 23-Feb-2016 |
| Analysis starting date: | 23-Feb-2016 |
| Analysis ending date: | 25-Feb-2016 |

| | | | |
|---|---|---|---|
| Arrival Temperature (°C) | 17.2 | Sample Weight | 70g |
| Sample Type | Solid | | |

| Dairy | Results | Unit | LOQ | LOD |
|---|---|---|---|---|
| # SU585 Allergen – Histamine | | | | |
| Histamine | <2.5 | mg/kg | | 2.5 |

SIGNATURE

Pathik Vyas
Technical Director

**EXPLANATORY NOTE**

≥ Greater than or equal to
< Less than
≤ Less than or equal to
N/A means Not applicable
▵ CNAS # DAKKS □CMA
☆ means the test is subcontracted within Eurofins group
◎ means the test is subcontracted outside Eurofins group
Not Detected means not detected at or above the Limit of Quantification (LOQ)
The result(s) relate(s) only to the item (s) tested.
This analytical report shall not be reproduced except in full, without written approval of the laboratory.
Eurofins General Terms and Conditions apply.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

eurofins

## Analytical Report Appendix C. Allergen Test for DHAPS™

| | | | |
|---|---|---|---|
| Sample Code | 502-2016-00009382 | Report date | 03-Mar-2016 |
| Certificate No. | AR-16-SU-008986-03 | | |

*This report invalidates all previous versions.

ECA HealthCare Inc.
Zhang Fan
1017 North Bldg.1839 Qixin Rd., Shanghai
201101 Shanghai
Fax +86-21-3358 0611

| | |
|---|---|
| Our reference: | 502-2016-00009382/ AR-16-SU-008986-03 |
| Client Sample Code: | 20141201 |
| Sample described as: | DHAPS™ (Marine Phosphatidylserine) |
| Sample Packaging: | Sealed plastic bag |
| Sample reception date: | 23-Feb-2016 |
| Analysis starting date: | 23-Feb-2016 |
| Analysis ending date: | 25-Feb-2016 |

| | | | |
|---|---|---|---|
| Arrival Temperature (°C) | 17.2 | Sample Weight | 70g |
| Sample Type | Solid | | |

| Dairy | Results | Unit | LOQ | LOD |
|---|---|---|---|---|
| # SU585 Allergen – Histamine | | | | |
| Histamine | <2.5 | mg/kg | | 2.5 |

SIGNATURE

Pathik Vyas
Technical Director

EXPLANATORY NOTE
≥ Greater than or equal to
< Less than
≤ Less than or equal to
N/A means Not applicable
▵ CNAS # DAKKS □CMA
☆ means the test is subcontracted within Eurofins group
● means the test is subcontracted outside Eurofins group
Not Detected means not detected at or above the Limit of Quantification (LOQ)
The result(s) relate(s) only to the item (s) tested.
This analytical report shall not be reproduced except in full, without written approval of the laboratory.
Eurofins General Terms and Conditions apply.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 14, LongShan Road, SND
Suzhou 215163
Jiangsu Province, P.R.China
Phone +86 512 6900 6566
Fax +86 512 6878 5966
www.eurofins.cn

# Certificate of Analysis

**Product Name:** DHAPS™ (Marine Phosphatidylserine)

Product Code: 1811701 Batch No.: 20150301 P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

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

**Product Characteristics:**

| | |
|---|---|
| Shelf Life | 24 months |
| Description | Yellow to brown cream form |
| Source | Marine Fish |
| Solvents Used | Ethanol & water |
| Country of Origin | China |

| Test Items | Specification | Test | Method |
|---|---|---|---|
| Identification | Positive | Conform | NMR |
| Phosphatidylserine | ≥30.0% | 31.21% | NMR/HPLC |
| Phosphatidyl acid | | 8.20% | 31P-NMR |
| Phosphatidyl Choline | | 1.50% | 31P-NMR |
| Lyso Phosphatidylserine | | 0.41% | 31P-NMR |
| Lyso phosphatidyl acid | | 1.11% | 31P-NMR |
| Phosphatidyl inositol | | 14.80% | 31P-NMR |
| Other phospholipids | | 19.40% | 31P-NMR |
| Glyceride (Tri-, di- and mono-) | | 12.10% | GC-FID |
| Calcium | | 2.80% | ICP-OES |
| Free L-serine | ≤1.0% | 0.30% | Ninhydrin |
| Loss on drying | ≤2.0% | 1.50% | Karl Fischer |
| Ash | ≤15% | 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** | | | |
| 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

# Certificate of Analysis

**Product Name:** DHAPS™ (Marine Phosphatidylserine)

Product Code: 1811701 Batch No.: 20150304 P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

MFG.DATE MAR. 12, 2015 EXP. DATE: MAR. 11, 2017

**Product Characteristics:**

| | |
|---|---|
| Shelf Life | 24 months |
| Description | Yellow to brown cream form |
| Source | Marine Fish |
| Solvents Used | Ethanol & water |
| Country of Origin | China |

| Test Items | Specification | Test | Method |
|---|---|---|---|
| Identification | Positive | Conform | NMR |
| Phosphatidylserine | ≥30.0% | 30.56% | NMR/HPLC |
| Phosphatidyl acid | | 8.70% | 31P-NMR |
| Phosphatidyl Choline | | 1.30% | 31P-NMR |
| Lyso Phosphatidylserine | | 0.52% | 31P-NMR |
| Lyso phosphatidyl acid | | 1.06% | 31P-NMR |
| Phosphatidyl inositol | | 14.50% | 31P-NMR |
| Other phospholipids | | 19.50% | 31P-NMR |
| Glyceride (Tri-, di- and mono-) | | 12.40% | GC-FID |
| Calcium | | 2.70% | ICP-OES |
| Free L-serine | ≤1.0% | 0.40% | Ninhydrin |
| Loss on drying | ≤2.0% | 1.40% | Karl Fischer |
| Ash | ≤15% | 14.50% | 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** | | | |
| 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

# Certificate of Analysis

**Product Name:** DHAPS™ (Marine Phosphatidylserine)

Product Code: 1811701 Batch No.: 20150401 P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

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

**Product Characteristics:**

| | |
|---|---|
| Shelf Life | 24 months |
| Description | Yellow to brown cream form |
| Source | Marine Fish |
| Solvents Used | Ethanol & water |
| Country of Origin | China |

| Test Items | Specification | Test | Method |
|---|---|---|---|
| Identification | Positive | Conform | NMR |
| Phosphatidylserine | ≥30.0% | 30.61% | NMR/HPLC |
| Phosphatidyl acid | | 8.20% | 31P-NMR |
| Phosphatidyl Choline | | 1.30% | 31P-NMR |
| Lyso Phosphatidylserine | | 0.53% | 31P-NMR |
| Lyso phosphatidyl acid | | 1.10% | 31P-NMR |
| Phosphatidyl inositol | | 14.20% | 31P-NMR |
| Other phospholipids | | 18.90% | 31P-NMR |
| Glyceride (Tri-, di- and mono-) | | 12.00% | GC-FID |
| Calcium | | 2.90% | ICP-OES |
| Free L-serine | ≤1.0% | 0.40% | Ninhydrin |
| Loss on drying | ≤2.0% | 1.40% | Karl Fischer |
| Ash | ≤15% | 14.80% | 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** | | | |
| 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

# Certificate of Analysis

**Product Name:** DHAPS™ (Marine Phosphatidylserine)

Product Code: 1811701 Batch No.: 20150403 P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

MFG.DATE APR. 11, 2015 EXP. DATE: APR. 10, 2017

**Product Characteristics:**

| | |
|---|---|
| Shelf Life | 24 months |
| Description | Yellow to brown cream form |
| Source | Marine Fish |
| Solvents Used | Ethanol & water |
| Country of Origin | China |

| Test Items | Specification | Test | Method |
|---|---|---|---|
| Identification | Positive | Conform | NMR |
| Phosphatidylserine | ≥30.0% | 31.11% | NMR/HPLC |
| Phosphatidyl acid | | 8.30% | 31P-NMR |
| Phosphatidyl Choline | | 1.20% | 31P-NMR |
| Lyso Phosphatidylserine | | 0.50% | 31P-NMR |
| Lyso phosphatidyl acid | | 1.01% | 31P-NMR |
| Phosphatidyl inositol | | 14.10% | 31P-NMR |
| Other phospholipids | | 18.70% | 31P-NMR |
| Glyceride (Tri-, di- and mono-) | | 12.00% | GC-FID |
| Calcium | | 2.80% | ICP-OES |
| Free L-serine | ≤1.0% | 0.30% | Ninhydrin |
| Loss on drying | ≤2.0% | 1.20% | Karl Fischer |
| Ash | ≤15% | 14.60% | 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** | | | |
| 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

# Certificate of Analysis

**Product Name:** DHAPS™ (Marine Phosphatidylserine)

Product Code: 1811701 Batch No.: 20150406 P/O NO.:

QUANTITY: 100KG PACKAGE: 10KG/CARTON

MFG.DATE APR. 20, 2015 EXP. DATE: APR. 19, 2017

**Product Characteristics:**

| | |
|---|---|
| Shelf Life | 24 months |
| Description | Yellow to brown cream form |
| Source | Marine Fish |
| Solvents Used | Ethanol & water |
| Country of Origin | China |

| Test Items | Specification | Test | Method |
|---|---|---|---|
| Identification | Positive | Conform | NMR |
| Phosphatidylserine | ≥30.0% | 30.80% | NMR/HPLC |
| Phosphatidyl acid | | 8.40% | 31P-NMR |
| Phosphatidyl Choline | | 1.10% | 31P-NMR |
| Lyso Phosphatidylserine | | 0.48% | 31P-NMR |
| Lyso phosphatidyl acid | | 1.05% | 31P-NMR |
| Phosphatidyl inositol | | 14.30% | 31P-NMR |
| Other phospholipids | | 19.10% | 31P-NMR |
| Glyceride (Tri-, di- and mono-) | | 12.20% | GC-FID |
| Calcium | | 2.70% | ICP-OES |
| Free L-serine | ≤1.0% | 0.30% | Ninhydrin |
| Loss on drying | ≤2.0% | 1.20% | Karl Fischer |
| Ash | ≤15% | 14.50% | 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** | | | |
| 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.**

**Zhang Fan**
1017 North Bldg.1839 Qixin Rd., Shanghai
Fax:+86-21-3358 0611

**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

*AR-16-SU-000449-01*

Sample

| | | | |
|---|---|---|---|
| Client Sample Description | | Sample Code | 50215S058320 |
| BioPS Phosphatidylserine | | 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

Pages 000072-000366 and pages 000369-000434 have been removed in accordance with copyright laws. Please see list of the references that have been removed on pages 000054-000058.

SUBMISSION END