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ORIGINAL SUBMISSION

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LIFE SCIENCES RESEARCH OFFICE

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Michael Falk, Ph.D.
Executive Director

January 25, 2012

Paulette Gaynor, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Pkwy.
College Park, MD 20740

Dear Dr. Gaynor:

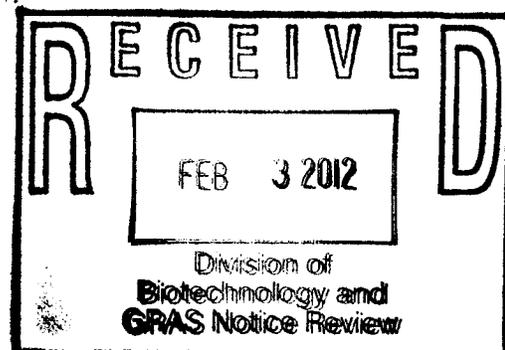
In accordance with proposed 21 CFR §170.36 (62 FR 18960; April 17, 1997), I am submitting in triplicate, as the representative of the notifier, ChemiNutra, Inc., 4463 White Bear Pkwy., Ste. 105, White Bear Lake, MN 55110, notice of a claim that the use of AlphaSize® Alpha-glycerol phosphoryl choline (A-GPC) as a food ingredient in conventional beverages and beverage bases, including coffee, tea, milk (liquid), milk powder, flavored milk and milk drinks, carbonated beverages, powdered beverages, meal replacements, and foods, including yogurt, grain-based bars, protein bars, ready-to-eat breakfast cereals, and snack foods, such as chewing gum, chocolates, and candies, when consumed at a level not to exceed 196.2 mg/person/day, is generally recognized as safe (GRAS) based on scientific procedures. An Expert Panel Report, providing the basis of the GRAS determination, the signed conclusions of the Expert Panel, and the *curricula vitae* of the three Expert Panel members are enclosed.

If you have any questions or concerns regarding this GRAS Notification, please contact me at 301-634-7030 or falkm@lsro.org. I look forward to receiving acknowledgement of receipt of this notice.

Sincerely,

(b) (6)

Michael Falk, Ph.D.
Executive Director
Life Sciences Research Organization (LSRO)



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Michael Falk, Ph.D.
Executive Director

January 25, 2012

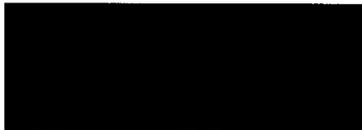
Paulette Gaynor, Ph.D.
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Sincerely,



Michael Falk, Ph.D.
Executive Director
Life Sciences Research Organization (LSRO)

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**Generally Recognized as Safe (GRAS) Determination for the
Use of AlphaSize® Alpha-Glycerolphosphoryl Choline**

**Submitted by
Life Sciences Research Organization, Inc.
9650 Rockville Pike
Bethesda, Maryland 20814
www.LSRO.org**

January 25, 2012

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I. GRAS Exemption Claim

ChemiNutra, Inc. through its agent, the Life Sciences Research Organization (LSRO), Inc. hereby notifies the Food and Drug Administration that AlphaSize® Alpha-glyceryl phosphoryl choline (A-GPC) has been determined to be generally recognized as safe (GRAS) by scientific procedures under the conditions of its intended use as described below and is, therefore, exempt from the requirements of premarket approval of the Federal Food, Drug, and Cosmetics Act.

Signed

(b) (6)

Michael C. Falk, Ph.D.
Executive Director
Life Sciences Research Organization, Inc.
9650 Rockville Pike,
Bethesda, MD 20814

Jan 25, 2012
Date

I.1 Name and Address of Notifier

Scott Hagerman,
ChemiNutra, Inc.
4463 White Bear Pkwy., Ste. 105
White Bear Lake, MN 55110

I.2 Agent of Notifier

Michael C. Falk, Ph.D.
9650 Rockville Pike
Bethesda, MD 20814
Telephone: 301-634-7030
Facsimile: 301-634-7876
E-mail: falkm@lsro.org

I.3 Common Name of GRAS Substance

The subject of this GRAS exemption claim is AlphaSize® alpha glyceryl phosphoryl choline (A-GPC). This material is also commonly referred to choline alfoscerate, GPC, or "Alpha-GPC" and is also known as alpha-glyceryl phosphatidylcholine, glycerophosphorylcholine, glycerophosphocholine, choline glycerophosphate, choline-hydroxide, GroPCho, l-A-glyceryl phosphorylcholine, and L-alpha-glycerylphosphorylcholine.

I.4 Conditions of Use

Life Sciences Research Organization
AlphaSize® Alpha-Glycerylphosphoryl Choline GRAS Determination

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The intended use of AlphaSize® A-GPC is as a nutrient in conventional beverages and beverage bases, including coffee, tea, milk (liquid), milk powder, flavored milk and milk drinks, carbonated beverages, powdered beverages, and meal replacements; foods including yogurt, grain-based bars, protein bars, ready-to-eat breakfast cereals; and snack foods such as chewing gum, chocolates, and candies. AlphaSize® A-GPC is not intended to be used in infant formula.

I.5 Basis of GRAS Determination

AlphaSize® AGPC has been determined to be safe on the basis of scientific procedures as described under 21 CFR §170.30(b) by panel of experts with the training and scientific expertise necessary to make such a determination comprising Edward Carmines, Ph.D., Richard Kraska, Ph.D., D.A.B.T., and Madhusudan Soni, Ph.D., F.A.C.N. LSRO conducted a comprehensive search of the literature on AGPC and summarized relevant published and unpublished data on A-GPC. The Expert Panel critically assessed the information summarized in a dossier prepared by LSRO and based on this and other information known to them determined the safety of A-GPC. The Expert Panel's determination of safety was based on extensive preclinical and clinical studies on AlphaSize® A-GPC, the use of AGPC as a dietary supplement, and the fact that A-GPC is a natural product found in breast milk. Use of AlphaSize® A-GPC is exempt under the Dietary Supplement Health and Education Act of 1994 (DSHEA). The Expert Panel is of the opinion that other scientists qualified to evaluate the body of data would also conclude that AlphaSize® A-GPC is GRAS by scientific procedures when used as proposed.

I.5 Availability of Information

The data and information that are the basis for the GRAS determination are available for the Food and Drug Administration to review and copying at reasonable times from the address below.

Michael C. Falk
Life Sciences Research Organization (LSRO)
9650 Rockville Pike
Bethesda, MD 20814

Telephone: 301-634-7030
Facsimile: 301-634-7876
E-mail: falkm@lsro.org

As an alternative, the data and information can be sent to FDA on request by contacting Dr. Falk.

II. Detailed Information About the Identity of the Substance

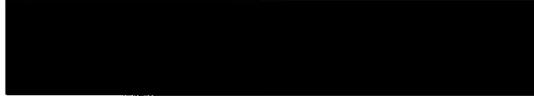
II.1 Identity

Life Sciences Research Organization
AlphaSize® Alpha-Glycerolphosphoryl Choline GRAS Determination

I. GRAS Exemption Claim

ChemiNutra, Inc. through its agent, the Life Sciences Research Organization (LSRO), Inc. hereby notifies the Food and Drug Administration that AlphaSize® Alpha-glyceryl phosphoryl choline (A-GPC) has been determined to be generally recognized as safe (GRAS) by scientific procedures under the conditions of its intended use as described below and is, therefore, exempt from the requirements of premarket approval of the Federal Food, Drug, and Cosmetics Act.

Signed,



Michael C. Falk, Ph.D.
Executive Director
Life Sciences Research Organization, Inc.
9650 Rockville Pike,
Bethesda, MD 20814

Jan 25, 2012
Date

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Scott Hagerman,
ChemiNutra, Inc.
4463 White Bear Pkwy., Ste. 105
White Bear Lake, MN 55110

I.2 Agent of Notifier

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Bethesda, MD 20814
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Facsimile: 301-634-7876
E-mail: falkm@lsro.org

I.3 Common Name of GRAS Substance

The subject of this GRAS exemption claim is AlphaSize® alpha glyceryl phosphoryl choline (A-GPC). This material is also commonly referred to choline alfoscerate, GPC, or "Alpha-GPC" and is also known as alpha-glyceryl phosphatidylcholine, glycerophosphorylcholine, glycerophosphocholine, choline glycerophosphate, choline-hydroxide, GroPCho, l-A-glyceryl phosphorylcholine, and L-alpha-glycerylphosphorylcholine.

I.4 Conditions of Use

Life Sciences Research Organization
AlphaSize® Alpha-Glycerylphosphoryl Choline GRAS Determination

1

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The intended use of AlphaSize® A-GPC is as a nutrient in conventional beverages and beverage bases, including coffee, tea, milk (liquid), milk powder, flavored milk and milk drinks, carbonated beverages, powdered beverages, and meal replacements; foods including yogurt, grain-based bars, protein bars, ready-to-eat breakfast cereals; and snack foods such as chewing gum, chocolates, and candies. AlphaSize® A-GPC is not intended to be used in infant formula.

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As an alternative, the data and information can be sent to FDA on request by contacting Dr. Falk.

II. Detailed Information About the Identity of the Substance

II.1 Identity

Life Sciences Research Organization
AlphaSize® Alpha-Glycerolphosphoryl Choline GRAS Determination

The GRAS substance is AlphaSize® Alpha-glyceryl phosphoryl choline (A-GPC) and the CAS number is 28319-77-9. The structure of A-GPC is shown below.

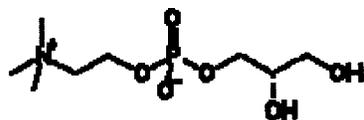


Figure 1. Structure of Glyceryl phosphoryl choline (A-GPC) 2-[(2R)-2, 3-dihydroxypropoxy]-hydroxy-phosphoryl]oxyethyl-trimethyl-azanium. The optical activity arises from the 2 carbon atom in the glycerin part of the molecule.

II.2 Common or Usual Name of the Substance

The common name of the substance is glycerylphosphoryl choline.

II.3 Method of Manufacture

Synthesis of the AlphaSize® products involves a non-enzymatic hydrolysis process involving a series of pH balanced food grade chemical reagents. Manufacturing of AlphaSize® products starts with a soy lecithin extract containing 35–38% phosphatidylcholine. For manufacturing of AlphaSize® 50P A-GPC, the soy lecithin extract is dissolved in alcohol and transesterified to provide the raw A-GPC and fatty acid esters (Chemi S.p.A., 2010). The reaction medium is extracted, and A-GPC is crystallized from the extraction solvent. The raw A-GPC is then dissolved and recrystallized to yield the partially purified product, which is then dissolved in water. The dissolved product undergoes micro-filtration and in process analysis. The A-GPC water solution is crystallized and taken through in-process analysis to yield crystalline A-GPC. It is then processed to yield AlphaSize® 50P, packaged, and submitted to final quality control analysis at which time the appropriate Certificate of Analysis is prepared. Finally, AlphaSize® 50P is blended with flow agents (dicalcium phosphate dihydrate, magnesium stearate, silicon dioxide, and magnesium silicate) purified by recrystallization and packaged (Chemi S.p.A., 2010). In the manufacturing of AlphaSize® 50WSP A-GPC, free fatty acids are removed from the sn1 and sn2 positions using pH-balanced hydrolysis in a reaction vessel to give 50–52% A-GPC (Chemi Nutra, 2010k). This is then blended with the flow agents mannitol and silicon dioxide. Manufacturing of AlphaSize® 100P A-GPC differs from the 50P process in that no flow agents are used (Chemi Nutra, 2010d).

II.4 Specifications for Food Grade AlphaSize® A-GPC

Technical Data Sheet 091106

AlphaSize® 50P

AlphaSize® 50P is a powdered A-GPC for use in nutritional supplements						
Description	Physical	Assay Values	Analytical	Assay Values	Microbiological	Assay Values
Typical composition	Consistency	White to off-white powder	α-Glycerolphosphorylcholine (A-GPC)	50 – 52 %	Total Plate Count	NMT 1,000 CFU/g
			Dicalcium phosphate dihydrate	43.5 – 49.5 %	Yeast and molds	NMT 100 CFU/g
			Magnesium stearate	0.5 – 1.5 %	<i>Escherichia coli</i>	Negative/g
			Silicon dioxide	0.5 – 1.5 %	Coliforms	Negative/g
			Magnesium Silicate	0.5 – 1.5 %	<i>Staphylococcus aureus</i>	Negative/g
					<i>Salmonella</i>	Negative/10 g
Storage and Handling	AlphaSize® 50P should be kept in the sealed shipping container at 50 – 77F (10 – 25C) avoiding light and moisture until ready for use. The container should be resealed immediately after use. The shelf life of AlphaSize® 50P is 60 months in the original unopened container.					
Regulatory Status	Labeling of AlphaSize® 50P should be as follows Alpha-Glyceryl Phosphoryl Choline, A-GPC					
Packaging	Standard packaging for AlphaSize® 50P is 10 kg net weight plastic containers					

Technical Data Sheet 091106

AlphaSize® 50WSP

AlphaSize® 50WSP is a water soluble powdered A-GPC for use in nutritional supplements						
Description	Physical	Assay Values	Analytical	Assay Values	Microbiological	Assay Values
Typical composition	Consistency	White to off-white powder	α-Glycerolphosphorylcholine (A-GPC)	50 – 52 %	Total Plate Count	NMT 1,000 CFU/g
			Mannitol	47.0 – 49.5 %	Yeast and molds	NMT 100 CFU/g
			Silicon dioxide	0.5 – 1.5 %	<i>Escherichia coli</i>	Negative/g
					Coliforms	Negative/g
					<i>Staphylococcus aureus</i>	Negative/g
					<i>Salmonella</i>	Negative/10 g
Storage and Handling	AlphaSize® 50WSP should be kept in the sealed shipping container at 50 – 77F (10 – 25C) avoiding light and moisture until ready for use. The container should be resealed immediately after use. The shelf life of AlphaSize® 50WSP is 60 months in the original unopened container.					
Regulatory Status	Labeling of AlphaSize® 50WSP should be as follows Alpha-Glyceryl Phosphoryl Choline, A-GPC					
Packaging	Standard packaging for AlphaSize® 50WSP is 10 kg net weight plastic containers					

Technical Data Sheet 091106

AlphaSize® 100P

Description	AlphaSize® 100P is a high purity A-GPC for use in nutritional supplements					
Typical composition	Physical	Assay Values	Analytical	Assay Values	Microbiological	Assay Values
	Consistency	White to off-white colored, highly soluble crystalline powder	α-Glycerolphosphorylcholine (A-GPC)	98 – 100 %	Total Plate Count	NMT 1,000 CFU/g
					Yeast and molds	NMT 100 CFU/g
					<i>Escherichia coli</i>	Negative/g
					Coliforms	Negative/g
					<i>Staphylococcus aureus</i>	Negative/g
					<i>Salmonella</i>	Negative/10 g
Storage and Handling	AlphaSize® 100P should be kept in the sealed shipping container at 50 – 77F (10 – 25C) avoiding light and moisture until ready for use. The container should be resealed immediately after use. The shelf life of AlphaSize® 50WSP is 60 months in the original unopened container.					
Regulatory Status	Labeling of AlphaSize® 100P should be as follows: Alpha-Glyceryl Phosphoryl Choline, A-GPC					
Packaging	Labeling of AlphaSize® 100P is per customer request					

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II.5 Basis of GRAS Determination

The Expert Panel has determined that AlphaSize® Alpha-glycerol phosphoryl choline (A-GPC) is GRAS by scientific procedures under 21 CFR §170.3(b) when used as intended. A-GPC is not intended to be used in infant formula. The mean dietary intake from foods with consumption data available is estimated to be 108.44 ± 3.46 mg/person/d for all users. The 90th percentile is estimated to be 196.15 ± 4.15 mg/person/d for all users. The mean dietary intake for foods with consumption data available is estimated to be 1.86 ± 0.06 mg/kg bw/d. Consumption by individuals in the 90th percentile is estimated to be 3.53 ± 0.10 mg/kg bw/d. The Expert Panel determined that the proposed use of AlphaSize® A-GPC intended to be added as a nutrient to the specified foods and beverages at the specified levels to result in consumption of no more than 196.2 mg/person/day is considered GRAS by scientific procedures established under 21 CFR §170.3(b).

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EXPERT PANEL REPORT

III. INTRODUCTION

III.1. Objective

At the request of ChemiNutra, Inc., (ChemiNutra), the Life Sciences Research Organization, Inc., (LSRO) has undertaken a comprehensive independent safety evaluation to ascertain whether the use of AlphaSize® Alpha-glycerol phosphoryl choline (A-GPC) as a nutrient in conventional beverages and beverage bases, including coffee, tea, milk (liquid), milk powder, flavored milk and milk drinks, carbonated beverages, powdered beverages, meal replacements, and foods including yogurt, grain-based bars, protein bars, ready-to-eat breakfast cereals, and snack foods such as chewing gum, chocolates, and candies at levels ranging from 10–100 mg/serving per day to be Generally Recognized as Safe (GRAS).

ChemiNutra provided LSRO with background information that addressed the safety and toxicity of A-GPC; the levels at which it occurs in food; the history of A-GPC consumption; and details about the composition, specifications and method of preparation of AlphaSize® A-GPC. Information about the amount of A-GPC that has been safely consumed, *i.e.*, “doses” or use levels is essential to extrapolate safe exposures for AlphaSize® A-GPC. As a result, ChemiNutra was also asked to provide information about past and present food use of A-GPC and other products. The information provided by ChemiNutra was supplemented with literature identified in an independent search of scientific and regulatory literature through July, 2010. The composite animal safety and toxicity studies and human studies providing information about exposure are ultimately the specific scientific underpinnings for a safety assessment.

IV. REGULATORY HISTORY OF THE INGREDIENT

A-GPC is a hydrolysis product of lecithin, which is a ubiquitous natural constituent of biological organisms and human food. Lecithin is considered to be GRAS by US Food and Drug Administration (FDA) (21 CFR 184.1400) (US Code of Federal Regulations, 2006). It was reviewed by the LSRO Select Committee on GRAS Substances (SCOGS) in report #106 (Life Sciences Research Office, 1979).

Hydrolyzed lecithin has been the subject of GRAS Notifications (GRN) to FDA. In 2004, GRN 000134, pursuant to 21 CFR 170.30, the C-Fraction Soy Protein Hydrolyzate with Bound Phospholipids was determined to be GRAS by scientific procedures based on information provided by Kyowa Hakko (USA) (CFSAN/FDA, 2004). In 2006, GRN 000186, in accordance with 21 CFR 170.30, the Soy Lecithin Phosphatidylserine Complex was determined to be GRAS on the basis of scientific procedures and information provided by Lipogen (Israel) (Lipogen Products Ltd, 2006). In 2008, GRN 000226, pursuant to 21 CFR 170.30, Krill-based Lecithin was determined to be GRAS

on the basis of scientific procedures and information provided by Enzymotec (Israel) (CFSAN/FDA, 2008).

FDA rejected a new dietary ingredient notification (NDIN) submitted on behalf of Lucas Meyer, Inc., (Decatur, Illinois), for Leci-GHA™ (US Food and Drug Administration, 1999). Leci-GHA™, was described as a phospholipid compound rich in A-GPC that was intended to be used as a dietary supplement (FDA, 1999 NDI notification rpt0060 Leci-GHA). FDA indicated that the NDI was rejected for lack of chemistry, oral exposure, and safety data specific to Leci GHA™. The manufacturer's claim of safety of Leci-GHA™ rested mainly on data for choline alfoscerate also known as A-GPC and some of the data were confidential unpublished information on A-GPC (Italfarmaco, 1997). FDA responded that Leci GHA™ contains substances in addition to choline alfoscerate and the manufacturer failed to note the percent enrichment of Leci-GHA™ with choline alfoscerate, thereby, preventing comparison, with certainty, the recommended dosage of Leci-GHA with the dosages of choline alfoscerate used in the studies the manufacturer described in the submission. FDA also noted that the submission for Leci GHA™ contained "no quantitative estimates of the actual human diet exposure to choline alfoscerate" and the submission "did not address differences, if any, in the absorption or bioavailability of choline alfoscerate in foods or the new dietary ingredient". FDA stated that, as a result, "no comparisons between typical dietary exposure to choline alfoscerate and the exposure to this substance that would result from the use of the proposed dietary supplement can be reliably made" (US Food and Drug Administration, 1999).

FDA also had specific criticisms of the choline alfoscerate data submitted by the manufacturer of Leci-GHA™ (Italfarmaco, 1997), which includes a subset of the data for ChemiNutra's A-GPC. FDA noted that the only study that described effect of A-GPC on patients with brain injuries, from cerebral ischemic events, multi-infarct dementia, vascular dementia, or senile organic brain syndrome administered choline alfoscerate by the oral route reported multiple side effects, such as headache, confusion/dizziness, erythema, and excitation/insomnia. Although the authors of the choline alfoscerate studies generally considered these side effects to be mild effects that occurred at a relatively low incidence, FDA questioned whether these studies can support a conclusion that, when used in a dietary supplement as proposed, Leci-GHA can be reasonably expected to be safe. Another criticism was that "most of the studies were conducted to study the pharmacological effects or efficacy of intravenously administered or injected choline alfoscerate in sick patients" and "none of the studies submitted were designed nor intended to examine the adverse or toxicological effects of choline alfoscerate in healthy people; instead, the dietary ingredient was used as a therapy in studies of persons with serious diseases". FDA noted that such studies "have limited utility for determining whether the long-term use of a substance as an ingredient in dietary supplements is safe because they do not provide dose response data or measurements of endpoints of toxicological concern. Third, "the findings from the studies reported several side effects that reflect changes in cognitive and behavioral function", and finally "all of these findings seem to point to choline alfoscerate contributing to changes in central nervous system neurochemical function. Although

some of these changes may be of potential clinical significance in persons with the conditions described in the studies, the significance of these central nervous system effects in otherwise healthy persons of different age groups, especially children, can not be determined from these studies. In fact, the studies are designed in a manner that limits the use of the findings obtained from them as an adequate basis to conclude that the reported central nervous system actions of choline alfoscerate are not of concern to healthy persons using dietary supplements containing it for an extended period of time.”

“The submission also contained the data from two studies in healthy young subjects that were conducted for 7 and 10 days and reported some side effects that the authors noted as being “mild”, however, those studies were not designed to investigate potential adverse effects. The findings from experimental exposures of such short duration do not provide an adequate basis to conclude that no untoward effects would be expected from the oral consumption of the test substance for longer periods of time, such as would be expected if it was used as a dietary supplement. FDA noted that “It is difficult to determine the exact nature of the toxicological and/or clinical significance of the choline alfoscerate-induced physiological and behavioral changes because the primary data was not provided...”and “...more information is needed about the nature and degree of the adverse effects seen in animals in order to have more certainty in the safety assessment.” Subsequent to the submission of the NDIN, several reports on safety of A-GPC have appeared.

A-GPC is sold in the EU as a drug under the trade name Gliatilin® and is reportedly under development as a drug to treat memory impairment in the US.

V. DESCRIPTION AND BACKGROUND OF ALPHASIZE® ALPHA-GLYCERYL PHOSPHORYL CHOLINE (A-GPC)

V.1. Chemical Name

A-GPC is the acronym for glyceryl phosphoryl choline, and its chemical structure appears in Figure 2. This material is also commonly referred to choline alfoscerate, GPC, or “Alpha-GPC” but is also known as alpha-glyceryl phosphatidylcholine, glycerophosphorylcholine, glycerophosphocholine, choline glycerophosphate, choline-hydroxide, GroPCho, I-A-glyceryl phosphorylcholine, and L-alpha-glycerylphosphorylcholine. The CAS number is 28319-77-9.

V.2. Chemical Structure

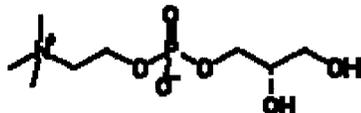


Figure 2 . Structure of Glyceryl phosphoryl choline (A-GPC) 2-[(2R)-2, 3-dihydroxypropoxy]-hydroxy-phosphoryl]oxyethyl-trimethyl-azanium. The optical activity arises from the 2 carbon atom in the glycerin part of the molecule.

A-GPC is a semi-synthetic derivative of phosphatidylcholine (Figure 3) and is a natural component of human breast milk. An elaborate chemical synthesis was reported in European Patent 0486100 Manufacturing Process (Puricelli, 1992).

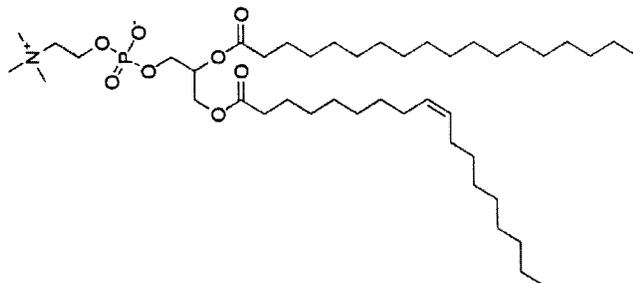


Figure 3. Structure of Phosphatidyl Choline

Preparation of A-GPC from lecithin is reported in US patent 5315023 (De Ferra et al., 1994). Lecithin is a mixture of phospholipids derived from vegetable oils such as soybean, rapeseed, or sunflower. Fractionation of soybean lecithin yields a material rich in phosphatidylcholine (van Nieuwenhuyzen & Tomás, 2008) The fatty acid composition varies with the oil source; stearyl and oleyl side chains are shown, but combinations with palmoyl, linoleyl and linolenoyl side chains are possible. The fatty acid groups are removed by transesterification to yield the desired A-GPC.

V.3. Biological Functions of Choline

Choline is a precursor of acetylcholine, phospholipids (such as phosphatidylcholine and sphingomyelin), and betaine, the methyl donor (Institute of Medicine, 1998). Choline has been designated an essential nutrient (Institute of Medicine, 1998). Choline is critical for the normal function of all cells. It helps to maintain the structural integrity of cell membranes and is important for methyl metabolism, cholinergic neurotransmission, and transmembrane signaling as well as lipid and cholesterol transport and metabolism (Institute of Medicine, 1998; Zeisel & Blusztajn, 1994). Choline deficiency can result in fatty liver (Buchman et al., 1995; Zeisel et al., 1991), hepatic carcinoma (Chandar &

Lombardi, 1988; Ghoshal & Farber, 1984), and promotion of apoptosis in some cell lines (Cui et al., 1996) and the fetal brain (Albright et al., 1999). Consumption of a diet deficient that is deficient in choline, but includes limited consumption of methionine and folate, for weeks or months results in hepatic, renal, pancreatic, memory, and growth disorders of most mammals (Zeisel & Blusztajn, 1994).

A-GPC is a component of breast milk and is an important source of choline required by infants for organ growth and membrane biosynthesis (Holmes et al., 2000; Holmes-McNary et al., 1996; Ilcol et al., 2005). It is also present in brain tissue as a product of phospholipid metabolism. Decreased risk of neural tube defect-affected pregnancies has been observed with higher periconception consumption of choline for all neural tube defects and for spina bifida and anencephaly (Shaw et al., 2004). Pregnancy may decrease rats' ability to obtain adequate choline from the diet (Zeisel et al., 1995; Zeisel, 1997).

The neurodegenerative diseases Alzheimer's disease and vascular dementia present with cognitive loss and dementia. In Alzheimer's disease, formation of amyloid peptides from amyloid precursor protein and deposition of amyloid plaques contribute to progressive deterioration (Selkoe & Schenk, 2003). Cellular calcium overload, lipid peroxidation, and membrane breakdown are also thought to contribute to the later stages of neuronal breakdown (Mattson & Chan, 2001). Growden et al. (1996) reported that elevated levels of A-GPC in cerebral spinal fluid correspond to the presence of Alzheimer's disease. Individuals with Alzheimer's disease have significantly increased phosphatidylcholine hydrolysis in the brain and elevated levels of A-GPC compared to age-matched cognitively normal individuals. This is due to activation of calcium-dependent phospholipase A₂ (Walter et al., 2004). Autopsies of individuals with Alzheimer's disease revealed brain choline levels that are 40–50% lower than levels in normal brains. The progression of vascular dementia is characterized by the lack of oxygen and nutrients, reduction of ATP, loss of membrane potential and release of massive amounts of glutamate (Walter et al., 2004). Supplementation with A-GPC has been proposed as a method to increase choline delivery in order to improve disorders with a cognitive deficit. Patients with mild to moderate Alzheimer's disease showed cognitive improvement after treatment with A-GPC (De Jesus Moreno Moreno, 2003). Additional studies showing the mental health benefits of A-GPC are summarized by (Kidd, 2008).

Because acetylcholine (ACh) may modulate the growth hormone (GH) response to growth hormone releasing hormone (GHRH), choline may also function as a growth hormone secretagogue (Physicians Desk Reference, 2008). Ceda et al. (1992) reported that A-GPC administration elevated GH response to GHRH in young and elderly subjects.

Jager et al. (2007) reviewed information on supplementation with phospholipids on sports performance. They concluded that the data are equivocal; however, acute oral supplementation with 0.2 g phosphatidylcholine per kg body mass prior to activity or

exercise enhances performance in various sporting activities in which exercise used up circulatory choline.

Intraperitoneal, and oral doses 80 μ M/kg A-GPC do not compete in any significant way *in vitro* with the cholinergic receptor. A-GPC taken through these routes is present in significant levels in the central nervous system and plasma and brain levels over time (Trabucchi et al., 1986). A-GPC increases striatal dihydroxyphenylacetic acid concentration one hour after treatment, which lasts for at least 4 hours and increases the capacity of striatal slices to release dopamine *in vitro* after a depolarizing stimulus, suggesting that it has cholinomimetic properties (Trabucchi et al., 1986). A single 50 mg/kg oral dose of choline generated a detectable change in brain composition in 4 healthy adult men approximately 3 h after choline ingestion (Stoll et al., 1995). In the same study a 200 mg/kg dose produced a proportionally larger elevation in brain choline resonance. Sigala et al. (1992) reported that A-GPC increased hippocampal cholinergic transmission in the rat. "A-GPC has been characterized as a centrally acting parasympathomimetic drug in international pharmacopeias (Reynolds, 1996) and in the Chemical Therapeutic Anatomical Classification" (p. 2443) (Parnetti et al., 2001; World Health Organization, 2003).

A-GPC may have a beneficial effect during exercise by increasing ACh release and promoting muscle contraction (Gatti et al., 1992).

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V.4. Metabolism and Distribution of A-GPC

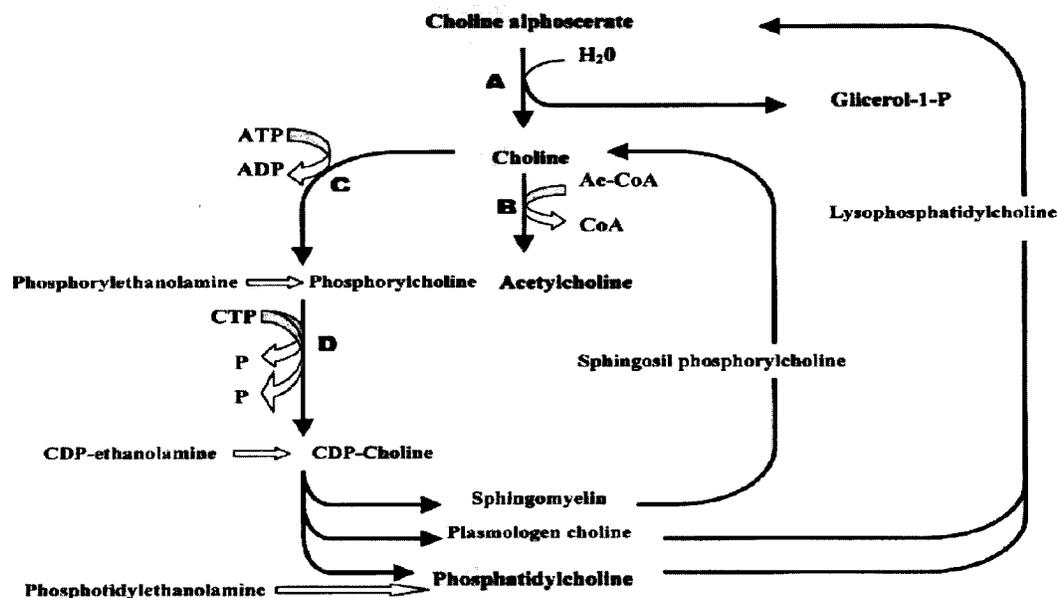


Figure 4. Choline anabolic pathways. (a) Glyceryl-phosphorylcholine diesterase; (B) Choline Acetyltransferase; (C) choline kinase; (D) Phosphocholine acydl transferase. (Adapted from (Amenta et al., 2001).

The metabolic pathway of A-GPC is presented in Figure 4. After oral consumption, A-GPC is converted to phosphorylcholine, which is metabolically active. Phosphorylcholine travels to the cholinergic synaptic endings and can elevate ACh synthesis and release (Abbiati et al., 1991; Lopez et al., 1991; Trabucchi et al., 1986). The increased choline that results from administration of A-GPC disappears from plasma relatively quickly, within 6 hours (Gatti et al., 1992). Animal studies have revealed that this is due to a deep penetration within the tissues (Abbiati et al., 1993; Di Perri et al., 1991; Frattola et al., 1991). The deep penetration results in brain levels remaining increased for a longer period of time (up to 30 hours after a 300 mg/kg oral dose). The improved cognitive effects seen in animal models are thought to be due to the increase in brain choline levels (Lopez et al., 1991), scopolamine treated normal individuals (Canal et al., 1991), and individuals with impaired learning and memory functions (Di Perri et al., 1991; Frattola et al., 1991).

V.5. Manufacturing Process of A-GPC

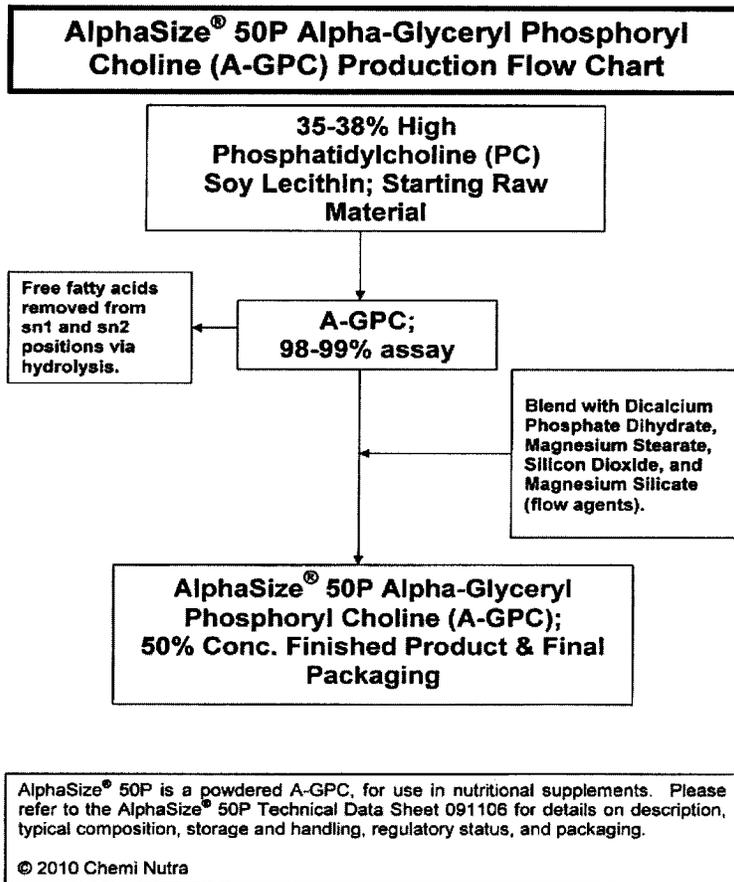
ChemiNutra provided flow charts for the manufacture of AlphaSize® 50P A-GPC (Figure 5) (Chemi Nutra, 2010j), AlphaSize® 50WSP A-GPC (Figure 6) (Chemi Nutra, 2010k), and AlphaSize® 100P A-GPC (Figure 7) (Chemi Nutra, 2010d).

Synthesis of the AlphaSize® products involves a non-enzymatic hydrolysis process involving a series of pH balanced food grade chemical reagents. Manufacturing of

AlphaSize® products starts with a soy lecithin extract containing 35–38% phosphatidylcholine. For manufacturing of AlphaSize® 50P A-GPC, the soy lecithin extract is dissolved in alcohol and transesterified to provide the raw A-GPC and fatty acid esters (Chemi S.p.A., 2010). The reaction medium is extracted, and A-GPC is crystallized from the extraction solvent. The raw A-GPC is then dissolved and recrystallized to yield the partially purified product, which is then dissolved in water. The dissolved product undergoes micro-filtration and in process analysis. The A-GPC water solution is crystallized and taken through in-process analysis to yield crystalline A-GPC. It is then processed to yield AlphaSize® 50P, packaged, and submitted to final quality control analysis at which time the appropriate Certificate of Analysis is prepared. Finally, AlphaSize® 50P is blended with flow agents (dicalcium phosphate dihydrate, magnesium stearate, silicon dioxide, and magnesium silicate) purified by recrystallization and packaged (Chemi S.p.A., 2010). In the manufacturing of AlphaSize® 50WSP A-GPC, free fatty acids are removed from the sn1 and sn2 positions using pH-balanced hydrolysis in a reaction vessel to give 50–52% A-GPC (Chemi Nutra, 2010k). This is then blended with the flow agents mannitol and silicon dioxide. Manufacturing of AlphaSize® 100P A-GPC differs from the 50P process in that no flow agents are used (Chemi Nutra, 2010d).

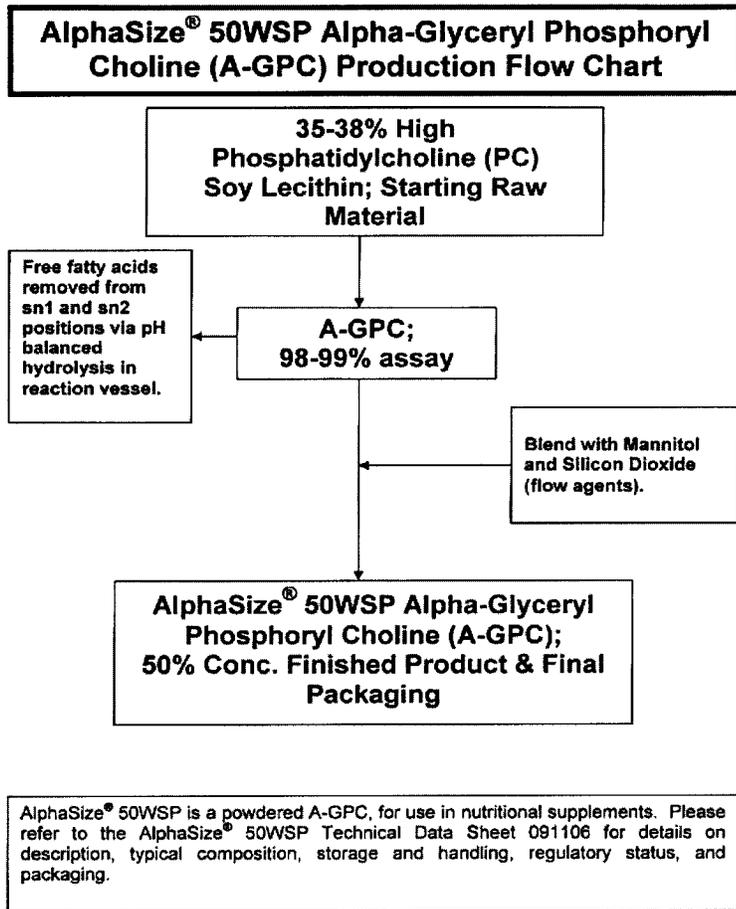
ChemiNutra stated that their products are manufactured entirely in their US FDA-inspected production facilities, which operate under food Good Manufacturing Practices. The company provided the Establishment Inspection Report for the active pharmaceutical ingredient manufacturing facility in Patrica, Italy (Dietrick & CDER, 2004). Additional documentation was provided for the site in Bahia, Brazil (ITF Chemical, 2009).

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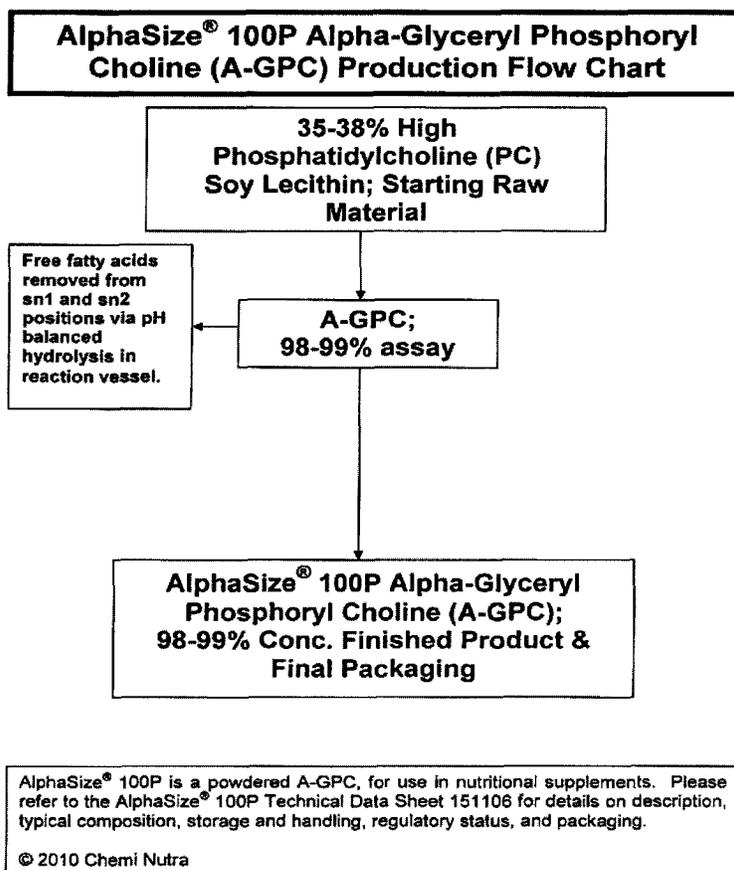
Figure 5. AlphaSize® 50P Alpha-Glycerol Phosphoryl Choline Production Flow Chart



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Figure 6. AlphaSize® 50WSP Alpha-Glyceryl Phosphoryl Choline Production Flow Chart

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Figure 7. AlphaSize® 100P Alpha-Glyceryl Phosphoryl Choline Production Flow Chart

V.6. Product Purity and Specifications

Technical data sheets are provided for AlphaSize® 50P, 50WSP, and 100P in Figures 8, 9, and 10.

The active ingredient, A-GPC, is claimed to be present at a level of 50.0–52.0% for AlphaSize® 50P and 50WSP. Both of these products are described as white to off-white powders. AlphaSize® 100P is a white to off-white colored, highly soluble, crystalline powder in which A-GPC is said to be present at a level of 98–100%. Assay value ranges are given for the inert fillers and flow agents in each product. Certificates of analysis have been provided for each product and a spectral analysis of the 50P product is also included in **Appendix A**.



Technical Data Sheet
091106

AlphaSize® 50P

Alpha-Glycerol Phosphoryl Choline (A-GPC)

Description: AlphaSize® 50P is a powdered A-GPC, for use in nutritional supplements.

Typical Composition:

	<u>Assay Values</u>
<u>Physical</u>	
Consistency	White to off-white powder
<u>Analytical</u>	
α-Glycerolphosphorylcholine (A-GPC)	50 – 52 %
Dicalcium phosphate dihydrate	43.5 – 49.5 %
Magnesium stearate	0.5 – 1.5 %
Silicon dioxide	0.5 – 1.5 %
Magnesium Silicate	0.5 – 1.5 %
<u>Microbiological</u>	
Total Plate Count	NMT 1,000 CFU / g
Yeast and molds	NMT 100 CFU / g
Escherichia Coli	Negative / g
Coliforms	Negative / g
Staphylococcus aureus	Negative / g
Salmonella	Negative / 10 g

Storage And Handling:

AlphaSize® 50P should be kept in the sealed shipping container at 50 – 77F (10 – 25C), avoiding light and moisture, until ready for use. The container should be resealed immediately after use. The shelf life of AlphaSize® 50P is 60 months, in the original unopened container.

Regulatory Status:

Labeling of AlphaSize® 50P should be as follows: Alpha-Glycerol Phosphoryl Choline, A-GPC

Packaging:

Standard packaging for AlphaSize® 50P is 10 kg net weight plastic containers.

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Figure 8. AlphaSize® 50P Alpha-Glycerol Phosphoryl Choline Technical Data Sheet

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Technical Data Sheet
091106

AlphaSize® 50WSP

Alpha-Glycerol Phosphoryl Choline (A-GPC)

Description: AlphaSize® 50WSP is water soluble powdered A-GPC, for use in nutritional supplements.

Typical Composition:

<u>Physical</u>	<u>Assay Values</u>
Consistency	White to off-white powder
<u>Analytical</u>	
α -Glycerophosphorylcholine (A-GPC)	50.0 – 52.0 %
Mannitol	47.0 – 49.5 %
Silicon dioxide	0.5 – 1.0 %
<u>Microbiological</u>	
Total Plate Count	NMT 1,000 CFU / g
Yeast and molds	NMT 100 CFU / g
Escherichia Coli	Negative / g
Coliforms	Negative / g
Staphylococcus aureus	Negative / g
Salmonella	Negative / 10 g

Storage And Handling:

AlphaSize® 50WSP should be kept in the sealed shipping container at 50 – 77F (10 – 25C), avoiding light and moisture, until ready for use. The container should be resealed immediately after use. The shelf life of AlphaSize® 50WSP is 60 months, in the original unopened container.

Regulatory Status:

Labeling of AlphaSize® 50WSP should be as follows: Alpha-Glycerol Phosphoryl Choline, A-GPC

Packaging:

Standard packaging for AlphaSize® 50WSP is 10 kg net weight plastic containers.

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Figure 9. AlphaSize® 50WSP Alpha-Glycerol Phosphoryl Choline Technical Data Sheet



Technical Data Sheet
151106

AlphaSize® 100P

Alpha-Glycerol Phosphoryl Choline (A-GPC)

Description: AlphaSize® 100P is a high purity A-GPC, for use in nutritional supplements

Typical Composition:

Physical

Consistency

Assay Values

White to off white colored, highly soluble, crystalline powder

Analytical

α-Glycerolphosphorylcholine (A-GPC) 98 – 100 %

Microbiological

Total Plate Count

NMT 1,000 CFU / g

Yeast and molds

NMT 100 CFU / g

Escherichia Coli

Negative / g

Coliforms

Negative / g

Staphylococcus aureus

Negative / g

Salmonella

Negative / 10 g

Storage And Handling:

AlphaSize® 100P should be kept in the sealed shipping container at 50 – 77F (10 – 25C), avoiding light and moisture, until ready for use. The container should be resealed immediately after use. The shelf life of AlphaSize® 100P is 60 months, in the original unopened container.

Regulatory Status:

Labeling of AlphaSize® 100P should be as follows: Alpha-Glycerol Phosphoryl Choline; A-GPC

Packaging:

Standard packaging for AlphaSize® 100P is per customer request

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Figure 10. Alphasize® 100P Alpha-Glycerol Phosphoryl Choline Technical Data Sheet

ChemiNutra also provided the heavy metal levels for Alphasize® 50P (Covance, 2009). A maximum level of 1 ppm is recommended. ChemiNutra noted that the heavy metal analysis was performed routinely, effective January 1, 2010.

Available data on analyses of the 50 P product are summarized below (Tables 1).

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Table 1. AlphaSize 50P analyses

Batch Number	09P0350		10P0107		10P0108		10P0286		09P0562	
	Quality Specifications	Results								
Description	White to off white powder	Conform								
Identification	TLC (Positive)	Positive								
% GPC	50 – 52%	50.6%	50 – 52%	51%	50 – 52%	50%	50 – 52%	50%	50 – 52%	51.3%
Microbial Contamination										
Total Plate Count	NMT 1,000 CFU/g	LT 100								
Total Yeast/Mold	NMT 100 CFU/g	LT 100								
<i>Escherichia coli</i>	negative/g	Negative								
<i>Staphylococcus aureus</i>	negative/g	Negative								
Coliforms	negative/g	Negative								
Salmonella	negative/10g	Negative								
Typical Composition (per 100g)										
	Dicalcium Phosphate Dihydrate 43.5 – 48.5%	46.8%	Dicalcium Phosphate Dihydrate 43.5 – 48.5%	46.9%	Dicalcium Phosphate Dihydrate 43.5 – 48.5%	47.0%	Dicalcium Phosphate Dihydrate 43.5 – 48.5%	46.8%	Dicalcium Phosphate Dihydrate 43.5 – 48.5%	47.5%
	Magnesium Stearate 0.5 – 1.5%	0.8%								
	Silicon dioxide 0.5 – 1.5%	1.0%								
	Magnesium silicate 0.5 – 1.5%	1.0%								
Heavy Metals (ICP Mass Spectrometry)										
	Arsenic	93.0 ppb	Arsenic	94.9 ppb	Arsenic	96.4 ppb	Arsenic	109 ppb	Arsenic	108 ppb
	Cadmium	11.2 ppb	Cadmium	11.3 ppb	Cadmium	12.4 ppb	Cadmium	14.2 ppb	Cadmium	17.0 ppb
	Lead	19.7 ppb	Lead	18.4 ppb	Lead	20.0 ppb	Lead	24.7 ppb	Lead	24.5 ppb
	Mercury	<10.0 ppb								
Particle Size (Sieved)	80.0% +/- 1.0% pass #80; 70.0% +/- 1.0% pass #100	80.0% pass #80; 71.0% pass #100	80.0% +/- 1.0% pass #80; 70.0% +/- 1.0% pass #100	80.6% pass #80; 71.2% pass #100	80.0% +/- 1.0% pass #80; 70.0% +/- 1.0% pass #100	80.0% pass #80; 70.9% pass #100	80.0% +/- 1.0% pass #80; 70.0% +/- 1.0% pass #100	79.7% pass #80; 70.4% pass #100	80.0% +/- 1.0% pass #80; 70.0% +/- 1.0% pass #100	80.6% pass #80; 70.9% pass #100

000030

LSRO requested results for the fillers or flow agents. ChemiNutra replied by stating that food grade ingredients are used in A-GPC formulation as seen in (Chemi Nutra, 2010a; Chemi Nutra, 2010b; Chemi Nutra, 2010c). Assay of the AA-GPC is conducted using a non-aqueous titration method. Identification is *via* TLC.

The products are certified to be free of allergens (Chemi S.p.A., 2005a), potential for BSE transmission (Chemi S.p.A., 2005c), GMO (Chemi Nutra, 2010g), pesticides (Chemi S.p.A., 2002b), aflatoxins, heavy metals, dioxins (Chemi S.p.A., 2005b), banned substances (Chemi Nutra, 2010f; Chemi S.p.A., 2002b), and animal by-products (Chemi S.p.A., 2001b; Chemi S.p.A., 2007). These certifications are based on the certified quality of the raw materials and their controlled handling and storage. ChemiNutra also asserts compliance with California's Proposition 65 (Chemi Nutra, 2010e). They have provided statements on pesticides (Chemi Nutra, 2010i) and organic solvent residues (Chemi Nutra, 2010h). The company stated that the suppliers of 35–38% high phosphatidylcholine lecithin to Chemi S.p.A. provide routine analytical documentation on residual pesticides in their material. Information about residual pesticides is also found in the technical data sheets.

A-GPC does not meet the requirements for the Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications for lecithin or partially hydrolyzed lecithin. These specifications apply to fatty-acid-containing phospholipids and A-GPC is not a fatty acid ester.

ChemiNutra states that an essentially pure working standard of 99+ % A-GPC is used to verify purity and any minuscule presence of phosphates does not interfere with the quantification of A-GPC.

An A-GPC 85% fluid exists for OTC and pharma uses in Italfarmaco's injectable and oral A-GPC called Gliatilin. ChemiNutra provided documentation of this 85% A-GPC, which is A-GPC 100 standardized with 15% water to make it fluid (Chemi S.p.A., 2009). They describe this as essentially their base 100% A-GPC out of the reactor mixed with water 100% A-GPC base called AlphaSize 100P that is also used to manufacture AlphaSize 50P and AlphaSize 50WSP.

Material safety data sheets for AlphaSize[®]50P and 50WSP are found in **Appendix B** (Chemi S.p.A., 2001a; Chemi S.p.A., 2002a).

V.6.1 Stability data

The technical data sheets for AlphaSize[®] products state that the products should be stored in the sealed shipping container at 50–77 F away from light and moisture. The container should be resealed immediately after use. The shelf life of the products is 60 months in the original unopened container. ChemiNutra stated that the retest date of 5 years, as shown on top center on the Certificate of Analysis for AlphaSize[®] 50P, AlphaSize[®] 50WSP, or AlphaSize[®] 100P is related to the fact that A-GPC is very stable, and actually exceeds the shelf life of most food and dietary supplement ingredients.

000031

ChemiNutra described A-GPC as governed by its chemical constituency as being very stable and nearly inert. Possible break down (*sic.*) compounds of A-GPC consist primarily of glycerophosphoric acid (glycerophospholipid) and choline, and generation of high levels of these compounds would have to be via forced hydrolysis caused by (intentional) extreme temperature, acid, and / or catalytic induced conditions.

VI. INTENDED USE/DIETARY EXPOSURE

VI.1. Natural Occurrence in Food

Choline is found in a variety of foods, mostly in the form of phosphatidylcholine in membranes. Milk, liver, eggs, wheat germ, and peanuts are rich sources of choline (Institute of Medicine, 1998; Zeisel, 1981). Zeisel (1981) reported that choline exists in free and esterified forms as phosphocholine glycerophosphocholine, phosphatidylcholine, and sphingomyelin.

VI.2. Institute of Medicine Adequate Intake and Tolerable Upper Limit Levels

The Institute of Medicine determined an adequate intake (AI) for choline of 550 mg/day for adult males, 425 mg/day for adult females, 450 mg/day for pregnant women and 550 mg/d for nursing mothers (Institute of Medicine, 1998) based on the amount necessary to prevent liver damage and fatty liver (Zeisel et al., 1991). These levels are equivalent to 7 mg/kg bw/day for men and women. However, 25% of pregnant women have intakes that are less than one-half of this amount (Shaw et al., 2004). Fischer et al. (2005) measured the choline content of diets consumed *ad libitum* by healthy adults housed in a clinical research center. They determined that men and women consumed similar levels of choline (8.4 and 6.7 mg/kg/d respectively; $P = 0.11$); these observed levels are similar to the AI for choline.

The Tolerable Upper Limit is 3,500 mg/d for adults, 1,000 mg/day for children between ages 1 and 8, and 2,000 mg/day for children between age 9 and 13. Table 2 shows the AI and tolerable upper limit for choline for different age groups and life stages (Institute of Medicine, 1998).

The Institute of Medicine describes the critical adverse effect from high choline intake as hypotension, cholinergic side effects (*e.g.*, sweating and diarrhea) and fishy body odor (Institute of Medicine, 1998).

A-GPC is used as a supplement with suggested daily intake of 250–500 mg, 2–3 times/day as a stand-alone product or in multi-ingredient formulations as 100–400 mg, 2–3 times/day (BioSynergy Nutraceuticals, 2009). According to the Physicians Desk Reference, intake of A-GPC ranges between 500–1,000 mg/day with 40% as choline (Physicians Desk Reference, 2008).

Table 2. Dietary reference intake values for choline

Population	Age	Adequate Intake	Tolerable Upper Limit
AI for infants	0–6 months	125 mg/day, 18 mg/kg,	Not possible to establish*
	6–12 months	150 mg/day	
AI for children	1–3 years	200 mg/day	1,000 mg/day
	4–8 years	250 mg/day	1,000 mg/day
	9–13 years	375 mg/day	2,000 mg/day
AI for males	14–18 years	550 mg/day	3,000 mg/day
	19 years and older	550 mg/day	3,500 mg/day
AI for females	14–18 years	400 mg/day	3,000 mg/day
	19 years and older	425 mg/day	3,500 mg/day
AI for pregnancy	All ages	450 mg/day	Age appropriate UL
AI for lactation	All ages	550 mg/day	Age appropriate UL

*Source of intake should be food and formula only
Adapted from Institute of Medicine (1998).

VI.2.1 Proposed foods and levels of intended use

ChemiNutra has proposed the use of A-GPC in carbonated beverages, powdered drink mixes, meal replacements, coffee, tea, milk (fluid and powdered), flavored milk/milk drinks, conventional beverages, grain and protein bars, baked goods, chocolate, candies, and chewing gum. The proposed levels of use in these foods are shown in Table 3.

Table 3. Proposed foods and levels of intended use of A-GPC

Proposed Food or Beverage	mg/serving	Serving size
Carbonated beverages	20	240 ml
Meal replacement liquid	100	240 ml
Powdered beverage	100	1 packet / 1 or 2 tablespoon(s)
Coffee	10	240 ml
Tea	10	240 ml
Milk fluid	20	240 ml
Flavored milk/milk drink	20	240 ml
Yogurt	40	225 g
Powdered milk	10	1/3 cup
Ready-to-eat breakfast cereals weighing < 20 g per cup	20	15 g
Ready-to-eat breakfast cereals weighing ≥ 20g and <43 g per cup or high fiber cereals containing 28g or more fiber per 100g	20	30 g
Ready-to-eat breakfast cereal weighing ≥ 43 g per cup or biscuit types	20	55 g
Grain-based bars	100	40 g
Protein bars	100	40 g
Chocolate	20	40 g
Candies	20	40 g
Chewing gum	20	3 g

VI.2.2 Estimated daily intake analysis

In order to estimate dietary exposure, the available USDA dietary exposure data were reviewed for these foods. The estimated intake of A-GPC was calculated using NHANES 2005–2006 data. Tables 4 and 5 show estimated exposure assuming that A-GPC is used at the proposed levels in mg/d and mg/kg bw/day, respectively. Mean dietary intake from foods with consumption data available is estimated to be 108.44 ± 3.46 mg/person/d for all users. The 90th percentile is estimated to be 196.15 ± 4.15 mg/person/d for all users. Mean dietary intake for foods with consumption data available is estimated to be 1.86 ± 0.06 mg/kg bw/d. Consumption by individuals in the 90th percentile is estimated to be 3.53 ± 0.10 mg/kg bw/d.

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Table 4. Summary of Estimated Combined Daily Intake of A-GPC from Selected Beverages, Coffee and Tea, Dairy Products, Grain Products, Protein Bars, and Snack Foods in the US by Population Group (NHANES 2005–2006 Data)

Age (Years)	Sex	% Users	Actual # Total Users	All-Person Consumption (mg)				All-Users Consumption (mg)			
				Mean	SE	90 th Percentile	SE	Mean	SE	90 th Percentile	SE
0–2	Male/ Female	72.19	667	50.56	2.13	104.04	6.38	63.92	2.31	115.08	7.90
3–11	Male/ Female	94.59	1520	80.49	2.67	146.86	5.93	84.22	2.70	149.02	6.48
12–18	Male	93.30	864	116.79	7.82	208.35	15.55	122.52	8.13	211.87	15.37
12–18	Female	91.79	861	87.18	4.55	169.31	11.01	93.52	4.70	169.85	13.20
19+	Male	95.28	2181	118.39	4.56	223.39	9.45	122.37	4.67	226.03	9.53
19+	Female	94.80	2353	102.43	7.40	182.99	6.42	106.57	7.66	184.02	6.29
Total Population	Male/ Female	92.14	8446	103.51	3.33	192.76	4.07	108.44	3.46	196.15	4.15

Specific food uses and carbonated beverages, meal replacements (liquid), powdered beverages, coffee, tea, milk (fluid), flavored milk/milk drink, yogurt, powdered milk, ready-to-eat breakfast cereals, grain-based bars, protein bars, chocolate, candies and chewing gum.

Table 5. Summary of Estimated Daily per Kilogram Intake of A-GPC from Selected Beverages, Coffee and Tea, Dairy Products, Grain Products, Protein Bars, and Snack Foods in the US by Population Group (NHANES 2005-2006 Data)

Age (Years)	Sex	% Users	Actual # Total Users	All-Person Consumption (mg)				All-Users Consumption (mg)			
				Mean	SE	90 th Percentile	SE	Mean	SE	90 th Percentile	SE
0-2	Male/ Female	72.20	665	4.10	0.17	8.55	0.39	5.18	0.18	9.27	0.56
3-11	Male/ Female	94.57	1515	3.16	0.11	6.20	0.38	3.30	0.11	6.37	0.39
12-18	Male	93.38	861	1.82	0.12	3.15	0.28	1.91	0.13	3.36	0.28
12-18	Female	91.74	855	1.47	0.08	3.03	0.16	1.57	0.08	3.12	0.16
19+	Male	95.26	2151	1.38	0.05	2.80	0.12	1.43	0.05	2.83	0.12
19+	Female	94.83	2331	1.49	0.14	2.52	0.10	1.55	0.14	2.57	0.11
Total Population	Male/ Female	92.14	8378	1.77	0.06	3.46	0.08	1.86	0.06	3.53	0.10

Specific food uses and carbonated beverages, meal replacements (liquid), powdered beverages, coffee, tea, milk (fluid), flavored milk/milk drink, yogurt, powdered milk, ready-to eat breakfast cereals, grain-based bars, protein bars, chocolate, candies and chewing gum.

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Usage of A-GPC at the proposed levels would result in total-choline mean intakes of 8.43 ± 0.05 and 8.55 ± 0.14 mg/kg bw/day for adult males and females, respectively, and 9.83 ± 0.12 and 9.57 ± 0.11 mg/kg bw/day for individuals in the 90th percentile. Table 6 shows the levels of consumption when choline intake and A-GPC intake are considered together.

Table 6. Mean and 90th percentile sums of adequate intake of choline and proposed levels of A-GPC for choline

Population	AI of Choline (mg/kg bw/day)	Proposed levels of A-GPC (Mean) (mg/kg bw/day)	AI of Choline + Proposed Levels of A-GPC (Mean) (mg/kg bw/day)	Proposed Levels of A-GPC (90 th percentile) (mg/kg bw/day)	Total AI of Choline + Proposed Levels of A-GPC (90 th percentile) (mg/kg bw/day)
Adult Males	7	1.43 ± 0.05	8.43 ± 0.05	2.83 ± 0.12	9.83 ± 0.12
Adult Females	7	1.55 ± 0.14	8.55 ± 0.14	2.57 ± 0.11	9.57 ± 0.11

VII. INFORMATION PERTAINING TO SAFETY

VII.1. Expert Reviews of Lecithin

A-GPC is chemically similar to lecithin, and is likely one of its metabolites. Therefore, expert reviews on the safety of lecithin in food are relevant to this discussion. The following excerpts are quoted from SCOGS (Life Sciences Research Office, 1979) and JECFA (1974) reviews on lecithin. Both entities note the absence of traditional toxicological studies on lecithin but endorse a strong presumption of safety based on its phospholipid structure and its normal occurrence in the diet.

- SCOGS opinion (Life Sciences Research Office, 1979)

Food grade lecithin is a complex mixture of substances derived from the processing of soybean, corn, or safflower oil. Almost all of the lecithin of commerce is derived from soybeans. Phosphoglycerides, the major constituents of lecithin, are present throughout the body as chief components of cell membranes; significant amounts are also present in bile and plasma. The major phosphoglycerides found in soy lecithin can be catabolized and also synthesized de novo in mammalian systems. Commercial lecithin may contain up to 35 percent triglycerides; these compounds occur naturally in the diet and are also catabolized and synthesized in man. The average daily consumption of lecithin added to foods by manufacturers in 1970, based on the total amount reported to be used, was 92 mg, amounting to about 1.5 mg per kg body weight for adults. The corresponding figure for lecithin bleached with hydrogen peroxide was probably less than 4mg, about 0.07 mg per kg. Thus, the lecithin added to foods amounts to only 2 to 10 percent of the 1 to 5 g of phosphoglycerides consumed daily as natural constituents of the diet. A 2 year feeding study with rats given 1400 mg lecithin per kg bodyweight daily (equivalent to a human dose of about 84 g daily) showed no adverse effects except for an increased incidence of parathyroid hyperplasia. The parathyroid hyperplasia seen in the rats probably resulted from the increased phosphate load in the diet. No adverse effects have been noted in volunteers taking 20g or more of lecithin daily for several months. The Select Committee is not aware of any animal feeding studies with "food grade" bleached lecithin. Similarly, there appear to be no studies identifying the reaction products of lecithin bleached with hydrogen peroxide. However, in another report, the Select Committee reviewed studies of animals fed compounds, which conceivably could form as a result of hydrogen

peroxide oxidation of unsaturated fatty acids. Limited feeding studies indicate these compounds are not carcinogenic when given orally and are toxic only at doses orders of magnitude greater than could be expected from the addition to food of lecithin bleached with hydrogen peroxide. No specifications are listed in the Food Chemicals Codex for the peroxide value of lecithin bleached with hydrogen peroxide; the Select Committee believes such specifications should be developed. In the light of these considerations, the Select Committee concludes that: There is no evidence in the available information on lecithin and lecithin bleached with hydrogen peroxide that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future.

- JECFA evaluation (JEFCA, 1974)

Lecithin is an essential constituent of all cells of the human body. The organism is able to synthesize phosphatides and the pathway of catabolism of lecithin in the organism is well-known. The average diet provides a daily intake of several grams of lecithin (approximately 1–5 g).

Although fewer toxicological studies have been conducted than would normally be required for substances used as food additives, it is considered that nutritional and clinical experience with lecithin is sufficiently extensive to compensate for the incompleteness of the experimental data. Since many observations have been made in man it is not considered necessary to calculate the safe intake level from animal experiments.

VII.2. Toxicology Studies Conducted by ChemiNutra

Summaries of several unpublished toxicology studies performed on A-GPC were provided by ChemiNutra (De Caro, 1986). A subset of these studies was published in Brownawell et al. (2011).

VII.2.1 Acute toxicity

VII.2.1.1 Mice

The acute oral toxicity of A-GPC was investigated in male and female Swiss mice (6 mice/sex/group) at doses of 2,500, 5,000 and 10,000 mg/kg. Behavior was observed carefully for 6 hours post-administration and then once *per day*. Post-mortems were performed on all dead animals and survivors sacrificed at the end of the two-week observation period. Mice in the 10,000 mg/kg group experienced severe prolonged reduced activity for 12–36 hours. Reduced activity was low and transient for the low dose and was generally mild in animals receiving the intermediate dose. Lethality in mice (33% males, 50% females) was seen at 10,000 mg/kg body weight, the highest dose administered. No deaths occurred in the 2,500 and 5,000 mg/kg groups.

The acute toxicity of intravenous doses of A-GPC delivered *via* the tail vein within 10 seconds was studied in Swiss mice. Male and female mice (6 mice/sex/dose group) received doses of 521, 729, 1,020, 1,429, or 2,000 mg/kg A-GPC. The 2,000 mg/kg dose was lethal to all animals within 24 hr of dosing. The mortality for male and female mice, respectively, was 0% and 0% at the 521 mg/kg dose, 0% and 16.6% at the 729

mg/kg dose (8.3% mortality M + F), 33.3% and 66.6% at the 1,020 mg/kg dose (50% mortality M + F), and 50 and 66.6% at the 1,429 mg/kg dose (58.3% mortality M + F). Some animals experienced convulsions prior to their death. For survivors, symptoms of toxicity included reduced or absent motility, reduced activity, and bradypnea or dyspnea, which was dose-dependent in severity and duration. The signs lasted for 24–48 hours for animals that received the higher doses. Weight loss or retardation of growth was observed in the first week but recuperation occurred the next week. The LD₅₀ was 1,267 mg/kg for males (C.I. = 1,056–1,520), 1,027 mg/kg for females (C.I. = 837–1,260), and 1,143 mg/kg for male and female mice (C.I. = 992–1,316).

The acute toxicity of A-GPC was studied at doses of 781, 1,093, 1,531, 2,143, and 3,000 mg/kg administered intraperitoneally to male and female Swiss mice (6 mice/sex/dose group). Toxic symptoms included reduced activity that was dose-dependent in terms of severity and duration. Some animals also displayed writhing movements, indicating local pain. The mortality rates were 0% for both males and females at 781 mg/kg, 0% males and 16.6% females (8.3% M + F) at 1,093 mg/kg, 33.3% male and 33.3% females (33.3% M + F) at 1,531 mg/kg, and 50% males and 50% females (50% M + F) at 2,143 mg/kg, within 14 d of receiving A-GPC. The 3,000 mg/kg dose was lethal for 83.3% of male mice and all female mice (91.6% M + F). The LD₅₀ was 2,053 mg/kg for males, 1,809 mg/kg for females, and 1,927 mg/kg for males and females.

VII.2.1.2 Rats

The acute oral toxicity of A-GPC was investigated in male and female Sprague Dawley[®] rats at doses of 2,500, 5,000, and 10,000 mg/kg (6 rats/sex/dose). No animals died within 14 d of receiving 2,500 or 5,000 mg/kg A-GPC doses. Mortality rates for the 10,000 mg/kg groups during the same time period were 16.6% and 33.3% for male and female rats, respectively, and 25% for overall mortality. The 10,000 mg/kg dose produced reduced activity that was generally severe and of varying duration (3–24 hours). Lower doses of A-GPC resulted in a less severe, shorter duration (1–6 hours) effects. The LD₅₀ was estimated as >10,000 mg/kg body weight. Necropsies did not reveal any changes attributable to A-GPC.

The acute intravenous toxicity of A-GPC administered *via* tail vein within 10 seconds was assessed in male and female Sprague Dawley[®] rats (6 rats/sex/dose) at doses of 781, 1,093, 1,531, 2,143, and 3,000 mg/kg. Symptoms of toxicity and the timing of death were similar to those for mice however A-GPC was slightly less toxic to rats than to mice. Mortality rates were 0% for male and female rats at 781 mg/kg; 16.6% for males, females, and both sexes at 1,093 mg/kg; 50% for males, females, and both sexes at 1,531 mg/kg; 66.6% for males, 83.3% for females, and 75% for both sexes at 2,143 mg/kg; and 100% for both sexes at 3,000 mg/kg. The LD₅₀ for male rats was calculated to be 1,621 mg/kg (C.I. = 1,323–1,986), 1,531 mg/kg for female rats (C.I. = 1,269–1,848), and 1,575 mg/kg for both sexes (C.I. = 1,372–1,809). Necropsies showed no changes attributed to administration of A-GPC.

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The acute intraperitoneal toxicity of A-GPC was assessed in male and female Sprague Dawley® rats using doses of 781, 1,093, 1,531, 2,143, and 3,000 mg/kg AA-GPC. Mortality rates were 0% for males and females at 781 and 1,093 mg/kg, 16.6% for males, females, and both sexes at 1,531 mg/kg, 33.3% for males, 50% for females, and 41.6% for both sexes at 2,143 mg/kg, and 100% for both sexes at 3,000 mg/kg. The LD₅₀ was 2,125 (C.I. = 1,797–2,513) mg/kg for male rats, 2,017 (C.I. = 1,722–2,362) mg/kg for female rats, and 2,073 (C.I. = 1,849–2,323) mg/kg for both sexes. Autopsies showed no changes attributable to A-GPC.

The LD₅₀ for the rodent studies are summarized in Table 7.

Table 7. Summary of Acute Toxicity in Rodents

Animal	Route	LD ₅₀ mg/kg (confidence interval)	
		Males	Females
Mouse	i.v.	1,267 (1,056–1,520)	1,027 (837–1,260)
Mouse	i.p.	2,053 (1,644–2,564)	1,809 (1,459–2,243)
Mouse	p.o.	>10,000	10,000
Rat	i.v.	1,621 (1,323–1,986)	1,531 (1,269–1,848)
Rat	i.p.	2,215 (1,797–2,513)	2,017 (1,722–2,362)
Rat	p.o.	>10,000	>10,000

i.v., intravenous; i.p., intraperitoneal; p.o., per os (orally)

Adapted from (De Caro, 1986) and Brownawell et al. (2011).

VII.2.1.3 Dogs

The acute oral toxicity was determined by Maximum Tolerated Dose (MTD) technique in beagle dogs of both sexes. Doses of 1,000 and 3,000 mg/kg were administered orally to 2 males and 2 females (3 ml/kg dose volume in distilled water). The animals were observed carefully during the six hours after administration and daily for 2 weeks. No mortality was observed at either dose. Mild reduced activity lasting 3–6 hours following administration of the highest dose was the only symptom observed; there was no effect on animal weight during the course of the study. The oral LD₅₀ was estimated to be >3,000 mg/kg.

The same 4 dogs also received 200 mg/kg and 500 mg/kg A-GPC doses (intramuscular – bilateral injection in the gluteofemoral region) in a dose volume of 1.5 ml/kg distilled water. The animals were observed carefully during the first six hours after administration of A-GPC and daily for two weeks. Animals in the 200 mg/kg group experienced mild reduced activity for 1–3 hours. One animal that received the 500 mg/kg body weight dose experienced more severe effects with bradypnea that lasted about 24 hours. The dogs in the higher-dose group also showed signs of pain such as yelping and licking of the injection area; this response was attributed to excessive concentration of the test solution. Mild reduced activity lasting 3–6 hours following administration of the highest dose was observed. No mortality was observed at either dose. The intramuscular LD₅₀ was estimated to be >500 mg/kg.

VII.2.2 Subchronic Toxicity

VII.2.2.1 Rats

VII.2.2.1.1 Oral route

Eighty Sprague Dawley® rats were divided into 4 groups (n = 10 rats/sex/dose group). Rats received distilled water (control), 100 mg/kg/d A-GPC, 300 mg/kg/d A-GPC, or 1,000 mg/kg/d A-GPC at the same time of day for 4 weeks. Oral administration of A-GPC at doses of 100 and 300 mg/kg body weight to Sprague Dawley® rats for 4 weeks did not result in any sign of general toxicity or change in animal behavior. There was no significant difference in body weights. No significant variations in hematology (hematocrit value, red blood cell [RBC] count, white blood cell [WBC] count, and WBC differential counts), blood chemistry (glucose, blood urea nitrogen [BUN], creatinine, proteins, serum glutamic oxaloacetic transaminase [SGOT], serum glutamic pyruvic transaminase [SGPT], and alkaline phosphatase) assessments, or urinalysis, including specific gravity, pH, proteins, glucose, ketone bodies, bilirubin, and urobilinogen, were observed at any dose, nor was there variation between groups in gross anatomy or histopathology. The only symptom observed at the 1,000 mg/kg/d dose was reduced activity, during the second half of the treatment period, that occurred in almost all female rats and half of male rats with variable intensity and duration. One male rat died during the course of the study due to a gavage error. Oral administration of rats with 100, 300 or 1,000 mg/kg/day A-GPC for 4 weeks resulted in no toxicological changes.

VII.2.2.1.2 Subcutaneous route

The toxicity of subcutaneously administered A-GPC at doses of 50, 150, or 500 mg/kg body weight/d to Sprague Dawley® rats (n = 10 rats/sex/dose group) for 4 weeks was investigated. The final dose volume was 3 ml/kg for all doses and the vehicle was distilled water for the highest dose and saline for the two lower doses. A-GPC had no significant effect on weight gain after 4 weeks at any dose. Hematological and biochemical assessments revealed no differences between animals treated with A-GPC and control animals of the same sex. Gross autopsies of heart, lungs, liver, spleen, kidneys, adrenals, and gonads and histological studies of brain, hypophysis, thymus, trachea, thyroid, esophagus, stomach, small gut, large gut, pancreas, bladder, prostate, uterus, eyes, spinal cord, ribs, femur, lymphatic ganglia, mammary glands, salivary glands, lymph nodes, skin and subcutaneous tissues indicated no abnormal changes. Some animals experienced skin thickening and induration at the injection site and had histological evidence of recent bleeding or hemorrhagic reabsorption with or without signs of dermal inflammation. These occurrences tended to be more frequent in rats in the highest-dose group, but a correlation between these occurrences and A-GPC could not be made because of the small difference. Occurrence of any pathological or borderline variations in test value was not dose-dependent. Animals receiving the 500 mg/kg dose experienced sedation as evidenced by decreased motility, poor reactivity and ptosis of the eyelids. These symptoms typically developed within an hour of dosing, and were generally mild and of variable duration (3–8 hours). No animals died during

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the course of the study and no differences between controls and A-GPC treated rats were observed.

VII.2.2.2 Dogs

VII.2.2.2.1 Oral route

Beagle dogs of both sexes were divided into 4 groups of 1 male and 1 female and were administered the following treatments by oral gavage (dose volume 1 ml/kg) for 4 weeks at the same time of day until the day before the animals were sacrificed: distilled water, 75 mg/kg A-GPC, 150 mg/kg A-GPC, and 300 mg/kg A-GPC. No abnormal autonomic and behavioral changes were observed in the 75 and 150 mg/kg groups. Mild reduced activity in the 300 mg/kg group was observed during the last week of treatment and was more pronounced in the female dog. Normal body weight and food intake was observed in all groups, and there were no drug-related differences between groups in hematology, blood chemistry, urinalysis, and in the absolute and relative organ weights. All animals survived the treatment. The authors attributed the few changes that occurred to spontaneous pathology.

VII.2.2.2.2 Intravenous route

One male and one female dog each received one of the following: 30 mg/kg A-GPC, 60 mg/kg A-GPC, 120 mg/kg A-GPC, or saline (control) in the morning for 4 weeks until the day before they were sacrificed. The female beagle that received the 30 mg/kg body weight dose of A-GPC occasionally experienced mild and transient sedation in the second and third weeks of the study, but was otherwise normal in terms of general health and behavior. The dogs in the 60 mg/kg and 120 mg/kg groups immediately became depressed on receiving A-GPC. The depression lasted for 1–3 hours and 3–5 hours, respectively. The authors described this state of depression as moderately dose-dependent. General health was otherwise normal. There was no difference in body weight, food consumption, hematology, blood chemistry, urinalysis, histology, and in the absolute and relative organ weights. Some dogs experienced mild phlebic reactions at the injection site, but these were not correlated with dose of A-GPC. Any other abnormal changes were considered by the authors to be due to spontaneous pathology.

VII.2.3 Chronic Toxicity

VII.2.3.1 Rats

VII.2.3.1.1 Oral route

Brownawell et al. (2011) reported studies on oral toxicity of A-GPC in rats. One hundred forty-four Sprague Dawley[®] rats were divided into four treatment groups with 18 males and 18 females *per* group. The rats were administered the following treatments by gastric tube once per day for 26 weeks: distilled water (controls), 100 mg/kg A-GPC, 300 mg/kg A-GPC, or 1,000 mg/kg A-GPC in a final dose volume of 5 ml/kg. At the end

of the dosing period, a quota of animals in the 1,000 mg/kg and control groups were chosen as recovery animals and were observed for an additional 4 weeks to determine if effects observed after 26 weeks were reversible. General health and behavior of the animals was assessed daily during the pretest and the dosing phases of the study; animal weight was measured weekly for the first 3 months and then once every 2 weeks. Food consumption was measured at 2-week intervals during the first 3 months and at 4-week intervals after the first 3 months. Blood samples were collected from the retro-orbital plexus under fasting conditions for hematology and clinical chemistry analyses during week 13. Urine samples were also collected. Hematology measures included hematocrit, hemoglobin, erythrocyte count, platelet count (during weeks 13 and 26) total and differentiated leukocytes, prothrombin time (week 26). Measurements for clinical chemistry included glucose, BUN, creatinine, AST, ALT, alkaline phosphatase, total serum proteins, bilirubin, cholesterol, triglycerides, sodium, and potassium). Measurements for urinalysis included specific weight, pH, protein, bilirubin, and blood. Necropsies were conducted and organ weights were measured.

VII.2.3.1.1.1 Mortality, behavior, and general health

Ten rats died during the course of the study (approximately 7% mortality rate). Two rats in the control group died; the causes of death were pulmonary infection and perforated gastric ulcer. Four rats in the 100 mg/kg group died; 2 deaths were due to gavage errors and 2 were due renal necrosis. Two rats in the 300 mg/kg group died. One died from gavage error and another from unknown causes and 2 in the 1000 mg/kg group died from pulmonary infection. No deaths were attributed to A-GPC treatment. In the 1,000 mg/kg group, reduced motor activity and reduced reactivity to stimulation were observed from week 3-4 of treatment through the end of study. These symptoms surfaced within 1 or 2 hours of receiving A-GPC, lasted for 3-5 hours, and were of variable, but mild to moderate severity. No behavioral effects were evident in rats that received the 100 and 300 mg/kg A-GPC doses. There were instances of semi-liquid stools in boxes housing control animals and those that received the 100 mg/kg dose, but not in the boxes of animals who were given the other doses. Alopecia and thinning of the fur occurred with similar frequency across groups (2 in the control group, 2 in the 100 mg/kg group and 3 in the 300 and 1,000 mg/kg groups) during the final 8 weeks of the study.

VII.2.3.1.1.2 Animal weight and feed consumption

No clinically significant differences in food intake or weight gain during the course of the study were observed in the groups of male and female rats that received 100 and 300 mg/kg doses of A-GPC. A significant difference in body weight of rats in the 1,000 mg/kg group compared with control was observed from the 6th week of treatment through the end of the study ($P < 0.01$ for males and $P < 0.05$ for weeks 6-14 for females and $P < 0.01$ for weeks 18-26 for females), which was due to reduced food consumption. A significant reduction in food intake compared with controls began during Week 4 of treatment ($P < 0.05$) and occurred at every time point except at Week 14 for males and Weeks 14 and 26 for females. Food consumption reverted to normal during the post-study recovery period for male rats and by week 26 for female rats. During

Week 12, food consumption was significantly higher in the female 100 mg/kg group than in controls (22.8 ± 0.4 vs. 20.9 ± 0.4 g/animal/d; $P < 0.05$). During Week 26, male rats in the 300 mg/kg group had a lower weight gain than controls (24.9 ± 1.1 vs. 28.3 ± 0.7 ; $P < 0.05$).

VII.2.3.1.1.3 Clinical findings

No significant clinical findings were observed after 13 weeks of oral treatment with A-GPC. At Week 13 there were no significant differences in levels of hemoglobin, hematocrit, RBC, WBC, monocytes, eosinophils, and basophils for male rats. There were significant differences between the 100 mg/kg group and control group of male rats for lymphocytes ($71.89 \pm 1.64\%$ vs. $75.11 \pm 0.79\%$; $P < 0.05$) and neutrophils ($24.33 \pm 1.28\%$ vs. $21.22 \pm 0.68\%$; $P < 0.05$). At Week 13, there were no significant differences in levels of hemoglobin, hematocrit, WBC, monocytes, eosinophils, and basophils for female rats. RBC and neutrophil levels for female rats were significantly different for the 100 mg/kg group compared with controls ($7.80 \pm 0.32 \times 10^6/\text{cu mm}$ vs. $7.02 \pm 0.08 \times 10^6/\text{cu mm}$ and $22.38 \pm 0.82\%$ vs. $20.40 \pm 0.54\%$ ($P < 0.05$), respectively. In the male 300 mg/kg group, there were significantly increased levels of urea (31.73 ± 1.40 vs. 25.98 ± 1.43 mg %; $P < 0.05$), while in the female 1,000 mg/kg group a significant decrease in creatinine (0.607 ± 0.04 vs. 0.829 ± 0.07 mg %; $P < 0.05$) was observed. At 26 weeks there were no significant differences in levels of hemoglobin, hematocrit, RBC, WBC, lymphocytes, neutrophils, monocytes, eosinophils, basophils, and prothrombin times either in the male or female 1,000 mg/kg groups, or for any of the other female rat dose groups. This was also the case for the male rats with the exception of RBC levels, which were significantly higher for 300 mg/kg than for controls ($8.40 \pm 0.24 \times 10^6/\text{cu mm}$ vs. $7.63 \pm 0.27 \times 10^6/\text{cu mm}$; $P < 0.05$); and platelets, which were significantly higher for 100 mg/kg than for controls ($859.2 \pm 18.7 \times 10^3/\text{cu mm}$ vs. $798.8 \pm 15.1 \times 10^3/\text{cu mm}$; $P < 0.05$). In the highest-dose group, significantly decreased plasma triglycerides were measured in males (38.79 ± 2.13 vs. 46.27 ± 1.95 mg %; $P < 0.05$) and females (36.11 ± 1.87 vs. 44.76 ± 2.30 mg%; $P < 0.05$), and decreased plasma bilirubin (0.218 ± 0.02 vs. 0.449 ± 0.04 mg%; $P < 0.05$), and SGPT (24.38 ± 1.00 vs. 29.26 ± 1.21 mg %; $P < 0.01$) were measured in females. By the end of the recovery period, all values returned to normal. Necropsy and histopathological findings were within normal limits at all dose levels.

VII.2.3.1.2 Subcutaneous route

The chronic toxicity of subcutaneous (laterodorsal) injections of A-GPC was investigated in 144 Sprague Dawley® rats (1986). The rats were divided into 4 groups, (18 male and 18 females). Groups received 50 mg/kg, 150 mg/kg, or 300 mg/kg A-GPC, or saline (control), daily for 26 weeks.

VII.2.3.1.2.1 Symptoms and mortality

Five animals died during the study: 1 female from the control group, 1 male and 1 female from the 50 mg/kg group, 1 male from the 150 mg/kg group and 1 male from the 300 mg/kg dose. These animals lacked a common organic toxicity.

VII.2.3.1.2.2 Behavior and general health

Injection with 500 mg/kg of A-GPC resulted in sedation that was more prolonged during the second month of the study, lasting for 3–8 hours after dosing in the first month and in some cases lasting for 12 hours in later stages of the study. The general health of the animals, as evidenced by skin trophism and the condition of the mucosal linings, was generally good for the lower-dose groups; however, the high-dose groups experienced some deterioration in their general health. Across all groups there were incidences of passage of loose or semi-liquid stools and hair loss from the back and stomach.

VII.2.3.1.2.3 Animal weight and feed consumption

With the exception of Weeks 6 and 8, when male rats in 150 mg/kg group had a significantly higher ($P < 0.01$) weight gain than controls, rats that received the 50 and 150 mg/kg A-GPC doses were not significantly different from controls. In contrast, the body weight of rats that received the 500 mg/kg dose were significantly different ($P < 0.01$) from controls from week 6 through week 26. The reduction in weight gain was attributed to lower food consumption after A-GPC treatment.

VII.2.3.1.2.4 Clinical findings

At Week 13, male rats in the 50 and 500 mg/kg groups had significantly higher percentages of eosinophils compared with controls (2.00 ± 0.37 and 2.00 ± 0.33 vs. 1.00 ± 0.26 , respectively; $P < 0.05$), but there were no differences between A-GPC treated female rats and controls. These values were within the normal range. Levels of hemoglobin, hematocrit, RBC, WBC, lymphocytes, neutrophils, monocytes, and basophils were not significantly different from controls for males or females. Also at Week 13 there was no significant difference in urea, creatinine, SGOT, or SGPT levels between groups. At Week 26 there were no significance differences in hematology measures among males or female rats. Biochemical assessments revealed no significant differences among male rats in the 50 and 150 mg/kg groups, or females in the 50 mg/kg group. Significantly lower levels of triglycerides (38.60 ± 2.53 vs. 50.15 ± 1.58 mg %; $P < 0.01$) and alkaline phosphatase (27.70 ± 0.82 vs. 33.00 ± 0.93 IU/L; $P < 0.01$) were reported for the 500 mg/kg group compared to controls. Female rats in the 150 mg/kg and 500 mg/kg groups had a significantly lower level of cholesterol than control rats (45.11 ± 1.33 mg % and 48.34 ± 1.86 mg % vs. 53.64 ± 1.83 mg %; $P < 0.01$ and 0.05 , respectively). Female rats in the 50 mg/kg group had higher levels of urea (31.64 ± 1.85 mg % vs. 25.72 ± 1.59 mg %; $P < 0.05$) and creatinine (0.948 ± 0.06 mg % vs. 0.657 ± 0.05 mg %; $P < 0.01$). At Week 26 there were no significant differences in urinalysis measures for male and female rats and organ weights for male rats; however, the hearts of female rats in the 500 mg/kg group weighed significantly less than those of control rats (751.5 ± 13.6 vs. 840.2 ± 26.7 mg %; $P < 0.05$). Reduced

heart weight was attributed to a lower total vasculature resulting from the reduced weight in this group. Histological assessment conducted on the skin and subcutaneous tissue at the injection site revealed changes within the normal range. There was an increased occurrence of pulmonary inflammation in animals who received the highest dose of A-GPC. The author stated that this likely resulted from a general deterioration of animals or an increased prevalence of concomitant infections. In the high-dose group there was a higher frequency of small subcutaneous inclusions with focal bleeding and inflammatory cell crowding. The authors noted that this suggests a potential effect of the test product beyond the mechanical action of the injection.

VII.2.3.2 Dogs

VII.2.3.2.1 Oral toxicity

Twenty-four beagle dogs were randomly divided into 4 groups (3 dogs/sex/group) and administered daily the following treatments by oral gavage for 26 weeks: distilled water (controls), 75 mg/kg A-GPC, 150 mg/kg A-GPC, 300 mg/kg A-GPC. Distilled water was used as the vehicle in a 1 ml/kg dose volume. The dogs had been vaccinated against rabies, canine distemper, viral hepatitis, and leptospirosis and had undergone a 3-week period of acclimation and observation. The dogs were administered treatment in the morning and fed in the afternoons. The general condition of the animals was checked daily and they were weighed monthly for the first 3 months and at the end of the study. The investigators collected venous blood samples under fasting conditions prior to the beginning of the study and the end of the 13 and the 26th weeks for hematological and clinical chemistry analyses. Urine samples were also collected at these time points. Necropsies were conducted on each animal at the end of the study and histopathological examination of select tissues was conducted.

VII.2.3.2.1.1 Behavioral symptoms and mortality

No animals died during the course of the study and no effects on behavior were noted for the 75 and 150 mg/kg groups. In the 300 mg/kg group, moderate sedation (lasting 2–5 hours, starting in the second week of the trial) was observed for some animals.

VII.2.3.2.1.2 Weight

A decrease in food intake and a reduction in body weight gain were observed in the high-dose group but not the other groups, with a significant difference being observed only at 13 weeks. There was a significant difference in weight in the male 300 mg/kg group compared to controls (9.20 ± 0.35 vs. 10.40 ± 0.20 kg; $P < 0.01$) at Week 13, but not for female rats in the control or any A-GPC groups at Weeks 0, 4, 8, or 26. Some of the animals in the 300 mg/kg group experienced anorexia.

VII.2.3.2.1.3 Clinical findings

Clinical chemistry tests performed on Week 13 showed a significant increase in plasma cholesterol (155.5 ± 5.44 vs. 127.8 ± 6.66 mg %; $P < 0.05$) and decrease in alkaline phosphatase (34.07 ± 0.86 vs. 40.68 ± 0.66 ; $P < 0.01$) in the 150 mg/kg group compared to the control group. In the 300 mg/kg/d group, significant decreases in plasma bilirubin (0.210 ± 0.05 vs. 0.472 ± 0.06 mg %; $P < 0.01$), plasma triglycerides (37.90 ± 2.86 vs. 57.55 ± 5.24 mg %; $P < 0.01$), and alkaline phosphatase (28.58 ± 0.53 vs. 31.33 ± 1.39 mg %; $P < 0.01$) compared to control were observed at Week 26. Data for clinical chemistry suggested reduced liver function. However, no dose-effect relationship was evident. Week 26 also evinced a statistically significant decrease in triglyceride levels between the control and 75 mg/kg groups (57.55 ± 5.24 vs. 43.58 ± 4.76 ; $P < 0.05$). No other treatment-related changes or abnormalities were observed.

VII.2.3.2.1.4 Gross anatomy and histology

There was no significant difference in the weights of any of the organs studied: brain, heart, lung, liver, spleen, kidney, adrenals, testes, ovaries, and thymus. There were dose-related decreases in weights of the liver and the heart that were not statistically significant.

VII.2.3.2.2 Intramuscular toxicity

Daily intramuscular A-GPC injections administered to male and female beagle dogs for 26 weeks were investigated for toxicity. The 4 treatment groups (3 dogs/sex/dose group) were: 30 mg/kg, 60 mg/kg, 120 mg/kg and saline (control). General health and behavior was assessed each day at the time of injections; animals were weighed once per week for the 3 months, and then once every 2 weeks. Daily food consumption and hematology measures (including hemoglobin assay, hematocrit, RBC, WBC, WBC differential count, platelet count, and prothrombin time) were measured. Blood biochemistry measures (including glucose, BUN, creatinine, proteins, cholesterol, triglycerides, bilirubin, SGOT, SGPT, serum alkaline phosphatase, sodium, and potassium) were assessed at Weeks 13 and 26. Urinalysis included measures of specific gravity, pH, glucose, proteins, ketone bodies, bilirubin, and blood. Gross autopsies of all animals were conducted and the brain, heart, lungs, liver, spleen, kidneys, adrenals, gonads, and thymus were weighed. Histological examination were conducted of the following organs: hypophysis, trachea, thyroid, esophagus, stomach, small gut, large gut, pancreas, bladder, prostate, uterus, eyes, spinal cord, ribs, femur, lymphatic ganglia, mammary glands, salivary glands, lymph nodes, gallbladder, and the tissue at and around the injection sites.

VII.2.3.2.2.1 Behavioral symptoms and mortality

All animals survived the treatment. There was no intolerance and no dose-response relationship between pathological occurrences (such as acute conjunctivitis, retroscapular abscess, loose stools or diarrhea or vomiting) and treatment dosages. Dogs receiving the highest dose experienced a mild decrease in spontaneous activity; however, reflexes and reactivity to stimulation remained intact.

VII.2.3.2.2 Weight and food consumption

Animals treated with A-GPC had consistently lower weights than controls but these differences were not statistically significant. The authors suggest that that a larger sample size would have made this difference statistically significant. Six animals experienced anorexia.

VII.2.3.2.3 Clinical findings

At Week 13, there was a significant difference in glucose levels between control and 30 mg/kg groups (98.23 ± 3.10 vs. 111.2 ± 3.57 mg %; $P < 0.05$). There was no significant between-group difference in any hematology measure. At Week 26 there were significant differences in bilirubin between the 120 mg/kg and control groups (0.252 ± 0.03 vs. 0.413 ± 0.04 mg %; $P < 0.01$). In addition, alkaline phosphatase levels in the 60 mg/kg (30.23 ± 1.35 IU/L; $P < 0.05$) and 120 mg/kg groups (27.65 ± 1.05 IU/L; $P < 0.01$) were significantly lower than in the control group (34.42 ± 1.34 IU/L).

VII.2.3.2.4 Gross anatomy and histology

Liver weight of the 60 mg/kg group was significantly lower than that of the control group (283.0 ± 4.98 g vs. 308.3 ± 11.5 g; $P < 0.05$). The organ weights of the 30 and 120 mg/kg groups were not significantly different from control animals

VII.2.3.2.3 Reproductive toxicity

VII.2.3.2.3.1 Fertility and general reproduction

Sprague Dawley[®] rats were divided into 4 treatment groups: control (saline), 50 mg/kg A-GPC, 150 mg/kg A-GPC, and 500 mg/kg/d A-GPC s.c. The vehicle was distilled water for the high-dose group and saline for the other doses; the dose volume was 3 ml/kg. Twenty four male and female Sprague Dawley[®] rats were included in each treatment group. Male rats received subcutaneous injections once daily with A-GPC or control for 9 weeks and throughout the mating period. Female rats received subcutaneous injections for 4 weeks before mating and until pregnancy was confirmed by detection of a vaginal plug or identification of spermatozoa in the vaginal smear. The mortality, general health and behavior, weight, and number of fertile matings were analyzed for generation P₁. Approximately 50% of the pregnant P₁ females from each dose group were sacrificed at 20 days of pregnancy and a laparotomy and Caesarian section were conducted. The number of female rats carrying live fetuses, dead fetuses, live and dead fetuses, and completely reabsorbed embryos was determined. The number of *corpora lutea*, implant sites, reabsorbed embryos, and fetuses were determined; the percentages of pre-implant losses (*i.e.*, the percent ratio of the number of implants and that of *corpora lutea* to that of implants) and post-implant losses (*i.e.*, the percent ratio of the number of live fetuses to that of implants) were determined. The number, weight and sex of fetuses and number of live and dead fetuses were determined. Viable

fetuses were those that weighed more than 70% of the mean for the litter and showed spontaneous respiration and or spontaneous reflex moves at extraction.

VII.2.3.2.3.1.1 P₁ generation

No male animals died prior to mating, but one female from the 500 mg/kg group died after 10 days of treatment. Animals in the 500 mg/kg/d group exhibited mild sedation beginning in Week 5 and had a significantly lower growth rate in the weeks prior to mating (Week 9: 343.5 ± 2.4 vs. 355.2 ± 1.7 ; $P < 0.01$). One female rat in the 50 mg/kg group exhibited true sterility. The male fertility index was 100% for all groups; the female fertility index was 95.8% in the 50mg/kg group and 100% for all other groups. Treatment had no significant effects on the number of *corpora lutea*, implant sites, reabsorbed embryos, or total number of fetuses extracted. All treatment groups were homogenous in terms of pre-implant losses (controls: 15.3%; 50 mg/kg: 15.7%; 150 mg/kg: 14.6%; 500 mg/kg: 17.3%) and post-implant losses (controls: 6.0%; 150 mg/kg: 5.7%; 150 mg/kg: 7.4%; 500 mg/kg: 7.5%). Early and complete reabsorption of embryos occurred for one rat in the control group and one rat in the high-dose group. There was no significant difference between groups in fetal birth weights and weights at weaning time. Fetal viability was homogenous across groups in fetal litter size, deaths of newborn rats at post-partum days 0, 4, and 21, and the viability indices (percent ratio of live newborns on Day 4 to those on Day 0) and nursing index (ratio of live baby rats on Day 21 vs. Day 4)

VII.2.3.2.3.1.2 F₁ generation

Data for stillborn fetuses and fetal weight at birth indicated no fetal toxicity of A-GPC. One major malformation was identified in a fetus whose mother was treated with 50 mg/kg; otherwise there was no effect on gamete quality and minor abnormalities occurred with similar frequency between the treatment groups. There was one major malformation in the control group and another in the high-dose group but this had no effect on development as assessed up to weaning time. Both male and female F₁ rats had fertility indices of 100%. All female F₁ rats had normal pregnancies except one in the control group and another in the 500 mg/kg group. F₁ females sacrificed after delivery were not significantly different in terms of the number of *corpora lutea*, implant sites, reabsorbed embryos, and total fetuses. F₂ litters showed no significant differences with regard to the number of live and still births, sex distribution or fetal weight. One malformation was found in the low-dosage group and the intermediate-dosage group.

VII.2.4 Teratogenesis

VII.2.4.1 Rats

The teratogenicity of A-GPC was investigated by mating 1 virgin male Sprague Dawley[®] rat of proven fertility with 3 female Sprague Dawley[®] rats. Day 0 was the day after observation of a positive vaginal smear (first day of pregnancy). Mated females were assigned to 1 of 4 treatment groups (24/dose group): control (saline), 50 mg/kg A-GPC,

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150 mg/kg A-GPC, and 500 mg/kg A-GPC in a dose volume of 3 ml/kg for all doses. Mated female rats received daily subcutaneous injections from the sixth to the fifteenth day of pregnancy, *i.e.*, the organogenesis period, at the same time of day. The general status behavior and mortality

The pregnant rats remained in good general health and there were no significant differences in animal weight between the 4 groups. Rats treated with A-GPC exhibited mild sedation occasionally that subsided during the final days of pregnancy and after treatment was discontinued on Day 16. On Caesarian section it was noted that fetus reabsorption occurred in the control, 50 mg/kg A-GPC, and 500 mg/kg groups. There were no significant differences in *corpora lutea* and implant sites and in pre- and post-implants losses between groups. A-GPC did not appear to affect the course of pregnancy post-implantation.

One fetus died in the control and 50 mg/kg groups, as did three in the 150 mg/kg group. No fetuses in the high-dose group died. There were no significant differences in fetal weight between the various treatment groups. Major fetal malformation was observed in the control and the 150 mg/kg groups (n = 1/group) and there was no significant difference in the frequency of minor malformations of skeletal formation. There was no significant difference across treatment groups in the number of fetuses with minor defects.

VII.2.4.1.1 Perinatal and postnatal toxicity

Female Sprague Dawley® rats that had been mated with male Sprague Dawley® rats of proven fertility were divided into 4 treatment groups: control physiological saline 50 mg/kg, 150 mg/kg, and 500 mg/kg A-GPC. The vehicles used were distilled water for the high dose and dilution with physiological saline in the other doses; dose volume was 3 ml/kg for all doses. Each treatment group included 15 pregnant rats that received daily subcutaneous injections of A-GPC at the same time of day from Day 15 of pregnancy to Day 21 postpartum. One of the fifteen animals in the 50 mg/kg group did not conceive. Complete reabsorption of all embryos occurred in one animal in the control group and one in the 150 mg/kg group. There were no significant differences in body weight between treated and control rats during the latter part of pregnancy or during the nursing period (treatment period). Rats that received the 500 mg/kg exhibited sedation more frequently and more intensely during the final two weeks of the nursing period. The effect was mild and did not compromise the quality of parental care. No behavioral effects were noted in rats that received the lowest A-GPC dose and cannibalization of the litter was not observed.

VII.2.4.1.1.1 F₁ litters

There was no significant difference between treatment groups and controls with regard to litter size, sex distribution, and mean weight *per* litter. There appeared to be no effect of A-GPC on prenatal mortality. The authors decreased the number of newborn animals *per* litter by randomizing to not more than 10 to create similarity for nursing and to

“increase the significance of mortality and animal growth data” (p 322) (Italfarmaco, 1997). Animal mortality was variable; rats in the control group and those given the 500 mg/kg dose had the highest mortality. The authors interpreted the data as evidence of no test product toxicity in the peri- or post-natal period, and stated that this was supported by the viability and nursing indices. There was no significant difference between the treated and control rats in mean weight at birth and successive measurements. Fur growth, eruption of the incisor teeth, and eye and ear opening were not significantly different between treated groups and control animals. No significant difference between groups was observed for neurological or sensory development of pups in principal reflexes (tail reflex, righting reflex, grabbing reflex, pinna reflex) and auditory (startle response) and visual function (visual placing).

VII.2.4.1.1.1 Fertility of F_1 matings

One female rat in the 50 mg/kg group was sterile, as evidenced by her failure to conceive on the first and second matings. In the 150 mg/kg group, one female rat experienced reabsorption of all embryos and another died from unknown causes during pregnancy. All other pregnancies were carried to Day 20 when Caesarean sections were performed.

VII.2.4.1.1.2 F_1 mother and F_2 fetuses

Caesarean section of F_1 females showed no significant differences in number of *corpora lutea*, implant sites, or reabsorbed embryos. There were also no significant differences in the total number of fetuses extracted, the number of live and dead fetuses, the mean fetal weight, or the sex distribution of fetuses. There was no correlation between pre- and post-implant losses and treatments given to F_1 mothers.

VII.2.4.2 Rabbits

Sixty New Zealand white female rabbits that had been mated with male rabbits of proven fertility were equally divided into 4 treatment groups: control (saline), 30 mg/kg A-GPC, 60 mg/kg A-GPC, and 120 mg/kg A-GPC. The rabbits received daily intravenous (marginal vein of the ear) injections of A-GPC or saline from Day 6 to Day 18 of pregnancy, *i.e.*, the period of fetal organogenesis. Injection with A-GPC (dose volume 0.6 ml/kg) had no effect on the health of pregnant rabbits or the course of the pregnancy. The highest dose of A-GPC elicited depression and poor reactivity lasting 0.5–4 hours, the 60 mg/kg dose elicited sedation that quickly passed, while the 30 mg/kg dose elicited no behavioral effects. There were no significant differences in weight between control and treated rabbits. There were no significant differences in the number of *corpora lutea* between control and treated groups. There was no statistically significant difference between treated groups in the number fetuses *per* litter. One rabbit in the control group lost a fetus. The number of partial and complete reabsorptions and post-implant losses was higher for the control and 60 mg/kg groups than for the 30 mg/kg and 120 mg/kg groups. There were a significantly higher number of pre-implant

losses in the treated groups than in the control group. This is unrelated to the A-GPC injections because implantation occurred prior to injection.

On Caesarian section of rabbits treated with A-GPC, 5 dead fetuses were found. Pregnant females treated with the intermediate and high doses lost 2 fetuses while those treated with the low dose lost one fetus. The authors suggest that this was incidental because of the poor correlation between the dose of A-GPC, death of fetuses, and similarity between fetal weights. A single major malformation was observed in the 30 and 60 mg/kg groups, but none was detected in the 120 mg/kg group. No correlation was noted between treatment and the frequency of minor malformations and skeletal variations.

VII.2.5 Pharmacology

VII.2.5.1 Rabbits

VII.2.5.1.1 Cardiocirculatory effects

Food was withheld for 16 hours from white New Zealand rabbits; they were given free access to drinking water. The A-GPC dose groups for the study were: 20, 60, and 200 mg/kg administered intravenously at 30-minute intervals at a rate of 1 mL/min. There were 4 animals/ dose. 200 mg/kg A-GPC was administered intramuscularly (bolus injection to the gluteofemoral region), and 1,000 mg/kg administered intraduodenally in a dose volume of 5 ml/kg. There were 4 animals per dose and dose route. The vehicle was distilled water for intraduodenal dosing and physiological salt solution for parenteral dosing. The dose volumes were 1 ml/kg for the intravenous and intramuscular routes and 5 ml/kg for the intraduodenal route. The 200 mg/kg intramuscular injection had no "important" effects on systolic blood pressure, diastolic blood pressure, or heart rate over a 3-hour period after dosing. Mean arterial pressure decreased compared to basal beginning at 90 min post-dosing, and heart rate increased at the same time point; but because there was no significant difference in these changes for control and A-GPC treated animals, the authors concluded that they were "spontaneous hemodynamic modifications in the experimental conditions of the test" (p. 346) (Italfarmaco, 1997). The 1,000 mg/kg intraduodenal dose had no effect on the cardiovascular system; the changes observed were similar to those observed when A-GPC was administered intramuscularly. Among the intravenous doses, 20 mg/kg did not significantly affect the parameters assessed; 60 mg/kg elicited transient hypotension of low magnitude, with a statistically significant diastolic component that lasted for 10 minutes along with a significant compensatory tachycardia that was transient; and 200 mg/kg elicited a stronger hypotensive effect that lasted longer than the effect elicited at the lower doses. At peak time (approximately 1 minute after the intravenous injection ended) the mean reduction was 18 mmHg. This effect was significant for the entire 2-hour observation period.

VII.2.5.1.2 Autonomic nervous system effects

Food was withheld from 5 male New Zealand white rabbits for 16 hours with free access to drinking water. The rabbits were given intravenous doses of 60 mg/kg A-GPC. The rabbits were anaesthetized with an intravenous injection of 1 g/kg ethyl urethane and 50 mg/kg diallylbarbituric acid. Rabbits underwent tracheal intubation to facilitate spontaneous breathing, catheterization of the femoral artery for measurement of blood pressure, and catheterization of a femoral vein for injecting the sympathomimetic and parasympatomimetic substances and A-GPC. After blood pressure was stabilized, the rabbits were given the following treatments at 5 minute intervals: 1.5 µg/kg norepinephrine, 0.1 µg/kg isoprenaline, and 1 µg/kg acetylcholine (ACh). These treatments were administered again in the same order 15 minutes after A-GPC was administered. The maximum change in mean arterial pressure in response to norepinephrine, isoprenaline, and ACh were measured prior to and after treatment with A-GPC. T-tests for paired data were used for statistical analysis. Treatment with A-GPC did not affect the hypertensive response to norepinephrine or the hypotensive response to isoprenaline. Treatment with A-GPC significantly increased the hypotensive response to ACh. The authors considered that because the increase was small it was not true potentiation of the effect of A-GPC on the action of Ach. They concluded that A-GPC dose not interfere with the peripheral adrenergic or cholinergic regulation of vascular tone.

VII.2.5.2 Rats

VII.2.5.2.1 Gastrointestinal motility

Acute oral doses of 100 mg/kg A-GPC, 300 mg/kg A-GPC, 1,000 mg/kg A-GPC, or saline (control) were given to male Sprague Dawley® rats (10/dose group) weighing 240–260 g that had been starved for approximately 16 hours with free access to drinking water. The effect of A-GPC on gastric emptying (Stickney et al., 1955) time when given 60 minutes before a “black” meal consisting of 2 ml of a 10% suspension of animal charcoal in 0.5% methylcellulose. Animals were sacrificed 40 minutes after the meal and the percent length of the ileum traveled by the test material was assessed for each treatment. A-GPC at all doses had no significant effect on the gastrointestinal transit of the meal. Percent length of small intestine traveled by the test meal in 40 minutes were 66.64 ± 2.19 (controls), 67.53 ± 2.84 (100 mg/kg A-GPC), 66.57 ± 2.99 (300 mg/kg A-GPC), and 65.85 ± 2.41 (1,000 mg/kg A-GPC). The authors concluded the A-GPC had “no direct effects on the gastrointestinal musculature and does not interfere with the neurohumoral mechanisms that regulate gastric emptying or intestinal transit” (p. 361) (Italfarmaco, 1997).

VII.2.5.2.2 Learning active avoidance responses in rats

Male Wistar rats were used to investigate the effect of a daily dose of A-GPC administered subcutaneously (laterodorsal) or orally. Rats received 15, 30, 60, or 120 mg/kg A-GPC or saline daily. There were 15 animals in each dose group. A-GPC was dissolved in saline for the subcutaneous dosing and in distilled water for the oral dosing at a volume of 5 ml/kg for both delivery routes. Treatments were given once *per day*, 30

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minutes after each testing session, for the 3 days preceding the start of training and throughout the 12-day training period. The shuttle-box method was used to assess the influence of A-GPC on active avoidance behavior. The shuttle box (Schindler et al., 1984) is a box divided into two compartments with a seesaw metal floor wired to an electricity source. Animal training involves daily completion of a random sequence of 20 trial runs during which the rats must learn to avoid electric shocks (80 V, 1 mA, 3 sec) from the floor of the shuttle box by moving to the other compartment of the box within 6 sec of delivery of a signal with a light and a sound component. The time interval between runs is 30 ± 20 sec. Before the trial, the rats were subjected to a series of 10 test runs; rats that who had at least one positive test response between the 11th and 20th runs were selected for the study. The number of correct responses by each rat per day and the latency time of each response (maximum 9 sec, *i.e.*, 6 sec of signal plus 3 sec of shock) were recorded. The number of animals *per* group that showed correct learning (a minimum of 8 correct responses in the last 10 trial runs of each series) and the sum of the latency times *per* animal and *per* series were determined. Data from the frequency of correct responses on the days of training were analyzed using the logistic equation in DeLean et al. (1978), to determine the time at which 50% of the animals of each group showed correct learning. The aggregated latency times of 6, 9, and 12 days of training were processed by variance analysis and the Dunnett test.

VII.2.5.2.2.1 Oral route

A-GPC facilitated learning at the 30 mg/kg dose and exhibited a dose response relationship (7.0, 5.2, 4.4, and 3.5 days, low to high doses respectively [I assume], compared to 6.6 days for rats fed saline. There was no significant difference between groups in latency times of responses on days 0 or 3. The latency time of responses (means \pm SD, calculated on 15 rats/group) on Day 6 was significantly higher for the 60 mg/kg (55.1 ± 19.6 sec, $P < 0.05$) and 120 mg/kg (45.6 ± 14.6 sec; $P < 0.01$) groups than for controls. On Day 9 there were significant differences in latency times between the 60 mg/kg (38.6 ± 12.5 sec; $P < 0.01$) and the 120 mg/kg (37.1 ± 8.5 sec; $P < 0.01$) dose compared to controls (64.7 ± 20.6 sec). There was no significant difference between groups on Day 12.

VII.2.5.2.2.2 Subcutaneous route

The responses were slightly faster for subcutaneous than for oral dosing. On Day 3, the mean latency time was lower for rats given the 120 mg/kg dose compared to the controls (92.0 ± 20.6 sec *v.*, 129.1 ± 21.4 sec; $P < 0.05$), but there was no significant difference between the 30 and 60 mg/kg groups and controls. On Day 6 there was a significant difference between the 30, 60 and 120 mg/kg groups compared with controls (means \pm S.D. = 66.0 ± 19.7 sec, $P < 0.05$; 46.7 ± 15.7 sec, $P < 0.01$; 49.2 ± 12.5 sec, $P < 0.01$ *vs.* 83.5 ± 20.2 sec, respectively). On Day 9 the mean latency time of response for all rats receiving treatment were significantly higher than for controls ($P < 0.01$). Values for the 30, 60 and 120 mg/kg groups compared to controls were 49.9 ± 10.1 sec, 36.1 ± 8.4 sec, 34.7 ± 5.7 sec *vs.* 75.1 ± 12.4 sec. There was no significant difference between groups by Day 12. The mean time necessary for complete learning in 50% of

rats (TA_{50}) was significantly higher for treated rats than for controls ($P < 0.01$). Values were 6.4 days for controls, 6.8 days for 15 mg/kg, 4.6 days for 30 mg/kg, 3.7 days for 60 mg/kg, and 3.2 days for 120 mg/kg.

VII.2.5.2.3 Maintenance of passive avoidance response in rats

Administration of the muscarinic antagonist scopolamine to healthy young rats results in a cognitive effect similar to that of adult-onset dementia (Drachman & Leavitt, 1974). Male Wistar rats were used to investigate the effects of A-GPC administered *via* oral gavage or subcutaneous injection (laterodorsal region) in the loss of acquired training due to scopolamine, which induces cholinergic depletion, or by exposure to hypoxia, which impairs brain function in a more general way. There were 10 animals *per* treatment group (control, 15, 30, 60 and 120 mg/kg). The vehicle for subcutaneous dosing was saline and distilled water for oral dosing; the dose volume was 3 ml/kg for all doses for both routes of administration. Treatments with A-GPC were administered one hour prior to injection of scopolamine or 45 min before hypoxia was induced. The tests were conducted in a shuttle box with two compartments. One of the compartments was clear and the other was blackened. The compartments were separated by a sliding door, which the operator controlled to allow the animal to travel between compartments. Initially, the rat was positioned in the clear part of the box and the trap door was closed. After 10 sec the trap door was opened and when the rat moves to the dark side of the box within 10 to 20 sec the trap door was shut. After 3 sec an electric shock (DC 0.75 mA) lasting for 3 sec was delivered through the floor of the box and the rat was removed from the box. Twenty-four hours were allowed to elapse before the second session in which the trap door was left open when the rat moved to the dark side of the box and no electric shock was delivered. The investigators determined that after the first session, rats did not move into the dark part of the box for at least 180 sec, but if given a 2 mg/kg subcutaneous injection of scopolamine 15 min before retesting on Day 2, the rat would typically move to dark part of the box within 50–60 sec. This is also the case for animals kept in a gas chamber with reduced oxygen content (7%) for 15 min before and after the first session.

Pretreatment of rats with A-GPC significantly decreased the loss avoidance behavior, *i.e.*, A-GPC pretreatment increased latency time for shuttling into the dark side of the box. Administration of subcutaneous doses of 15, 30, 60, or 120 mg/kg A-GPC resulted in latency times of 83.9 ± 6.5 sec, 91.6 ± 4.5 sec, 117.5 ± 6.1 sec, and 142.9 ± 5.8 sec, respectively, compared to control (58.8 ± 6.7 sec, $P < 0.01$). Oral doses of 15, 30, 60, and 120 mg/kg resulted in latency times of 59.4 ± 10.1 , 84.3 ± 9.6 , 94.5 ± 6.8 , 123.8 ± 0.6 sec respectively and a latency time of 55.8 ± 5.9 sec for controls. When the animal experienced hypoxia-induced amnesia, subcutaneous doses of 30, 60 and 120 mg/kg of A-GPC resulted in significantly shorter latency times (94.6 ± 6.1 , 116.5 ± 8.3 , and 146.9 ± 6.1 sec, respectively; $P < 0.01$), compared to control rats (54.9 ± 7.6 sec). However, latency time for the rats given 15 mg/kg doses was 77.9 ± 9.6 sec. Latency times when given oral doses of A-GPC were significantly higher for the rats given 60 or 120 mg/kg A-GPC (96.6 ± 6.7 and 130.9 ± 8.2 sec, respectively) compared to control rats ($61.7 \pm$

5.4 sec), and there was no significant difference (compared to control) between rats given 15 or 30 mg/kg A-GPC (52.2 ± 4.5 and 80.6 ± 6.9 sec, respectively).

Govoni et al. (1992) investigated the effect of A-GPC on ACh release and passive avoidance in adult male Wistar rats. The step-down apparatus was used in the study. Animals that stepped down from the grid (not electrified) within two minutes were chosen for training. The next day, training took place. During training, the latency to step down from the platform onto the grid and receive an electric foot shock (0.8 mA for 5 sec; animal test cage grid floor shocker, Coulbourn Instrument) was measured and the animal returned to its cage. Twenty-four hours later, the animals were tested as before (retention trial) and latency to step down onto the grid was measured, but the cutoff time was 600 sec. The rats were treated with 100 (n = 7), 300 (n = 34), 600 (n = 15) or 1,000 (n = 9) mg/kg A-GPC 5 hr before training. A-GPC antagonized the amnesic effect of 0.75 mg/kg *i.g.* scopolamine in the passive avoidance behavior of rats. The maximum effect occurred with the 600 mg/kg *i.g.* dose and lasted for approximately 30 hours. ACh release from the hippocampus occurred with the 75 mg/kg *i.g.* dose and the maximum response was obtained with 300 mg/kg (147% of control values) 3 hr after administration. The effect in the cortex was shorter than and not as strong as the effect in the hippocampus. Twenty-two days of treatment with 100 or 300 mg/kg *i.g.* antagonized scopolamine-induced amnesia. The 300 mg/kg dose increased ACh release from the hippocampus (+271%) and cortex (+57%).

VII.2.5.2.4 Effect on brain choline and acetylcholine levels

Lopez et al. (1991) examined the effect of A-GPC on scopolamine-induced amnesia and brain acetylcholine in adult male Wistar rats. The animals were housed in plastic cages in groups of 4 for at least 7 days. Step down passive avoidance was studied using the step-down apparatus derived from Kubanis et al. (1982) and included an aluminum platform attached to one end wall and about 7 cm above the apparatus floor. The floor consisted of a grid that is electrified when the rat steps down. Rats that stepped down within two minutes underwent training. During training, the rats were placed on the platform and the latency time to step-down was recorded. Once the animal stepped down to the floor of the cage, it was shocked and returned to its cage. Twenty-four hours after training the animal was tested and the latency time was recorded, with a cut-off time of 600 sec. Rats were given 100, 300, 600, or 1,000 mg/kg *i.g.* 5 hr before training. A different group of rats received 600 mg/kg A-GPC AT 1, 3, 5, 20, 30, and 48 hours before training and received scopolamine 0.75 mg/kg *s.c.* 30 min before training. Control animals received 1 mL tap water *i.g.* and 0.1 mL of physiological saline *s.c.* A-GPC reversed amnesia induced by 0.75 mg/kg *s.c.* scopolamine in the passive avoidance test, with the peak effect seen with 600 mg/kg ($P < 0.01$ compared with scopolamine) 5 hours prior to training; the effect lasted for up to 30 hours. The 300 mg/kg dose also had a significantly lower latency time ($P < 0.01$ compared with scopolamine) than animals treated with scopolamine alone, but the 100 and 1,000 mg/kg doses did not have significantly reduced latency. When A-GPC was administered 3 hr at doses of 300, 600, 1,000, and 2,000 mg/kg before sacrifice and scopolamine was administered 1.5 hr prior to sacrifice, A-GPC partially reduced the reduction in brain

ACh levels in the hippocampus (26%) and cerebral cortex (32%) that was caused by scopolamine in the passive avoidance study, but had no effect in the striatum.

Sigala et al. (1992) investigated the effect of A-GPC on scopolamine-induced amnesia and hippocampal cholinergic transmission in male Sprague Dawley® rats. Rats were trained using the step-down passive avoidance apparatus with a plastic platform and a steel rod grid floor and were given a shock when they stepped down from the plastic platform onto the grid floor. A consolidation test was conducted 3 hours after training and the retention test was conducted after 24 hours. An oral dose of A-GPC administered 3 hours prior to the behavioral test prevented induction of the learning impairment by scopolamine that was given 30 minutes before a passive avoidance response. A-GPC also reversed retrograde amnesia induced when scopolamine was given immediately after acquisition training

VII.2.5.3 Italfarmaco study

A study commissioned by Italfarmaco (1997) examined the effect of administration of subcutaneous doses of A-GPC in a 5 ml/kg dose volume on brain choline and acetylcholine levels in rats. Male Sprague Dawley® rats (8 rats/dose group) were fasted for 16 hours with access to drinking water, and then administered saline (control), 1 mg/kg scopolamine, 200 mg/kg A-GPC, or 200 mg/kg A-GPC plus 10 mg/kg scopolamine. A-GPC was injected subcutaneously 6 hours before the animals were sacrificed and scopolamine was administered 60 min before the animals were sacrificed to deplete brain levels of choline and ACh. In another study, A-GPC was subcutaneously injected 6 hours before the animals were sacrificed and atropine (20 mg/kg) was administered 60 minutes before the animals were sacrificed to deplete brain levels of choline and ACh. After the animals were sacrificed their brains were collected and the cortex separated and homogenized with 5 volumes of cold acetone containing 15% formic acid 1N. Levels of ACh and choline were assessed using gas chromatography using a nitrogen and phosphorus detector (Kosh et al., 1979).

Treatment with 1 mg/kg scopolamine significantly decreased levels of ACh compared to control (13.01 ± 1.09 vs. 19.65 ± 1.13 nM/g tissue; $P < 0.01$) and choline compared to control (13.45 ± 1.55 vs. 20.99 ± 1.24 nM/g tissue; $P < 0.05$). There were no significant differences in ACh levels when A-GPC was given alone (19.72 ± 1.29 nM/g tissue) or with scopolamine (16.21 ± 0.87 nM/g tissue). There was a significant difference compared with controls when 200 mg/kg A-GPC was given with 10 mg/kg scopolamine (33.15 ± 2.24 nM/g tissue). There was a significant difference in choline levels when scopolamine was administered with A-GPC compared with when scopolamine was administered alone (23.64 ± 2.75 vs. 13.45 ± 1.55 nM/g tissue; $P < 0.01$).

In the atropine study, there was no significant difference in ACh levels compared to control when A-GPC alone was given to the rats, however, there was a significant difference in values when atropine alone was given to rats compared to control (9.82 ± 1.07 vs. 20.71 ± 1.27 nM/g tissue; $P < 0.01$). When A-GPC and atropine were given together, there was a significant difference in ACh levels compared to atropine alone

(14.49 ± 0.93 vs. 9.82 ± 1.07 nM/g tissue; $P < 0.05$). Levels of choline were not significantly lower after atropine treatment (19.50 ± 0.91 nM/g tissue) compared to levels after choline treatment, compared to control (19.50 ± 0.91 nM/g tissue). Treatment with A-GPC resulted in a significantly lower level of choline (30.21 ± 1.80 nM/g tissue; $P < 0.01$) compared with control. The combination of A-GPC and atropine resulted in a significantly higher amount of choline compared to atropine alone (32.27 ± 1.04 vs. 19.50 ± 0.91 nM/g tissue; $P < 0.01$).

VII.2.5.3.1 Explorativeness in rats

Sprague Dawley[®] rats weighing 180–200 g were starved for 14–16 hours but were given free access to water. A-GPC in saline at doses of 50, 100, 200, or 400 mg/kg was administered orally or subcutaneously to 10 animals/dose/route of administration 60 min before testing. The dose volume was 5 ml/kg for all doses and administration routes. Testing for explorativeness involved placing the rats in a plexiglass box with 2 photoelectric cells wired to equipment for measuring animal movements. The animals were tested individually for 30 min and the number of interruptions of the photocell beam due to the movement of the rat was recorded. The same number of animals *per* group was tested for each administration route in random order on each day. Subcutaneous injections elicited significantly decreased explorativeness for rats that received dose of 200 mg/kg (-32.2%) and 400 mg/kg A-GPC (-54%), but there was no dose-effect relationship. The number of movements for animals injected subcutaneously with 50, 200, and 400 mg/kg were significantly lower than those of the control animals (53.9 ± 3.9 , $P < 0.05$; 48.1 ± 4.3 , $P < 0.01$; 32.6 ± 4.6 , $P < 0.01$; vs. 70.9 ± 5.1 , respectively), but not for the 100 mg/kg group (57.8 ± 5.2). The number of movements for animals given an oral dose of 400 mg/kg was significantly lower than that for the controls (34.1 ± 3.6 vs. 55.5 ± 6.3 , $P < 0.05$), but not for animals given 50, 100, or 200 mg/kg (58.0 ± 5.6 , 58.6 ± 3.6 , and 42.9 ± 4.6 , respectively).

VII.2.5.3.2 Cognitive mechanisms in rats

Drago et al. (1992) investigated the effect of A-GPC on cognitive mechanisms in aged male rats. Two groups of Sprague Dawley[®] rats were tested: 24-month-old rats that showed a deficit in learning and memory, and rats in which amnesia was induced using bilateral injections of 1 μ g/1 μ L kainic acid (dissolved in saline) into the nucleus basalis magnocellularis. Rats were given 100 mg/kg A-GPC *i.p.* daily for 20 days, with the last dose being given 1 hour before behavioral testing. Control rats were treated with a saline placebo. The learning criterion was five consecutive shuttle-box conditioned avoidance responses (CARs). Compared with saline-treated older rats, the A-GPC-treated animals had significantly higher numbers of CARs (14.7 ± 0.5 , $n = 6$, vs. 7.4 ± 0.6 , $n=5$; $P < 0.05$) and percentages of learners (50.0 , $n = 6$ vs. $n=5$; $P < 0.05$). There was a significant difference in median latency between A-GPC-treated rats and older rats treated with saline (57 sec vs. 25 sec; $P < 0.05$). After rats with bilateral lesions of the nucleus basalis magnocellularis were treated with A-GPC, the number of CARs, percentage of learners, and retention of passive avoidance increased significantly compared to vehicle-injected lesioned animals.

VII.2.5.3.3 Neurotoxic activity in rats

The neurotoxicity of orally and subcutaneously administered A-GPC was investigated in male and female Sprague Dawley® rats that had been starved for 12–14 hours with free access to drinking water (De Caro, 1986; Italfarmaco, 1997). A-GPC was administered in a 5 ml/kg dose volume in a saline vehicle for subcutaneous dosing and in distilled water for oral dosing. Doses of 50, 100, 200, and 400 mg/kg A-GPC were administered to 10 animals (5 rats/sex/dose group) 60 minutes before each test. The rota-rod test (U Basile Co.) was used. Before testing, rats were trained to walk on a particular region of the rota-rod device while it was turning at 10 rpm, for 60 seconds without falling. Training was 3 consecutive runs lasting 60 seconds each that were repeated at 30 minute intervals. Animals that did not learn to navigate the rota-rod without falling off in the first 5 seconds were replaced. After the required number of animals was trained they received their allotted treatment and were retested. The number of rats falling off the rota-rod before the defined period of time had elapsed was recorded in order to calculate a neurotoxic dose for 50% of the animals. There were no neurotoxic effects of any doses of A-GPC for either route of administration.

VII.2.5.4 Other animal studies

VII.2.5.4.1 Recovery from ischemia/stroke

Onishchenko et al. (2008) compared the morphological changes occurring at the focus of experimental ischemic stroke in adult mongrel male rats treated with A-GPC (choline alfoscerate) and other drugs after surgery. Acute ischemia was induced in 18 rats by making transient cerebrovascular lesions in the right brain hemisphere and clipping the stem of the innominate artery, thereby stopping blood flow to the right carotid and vertebrobasilar basins for 40 minutes. After the wound was sutured, animals were daily given either 45 mg/kg A-GPC, 65 mg/kg cerebrolysin, 60 mg/kg pirecetam, 1 mg/kg vinpocetin, isotonic saline, or no treatment (n = 3 rats/group). After surgery, all animals experienced left-sided hemiparesis with hemiplegia and ataxia, accompanied by hypodynamia, inhibition, and refusal of food and water. Animals treated with A-GPC post surgery were the first to experience activation of behavior and 60–80 minutes later showed no signs of cerebrovascular impairment. The A-GPC-treated animals almost immediately resumed consumption of food and water and showed the most complete post-ischemic recovery of brain tissue. In all groups except those treated with A-GPC, light microscopy revealed spasm of arterioles and venous congestion. Cerebellar piriform cells of animals treated with A-GPC were better preserved than those in the other groups. The nuclear and cytoplasmic structure of rats treated with A-GPC was not different from intact rat. The synaptic apparatus was close to normal, trophic and myelin-forming oligodendrocytes were often observed in the active morphofunctional state.

Ciriaco et al. (1992) investigated the effect of A-GPC on rat hippocampal mossy fibres after monolateral lesioning of the nucleus basalis magnocellularis (NBM). Lesioning of

the NBM, the nucleus that sends cholinergic projections to the fronto-parietal complex, produces ultrastructural changes. A-GPC restored the intensity of staining in the mossy fibre area and reduced the loss of presynaptic buttons and the number of impaired buttons.

VII.2.5.4.2 Retinal Processing

Antal et al. (1999) studied the effects of L-A-GPC on pattern electroretinogram in rhesus monkeys in order to determine the effect on retinal spatial frequency tuning. Between 10 and 30 minutes after two young adult male rhesus monkeys were given an intramuscular injection of 85 mg/kg A-GPC, the amplitude of the pattern electroretinogram that represents the summed neuronal activity of the ganglion cells increased significantly. They also reported that in addition to general facilitating properties, cholinergic substances may have specific tuning functions at the retinal processing level.

VII.2.5.5 Mutagenicity/genotoxicity studies

Tests of the mutagenicity and toxicity of A-GPC were reported in Brownawell et al. (2011) and Dubini (1984).

VII.2.5.5.1 Ames test

The Ames test was performed with *Salmonella typhimurium* indicator strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100. Testing was performed with and without metabolic activation. Concentrations of A-GPC tested were 10, 100, 300, 1,000, 3,000, or 10,000 µg/plate. In none of the 5 test strains did A-GPC cause an increase in number of reverted colonies above the control level.

VII.2.5.5.2 Gene Conversion test

A-GPC did not alter the mitotic gene conversion of *Saccharomyces cerevisiae* (genes for "Ade 2" and "Trp 5"). Testing was carried out with and without a mammalian metabolic activator, and the concentrations of A-GPC used were 10, 100 and 1,000 µg/mL.

VII.2.5.5.3 Yeast Forward Mutation (Gene Mutation) test

A-GPC did not alter the frequency of spontaneous forward mutations of *Schizosaccharomyces Pombe* strain P₁ at concentrations of 30, 100, 300, 1,000, or 3,000 µg/mL. Tests were carried out in the presence and absence of a metabolic activation system.

VII.2.5.5.4 Host Mediated assay

The mutagenic potential of A-GPC and its metabolites has been investigated *in vivo* using female Swiss[®] mice, which were administered A-GPC at exposures of 3, 30, and

300 mg/kg by subcutaneous route, three animals *per* dose. Treatments were given twice with an interval of 24 hours, and *Saccharomyces cerevisiae* strain D4 was administered intraperitoneally immediately after the second treatment. The results showed that the pre-treatment with A-GPC up to 300 mg/kg s.c. did not alter the frequency of gene conversion of *S. cerevisiae*.

VII.2.5.5.5 Micronucleus Test-Mice

The mutagenic potential of A-GPC *in vivo* was assessed in mammalian cells by testing the effect of A-GPC on the normal variation range of micronucleated polychromatic erythrocytes in the bone marrow of male and female Swiss mice. Two doses of 30, 100, or 300 mg/kg administered at 24 hour intervals did not induce bone micronuclei in bone marrow erythrocytes of the mice.

VII.2.5.5.6 Chromosome Aberration test

Lymphocyte cultures were incubated with A-GPC at the concentrations of 1, 10, 100, and 1,000 µg/ml, either in the presence or absence of a metabolic activator. The frequency of chromosomal aberration observed in human lymphocytes incubated with A-GPC up to a concentration of 1,000 g/kg was not significantly different than those found in controls.

VII.2.5.5.7 Other *In Vitro* studies

Bramanti et al. (2008) investigated the effect of L-A-GPC on transglutaminase activity and expression in primary astrocyte cultures where astrocytes were obtained from brains of 1–2-day-old albino rats. Transglutaminase is a calcium-dependent protein that plays a role in transduction, differentiation, and apoptosis during fetal development. Cultures were exposed to A-GPC for 6, 12, or 24 h. At 0.1 µM and 1 µM concentrations, A-GPC resulted in elevated TG activity in primary astrocyte cultures as shown by confocal laser scanning microscopy analysis. Western blot analysis showed that within 24 h of applying 1 µM A-GPC, transglutaminase antibodies increased the authors suggest that this indicates the critical role of A-GPC in various stages of astroglial cell proliferation and differentiation in cell cultures.

VIII. HUMAN STUDIES AND EXPERIENCE

Ziegenfuss et al. (2008) found that a single 600 mg dose of A-GPC (as AlphaSize®), when administered 90 min prior to resistance exercise, increased serum growth hormone (GH) and peak bench press force. Seven men (mean age 30.1 ± 7.3 years) with at least 2 years of resistance training experience ingested 600 mg A-GPC or placebo 90 min prior to resistance exercise. Serum samples were obtained before and at 0, 5, 15, 30, 90, and 120 minutes post-exercise. A-GPC (as AlphaSize®) increased peak GH 44-fold (0.19 ± 0.06 to 8.4 ± 2.1 ng/ml) compared to baseline. Placebo increased peak GH 2.6-fold (from 1.9 ± 0.8 to 5.0 ± 4.8 ng/ml). The peak bench press force was higher for individuals treated with A-GPC (933 ± 98 N) compared to placebo

(818 ± 77 N, $P < 0.02$). A-GPC had no significant effect on peak power, rate of force development, resting metabolic rate, heart rate, or blood pressure. No adverse effects were reported.

A double-blind, multicenter clinical trial was conducted by de Jesus Moreno Moreno (2003) in Mexico to determine if treatment with A-GPC caused cognitive improvement in patients with mild to moderate dementia of the Alzheimer's type. A total of 261 patients were either treated with 1200 mg/d of A-GPC (400 mg capsules, 3 times/day) or placebo for 180 d. The mean age \pm S.D. of the subjects in the A-GPC group ($n = 132$, 105 women and 27 men) was 72.2 ± 7.5 years; the mean age \pm S.D. of the placebo group subjects ($n = 129$, 94 women and 35 men) was 71.7 ± 7.4 years. Two hundred twenty-nine patients completed the study: 114 in the A-GPC group and 114 in the placebo group. Most behavioral parameters assessed after 90 and 180 days of treatment were improved compared to placebo. Fifteen minor drug-related adverse effects were noted: 10 episodes of constipation and 5 episodes of nervousness. These effects were mild and did not warrant discontinuation of treatment.

A randomized controlled study by Parnetti et al. (1993) in Italy compared the effects of A-GPC and ST200 (acetyl-L-carnitine) in 126 patients with probable mild to moderate senile dementia of Alzheimer's type (SDAT) for 6 months. After a 2-week washout period from other treatments, 65 patients received oral A-GPC (800 mg at 8 am and 400 mg at 4 pm) and 61 patients received oral ST200 (1,000 mg at 8 am and 500 mg at 4 pm) for 6 months. The efficacy of the drugs was evaluated by means of psychometric tests and behavioral scales. Significant improvements were seen in most neurophysiological parameters in patients who received A-GPC, including verbal memory (assessed by Rey's 15-word test) and intellectual and emotional impairments. Similar improvements occurred in patients who received ST200, but to a lesser extent. Both drugs were well-tolerated. Adverse effects observed in the A-GPC group included insomnia, gastralgia, and restlessness in 1 patient each. These effects did not require drug withdrawal.

A clinical study in 817 elderly patients (age 61–95, mean age 73 years, 412 males and 405 females) from northern, central, and southern Italy with multi-infarct dementia (MID), primary degenerative dementia (PDD), or mixed dementia (MD) was performed by Ban et al. (1991) using 1,200 mg A-GPC daily for 6 months. A-GPC was supplied in 400 mg soft gelatin capsules and was administered as one capsule 3 times daily. Patients were between stages 2 and 5 on the Global Deterioration Scale (GDS) of Reisberg et al. (1988) and a score of 23 or less on the Mini Mental State Examination (MMSE) of Folstein et al. (1975). The GDS is "a 7-point rating instrument for staging the magnitude of cognitive and functional capacity in normal aging, age-associated memory impairment (AAMI) and primary degenerative dementia" (Reisberg et al., 1988). The MMSE is a scored form for cognitive mental status examination that emphasizes on the cognitive aspects of mental function (Folstein et al., 1975). Favorable changes were observed with A-GPC treatment according to psychopathological scoring, performance scales, and social behavior. There were 14 different side effects observed in 34 patients, including agitation (13 patients), heartburn (7 patients), nausea (5 patients),

headache and insomnia (3 patients) and orthostatic hypotension (2 patients). None of these effects warranted discontinuation of treatment.

Canal et al. (1991) investigated the effect of L-A-GPC on memory impairment induced by scopolamine in 32 healthy volunteers: 18 men and 14 women with a mean age \pm S.D. of 26.5 ± 4.5 years. Scopolamine is a drug that has been shown to cause transitory memory impairment in healthy individuals similar to that observed in normal aging *via* interference with cholinergic transmission in the central nervous system. For 10 d, volunteers were either orally administered 1200 mg/d L-A-GPC or placebo. On the 11th day, volunteers from each group were given either scopolamine (0.5 mg, i.m.) or placebo. Before the scopolamine or placebo injection and at 0.5, 1, 2, 3, and 6 hours after, the subjects were given attention and mnemonic tests. Results showed that A-GPC was able to antagonize the scopolamine-induced impairment of attention and memory and enhance performance when given with placebo. No adverse effects due to A-GPC administration were reported.

Frattola et al. (1991) compared the effects of A-GPC and cytidine diphosphocholine (CDP-choline) in treatment of multi-infarct dementia. Multi-infarct dementia is a syndrome caused primarily by focal or generalized decrease in blood supply to various areas of the brain resulting in hypoxia or anoxia (ischemia/infarction) and neurologic damage (Scheinberg, 1988). One hundred twenty-six individuals with mild to moderate multi-infarct dementia were allowed a two-week washout period for any other drugs and then were treated with 1 g A-GPC i.m. (n = 59 subjects) or CDP-choline i.m. (n = 58 subjects) daily for 90 days. At 45 and 90 days after the start of the treatment, subjects were evaluated using the Sandoz Clinical Assessment for Geriatric Patients, the Modified Parkside Behavior Rating Scale, the memory logic test of the Wechsler Memory Scale, and the word fluency test. Thirty-nine men and 20 women from the A-GPC group completed the study and 39 men and 19 women from the CDP-choline group completed the study. Five subjects in the CDP-choline group and four in the A-GPC group did not complete the study for reasons unrelated to the medication. Although both A-GPC and CDP-choline improved memory, cognition, and behavior, the effects of A-GPC occurred earlier and were more extensive for the Sandoz Clinical Assessment for Geriatric Patients and the word fluency test. The authors stated that no local or systemic adverse reaction had been reported and there were no clinically or statistically significant changes in systolic and diastolic blood pressure and heart rate or blood count, including differential, blood glucose, lipids, tests of liver and renal function were reported.

Muratorio et al. (1992) investigated the efficacy and tolerability of 1 g/d i.m. A-GPC on treatment of multi-infarct dementia. In this multicenter, unblinded, randomized, controlled clinical trial the effect of A-GPC was compared to that of cytidine diphosphocholine (CDP-choline) in patients with mild to moderate multi-infarct dementia. After a two-week washout period, 112 subjects ages 50 to 80 were treated with A-GPC or CDP-choline for 90 days and were then observed for 90 days to determine how long the effects of treatment lasted. Ninety-seven subjects (63 men and 34 women) completed the treatment component of the study. Forty-eight subjects were

treated with A-GPC and forty-nine were treated with CDP-choline. Attrition of 18 subjects was due primarily to poor compliance. Seventy-three patients completed the follow-up component of the study. The effectiveness of the drugs was assessed using the Blessed Dementia Scale and Blessed Information, Memory, Concentration test; the Sandoz Clinical Assessment Geriatric Rating Scale; the Wechsler Memory Scale; the Word Fluency test; the Token test; the Simple Drawing Copy; and the Rapid Disability Rating Scale 2 after 30 and 90 days and at the end of the follow-up period. Subjects who were given A-GPC had significantly improved cognitive function, behavior, and personality at the end of the treatment and follow-up periods compared with baseline. Treatment with CDP-choline only improved aphasia. The favorable effects of A-GPC lasted throughout the 3-month follow-up period.

Gatti et al. (1992) investigated the profile of free plasma choline levels in 12 healthy men age 20–29 years (mean \pm SEM = 26 \pm 2 years) after a single dose of A-GPC in 12 normal subjects and compared the effect to that of 1,000 mg citicoline, another choline precursor. In this randomized crossover study, free plasma choline levels were measured for three different treatments for each individual with at least one week between sessions, control day without drug, after 1,000 mg i.m. A-GPC (Gliatilin[®], Italfarmaco), and after 1,000 mg citicoline i.m. (Nicholi, Lederle). Subjects consumed a controlled diet that did not include choline-rich foods such as eggs, liver, soybeans, peanuts, rice, cocoa, chocolate, oatwheat, and fatty cheese. On study days, blood samples were collected to measure plasma choline over a 6-hour period. Blood samples were collected just prior to the injection and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, and 6 hours. They reported that during control days, the endogenous choline level was relatively stable and no significant differences were noted between any of the time points measured. There were no significant differences observed between the 0-time point measurement on the control day and the 0-time point on drug treatment days. By 6 hours, there was no significant difference between plasma choline levels and the 0-time point measurement. There was a significant difference between the peak concentration time (C_{max}) after treatment with A-GPC (mean \pm SE = 28.2 \pm 3.1 μ mol/L, range 14.5–45.3 μ mol/L) compared to citicoline (mean \pm SE = 14.0 \pm 1.2 μ mol/L, range 8.2–22.1 μ mol/L, $P < 0.01$). There was also a significant difference between A-GPC and citicoline in terms of area under the curve (AUC_{0-4h}) (mean \pm SE = 42.7 \pm 2.7 μ mol/L, range 27.7–58.2 μ mol/L) compared with citicoline (mean \pm SE = 22.0 \pm 1.6 μ mol/L, range 12.5–31.7 μ mol/L), $P < 0.001$. There were no significant differences in time of peak, elimination rate constant and terminal half-life.

Abbati et al. (1991) compared the efficacy of A-GPC to that of oxiracetam in a randomized controlled study. Forty subjects, with senile organic brain syndrome of medium severity according to DSM-III-R, were randomly assigned to receive 1,000 mg A-GPC or the same dose of oxiracetam for 12 weeks. Subjects were examined for neuropsychological and clinical parameters such as measurement of reaction time at 2, 4, 6, 8, and 12 weeks. Oxiracetam elicited early favorable changes that continued when the treatment was continued but disappeared after treatment was discontinued. Both treatments were said to have been well tolerated. The effect of A-GPC was slower than that of oxiracetam, but was continued up to 8 weeks after treatment ended.

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Barbagallo Sangorgio et al. (1994) conducted a multicenter open trial to examine the effect of A-GPC on mental recovery after cerebral ischemic attacks. The study was divided into two phases that lasted a total of 6 months. From days 1 to 28 subjects were given i.m. doses of 1,000 mg/d A-GPC; from Day 19 to the end of Month 6, subjects were given oral 1,200 mg/d doses of A-GPC. Subjects (n = 2,044) were 45–85 years old and had experienced a cerebral ischemic attack within the 10 days preceding enrollment in the study. Men comprised 55.5% of subjects and women, 45.5%. One hundred forty subjects (6.8%) did not complete the study. Forty-one subjects died during the course of the study for reasons that were not attributed to the treatment. Subjects were observed for adverse events. Mean blood pressure and heart rate did not change significantly. Fifty-one adverse events were reported by 44 of the subjects. 140 subjects withdrew from the study, 101 in the first phase and 38 during the second phase. The 51 adverse events that were reported were heartburn (n = 14), nausea/vomiting (n = 10), excitation/insomnia (n = 9), headache (n = 4), diarrhea (n = 3), dizziness (2), skin rash (n = 2), gastric bleeding (n = 1), increased γ -GT (n = 1), increased ALAT/ASAT (n = 1), confusion (n = 1), anemia and low vitamin B12 (n = 1), increased ALAT/ASAT (n = 1), supraventricular arrhythmia (n = 1), confusion (n = 1), anemia and low vitamin B12 (n = 1), and repeated drop attacks (n = 1). Most events were reportedly of mild or light severity and did not result in withdrawal from the study. Overall tolerability was said to be very good in both parts of the study and A-GPC enhanced cognitive improvement of individuals that had recently had strokes.

Ferini-Strambi et al. (1991) investigated the short term effects of choline alfoscerate on sleep in 8 healthy young men. Subjects were 20 to 31 years of age (mean 24.7 ± 3.8 years) and were not taking any medications prior to or during the study. On days 1 and 2, subjects adapted to the sleep lab and on days 3 and 4, baseline sleep parameters were measured. On days 5 to 11, they were treated with 400 mg of A-GPC or three times per day. On days 5-9, A-GPC was provided to subjects at home and on days 10 and 11, the subjects came into the laboratory where their sleep was measured. Subjects were kept awake during the day and were instructed to avoid major physical activity prior to laboratory study at nighttime. An 8-hour polysomnography including EEG, electrooculogram, chin electromyogram, and ECG was recorded. Measures of sleep included sleep latency, time awake after onset of sleep, total time awake, number of awakenings, number of sleep stage shifts, total sleep time, sleep stages (duration and percentage of stages 1, 2, and 3–4, and REM, REM latency, number and length of REM episodes, REM density (percent of 5-sec mini epochs with at least one REM). The authors reported no significant effect of A-GPC on baseline sleep induction and maintenance for one week, however, total time awake and number of awakenings reflected a trend towards a decrease compared with baseline. A-GPC did not significantly increase sleep efficiency and no difference in sleep stages occurred. A-GPC had no effect on the percentage of REM or the latency to the first REM period, however, the authors noted that it seemed to increase REM density. REM density returned to baseline level after A-GPC was discontinued. Table 8 summarizes critical clinical studies conducted on A-GPC.

Table 8. Summary of Clinical Studies on A-GPC

Study	Dose (mg/d)	Number of A-GPC Treated Subjects	Duration
Ziegenfuss et al. (2008)	600 (single dose)	7 total in study*	120 minutes
De Jesus Moreno Moreno (2003)	1,200	132	180 days
Ferini-Strambi et al. (1991)	1,200	8	6 days
Parnetti et al. (1993)	1,200	65	6 months
Ban et al. (1991)	1,200	817	6 months
Canal et al.(1991)	1,200	16	10 days
Gatti (1992)	1,000	12	1 day
Frattola (1991)	1,000	59	90 days
Barbagallo Sangorgio (1994)	1,000	1904	28 days
	1,200	1866	5 months

*Number of A-GPC treated subjects not specified.

A number of studies were conducted in Italy and published in Parnetti et al. (2001) (Table 9).

Table 9. Human Clinical Studies Written in Italian

Study	Dose (mg/day)	Number of Subjects ¹ with A-GPC	Duration
Palleschi et al. (1992)	Not able to discern	Not able to discern	6 months
Vezzetti & Bettini (1992)	1,200	30	3 months
Paciaroni & Tomassini (1993)	1,200	25	6 months
Tomasina et al. (1996)	Not able to discern	15	1

VIII.1 Published Brownawell et al. (2011) and Italfarmaco Unpublished Clinical Studies

Italfarmaco conducted a number of clinical trials and compared results with standard reference drugs such as citicoline and phosphatidylcholine or placebo. the discussions of these studies are based on summaries in the Investigator's Brochure (Italfarmaco, 1997).

Pirelli (Istitute di Patologia Speciale Medica e Metodologia Clinica, Università di Bari, Italy) investigated the efficacy of A-GPC when delivered *via* i.m. or orally in 86 elderly subjects with mental decline in a parallel, randomized placebo controlled study (Italfarmaco, 1997). Patients were assigned to three treatment groups. Group One included 30 patients: 20 males and 10 females with a mean age of 67.2 years. Group One subjects received OID for 20 d, 800 mg A-GPC i.m. followed by two 400 mg A-GPC capsules b.i.d. for 20d. Group Two included 30 patients: 13 males and 17 females with a mean age of 67.7 years. Subjects in this group received two 400 mg A-GPC oral capsules b.i.d. for 40 days. Group Three included 26 patients: 13 men and 13 women with a mean age of 66.2 years. These subjects received two oral placebo capsules b.i.d. for 40 d. A-

GPC was described as improving neurological symptoms and psychometric indices. There were no statistically significant differences between the injectable and oral treatment except for a trend toward a larger improvement with the injectable form of the drug at day 20. No information about adverse effects was reported and no detailed information about tolerability of the drug was provided.

Stramba-Badiale (Istituto Geriatrico "Camillo Golgi," Abbiategrasso, Milan, Italy) conducted a parallel randomized, controlled clinical trial of 1,000 mg A-GPC i.m. administered in patients with sequelae of cerebrovascular accidents. Subjects were randomly assigned to two treatment groups. Group one included 20 patients, 7 males and 13 females, with a mean age of 75.7 years (range 63-88 years). These subjects were given a single i.m. injection of 1,000 mg A-GPC daily for 15 consecutive days. Group two included 20 subjects, 5 males and 15 females mean age 79.3, range 55-91. Subjects in group two were given one 1,000 mg vial of citicoline once *per* day for 15 days. Tests for tolerability included laboratory tests such as hemoglobin assay, hematocrit value, red blood cell count, WBC count, BUN assay, glycemia, blood uric acid, and blood creatinine. It was reported that A-GPC treatment resulted in favorable alterations in post stroke signs and symptoms such as irritability, agitation, fatigability, attention deficit, emotional instability, apathy, depression, headache, dizziness and asthenia and improvement in the subjects' general status. Good tolerability and no signs or symptoms in laboratory investigations were reported.

Muiesan (Istituto di Clinica Medica Generale e Terapia Medica, Università di Brescia, Brescia, Italy) conducted a double blind parallel, randomized controlled clinical trial to compare the effectiveness of A-GPC to citicoline. Thirty subjects were randomly assigned to two treatment groups. Group One, which included 15 patients (3 males and 12 female, mean age 71.5 years) were administered 800 mg A-GPC as a single injection i.m. for 20 consecutive days. Group Two included 15 subjects, 5 males and 10 females with a mean age of 74.6, ranging from 62 to 88 years. These subjects received 1,000 mg citicoline daily for 20 consecutive days. Twelve subjects had initial mental deterioration of cerebrovascular origin. There was no significant difference in the effect on the treatment groups. Both drugs enhanced the subjects' ability to pay attention and to concentrate and improved cognitive functions. A-GPC did not elicit any adverse side effects locally or systemically and did not have any clinical interactions with other drugs such as digitalis, antihypertensives-diuretics, coronary vasodilators, aminophylline, and antiepileptic drugs.

Materazzo (Centro Studi, Ospedale di Manduria, Taranto, Italy) conducted a controlled clinical study in elderly patients with involution brain syndrome. In this double-blind, randomized clinical trial, the effect of A-GPC was compared to that of citicoline. Forty subjects were placed into two groups (20 in each group). Subjects in the first group received 1,000 mg/d A-GPC i.m. /day for 20 d and those in the second group received 1,000 mg citicoline i.m. /day for 20 days. The investigators reported that A-GPC consistently and significantly improved clinical symptoms and psychic function, but there was no mention of the tolerability of A-GPC.

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Pagliano (Divisione di Medicina Generale dell'Ospedale Viarana Besana Brianza) compared the therapeutic activity and tolerability of oral doses of 400mg A-GPC capsules and phosphatidylcholine in patients with senile mental decline. In this single-blind, controlled randomized clinical trial, 20 patients were randomly assigned to the A-GPC group (11 females, 9 males; 62–95 years) and 20 were assigned to the phosphatidylcholine group (13 females, 7 males; 79–88 years). A-GPC was said to improve anxiety significantly as measured by the Sandoz Clinical Assessment-Geriatric Scale. The reported that there were no side effects and no changes in laboratory test before and after treatment.

Guardamagna (Divisione Medica, Ospedale Generale di Erba, Como, Italy) compared the therapeutic activity and tolerability of A-GPC and phosphatidylcholine with senile mental decline. Twenty subjects were randomly assigned to receive one 400mg oral capsule of A-GPC TID for 20 days and twenty other subjects were randomly assigned to receive two 200 mg oral capsules of phosphatidylcholine TID for 20 days. A-GPC was said to be more effective than phosphatidylcholine. No side effects were reported and the overall tolerability was said to have been good for all subjects.

Vezzetti and Bettini (1992) (Medicine Division, Del Ponte Hospital, Varese, Italy) evaluated the therapeutic effects of A-GPC on mental decay using psychometric scales and reaction time recording result. Sixty subjects (30 men and 30 women, ages not reported) participated in the study. One group received A-GPC at a dose of 1,200 mg/d orally and the other group received a placebo (not specified) for 3 months. A-GPC improved performance at the conclusion of therapy on two tests and improved reaction time. No information was provided about the tolerability or adverse effects of A-GPC.

Schettini et al. (1993) investigated the effect of A-GPC on elderly subjects with primary degenerative deficiency. This study included 10 subjects, men and women above age 60 meeting the NINCDS-ADRDA criteria for Alzheimer's disease with negative brain CAT scans for focal lesions. Subjects were randomly assigned to receive i.m. 1,000 mg/d injections or placebo for 3 months.

VIII.2 Adverse Events for Gliatilin

Italfarmaco conducted a safety update from 1990 through 1996 for Gliatilin, a product of Italfarmaco S.p.A. (1996) that is available in Italy, Argentina, South Korea, and Poland as i.v./e.v. ampoules. In Italy two other products with the same active ingredient were available under the names Brezal® (Sandoz) and Delicit® LPB. The report stated that there were an estimated 705,000 individuals treated with Gliatilin between 1990 and 1996 and there were no reports of adverse events by individuals who prescribed the medication.

Italfarmaco conducted eight Phase IV clinical trials treating 4,285 subjects and treating with 1,000-1,200 mg Gliatilin®/d for 1 to 6 months. The adverse events reported in the studies are summarized in Table 10. Italfarmaco reported a 2.1% overall incidence of adverse events, none of which were considered serious. Central nervous system

complaints such as restlessness, insomnia, headache in 1% of subjects and gastrointestinal tract complaints such as sickness, emesis, and epigastria in 1 percent of patients were the most commonly reported.

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Table 10: Phase IV studies Gliatilin™ Safety Update 1990–1996

Indication	Treatment with Gliatilin™				Patients with adverse reactions	No. of adverse reactions
	No. of patients	Dose/day (mg)	Administration route	Therapy duration		
Senile Dementia of Alzheimer's type	60	1,200	os	6 months	1 (1.7%)	1
Multi infarct dementia	57	1,000	i.m.	3 months	0	0
Age-associated memory impairment	10	1,200	os	6 months	1 (10%)	1
Post-traumatic dementia	20	1,200	os	6 months	1 (5%)	1
Dementia of various types	15	1,000	i.m.	2 months	0	0
Post-stroke dementia	1,295	1,200	os	6 months	42 (3.2%)	45
Recovery of neuronal-psychical function in post-stroke dementia	770	1,000	i.m.	1 month	17 (2.2%)	20
		1,200	os	5 months		
	2,058	1,000	i.m.	1 month	27 (1.3%)	27
		1,200	os	5 months		
Total	4,285		i.m. os		89 (2.1%)	

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IX. DISCUSSION AND ANALYSIS

IX.1 GRAS Criteria

FDA defines “safe” or “safety” as it applies to food ingredients as:

“... reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance” (21 CFR 170.3(i)).

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance and appropriate safety factors. It is FDA’s operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, 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.

General recognition of safety through experience based on common use in food prior to January 1, 1958, shall be based solely on food use of the substance prior to January 1, 1958, and shall ordinarily be based upon generally available data and information” (21 CFR 170.3(a)).

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, *i.e.*, the so-called “common knowledge element,” in terms of the two following component elements:¹

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not necessarily unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as JECFA and the National Academy of Sciences.

¹See Footnote 1.

The apparent imprecision of the terms “appreciable”, “at the time” and “reasonable certainty” demonstrates that the FDA recognizes the impossibility of providing absolute safety, in this or any other area (Lu, 1988; Renwick, 1990).

IX.2 Review of Information on A-GPC

GRAS conclusions rely extensively on the combination of chemical composition data, safety/toxicity information, and anticipated consumer exposure levels. The GRAS assessment process requires establishing agreement within an Expert Panel to be convened that the salient data and regulatory issues of A-GPC will support the safety standard that there is reasonable certainty of no harm under the intended conditions of use. It should be noted that possible benefits of the food ingredient carry no weight in the assessment of safe dietary levels.

A-GPC is derived from soy lecithin. Similarly to lecithin, there is a high presumption of safety for the use of A-GPC in food. This presumption of safety was firmly expressed in the SCOGS and JECFA reviews of lecithin where there were limited toxicological data available. In contrast, there are reasonable amounts of toxicological data available for A-GPC. A subset of toxicity data conducted on A-GPC that included *in vitro* studies (bacterial reverse mutation, yeast forward mutation, yeast gene conversion, yeast host mediated gene conversion and mouse micronucleus assay), acute oral toxicity studies in rats, mice, and dogs, sub-chronic oral toxicity studies in rats, and chronic oral toxicity studies in rats and dogs has been published (Brownawell et al., 2011).

In evaluating the safety of A-GPC, the Expert Panel placed emphasis on chronic toxicity studies of A-GPC conducted on Sprague Dawley® rats and beagle dogs, especially two studies done by the oral route. These studies were described in Brownawell et al. (2011) and De Caro (1986) unpublished report. Rats in the low-dose (100 mg/kg A-GPC) and mid-dose (300 mg/kg A-GPC) dose groups exhibited no change in behavior and no differences in food consumption and body weight gain after treatment with oral A-GPC for 26 weeks. After 3-4 weeks of oral treatment with A-GPC, rats that received the high dose (1,000 mg/kg) of A-GPC experienced a variable, but generally mild to moderate, decrease in spontaneous motor activity and reactivity to stimulation within 1–2 hours of dosing. These effects persisted for 3–5 hours, however, the activity of the rats returned to normal during the recovery period. Rats in the 1,000 mg/kg dosing group also exhibited reduced food consumption and body weight gain. Alterations in clinical chemistry that may have been related to decreased activity and reduced body weight were observed. These alterations included reduced plasma triglycerides in both sexes and decreased plasma bilirubin, ALT, and creatinine. Body weight gain of animals in the high dose group was similar to that of controls by the end of the 4-week recovery period. Urinalysis, necropsy, and histopathological studies revealed no effects related to A-GPC treatment. Overall, the type and frequency of the pathologies were generally mild and similar across the experimental groups (Brownawell et al., 2011). Some of the 10 animal deaths in the study were due to problematic gavage manipulation. Other deaths, which lacked common organ toxicity, were not considered attributable to the dose of A-GPC.

In chronic studies with beagle dogs, administration of 75 mg/kg (low dose) and 150 mg/kg (moderate-range dose) A-GPC for 26 weeks did not affect behavior, body weight, or hematology, clinical chemistry or urinalysis measurements. Dosing of 6 beagle dogs with 75, 150, or 300 mg/kg A-GPC via the oral route resulted in no deaths (Brownawell et al., 2011). The 300 mg/kg (high) dose elicited a mild reduction in activity that lasted for 2–5 hours. Body weight gain was reduced at week 13. Increased plasma cholesterol and decreased alkaline phosphatase were observed at week 13, but not at week 26. Decreased liver and heart weights were observed, however, there were no histopathological correlates. Clinical chemistry analyses also revealed an increase in plasma bilirubin, triglycerides and alkaline phosphatase, which was suggestive of reduced liver function however, these changes may have been related to decreases in activity and body weight gain. Overall, these effects were minor. In the chronic studies in rats and dogs, the reduction in plasma triglycerides may be viewed as beneficial. The NOAEL for A-GPC is greater than 150 mg/kg in dogs and 300 mg/kg in rats.

Subchronic (4-week) studies in rats in which doses of 100, 300 and 1,000 mg/kg bw/d A-GPC were administered orally resulted in no toxicological effects. There were no significantly different changes in body weight gain, hematology, clinical chemistry, or urinalysis, and no A-GPC related effects on organ weight or histopathology. Only the 1,000 mg/kg dose of A-GPC elicited reduced activity; the lower doses (100 and 300 mg/kg) did not have this effect. In another subchronic rat study in which 50, 150, and 500 mg/kg A-GPC were administered subcutaneously for 30 days showed only the effect of CNS depression at the 500 mg/kg dose but no effects on other measures. Doses of 60 and 120 mg/kg A-GPC delivered to dogs intravenously each day for 4 weeks elicited reduced activity, but no doses resulted in harmful effects on growth, food consumption, tissue injury, or other signs of toxicity. Oral dosing of dogs with 75, 150 or 300 mg/kg A-GPC revealed no significant changes for the two lower doses but the 300 mg/kg dose gave rise to moderate sedation. While renal tubular degeneration was observed in a 90-day study due to high phosphorus intake (Schauss et al., 2009), no such effect was seen in A-GPC studies.

Acute toxicity studies in rats and dogs indicate low toxicity of A-GPC. Mortality was observed only with the 10,000 mg/kg of A-GPC. The LD₅₀ values were 1,143 mg/kg in mice and 1,575 mg/kg in rats for i.v. dosing, and 1,927 mg/kg in mice and 2,073 mg/kg in rats for i.p. dosing. The primary toxic effect observed was central nervous system depression with increasing dosage. Acute toxicity studies in beagle dogs resulted in no deaths. All the doses administered in studies (200 and 500 mg/kg i.m. and 1,000 and 3,000 mg/kg oral) elicited CNS depression with increasing severity and duration of effect with the higher doses.

Perinatal and postnatal toxicity studies in rats revealed that those given subcutaneous doses of 50, 150, and 500 mg/kg A-GPC carried their pregnancies to term and had no appreciable difference in weight gain compared to controls during the final stages of pregnancy and during the nursing period. Animals that received the highest dose exhibited mild sedation, but those receiving the lower doses did not demonstrate any

evidence of sedation. A-GPC had no effect on number of fetuses delivered, sex distribution, or the average weight for the litter size. Mortality of the litters was highest for control animals and for animals given the highest dose of A-GPC. There was no significant difference in mean weight at birth or at other time points compared to controls, or with respect to various developmental measurements. Rats receiving various doses of A-GPC had similar numbers of fetuses, live and dead fetuses, mean fetal weight and sex distribution, and there was no correlation between pre-implant and post-implant losses and treatment given to the dam. F₁ rats showed no significant differences in the number of corpora lutea or implant sites. These data suggest that A-GPC has low toxicity to pregnancy outcomes, physical neurological and sensory development of F₁ animals, fertility of F₁ rats, and findings at Caesarian sections for F₁ rats and F₂ fetuses.

Additional support for low toxicity of A-GPC comes from *in vitro* studies, which showed no evidence of mutagenicity of A-GPC Brownawell et al. (2011) and Dubini (1984). The bacterial reverse mutation test showed no significant increases in the number of revertant colonies after treatment with up to 10,000 µg/plate and A-GPC was not mutagenic in strains TA98, TA100, TA 1535 and TA1537 of *Salmonella typhimurium*. In the yeast mutation study, A-GPC did not change the frequency of spontaneous forward mutations of *S. pombe* P1 up to 3,000 µg/ml, with or without, microsomal activation. Similarly, A-GPC was not found to be mutagenic in the yeast conversion test, host-mediated yeast conversion test, the mouse micronucleus assay or the human lymphocyte assay (Brownawell et al., 2011)

A number of clinical studies have been conducted on A-GPC, some of which have not been published or are published in Italian and have been summarized based on other publications. Clinical studies have tested up to 1,200 mg/person/d A-GPC for up to 6 months. A number of studies have reported no adverse health effects of A-GPC (Canal et al., 1991; Frattola et al., 1991; Muratorio et al., 1992; Ziegenfuss et al., 2008) or mild effects (Ban et al., 1991; De Jesus Moreno Moreno, 2003; Parnetti et al., 1993) or overall tolerability was said to be good (Abbati et al., 1991; Barbagallo Sangiorgi et al., 1994). The large number of clinical trials where no or mild adverse health effects were observed have lead to a conclusion of safety at the dose of 1,200 mg/person/day.

A safety assessment of Gliatilin, the ChemiNutra product, reported a 2.1% overall incidence of adverse effects in eight Phase IV clinical trials including approximately 4,300 subjects, none of which were considered to be "serious." In addition to CNS effects such as restlessness, insomnia, and headache, issues with the GI tract were the most frequently reported concerns.

A-GPC is a metabolite of lecithin, which is a common component of food and is viewed as harmless. In addition, the daily requirement for choline is rather high. A-GPC is an endogenous material in humans and is also found in human breast milk. A-GPC is well characterized chemically and has very adequate toxicology and clinical data supporting its safety and published toxicological data on A-GPC are in press. An important determinant of safety is the maximum daily consumption level of a substance.

ChemiNutra has proposed the use of A-GPC in carbonated beverages (20 mg/serving, serving size –240 ml); liquid meal replacement (100 mg/serving, serving size – 240 ml); powdered beverages (100 mg/serving, serving size – 1 packet/ 1 or 2 tablespoon(s)); coffee (10 mg/serving, serving size – 240 ml); tea (10 mg/serving, serving size – 240 ml); milk (fluid) (20 mg/serving, 240 ml serving size); flavored milk/milk drink (20 mg/serving, serving size – 240 ml); yogurt (40 mg/serving, serving size – 225 g); powdered milk (10 mg serving, serving size - 1/3 cup); ready-to-eat breakfast cereals weighing < 20 g per cup (20 mg/serving, serving size – 15 g); ready-to-eat breakfast cereals weighing \geq 20 g per cup and < 43 g per cup or high fiber cereals containing 28g or more fiber per 100 g (20 mg/serving, serving size – 30 g); ready-to-eat breakfast cereals weighing \geq 43 g per cup or biscuit types (20 mg/serving, serving size – 55 g); grain-based bars (100 mg/serving, serving size – 40 g); protein bars (100 mg/serving, serving size – 40 g); chocolate (20 mg/serving, serving size – 40 g); candies (20 mg/serving, serving size – 40 g); and chewing gum (20 mg/serving, serving size –3 g). The mean estimated intake of A-GPC if used in the specified foods and beverages at the specified level is 108.4 mg/person/day or 1.86 mg/kg bw/day. The estimated intake of A-GPC if used in the specified foods and beverages at the specified level for a consumer in the 90th percentile is 196.2 mg/person/day or 3.5 mg/kg bw/day. This estimated intake is lower than the doses found to be safe in clinical studies in the doses were as high as 1,200 mg/person/day. No effect levels in the chronic animal toxicology studies ranged from 150 mg/kg bw/day (dogs) and 300 mg/kg bw/day (rats). The only effects observed in the chronic studies were mild (sedation). Although a 100-fold margin of safety gives increased confidence in safety of a substance, the estimated intake of A-GPC places exposure in the range of a 50-fold margin of safety. The Panel considers use of A-GPC at the proposed levels in the specified foods and beverages, resulting in an estimated daily intake of 196.2 mg/person/day for a consumer in the 90th percentile, to be GRAS. The Panel notes that there were no data provided about the safety of the use of A-GPC in infant formula, and in order to err on the side of caution, the Panel advises against use of A-GPC in infant formula.

IX.3 General Recognition of Safety of A-GPC

The Expert Panel has determined that the proposed use of Alphasize™ A-GPC intended to be added as a nutrient to the specified foods and beverages at the specified levels to result in consumption of no more than 196.2 mg/person/day is considered safe, with the exception of use in infant formula, by scientific procedures established under 21 CFR §170.3(b).

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X. Expert Panel Signatures



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Michael Falk, Ph.D.
Executive Director

The Expert Panel has determined, based on critical, independent evaluation of the available data and information, that AlphaSize® Alpha-glycerol phosphoryl choline (A-GPC), meeting the specifications cited, is Generally Recognized as Safe (GRAS) by scientific procedures, when used as an nutrient in conventional beverage and beverage bases, including coffee, tea, milk (fluid), powdered milk, flavored milk/milk drinks, carbonated beverages, powdered beverages, meal replacement liquids, foods including yogurt, grain-based bars, protein bars, ready-to-eat breakfast cereals, and snack foods including chocolates, candies, and chewing gum at levels ranging from 10–100 mg/serving per day and resulting in consumption of no more than 196.2 mg/person/day, excluding use in infant formula, would be Generally Recognized as Safe (GRAS), as long as it is produced in accordance with current Good Manufacturing Practice (21CFR§182.1 (b)) and used in an amount not to exceed the specified levels in the finished products.

Edward Carmines, Ph.D.
Principal, Carmines Consulting, LLC

Name: [Redacted]
Date: 3/24/11

Richard Kraska, Ph.D., D.A.B.T.
COO & Co-Founder,
Gras Associates, LLC
Vice President and Principal,
Kraska Consultants, Inc.

Name: [Redacted]
Date: 3/29/2011

Madhusudan G. Soni, Ph.D., FACN
Consulting Toxicologist
Soni and Associate, Inc.

Name: [Redacted]
Date: 3/30/11

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XII. APPENDICES A–B



QUALITY CONTROL DEPARTMENT
 MANUFACTURED IN CHEMI S.p.A.
 Veduggia, 5 - 03010 Patrica (FR) Italy
 (+39) 0775.2581
 (+39) 0775.258253

CERTIFICATE OF ANALYSIS N° 5029049

Page 1 of 1

Patrica: 01/07/2009

Code: FV5004

Manuf. date: 07/2009

Retest date: 07/2014

Product: AlphaSize 50P

Sinonym: sn-Glycero-3-phosphorylcholine (GPC 50%)

Formula: C8H20NO6P

M.W.: 257.22

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description	White to off white powder	Conform
Identification	TLC (Positive)	Positive
Glycerylphosphorylcholine (% GPC)	50 - 52%	50.6
Microbial contamination	Total plate count: NMT 1000 CFU/g	LT 100
	Yeast and molds: NMT 100 CFU/g	LT 100
	Escherichia Coli: negative/g	Negative
	Staphylococcus aureus: negative/g	Negative
	Coliforms: negative/g	Negative
	Salmonella: negative/10g	Negative
Typical composition (per 100g)	Dicalcium Phosphate Dihydrate:	
	43.5 - 48.5%	
	Magnesium stearate: 0.5 - 1.5%	
	Silicon dioxide: 0.5 - 1.5%	
	Magnesium silicate: 0.5 - 1.5%	

DATE

22/7/09

000087

QUALITY CONTROL
 (b) (6)

M. Irrera



QUALITY CONTROL DEPARTMENT
 MANUFACTURED IN CHEMI S.p.A.
 Via Vadisi, 5 - 03010 Patrica (FR) Italy
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 Fax (+39) 0775.258253

USA Business Unit & Address:
 Chemi Nutra
 4463 White Bear Parkway
 Suite 105
 White Bear Lake, MN55110
 USA
 P 651 404 0400
 F 651 407 0509

CERTIFICATE OF ANALYSIS N° 5029049/A

Page 1 of 1

Code: FV5004

Manuf. date: 07/2009

Patrica: 08/09/2009

Retest date: 07/2014

Product: AlphaSize 50P

Synonym: sn-Glycero-3-phosphorylcholine (GPC 50%)

Formula: C8H20NO6P

M.W.: 257.22

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description [Visual]	White to off white powder	Conform
Identification [TLC]	TLC (Positive)	Positive
Glycerolphosphorylcholine (% GPC) [Potentiometric HClO4 titration]	50 - 52%	50.6
Microbial contamination [USP]	Total plate count: NMT 1000 CFU/g	LT 100
	Yeast and molds: NMT 100 CFU/g	LT 100
	Escherichia Coli: negative/g	Negative
	Staphylococcus aureus: negative/g	Negative
	Coliforms: negative/g	Negative
	Salmonella: negative/10g	Negative
Typical composition (per 100g)	Dicalcium Phosphate Dihydrate: 43.5 - 48.5%	
	Magnesium stearate: 0.5 - 1.5%	
	Silicon dioxide: 0.5 - 1.5%	
	Magnesium silicate: 0.5 - 1.5%	

DATE

8/5/09

QUALITY CONTROL

(b) (6)

M. Irrera

000088



THE DEVELOPMENT SERVICES COMPANY

3301 Kinsman Boulevard
Madison, WI 53704

Chemi Nutra

4463 White Bear Pkwy
Suite 105
White Bear Lake Minnesota 55110 United States

Print Date: 23-Nov-2010 2:31 pm

Report Date: 23-Nov-2010

Report Number: 310269-0

Certificate of Analysis

Final Report

Client Sample Name:	AlphaSize 50 P	Covance Sample Number:	629329
Project ID	CHEMI_NUTR-20101119-0003	Receipt Date	19-Nov-2010
PO Number	111810/charge Visa	Receipt Condition	Ambient temperature
Lot Number	(b) (4)	Login Date	19-Nov-2010
		Storage Condition at Covance	Ambient temperature
		Number Compositd	1
		Disposal Instructions	Dispose 30 days after final reported

Analysis/Result	Result
Elements by ICP Mass Spectrometry	
Arsenic	93.0 ppb
Cadmium	11.2 ppb
Lead	19.7 ppb
Mercury	<10.0 ppb

Method References

Testing Location

Elements by ICP Mass Spectrometry (ICP_MS_S:11)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 993.14 (Modified).

Testing Location(s)

Covance Laboratories Inc.
3301 Kinsman Blvd
Madison WI 53704

Released on Behalf of Covance by

Doug Winters

Laboratory Director

For questions on this report, please
contact your Client Service Representative
at 608-242-2712 x4170

These results apply only to the items tested. This certificate of analysis shall not be reproduced, except in its entirety, without the written approval of Covance.

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CERTIFICATE OF ANALYSIS N° 5030247

Page 1 of 1

Code: FV5004

Manuf. date: 03/2010

Patrica: 19/03/2010

Retest date: 03/2015

Product: AlphaSize 50P

Synonym: sn-Glycero-3-phosphorylcholine (GPC 50%)

Formula: C8H20NO6P

M.W.: 257.22

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description [Visual]	White to off white powder	Conform
Identification [TLC]	TLC (Positive)	Positive
Glycerylphosphorylcholine (% GPC) [Potentiometric HClO4 titration]	50 - 52%	51
Microbial contamination [USP]	Total plate count: NMT 1000 CFU/g	LT 100
	Yeast and molds: NMT 100 CFU/g	LT 100
	Escherichia Coli: negative/g	Negative
	Staphylococcus aureus: negative/g	Negative
	Coliforms: negative/g	Negative
	Salmonella: negative/10g	Negative
Typical composition (per 100g)	Dicalcium Phosphate Dihydrate: 43.5 - 48.5%	
	Magnesium stearate: 0.5 - 1.5%	
	Silicon dioxide: 0.5 - 1.5%	
	Magnesium silicate: 0.5 - 1.5%	

Heavy Metals (ICP Mass Spectrometry)	
Arsenic	117 ppb
Cadmium	16.0 ppb
Lead	23.0 ppb
Mercury	<10.0 ppb

DATE

M. Irrera

QUALITY CONTROL
 (b) (6)
 M. Irrera

000090



QUALITY CONTROL DEPARTMENT
 MANUFACTURED IN CHEMI S.p.A.
 Via Vadisi, 5 - 03010 Patrica (FR) Italy
 Tel (+39) 0775.2581
 Fax (+39) 0775.258253

CERTIFICATE OF ANALYSIS N° 5030247

Code: FV5004

Manuf. date: 03/2010

Patrica: 19/03/2010

Retest date: 03/2015

Product: AlphaSize 50P

Synonym: sn-Glycero-3-phosphorylcholine (GPC 50%)

Formula: C8H20NO6P

M.W.: 257.22

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description [Visual]	White to off white powder	Conform
Identification [TLC]	TLC (Positive)	Positive
Glycerylphosphorylcholine (% GPC) [Potentiometric HClO4 titration]	50 - 52%	51
Microbial contamination [USP]	Total plate count: NMT 1000 CFU/g	LT 100
	Yeast and molds: NMT 100 CFU/g	LT 100
	Escherichia Coli: negative/g	Negative
	Staphylococcus aureus: negative/g	Negative
	Coliforms: negative/g	Negative
	Salmonella: negative/10g	Negative
Typical composition (per 100g)	Dicalcium Phosphate Dihydrate: 43.5 - 48.5%	
	Magnesium stearate: 0.5 - 1.5%	
	Silicon dioxide: 0.5 - 1.5%	
	Magnesium silicate: 0.5 - 1.5%	

Heavy Metals
 (ICP Mass Spectrometry)

LT 10 ppm

000091

DATE

M. Irrera

QUALITY CONTROL
 (b) (6)
 M. Irrera



THE DEVELOPMENT SERVICES COMPANY

3301 Kinsman Boulevard
Madison, WI 53704

Chemi Nutra

4463 White Bear Pkwy
Suite 105
White Bear Lake Minnesota 55110 United States

Print Date: 23-Nov-2010 2:32 pm

Report Date: 23-Nov-2010

Report Number: 310270-0

Certificate of Analysis

Final Report

Client Sample Name:	AlphaSize 50 P	Covance Sample Number:	629330
Project ID	CHEMI_NUTR-20101119-0003	Receipt Date	19-Nov-2010
PO Number	111810/charge Visa	Receipt Condition	Ambient temperature
Lot Number	(b) (4)	Login Date	19-Nov-2010
		Storage Condition at Covance	Ambient temperature
		Number Compositd	1
		Disposal Instructions	Dispose 30 days after final reported

Analysis/Result	Result
Elements by ICP Mass Spectrometry	
Arsenic	94.9 ppb
Cadmium	11.3 ppb
Lead	18.4 ppb
Mercury	<10.0 ppb

Method References

Testing Location

Elements by ICP Mass Spectrometry (ICP_MS_S:11)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 993.14 (Modified).

Testing Location(s)

Covance Laboratories Inc.
3301 Kinsman Blvd
Madison WI 53704

Released on Behalf of Covance by

Doug Winters

Laboratory Director

For questions on this report, please
contact your Client Service Representative
at 608-242-2712 x4170

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000092



QUALITY CONTROL DEPARTMENT
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 Via Vadis: 5 - 03010 Patrica (FR) Italy
 Tel: (+39) 0775 2561
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CERTIFICATE OF ANALYSIS N° 5030262

Page 1 of 1

Code: FV5004

Manuf. date: 03/2010

Patrica: 25/03/2010

Retest date: 03/2015

Product: AlphaSize 50P

Sinonym: sn-Glycero-3-phosphorylcholine (GPC 508)

Formula: C8H20NO6P

M.W.: 257.22

Batch N°: 10P0108

Tests	Quality specifications	Results
Description [Visual]	White to off white powder	Conform
Identification [TLC]	TLC (Positive)	Positive
Glycerylphosphorylcholine (% GPC) [Potentiometric HClO4 titration]	50 - 52%	50
Microbial contamination [USP]	Total plate count: NMT 1000 CFU/g	LT 100
	Yeast and molds: NMT 100 CFU/g	LT 100
	Escherichia Coli: negative/g	Negative
	Staphylococcus aureus: negative/g	Negative
	Coliforms: negative/g	Negative
	Salmonella: negative/10g	Negative
Typical composition (per 100g)	Dicalcium Phosphate Dihydrate: 43.5 - 48.5%	
	Magnesium stearate: 0.5 - 1.5%	
	Silicon dioxide: 0.5 - 1.5%	
	Magnesium silicate: 0.5 - 1.5%	

Heavy Metals

(ICP Mass Spectrometry)

LT 10 ppm

DATE

M. Irrera

000093

QUALITY CONTROL

(b) (6)

M. Irrera



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CERTIFICATE OF ANALYSIS N° 5030262

Page 1 of 1

Code: FV5004

Manuf. date: 03/2010

Patrica: 25/03/2010

Retest date: 03/2015

Product: AlphaSize 50P

Synonym: sn-Glycero-3-phosphorylcholine (GPC 50%)

Formula: C8H20NO6P

M.W.: 257.22

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description [Visual]	White to off white powder	Conform
Identification [TLC]	TLC (Positive)	Positive
Glycerylphosphorylcholine (% GPC) [Potentiometric HClO4 titration]	50 - 52%	50
Microbial contamination [USP]	Total plate count: NMT 1000 CFU/g	LT 100
	Yeast and molds: NMT 100 CFU/g	LT 100
	Escherichia Coli: negative/g	Negative
	Staphylococcus aureus: negative/g	Negative
	Coliforms: negative/g	Negative
	Salmonella: negative/10g	Negative
Typical composition (per 100g)	Dicalcium Phosphate Dihydrate: 43.5 - 48.5%	
	Magnesium stearate: 0.5 - 1.5%	
	Silicon dioxide: 0.5 - 1.5%	
	Magnesium silicate: 0.5 - 1.5%	

Heavy Metals

LT 10 ppm

(ICP Mass Spectrometry)

DATE

26/4/10

QUALITY CONTROL

(b) (6)

[Redacted Signature]

M. Irrera

000094



THE DEVELOPMENT SERVICES COMPANY

3301 Kinsman Boulevard
Madison, WI 53704

Certificate of Analysis
Final Report

Print Date: 23-Nov-2010 2:32 pm

Report Date: 23-Nov-2010

Report Number: 310271-0

Chemi Nutra

4463 White Bear Pkwy
Suite 105
White Bear Lake Minnesota 55110 United States

Client Sample Name: AlphaSize 50 P		Covance Sample Number: 629331	
Project ID	CHEMI_NUTR-20101119-0003	Receipt Date	19-Nov-2010
PO Number	111810/charge Visa	Receipt Condition	Ambient temperature
Lot Number	10PO108	Login Date	19-Nov-2010
		Storage Condition at Covance	Ambient temperature
		Number Compositied	1
		Disposal Instructions	Dispose 30 days after final reported

Analysis/Result	Result
Elements by ICP Mass Spectrometry	
Arsenic	96.4 ppb
Cadmium	12.4 ppb
Lead	20.0 ppb
Mercury	<10.0 ppb

Method References

Testing Location

Elements by ICP Mass Spectrometry (ICP_MS_S:11)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 993.14 (Modified).

Testing Location(s)

Covance Laboratories Inc.
3301 Kinsman Blvd
Madison WI 53704

Released on Behalf of Covance by

Doug Winters

Laboratory Director

For questions on this report, please
contact your Client Service Representative
at 608-242-2712 x4170

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000095



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 Via Madisi, 5 - 03010 Patrica (FR) Italy
 Tel. (+39) 0775.2581
 Fax (+39) 0775.258253

CERTIFICATE OF ANALYSIS N° 5030366/A

Code: FV5004

Manuf. date: 05/2010
 Retest date: 05/2015

Patrica: 04/06/2010

Product: AlphaSize 50P

Synonym: sn-Glycero-3-phosphorylcholine (GPC 50%)
 Formula: C8H20NO6P M.W.: 257.22

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description [Visual]	White to off white powder	Conform
Identification [TLC]	TLC (Positive)	Positive
Glycerolphosphorylcholine (% GPC) [Potentiometric HCl04 titration]	50 - 52%	50
Microbial contamination [USP]	Total plate count: NMT 1000 CFU/g	LT 100
	Yeast and molds: NMT 100 CFU/g	LT 100
	Escherichia Coli: negative/g	Negative
	Staphylococcus aureus: negative/g	Negative
	Coliforms: negative/g	Negative
	Salmonella: negative/10g	Negative
Particle size (Sieved)	100µm less than 40%	34
Heavy metal [USP (231)]	NMT 10 ppm	LT 10
Typical composition (per 100g)	Silicon dioxide: 0.5 - 1.5%	
	Magnesium silicate: 0.5 - 1.5%	
	Dicalcium Phosphate Dihydrate: 43.5 - 48.5%	
	Magnesium stearate: 0.5 - 1.5%	

Heavy Metals (ICP Mass Spectrometry) LT 10 ppm

DATE
 6/6/10

QUALITY CONTROL
 (b) (6)
 M. Herrera

000096

Chemi Nutra

4463 White Bear Pkwy
Suite 105
White Bear Lake Minnesota 55110 United States

Client Sample Name:	AlphaSize 50 P	Covance Sample Number:	629332
Project ID	CHEMI_NUTR-20101119-0003	Receipt Date	19-Nov-2010
PO Number	111810/charge Visa	Receipt Condition	Ambient temperature
Lot Number	10PO286	Login Date	19-Nov-2010
		Storage Condition at Covance	Ambient temperature
		Number Composited	1
		Disposal Instructions	Dispose 30 days after final reported

Analysis/Result	Result
Elements by ICP Mass Spectrometry	
Arsenic	109 ppb
Cadmium	14.2 ppb
Lead	24.7 ppb
Mercury	<10.0 ppb

Method References

Testing Location

Elements by ICP Mass Spectrometry (ICP_MS_S:11)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 993.14 (Modified).

Testing Location(s)

Covance Laboratories Inc.
3301 Kinsman Blvd
Madison WI 53704

Released on Behalf of Covance by

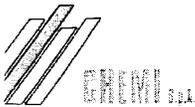
Doug Winters

Laboratory Director

For questions on this report, please
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at 608-242-2712 x4170

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000097



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 Fax (+39) 0775.258253

CERTIFICATE OF ANALYSIS N° 5029258

Page 1 of 1

Code: FV5004

Manuf. date: 10/2009

Patrica: 02/10/2009

Retest date: 10/2014

Product: AlphaSize 50P

Synonym: sn-Glycero-3-phosphorylcholine (GPC 50%)

Formula: C8H20NO6P

M.W.: 257.22

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description [Visual]	White to off white powder	Conform
Identification [TLC]	TLC (Positive)	Positive
Glycerylphosphorylcholine (% GPC) [Potentiometric HClO4 titration]	50 - 52%	51.3
Microbial contamination [USP]	Total plate count: NMT 1000 CFU/g	100
	Yeast and molds: NMT 100 CFU/g	LT 100
	Escherichia Coli: negative/g	Negative
	Staphylococcus aureus: negative/g	Negative
	Coliforms: negative/g	Negative
	Salmonella: negative/10g	Negative
Typical composition (per 100g)	Dicalcium Phosphate Dihydrate: 43.5 - 48.5%	
	Magnesium stearate: 0.5 - 1.5%	
	Silicon dioxide: 0.5 - 1.5%	
	Magnesium silicate: 0.5 - 1.5%	

DATE

15/10/09

(b) (6)

QUALITY CONTROL

M. Irrera

000098



THE DEVELOPMENT SERVICES COMPANY

3301 Kinsman Boulevard
Madison, WI 53704

Certificate of Analysis
Final Report

Print Date: 23-Nov-2009 4:20 pm
Report Date: 23-Nov-2009
Report Number: 170850-0

Chemi Nutra

4463 White Bear Pkwy
Suite 105
White Bear Lake Minnesota 55110 United States

Client Sample Name: AlphaSize 50P		Covance Sample Number: 330142	
Project ID	CHEMI_NUTR-20091119-0002	Receipt Date	19-Nov-2009
PO Number	111809	Receipt Condition	Ambient temperature
Lot Number	09PO562	Login Date	19-Nov-2009
		Storage Condition at Covance	-20 (+/- 10) Degrees Celsius
		Number Compositied	1
		Disposal Instructions	Dispose 30 days after final reported

Analysis/Result	Result
Elements by ICP Mass Spectrometry	
Arsenic	117 ppb
Cadmium	16.0 ppb
Lead	23.2 ppb
Mercury	<10.0 ppb

Method References

Testing Location

Elements by ICP Mass Spectrometry (ICP_MS_S:11)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 993.14 (Modified).

Testing Location(s)

Covance Laboratories Inc.
3301 Kinsman Blvd
Madison WI 53704

Released on Behalf of Covance by

Doug Winters

Laboratory Director

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at 608-242-2712 x4170

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000099



THE DEVELOPMENT SERVICES COMPANY

3301 Kinsman Boulevard
Madison, WI 53704

Chemi Nutra

4463 White Bear Pkwy
Suite 105
White Bear Lake Minnesota 55110 United States

Print Date: 23-Nov-2010 2:33 pm

Report Date: 23-Nov-2010

Report Number: 310273-0

Certificate of Analysis

Final Report

Client Sample Name: AlphaSize 50 P **Covance Sample Number:** 629333

Project ID	CHEMI_NUTR-20101119-0003	Receipt Date	19-Nov-2010
PO Number	111810/charge Visa	Receipt Condition	Ambient temperature
Lot Number	(b) (4)	Login Date	19-Nov-2010
		Storage Condition at Covance	Ambient temperature
		Number Compositied	1
		Disposal Instructions	Dispose 30 days after final reported

Analysis/Result	Result
Elements by ICP Mass Spectrometry	
Arsenic	108 ppb
Cadmium	17.0 ppb
Lead	24.5 ppb
Mercury	<10.0 ppb

Method References

Testing Location

Elements by ICP Mass Spectrometry (ICP_MS_S:11)

Covance Laboratories Inc.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 993.14 (Modified).

Testing Location(s)

Covance Laboratories Inc.
3301 Kinsman Blvd
Madison WI 53704

Released on Behalf of Covance by

Doug Winters

Laboratory Director

For questions on this report, please
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at 608-242-2712 x4170

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000100

Analysis report
CHN20529-5

Spectral Service



Laboratorium
für Auftragsanalytik GmbH

Spectral Service GmbH Post box 560122 D-50986 Köln

Chemi Nutra
Mr. Scott Hagerman
4463 White Bear Parkway - Suite 105
White Bear Lake, MN 55110
USA

Phone: +49/(0)2236/96947-0
Fax: +49/(0)2236/96947-11
eMail: Info@spectralservice.de
http: //www.spectralservice.de

Analysis method: quantitative ^{31}P -NMR spectroscopy according to SAA043

Instrument: Bruker Avance 300 MHz with autom. sample changer and BBI probe head

Internal standard: Phosphonoacetic acid Content [%]: 99,9 MW [g/mol]: 140,03

Initial weight test item [mg]: 87,86 Initial weight Internal standard [mg]: 40,01

Sample Ident.: AlphaSize 50P, Ch.-B.: 07P0107

Lab. No.:

Spectral Service Code: CHN20529-5

Results from: 09.08.2007

Phospholipid	Weight-%**)	Mol-%**)	MW [g/mol]
GPC	50,49	65,33	257,2
GPI	0,00	0,00	334,2
GPE	0,00	0,00	215,1
GPA	0,00	0,00	172,1
Phosphate (as H_3PO_4)	26,80	34,67	98,0
Sum	77,29	100,00	
Phosphorus	9,31		

*) = not observed, no signal assignment or < 0,1%
Limit of detection (S/N 10:1 Peak/Peak) = 0,5%
**) estimate (method not validated)

Cologne, 10. August 2007

(b) (6)

Thorsten Buchen
(Food chemist)

000101

CHN 20529-5

13.98
13.50
13.00

1.25
1.47

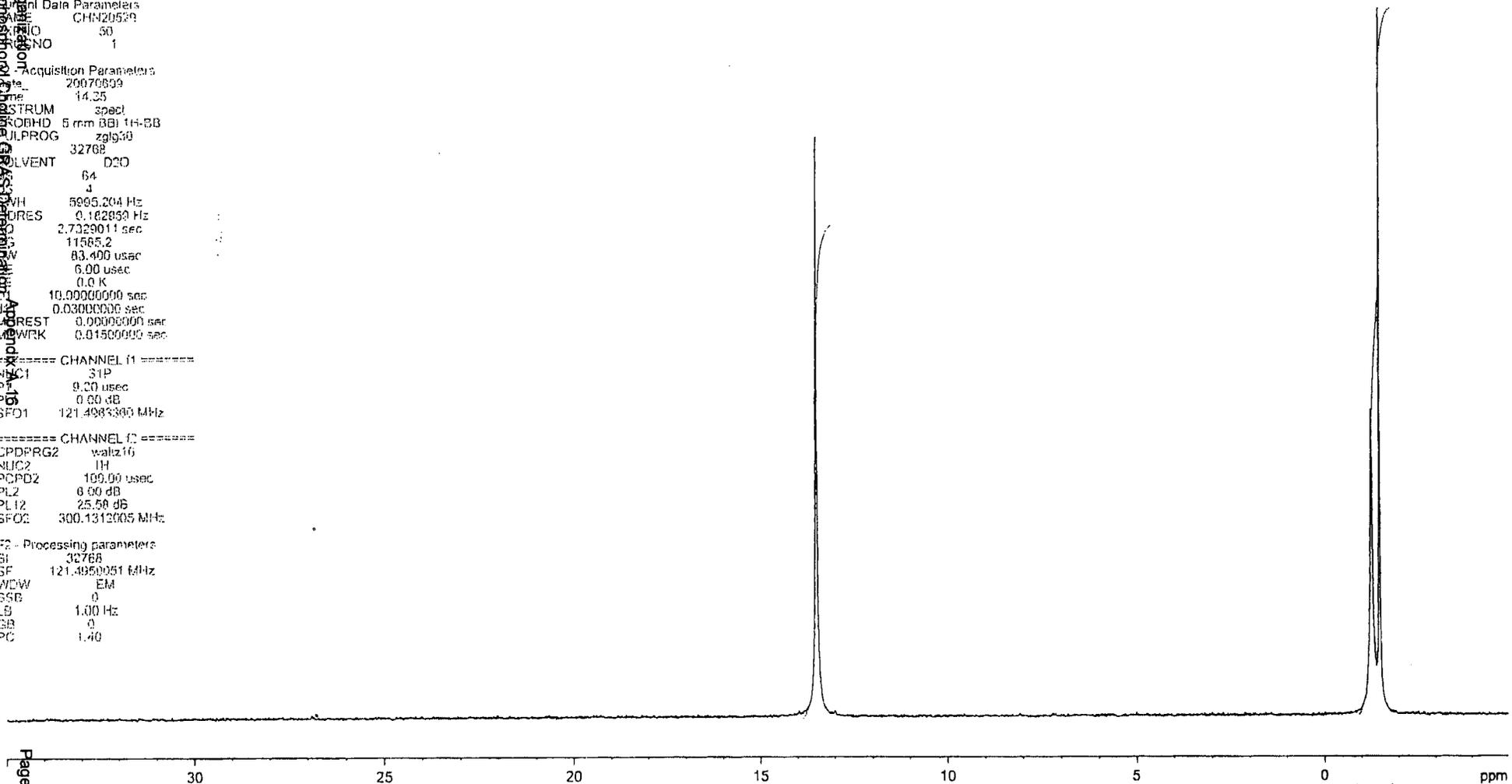
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 General Data Parameters
 CHN20529
 F1 50
 F2 1

Acquisition Parameters
 Date_ 20070609
 Time 14.35
 INSTRUM spect
 PROBHD 5 mm BBI H1-BB
 PULPROG zgpg30
 PC 32768
 SOLVENT D2O
 CH 64
 J 4
 NU1 5005.204 Hz
 FRES 0.162850 Hz
 AQ 2.7329011 sec
 SFO1 11565.2
 PC 83.400 usec
 PL1 6.00 usec
 PL2 0.0 K
 d1 10.0000000 sec
 d2 0.0300000 sec
 MREST 0.0000000 sec
 MWRK 0.0150000 sec

==== CHANNEL f1 =====
 NUC1 31P
 PL1 9.00 usec
 PL2 0.00 dB
 SFO1 121.4983300 MHz

==== CHANNEL f2 =====
 CPDPRG2 waltz16
 NUC2 1H
 PCPD2 100.00 usec
 PL1 8.00 dB
 PL2 25.50 dB
 SFO2 300.1312005 MHz

F2 - Processing parameters
 SI 32768
 SF 121.4950051 MHz
 WCW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40



13.98
13.50
13.00

1.25
1.47

000102



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CERTIFICATE OF ANALYSIS N° 5030314

Page 1 of 1

Code: FV5005

Manuf. date: 04/2010

Patrica: 21/04/2010

Retest date: 04/2015

Product: AlphaSize 50WSP

Synonym: sn-Glycero-3-phosphorylcholine (GPC 50%)

Formula: C8H20NO6P

M.W.: 257.22

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description [Visual]	White to off white powder	Conform
Identification [TLC]	TLC A e B (Positive)	Positive
Glycerolphosphorylcholine (% GPC) [Potentiometric HClO4 titration]	50 - 52%	50.3
Microbial contamination [USP]	Total plate count: NMT 1000 CFU/g	LT 100
	Yeast and molds: NMT 100 CFU/g	LT 100
	Escherichia Coli: negative/g	Negative
	Staphylococcus aureus: negative/g	Negative
	Coliforms: negative/g	Negative
	Salmonella: negative/10g	Negative
Typical composition (per 100g)	Mannitol: 47- 49.5%	
	Silicon dioxide: 0.5 - 1%	

Heavy Metals

LT 10 ppm

(ICP Mass Spectrometry)

000103

DATE

3/5/10

QUALITY CONTROL

(b) (6)

M. Irrera



QUALITY CONTROL DEPARTMENT
 MANUFACTURED IN CHEMI S.p.A.
 Via Radice, 8 - 03010 Patrica (FR) Italy
 Tel. (+39) 0775.2561
 Fax (+39) 0775.356253

0775253036

CERTIFICATE OF ANALYSIS N° 5030112

Page 1 of 1

Code: FV5005

Manuf. date: 01/2010

Patrica: 22/01/2010

Retest date: 01/2015

Product: AlphaSize 50WSP

Synonym: sn-Glycero-3-phosphorylcholine (GPC 50%)

Formula: C8H20NO6P

M.W.: 257.22

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description [Visual]	White to off white powder	Conform
Identification [TLC]	TLC A e B (Positive)	Positive
Glycerolphosphorylcholine (% GPC) [Potentiometric HCl04 titration]	50 - 52%	51
Microbial contamination [USP]	Total plate count: NMT 1000 CFU/g LF 10 Yeast and molds: NMT 100 CFU/g LF 10 Escherichia Coli: negative/g Staphylococcus aureus: negative/g Coliforms: negative/g Salmonella: negative/10g	Negative Negative Negative Negative Negative
Typical composition (per 100g)	Mannitol: 47- 49.5% Silicon dioxide: 0.5 - 1%	

DATE

QUALITY CONTROL

000104

(b) (6)

Page 99 of 136

M. Irrera



QUALITY CONTROL DEPARTMENT
 MANUFACTURED IN CHEMI S.p.A.
 Via Vadisi, 5 - 03010 Patrica (FR) Italy
 Tel (+39) 0775.2581
 Fax (+39) 0775.258253

USA Business Unit & Address:
 Chemi Nutra
 4463 White Bear Parkway
 Suite 105
 White Bear Lake, MN55110
 USA
 P. 651.404.0400
 F. 651.407.0509

CERTIFICATE OF ANALYSIS N° 5029042/A

Code: FV5005

Manuf. date: 06/2009

Patrica: 08/09/2009

Retest date: 06/2014

Product: AlphaSize 50WSP

Synonym: sn-Glycero-3-phosphorylcholine (GPC 50%)

Formula: C8H20NO6P

M.W.: 257.22

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description [Visual]	White to off white powder	Conform
Identification [TLC]	TLC (Positive)	Positive
Glycerylphosphorylcholine (% GPC) [Potentiometric HClO4 titration]	50 - 52%	50.4
Microbial contamination [USP]	Total plate count: NMT 1000 CFU/g	LT 100
	Yeast and molds: NMT 100 CFU/g	LT 100
	Escherichia Coli: negative/g	Negative
	Staphylococcus aureus: negative/g	Negative
	Coliforms: negative/g	Negative
	Salmonella: negative/10g	Negative
Typical composition (per 100g)	Mannitol: 47- 49.5%	
	Silicon dioxide: 0.5 - 1%	

000105

DATE

2/5/09

QUALITY CONTROL
 (b) (6)

M. Irrera



Certificate of analysis of Choline Alphoscerate batch 06PB0158



QUALITY CONTROL DEPARTMENT
MANUFACTURED IN ITALY

CERTIFICATE OF ANALYSIS N° 5020233/A

Page 1 of 1

Code: FV5000

Manuf. date: 02/2006

Patrica: 02/03/2006

Retest date: 02/2011

Product: GLYCERYLPHOSPHORYLCHOLINE

Sinonym: CHOLINE ALPHOSCERATE (GPC 85%)

Formula: C₈H₂₀NO₆P

M.W.: 257.2

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description	Highly viscous, transparent limpid liquid of semi-solid mass	Conform
Identification	TLC (Positive)	Positive
Solubility	Highly soluble in water and ethyl alcohol	Conform
solution (10% in Water)	Clear (Ph. Eur. 2.2.1.); not more colored than reference solution Y7 (Eu. Ph. 2.2.2.; Method II)	Conform
Specific Rotation (20°C, 589.3 nm)	-2.4° to -2.8° on d.b.	-2.7
pH (10% w/v Sol. in water)	5.0 - 7.0	5.5
Heavy metals	Not more than 10 ppm	LT 10
Sulphates	Not more than 200 ppm	Not Detectable
Chlorides	Not more than 200 ppm	LT 100
Free phosphates	Not more than 50 ppm	LT 50
Exposure to UV (254 nm)	Clear and not more intensely colored than reference solution Y7	Conform
Water content (K.F.)	14% to 16%	15.6
Related substances (TLC)	Single impurities NMT 0.5%	Not Detectable
	Total impurities NMT 1.0%	Not Detectable
Residual solvents	Methyl alcohol NMT 100 ppm	Not Detectable
Assay	98% - 102% on d.b.	100.3
Bacteria endotoxins (C=20% w/w)	< 87.7 EU/ml	< 87.7
Microbial contamination	Total plate count NMT 10 ² CFU/g	LT 10
	Molds and Yeasts NMT 10 CFU/g	LT 10
	Escherichia Coli NMT 1 CFU/g	LT 1
	Salmonella Spp. NMT 1 CFU/10 g	LT 1

Quality Assurance
CONFORME
2/3/06 (b) (6)

DATE

02/03/06

QUALITY CONTROL

(b) (6)

E. Pompiliè [QC/ Manager]

000106



Certificate of analysis of Choline Alphoscerate batch 06PB0163



QUALITY CONTROL DEPARTMENT
MANUFACTURED IN ITALY

CERTIFICATE OF ANALYSIS N° 5020241

Page 1 of 1

Code: FV5000

Manuf. date: 02/2006

Patrica: 28/02/2006

Retest date: 02/2011

Product: GLYCERYLPHOSPHORYLCHOLINE

Sinonym: CHOLINE ALPHOSCERATE (GPC 85%)

Formula: C8H20NO6P

M.W.: 257.2

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description	Highly viscous, transparent limpid liquid of semi-solid mass	Conform
Identification	TLC (Positive)	Positive
Solubility	Highly soluble in water and ethyl alcohol	Conform
Solution (10% in Water)	Clear (Ph. Eur. 2.2.1.); not more colored than reference solution Y7 (Eu.Ph. 2.2.2.; Method II)	Conform
Specific Rotation (20°C, 589.3 nm)	-2.4° to -2.8° on d.b.	-2.6
pH (10% w/v Sol. in water)	5.0 - 7.0	5.2
Heavy metals	Not more than 10 ppm	LT 10
Sulphates	Not more than 200 ppm	Not Detectable
Chlorides	Not more than 200 ppm	LT 200
Free phosphates	Not more than 50 ppm	LT 50
Exposure to UV (254 nm)	Clear and not more intensely colored than reference solution Y7	Conform
Water content (K.F.)	14% to 16%	14.5
Related substances (TLC)	Single impurities NMT 0.5%	Not Detectable
	Total impurities NMT 1.0%	Not Detectable
Residual solvents	Methyl alcohol NMT 100 ppm	Not Detectable
Assay	98% - 102% on d.b.	101
Bacteria endotoxins (C=20% w/w)	< 87.7 EU/ml	< 87.7
Microbial contamination	Total plate count NMT 10 ² CFU/g	LT 10
	Molds and Yeasts NMT 10 CFU/g	LT 10
	Escherichia Coli NMT 1 CFU/g	LT 1
	Salmonella Spp. NMT 1 CFU/10 g	LT 1

Quality Assessed
CONFORME
Date: 6/3/06 (b) (6)

000107 DATE
06/03/06

QUALITY CONTROL
(b) (6)
E. Pompilio [QC/ Manager]



Choline Alposcerate

our ref. DDR135.002

Certificate of analysis of Choline Alposcerate batch 06PC0189



QUALITY CONTROL DEPARTMENT
MANUFACTURED IN ITALY

CERTIFICATE OF ANALYSIS N° 5020263

Page 1 of 1

Code: FV5000

Manuf. date: 03/2006

Patrica: 06/03/2006

Retest date: 03/2011

Product: GLYCERYLPHOSPHORYLCHOLINE

Sinonym: CHOLINE ALPHOSCERATE (GPC 85%)

Formula: C8H20NO6P

M.W.: 257.2

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description	Highly viscous, transparent limpid liquid of semi-solid mass	Conform
Identification	TLC (Positive)	Positive
Solubility	Highly soluble in water and ethyl alcohol	Conform
lution (10% in Water)	Clear (Ph. Eur. 2.2.1.); not more colored than reference solution	
Specific Rotation (20°C, 589.3 nm)	-2.4° to -2.8° on d.b.	-2.4
pH (10% w/v Sol. in water)	5.0 - 7.0	6.0
Heavy metals	Not more than 10 ppm	LT 10
Sulphates	Not more than 200 ppm	Not Detectable
Chlorides	Not more than 200 ppm	LT 200
Free phosphates	Not more than 50 ppm	LT 50
Exposure to UV (254 nm)	Clear and not more intensely colored than reference solution Y7	Conform
Water content (K.F.)	14% to 16%	15.3
Related substances (TLC)	Single impurities	NMT 0.5% Not Detectable
	Total impurities	NMT 1.0% Not Detectable
Residual solvents	Methyl alcohol	NMT 100 ppm LT 100
Assay	98% - 102% on d.b.	100
Bacteria endotoxins (C=20% w/w)	< 87.7 EU/ml	< 87.7
Microbial contamination	Total plate count	NMT 10 ² CFU/g LT 10
	Molds and Yeasts	NMT 10 CFU/g LT 10
	Escherichia Coli	NMT 1 CFU/g LT 1
	Salmonella Spp.	NMT 1 CFU/10 g LT 1

Quality Assurance
 CONFORME
 13/3/06 F.M.C. (b) (6)

DATE

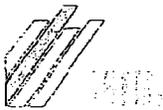
13/03/06

QUALITY CONTROL

(b) (6)

E. Pompilio [QC/ Manager]

000108



QUALITY CONTROL DEPARTMENT
MANUFACTURED IN ITALY

CERTIFICATE OF ANALYSIS N° 5023006

Page 1 of 1

Code: FV5003

Manuf. date: 12/2006

Patrica: 17/01/2007

Retest date: 12/2011

Product: L-Alfa-GLYCEROPHOSPHORYLCHOLINE

Sinonym: CHOLINE ALPHOSCERATE /GPC (CRYSTALLINE)

Formula: C8H20NO6P

M.W.: 257.2

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description	A white to off-white crystalline powder; highly soluble in water.	Conform
Identification	TLC (Positive)	Positive
Solution (5% in Water)	Clear (Ph. Eur. 2.2.1.); not more colored than reference solution	
	Y6 (Eu.Ph. 2.2.2.; Method II)	Conform
Water content (K.F.)	Not more than 2.0%	1.2
Assay (HClO4 0.1 N)	98.0% - 102.0% on d.b.	101.3
Microbial contamination	Total plate count NMT 1000 CFU/g	LT 100
	Yeast and molds: NMT 100 CFU/g	LT 100
	Escherichia Coli: negative/g	Negative
	Staphylococcus aureus: negative/g	Negative
	Coliforms: negative/g	Negative
	Salmonella Sp.: negative/10g	Negative

000109

DATE

15/12/07

QUALITY CONTROL
(b) (6)
M. Herrera



QUALITY CONTROL DEPARTMENT
 MANUFACTURED IN CHEMI S.p.A.
 Via Tadisi, 5 - 03010 Patrica (FR) Italy
 Tel. (+39) 0775.2581
 Fax (+39) 0775.258253

CERTIFICATE OF ANALYSIS N° 5030024/B

Code: FV5002

Manuf. date: 11/2009

Patrica: 17/12/2009

Retest date: 11/2014

Product: ALPHA SIZE 100P

Synonym: sn-Glycero-3-phosphorylcholine (GPC 100%)

Formula: C8H20NO6P

M.W.: 257.2

Batch N°: (b) (4)

Tests	Quality specifications	Results
Description	A white to off-white crystalline powder; highly soluble in water.	Conform
Identification	TLC (Positive)	Positive
Solution (5% in Water)	Clear (Ph. Eur. 2.2.1.); not more colored than reference solution Y6 (Eu.Ph. 2.2.2.; Method II)	Conform
Specific Rotation (20°C, 589.3 nm)	-2.4° to -2.8° on d.b.	-2.7
Heavy metals	Not more than 0.001%	LT 0.001
Water content (K.F.)	Not more than 1.0%	0.3
Chrom. purity (TLC)	Choline NMT 0.5%	LT 0.5
	Glycerol NMT 0.5%	LT 0.5
	Related substances NMT 0.5%	LT 0.5
	Single unknown NMT 0.1%	LT 0.1
	Total impurities NMT 1.5%	LT 1.5
Residual solvents	Report result	(1)
Assay (HClO4 0.1 N)	98.0% - 102.0% on d.b.	98.9
Moulds and Yeast (CFU/g)	Not more than 100 CFU/g	LT 10
Total plate count (CFU/g)	Not more than 1000 CFU/gr	70

Remarks: (1) Ethanol 0.24%

000110

DATE

(b) (6)

QUALITY CONTROL

M. Irrera

Material Safety Data Sheet

Firm Code: 505
Revised Date: 26/07/02

1. Identification Of The Substance/Preparation And Of The Company:

Identification of the product:

Product name: AlphaSize 50WSP

Chemical name: sn-Glycero-3-phosphorylcholine (GPC 50%)

Synonyms: GLYCERYLPHOSPHORYLCHOLINE 50%; Alpha-GPC 50%; FV5005

Manufacturer/supplier identification:

Company: CHEMI S.p.A., 03010 PATRICA (FR) ITALY

Contact for information: Safety Department

Emergency Tel. No.: 011 39 0775 2581

2. Composition/Information On Ingredients:

AlphaSize 50WSP 50-52%
MANNITOL 47-49, 5%
SILICON DIOXIDE 0,5-1 %

CAS No.: 28319-77-9

Molar mass: 257.22

EINECS number: 248-962-2

Molecular formula: C₈H₂₀N₀₆P

CHEMICAL CLASS: Phospholipid.

Therapeutic Category: Nootropic agent.

3. Hazards Identification:

- The powder can be irritant.
- Thermal decomposition or burning materials emits vapors of CO, CO₂, NO_x, and PO_x.
- This product is an active drug substance.

Individuals working with chemicals should consider all chemicals to be potentially hazardous even if their individual hazard may be uncharacterized or unknown.

000111

4. First Aid Measures: Remove from exposure. Remove contaminated clothing.

After skin contact: Wash with water.

After eye contact: Immediately flush with copious amounts of water for at least 15 minutes.

After ingestion: Wash out mouth with water, provided person is conscious.

After inhalation: Remove from exposure. If the person is not breathing give artificial respiration. If breathing is difficult give oxygen.

If irritation persists or signs of toxicity/hypersensitivity reaction occur, seek medical attention. Provide symptomatic/supportive care as necessary.

5. Fire-Fighting Measures:

Suitable extinguishing media: Use water spray, dry chemical, carbon dioxide or foam, as appropriate, for surrounding fire and materials.

Special fire fighting procedures: As with all fire, evacuate personnel to safe area. Fire fighters should use self-contained breathing equipment and protective clothing.

6. Accidental Release Measures:

Spill or release procedures: Refer to section 7 and 8 before handling the product. Recover product and place in appropriate container for disposal. Ventilate and wash area.

7. Handling And Storage:

Handling: Do not breathe dust.
Avoid prolonged or repeated exposure.
Avoid contact with eyes, skin and clothing.
Wash contaminated clothing before reuse.

Storage: Store in original container, tightly closed, in a cool (15/25°C) dry place, out of direct action of light. Provide for good ventilation of the room.

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8. Exposure Controls/Personal Protection:

Specific control parameter: TLV not established.

Personal protective equipment: (x) Approved respirators.
(x) Chemical resistant gloves.
(x) Safety goggles.
(x) Protective clothing.

Industrial hygiene: Do not eat, drink or smoke at work.
Wash thoroughly after handling.

Special precautions & comments: This material is a chemical/active drug substance. Standard good laboratory safety procedures and good manufacturing practices should be followed when handling this substance.

9. Physical And Chemical Properties:

Form: Powder.

Color: White to off-white.

Odor: Characteristic.

Boiling temperature: Not available.

Ignition temperature: Not applicable.

Flash point: Not applicable.

Explosion limits: Lower – not applicable.
Upper – not applicable.

Density (20°C): Not applicable.

Solubility in Organic solvent (20°C): Soluble in methanol.

Water (20°C): Soluble.

10. Stability And Reactivity:

Stability: Stable under normal use condition.

Incompatibilities: Strong oxidizing agents.

Conditions to be avoided: Light, air, moist, and excessive heat.

Hazardous decomposition products: When heated to decomposition it emits toxic fumes of CO, CO₂, NO_x, PO_x.

11. Toxicological Information:**- AlphaSize A-GPC:**

Acute toxicity assessed in mice, rats and dogs, both by oral and parenteral route, was very low. In no species could the oral LD50 be evaluated. Sub lethal doses in rodents and dogs (10 g/kg and 3 g/kg respectively) were found to be 584 and 175 times greater, respectively, than the predicted therapeutic daily dosage for a 70 kg man.

AlphaSize A-GPC did not affect mating behavior, fertility or performance in the tested animals. AlphaSize A-GPC is not teratogenic in rats or rabbits. Results obtained using experimental procedures both "in vivo" and "in vitro" clearly indicate that AlphaSize A-GPC is void of any mutagenic activity. In conclusion, AlphaSize A-GPC has a very low experimental toxicity and, therefore, is expected to be well tolerated in humans.

- MANNITOL

Acute toxicity: LD50 oral rat: 13500 mg/Kg

No toxic

12. Ecological Information:

No available data.

13. Disposal Consideration:

Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Observe all federal, state and local laws.

14. Transport Information:

Class RID-ADR-IMO-IATA: Not hazardous.

15. Regulatory Information:

Labeling according to EEC Directives: Not classified

Symbol: Not applicable

Classification: Not applicable

R-phrases: Not applicable

S-phrases: 22 – Do not breathe dust.

24/25 – Avoid contact with skin and eyes.

16. Other Information:**Bibliography:**

- Data Bank CHEMI S.p.A.

DISCLAIMER: This information is based on our current knowledge and is intended to describe the product for the purposes of health, safety and environmental requirements only. It should not therefore be construed as guaranteeing any specific property of the product.

000114

Material Safety Data Sheet

Firm Code: 447

Revised Date: 15/05/01

1. Identification Of The Substance/Preparation And Of The Company:

Identification of the product:

Product name: AlphaSize 50P
GLYCERYLPHOSPHORYLCHOLINE 50% POWDER

Chemical name: sn-Glycero-3-phosphorylcholine

Synonyms: GLYCERYLPHOSPHORYLCHOLINE 50% POWDER
Alfa-GPC 50%; FV5004

Manufacturer/supplier identification:

Company: CHEMI S.p.A., 03010 PATRICA (FR) ITALY

Contact for information: Safety Department

Emergency Tel. No.: 011 39 0775 2581

2. Composition/Information On Ingredients: AlphaSize 50P 50%

Dicalcium phosphate dihydrate 43.5-48.5%

CAS No.: 28319-77-9

Molar mass: 257.22

EINECS number: 248-962-2

Molecular formula: C₈H₂₀N₀O₆P

CHEMICAL CLASS: Phospholipid.

Therapeutic Category: Nootropic agent.

3. Hazards Identification:

- Can be irritant.
- Thermal decomposition or burning materials emits vapors of CO, CO₂, NO_x, and PO_x.
- This product is an active drug substance.
- Individuals working with chemicals should consider all chemicals to be potentially hazardous even if their individual hazard may be uncharacterized or unknown.

000115

4. **First Aid Measures:** Remove from exposure. Remove contaminated clothing.

After skin contact: Immediately wash with soap and copious amounts of water.

After eye contact: Immediately flush with copious amounts of water for at least 15 minutes.

After ingestion: Wash out mouth with water, provided person is conscious.

After inhalation: Remove from exposure. If the person is not breathing give artificial respiration. If breathing is difficult give oxygen. If irritation persists or signs of toxicity/hypersensitivity reaction occur, seek medical attention. Provide symptomatic/supportive care as necessary.

5. Fire-Fighting Measures:

Suitable extinguishing media: Use water spray, dry chemical, carbon dioxide or foam, as appropriate, for surrounding fire and materials.

Special fire fighting procedures: As with all fire, evacuate personnel to safe area. Fire fighters should use self-contained breathing equipment and protective clothing.

6. Accidental Release Measures:

Spill or release procedures: Refer to section 7 and 8 before handling the product. Recover product and place in appropriate container for disposal. Ventilate and wash area.

7. Handling And Storage:

Handling: Do not breathe dust.
Avoid prolonged or repeated exposure.
Avoid contact with eyes, skin and clothing.
Wash contaminated clothing before reuse.

Storage: Store in original container, tightly closed, in a cool (15/25°C) dry place, out of direct action of light. Provide for good ventilation of the room.

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8. Exposure Controls/Personal Protection:

Specific control parameter: TLV not established.

Personal protective equipment: (x) Approved respirators.
(x) Chemical resistant gloves.
(x) Safety goggles.
(x) Protective clothing.

Industrial hygiene: Do not eat, drink or smoke at work.
Wash thoroughly after handling.

Special precautions & comments: This material is a chemical/active drug substance. Standard good laboratory safety procedures and good manufacturing practices should be followed when handling this substance.

9. Physical And Chemical Properties:

Form: Powder.

Color: White to off-white.

Odor: Characteristic.

Boiling temperature: Not available.

Ignition temperature: Not applicable.

Flash point: Not applicable.

Explosion limits: Lower – not applicable.
Upper – not applicable.

Density (20°C): Not applicable.

Solubility in Organic solvent (20°C): Soluble in methanol.

Water (20°C): Soluble.

10. Stability And Reactivity:

Stability: Stable under normal use condition.

Incompatibilities: Strong oxidizing agents.

Conditions to be avoided: Light, air, moist, and excessive heat.

Hazardous decomposition products: When heated to decomposition it emits toxic fumes of CO, CO₂, NO_x, PO_x.

000117

11. Toxicological Information:**- Alpha-GPC:**

Acute toxicity assessed in mice, rats and dogs, both by oral and parenteral route, was very low. In no species could the oral LD50 be evaluated. Sub lethal doses in rodents and dogs (10 g/kg and 3 g/kg respectively) were found to be 584 and 175 times greater, respectively, than the predicted therapeutic daily dosage for a 70 kg man.

Alpha-GPC did not affect mating behavior, fertility or performance in the tested animals. Alpha-GPC is not teratogenic in rats or rabbits.

Results obtained using experimental procedures both "in vivo" and "in vitro" clearly indicate that alpha-GPC is void of any mutagenic activity.

In conclusion, alpha-GPC has a very low experimental toxicity and, therefore, is expected to be well tolerated in humans.

- Dicalcium phosphate dihydrate:

May be harmful by inhalation, ingestion or skin absorption.

Causes eye and skin irritation. Material is irritating to mucous membranes and upper respiratory tract. Exposure can cause gastrointestinal disturbance, nausea, headache and vomiting.

12. Ecological Information:

No available data.

13. Disposal Consideration:

Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Observe all federal, state and local laws.

14. Transport Information:

Class RID-ADR-IMO-IATA: Not hazardous.

15. Regulatory Information:***Labeling according to EEC Directives:***

Symbol: Xi

Classification: Irritant.

R-phrases: 36/37/38 – Irritating to eyes, respiratory system, skin.

S-phrases: 26 – In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

36 – Wear suitable protective clothing.

16. Other Information:***Bibliography:***

- Data Bank CHEMI S.p.A.

- Data Bank SIGMA-ALDRICH.

DISCLAIMER: This information is based on our current knowledge and is intended to describe the product for the purposes of health, safety and environmental requirements only. It should not therefore be construed as guaranteeing any specific property of the product.

000118

Pages 000119-000141 removed under Freedom of Information Exemption B6.

SUBMISSION END

000142