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Safety assessment of AGPC as a food ingredient

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ABSTRACT

α-Glycerylphosphorylcholine (AGPC) is a semi-synthetic derivative of lecithin. Following oral administration, it is converted to phosphatidylcholine, a metabolically active form of choline that is able to reach cholinergic synaptic endings where it increases acetylcholine synthesis and release. A series of studies were conducted to demonstrate the safety of AGPC. The oral LD50 was equal to or greater than 10,000 mg/kg in rats and mice. Deaths were preceded by convulsions in some animals. Dosing of dogs with up to 3000 mg/kg AGPC resulted only in reduced activity. Sub-chronic and chronic oral toxicity studies in rats (up to 1000 mg/kg/day) and beagles (up to 300 mg/kg/day) produced symptomology primarily consisting of reduced activity; slight decreases in food consumption and body weight gain; and slight reduction in liver weight, paralleled by significant decreases in plasma triglycerides, bilirubin, and alkaline phosphatase. There were no histopathological correlates. The *in vivo* and *in vitro* assays clearly indicated that AGPC was devoid of mutagenic activity. Based on these results, AGPC is not genotoxic *in vitro* or *in vivo*, exhibits low acute oral toxicity and, has an oral NOAEL of 150 mg/kg bw/day following 26 weeks oral exposure.

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1. Introduction

α-Glycerylphosphorylcholine (AGPC) is a semi-synthetic derivative of lecithin (see Fig. 1 for chemical structure). Following oral administration, it is converted to phosphorylcholine, a metabolically active form of choline able to reach cholinergic synaptic endings where it increases acetylcholine synthesis and release (Lopez et al., 1991; Trabucchi et al., 1986; Abbiati et al., 1991) (see Fig. 2). 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. The Institute of Medicine determined an adequate intake of 550 mg/day for adult males, 425 mg/day for adult females, 450 mg/day for pregnant women and 550 mg/day for nursing mothers (Institute of Medicine, 1998) that was based on the amount of choline 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. The IOM describes the critical adverse effect from high choline intake as 'hypotension, with corroborative evidence on cholinergic side effects (e.g., sweating and diarrhea) and fishy body odor' (Institute of Medicine, 1998). AGPC also contributes to anabolic processes responsible for membrane phospholipid and glycerolipid synthesis, thus positively influencing membrane fluidity. Investigators have examined the usefulness of AGPC in age-related dementias because these disorders are often associated with reduced cholinergic synthesis and impaired fluidity of neuronal membranes (Parnetti et al., 1993; De Jesus Moreno Moreno, 2003). AGPC may also influence the physiological response to exercise by altering acetylcholine release and promoting muscle contraction (Gatti et al., 1992). AGPC has been characterized as a centrally acting parasympathomimetic chemical in International Pharmacopeia and in the Chemical Therapeutic Anatomical Classification.

AGPC 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 (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 to FDA. In 2004, GRAS Notification 000134, pursuant to 21 CFR 170.30, the C-Fraction Soy Protein Hydrolyzate with Bound Phospholipids (CSPHP) was determined to be GRAS by scientific

Abbreviations: AGPC, α-glycerylphosphorylcholine; i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous; p.o., per os; NOAEL, no observable adverse effect level; MTD, maximum tolerated dose; SE, standard errors.

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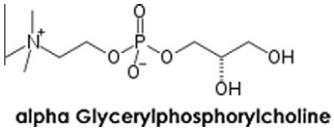

Fig. 1. Chemical structure of AGPC.

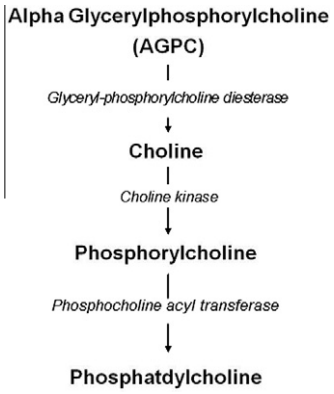

Fig. 2. Metabolism of AGPC (adapted from Amenta et al., 2001).

procedures based on information provided by Kyowa Hakko (USA) (CFSAN/FDA, 2004). In 2006, GRAS Notification 000186, in accordance with 21 CFR 170.30, the Soy Lecithin Phosphatidylserine Complex was determined to be GRAS on the basis of scientific procedures and information provided by Lipogen (Israel) (Lipogen Products Ltd., 2006). In 2008, GRAS Notification 000226, pursuant to 21 CFR 170.30, Krill-based Lecithin was determined to be GRAS on the basis of scientific procedures and information provided by Enzymotec (Israel) (CFSAN/FDA, 2008).

This paper summarizes the results of acute, sub-chronic, and chronic toxicity studies of oral and parenterally administered AGPC in rodents and dogs, as well as in standard *in vitro* and *in vivo* mutagenicity assays. These studies are intended to provide the general recognition of safety required for a GRAS determination.

2. Materials and methods

2.1. AGPC[1]

AGPC was supplied by Italafarmaco S.p.A. as an aqueous solution for gavage or injection in an ampoule containing an aqueous solution/suspension of 90% active substance. Each 4 mL AGPC ampoule contained 1 g AGPC, 27 mg sodium chloride, and 4 mL water. The following lots of AGPC were used in the studies: Lot 165F (analytical certificate 74A/83 dated 5 September 1983) and Lot 61G (analytical certificate 106A/B dated 25 September 1984). Throughout this report, the stated amounts of active substance represent the amount of substance actually administered.

2.2. Study conduct and GLP

These original research studies were conducted in accordance with OECD Guidelines for Testing Chemicals and GLP regulations outlined in Organisation for Economic Cooperation and Development Guidelines (Organisation for Economic Co-operation and Development, 2009). Animal toxicity studies were conducted by the Institute of Pharmacology at the University of Camerino (Italy). The mutagenicity studies were conducted by the Institute of Human Anatomy at the University of Catania (Italy) and the Institute of Microbiology, at the University of Milan (Italy). The sources of the individual reagents used in the assays are not known.

[1] AGPC is a registered drug in the European Union and is marketed under the trade name Gliatilin (choline alfoscerate).

2.3. Animal strains

Swiss mice and Sprague–Dawley rats were obtained from animal nurseries at the University of Camerino (Italy). Beagles were supplied and maintained by the firm Far.Al.Co. Service S.r.l. of Monza from their animal nurseries at Ornago (Milan, Italy).

2.4. Housing, care, and allocation

Animals were kept in constant temperature (20 ± 1 °C) and relative humidity (60 ± 5%) rooms. Rodents were housed in groups of the same sex in Makrolon boxes, and removed to individual boxes when necessary. Beagles were housed in groups of 2 or 3 (or individually when necessary) in brickwork boxes built in an enclosed ventilated and centrally heated room. All animals were maintained on appropriate diets of pelletized feed (Laboratorio Dottori Piccioni, Milan, Italy). House water was available *ad libitum*. Animals were randomly allocated to the studies after a 1 to 3 week quarantine period. Each animal was identified by cage and ear markings. The age of the animals at each study initiation was not available.

2.5. Acute toxicity studies

2.5.1. Rats and mice

The acute toxicity of AGPC was investigated in mice and rats of both sexes (6 male/6 female in each dosing group) receiving single administrations by intravenous, intraperitoneal, and oral routes. The appearance and behavior of the animals were observed for 6 h after dosing and then daily for 2 weeks. Deaths were recorded daily, and post mortem examinations were performed on all dead animals, as well as on the survivors at the end of the observation period.

2.5.2. Dogs

The acute toxicity was investigated as a Maximum Tolerated Dose (MTD) in young Beagle dogs of both sexes after intramuscular or oral dosing. The animals were observed for 6 h following dosing and then daily for 2 weeks.

2.6. Sub-chronic toxicity study

2.6.1. 4-Week oral rat

Eighty Sprague–Dawley rats were randomly divided into 4 groups of 10 males and 10 females each and orally administered (by gavage) the following treatments: controls, NaCl 0.9%; low-dose, 100 mg AGPC/kg/day; mid-dose, 300 mg AGPC/kg/day; high-dose, AGPC 1000 mg/kg/day. The volume of all treatments was 5 mL/kg. Daily clinical observation and weekly body weight measurements were conducted during the pre-treatment and active phases of the study. Following 4 weeks of AGPC treatment, urine samples were collected and blood drawn from the abdominal aorta under fasting conditions and general anesthesia. Hematology and limited clinical chemistry analyses were performed on all animals. A post-mortem examination including organ weights and histopathology was conducted on all animals at termination.

2.7. Chronic toxicity studies

2.7.1. 26-Week oral rat

One-hundred forty-four Sprague–Dawley rats were randomly divided into 4 groups of 18 males and 18 females each and dosed by gavage (5 mL/kg): controls, distilled water; low-dose, 100 mg AGPC/kg; mid-dose, 300 mg AGPC/kg; high-dose, 1000 mg AGPC/kg. Individual daily clinical observations were performed during both the pre-test and dosing phases of the study. Body weights were measured weekly during the first 3 months of treatment, and every 2 weeks thereafter. Food consumption was measured every 2 weeks during the first 3 months, and every 4 weeks thereafter. During the 13th week of treatment, blood samples were drawn from the retro-orbital plexus under fasting conditions for limited hematology and clinical chemistry evaluations. Blood and urine was collected from 10/sex/group after 26 week of treatment. Recovery animals (controls and high dose) were observed for 4 additional weeks. A full necropsy was performed following sacrifice under general anesthesia. The parameters evaluated included: body weight, organ weight, hematology (hematocrit, hemoglobin, erythrocyte count, platelet count (13th and 26th week), total and differential leukocytes, prothrombin time (26th week), clinical chemistry (glucose, BUN, creatinine, AST, ALT, alkaline phosphatase, total serum proteins, bilirubin, cholesterol, triglycerides, sodium, and potassium), and urinalysis (specific weight, pH, protein, bilirubin, blood). Histopathologic examinations were performed on all high dose and control animals and those showing gross lesions in the mid- and low-dose groups.

2.7.2. 26-Week oral dog

Twenty-four beagle dogs were randomly divided into 4 groups of 3 males and 3 females each and administered one of the following daily treatments by gavage (1 mL/kg): controls, distilled water; low-dose, 75 mg AGPC/kg; mid-dose, 150 mg AGPC/kg; high-dose, 300 mg AGPC/kg for 26 consecutive weeks. The dogs were dosed in the morning and fed in the afternoon. The animals were observed daily

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