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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.

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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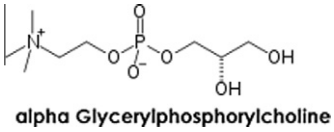

Fig. 1. Chemical structure of AGPC.

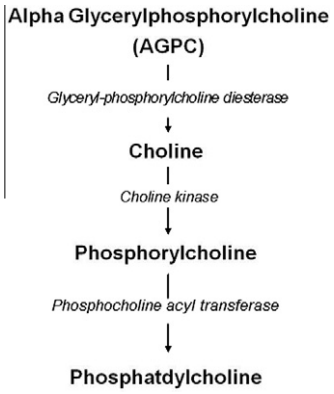

Fig. 2. Metabolism of AGPC (adapted from Amenta et al., 2001).

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

and body weights were measured at monthly intervals during the first 3 months of treatment, and then at the end of the study. Venous blood samples were collected under fasting conditions before study initiation and at the end of the 13th and 26th week for hematology and clinical chemistry evaluations. Urine samples were also collected at the same time points. A full necropsy was performed on all animals at study termination. The parameters examined included: body weight, organ weight, hematology (hematocrit, hemoglobin, erythrocyte count, platelet count, total and differential leukocytes, and prothrombin time), 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, and blood). Necropsy and select histopathological examination of certain tissues were also performed on all animals.

2.8. Mutagenicity

2.8.1. Bacterial reverse mutation

AGPC was evaluated for mutagenic activity in the bacterial reverse mutation test using standard *Salmonella typhimurium* direct plate incorporation method (Maron and Ames, 1983). The vehicle (DMSO) was used as the negative control and 2-acetylaminofluorene and N-methyl-N′-nitro-N-nitrosoguanidine mutagens were used as positive controls. The potential for mutagenicity was assessed in *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538 (Istituto Superiore de Sainta). The tester strains were incubated with AGPC (dissolved in DMSO) at concentrations of 100, 300, 1000, 3000, and 10,000 μg/plate in the presence and absence of the post-mitochondrial fraction of liver homogenates (S9) from rats pretreated with Aroclor® 1254.

2.8.2. Yeast forward mutation

AGPC was evaluated for mutagenic activity in the gene mutation assay using a standard plate method. The vehicle (phosphate buffer) was used as the negative control and dimetylnitrosoamine and methyl methanesulfonate were used as positive controls. The potential for mutagenicity was assessed in *Schizosaccharomyces pombe* (strain P1). Mutant colonies turn white, while the non-mutated ones are red. The yeast were incubated with AGPC (diluted in phosphate buffer) at concentrations of 30, 100, 300, 1000, and 3000 μg/ml in the presence and absence of the post-mitochondrial fraction of liver homogenates (S9) from rats pre-treated with Aroclor® 1254. The gene conversion frequency was determined based on the number of colonies present.

2.8.3. Gene conversion – yeast

AGPC was evaluated for mutagenic activity in the gene conversion assay using a standard the plate method. The vehicle (phosphate buffer) was used as the negative control and cyclophosphamide and N-methyl-N′-nitro-N-nitrosoguanidine were used as positive controls. The potential for mutagenicity was assessed in *Saccharomyces cerevisiae* (strain D4) with selective media for the gene *TRP 5* (tryptophan) or for the gene *ADE 3* (adenine). The yeast was incubated with AGPC (diluted in phosphate buffer) at concentrations of 100, 300, 1000, and 3000 μg/ml in the presence and absence of the post-mitochondrial fraction of liver homogenates (S9) from rats pre-treated with Aroclor® 1254. The gene conversion frequency was determined based on the number of colonies present.

2.8.4. Host mediated gene conversion in yeast

AGPC was evaluated for mutagenic activity in the yeast gene conversion assay using a host-mediated technique exposing the yeast to the test material in the peritoneum of rats. Rats were pretreated (s.c.) with AGPC for 2 days. Immediately after the second AGPC dose, the rats were injected with *S. cerevisiae* (strain D4). After 4 h the yeast were removed and plated on selective media. Cyclophosamide was the positive control being administered just before the yeast. The gene conversion frequency was determined based on the number of colonies present.

2.8.5. Micronucleus

The mutagenic potential of AGPC was assessed in mammalian cells by investigating the effect of AGPC on the normal variation range of micronucleated polychromatic erythrocytes in the bone marrow of male and female Swiss mice. AGPC at 30, 100, and 300 mg/kg was administered twice, via subcutaneous injection, at a 24 h intervals. Mitomycin C (7 mg/kg) was the positive control. Six hours after the last AGPC injection, the animals were killed and the femur removed. The number of micronucleated and polychromatic erythrocytes was counted.

2.9. Statistical methods

Results are expressed as mean values per group with appropriate standard errors (SE), unless otherwise specified. The data was evaluated by one-way variance analysis to test values for the homogeneity of experimental groups. All differences between treated and untreated control animals were tested for statistical significance using Dunnett's test. The LD_{50} was calculated by the method of Bliss (1956). The software package used is not known.

3. Results

3.1. Acute toxicity

3.1.1. Rodents

LD_{50} values for mice and rats by intravenous, intraperitoneal, and oral routes are shown in Table 1. Intravenous administration in mice produced lethal effects at 1020 mg/kg in males and 729 mg/kg in females. The 2000 mg/kg dose was lethal to all animals. All deaths occurred within 24 h. In some cases the death was preceded by convulsions. Observed effects included reduced or absent motility, reduced activity, and bradypnea or dyspnea. The effects were dose-dependent in severity and duration. In animals surviving higher dosages, reduced activity lasted 24 to 48 h, and was accompanied by loss of body weight during the first week with recovery in the second. Intraperitoneal dosing of mice produced mortality starting at 1531 mg/kg in males and 1093 mg/kg in females. The effects observed were the same as after intravenous dosing. They were also dose dependent in terms of severity and duration. Intravenous and intraperitoneal dosing of rats resulted in the same effects as in mice with slightly higher LD_{50} values. Oral dosing of mice with 10 g/kg resulted in 33% mortality in males and 50% in females. No deaths occurred at lower doses. At the lethal dose all animals displayed severe reduced activity and motility lasting 12–36 h. Reduced activity was mild at 5 g/kg and only slight and fleeting at 2.5 g/kg. Oral dosing of rats with 10 g/kg resulted in 16% mortality in males and 33% in females. No deaths occurred at lower doses. Reduced mobility and activity were severe at the lethal dose lasting from 3 to 24 h. The effects were less severe and of a shorter duration (1–6 h) at the lower doses. Necropsies on animals dying or after 2 weeks did not reveal any AGPC related changes. As expected, the i.v. LD_{50} was less than the i.p., which was less than the oral. There were no species or sex differences.

3.1.2. Dogs

Administration of AGPC, either intramuscularly (200 or 500 mg/kg) or orally (1000 or 3000 mg/kg), did not cause any deaths. Mild to no reduced activity was observed following administration of the lowest doses. Mild reduced activity, lasting between 3 and 24 h, was observed after the highest doses. The intramuscular and oral LD_{50} values were estimated to be >500 mg/kg and >3000 mg/kg, respectively.

3.2. Sub-chronic toxicity

3.2.1. 4-Week oral rat

Oral administration of 100 and 300 mg/kg AGPC for 4 weeks did not alter animal behavior or produce any signs of general toxicity. Reduced activity, with intensity varying from animal to animal, was generally observed in the groups of rats administered the highest dose (1000 mg/kg). There were no significant differences in body weights (Fig. 3). There were also no significant changes in hematology and clinical chemistry (Table 2) or urinalysis

Table 1
Acute toxicity in rodents.

Species	Route	LD_{50} mg/kg (confidence interval)	
		Males	Females
Mouse	i.v.	1267 (1056–1520)	1027 (837–1260)
Mouse	i.p.	2053 (1644–2564)	1809 (1459–2243)
Mouse	p.o.	>10,000	10,000
Rat	i.v.	1621 (1323–1986)	1531 (1269–1848)
Rat	i.p.	2215 (1797–2513)	2017 (1722–2362)
Rat	p.o.	>10,000	>10,000

i.v., Intravenous; i.p., Intraperitoneal; p.o., per os.

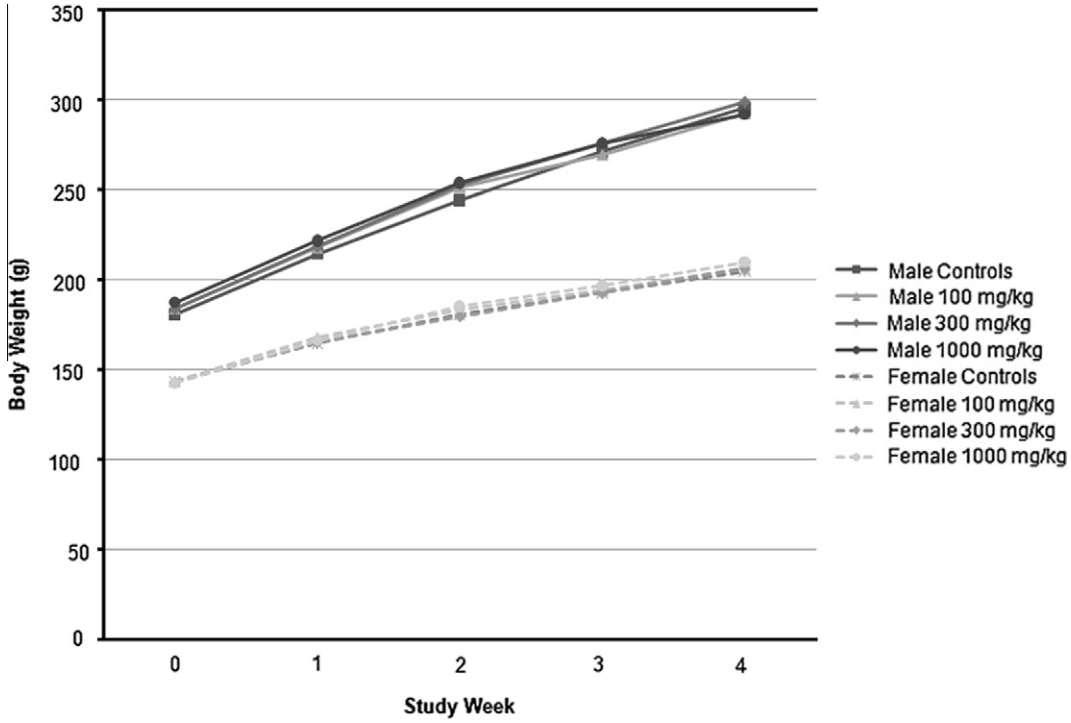

Fig. 3. Sub-chronic oral toxicity of AGPC in male and female rats at 4 weeks: Body weight.

Table 2
Clinical chemistry and hematology results in rats after 4 weeks of oral dosing with AGPC in rats.

Examination	Males				Females			
	Controls	100 mg/kg	300 mg/kg	1000 mg/kg	Controls	100 mg/kg	300 mg/kg	1000 mg/kg
Glucose (mg%)	93.60 ± 2.10	92.40 ± 1.97	91.80 ± 1.59	90.44 ± 2.58	87.70 ± 1.79	90.40 ± 2.05	91.10 ± 2.26	92.50 ± 1.42
Urea (mg%)	24.60 ± 1.27	23.40 ± 1.02	23.60 ± 1.10	24.89 ± 1.14	23.40 ± 0.92	25.00 ± 0.84	24.80 ± 1.33	24.70 ± 0.96
Creatinine (mg%)	0.764 ± 0.05	0.736 ± 0.06	0.775 ± 0.05	0.738 ± 0.05	0.681 ± 0.04	0.701 ± 0.04	0.800 ± 0.09	0.679 ± 0.04
Protein (g%)	6.38 ± 0.10	6.49 ± 0.13	6.27 ± 0.07	6.52 ± 0.11	6.31 ± 0.11	6.36 ± 0.09	6.29 ± 0.08	6.36 ± 0.06
AST (I.U./L)	50.60 ± 2.56	45.80 ± 1.93	45.40 ± 2.26	45.44 ± 1.74	37.60 ± 2.22	40.10 ± 1.54	40.70 ± 1.46	40.00 ± 1.73
ALT (I.U./L)	23.80 ± 1.31	24.20 ± 1.14	25.90 ± 0.64	26.00 ± 0.94	23.40 ± 1.20	24.20 ± 1.15	22.90 ± 1.28	23.50 ± 1.05
Alkaline phosphatase (I.U./L)	32.90 ± 1.47	33.10 ± 1.62	31.90 ± 1.47	34.78 ± 1.73	37.40 ± 1.48	39.10 ± 1.82	39.00 ± 1.22	38.30 ± 1.37
Hemoglobin (g%)	15.25 ± 0.21	15.07 ± 0.23	15.50 ± 0.15	15.00 ± 0.23	14.69 ± 0.18	15.04 ± 0.12	14.87 ± 0.22	14.95 ± 0.16
Hematocrit (%)	46.40 ± 0.87	45.50 ± 1.12	47.50 ± 0.89	45.44 ± 1.08	43.20 ± 0.96	44.90 ± 0.71	43.50 ± 0.97	44.40 ± 0.95
Erythrocytes (10^6/mm^3)	7.71 ± 0.11	7.67 ± 0.15	7.75 ± 0.12	7.57 ± 0.12	7.34 ± 0.12	7.45 ± 0.08	7.32 ± 0.13	7.43 ± 0.11
Leukocytes (10^3/mm^3)	8.17 ± 0.30	8.19 ± 0.28	8.41 ± 0.47	8.54 ± 0.31	8.34 ± 0.32	8.12 ± 0.28	8.85 ± 0.42	8.10 ± 0.29
Lymphocytes (%)	76.00 ± 1.32	75.10 ± 1.51	75.90 ± 1.34	77.55 ± 1.39	76.40 ± 1.33	78.00 ± 1.06	76.70 ± 1.44	76.40 ± 0.91
Neutrophils (%)	20.70 ± 1.12	21.30 ± 1.26	20.60 ± 1.11	19.00 ± 1.12	20.70 ± 1.19	19.10 ± 1.03	20.00 ± 1.12	20.20 ± 0.85
Monocytes (%)	1.50 ± 0.29	1.50 ± 0.38	1.90 ± 0.33	1.56 ± 0.28	1.10 ± 0.22	1.40 ± 0.29	1.60 ± 0.43	1.60 ± 0.25
Eosinophils (%)	1.60 ± 0.21	1.60 ± 0.25	1.50 ± 0.29	1.56 ± 0.39	1.70 ± 0.32	1.30 ± 0.25	1.50 ± 0.32	1.70 ± 0.20
Basophils (%)	0.20 ± 0.13	0.50 ± 0.21	0.20 ± 0.13	0.33 ± 0.16	0.10 ± 0.09	0.30 ± 0.20	0.20 ± 0.13	0.10 ± 0.09

(data not shown). Organ weights (Table 3) and histopathology (Table 4) did not show any treatment related effects. Treatment of rats at a dose up to 1000 mg/kg/day for 4 weeks did not produce any toxicological changes.

3.3. Chronic toxicity

3.3.1. 26-Week oral rat

There were 10 deaths during the study: 2 controls (causes: pulmonary infection and perforated gastric ulcer); 4 in the 100 mg/kg group (2 gavage errors and 2 with renal necrosis); 2 in the 300 mg/kg group (gavage error and unknown cause); and 2 in the 1000 mg/kg group (both pulmonary infection). None of the deaths were attributed to treatment. Reduction in spontaneous motor activity and of reactivity to stimulation was observed in animals receiving the high-dose of AGPC (1000 mg/kg) starting after 3–4 weeks of treatment. The symptoms appeared within 1–2 h of dosing and continued for 3–5 h thereafter. There was considerable variability from one animal to another but the severity was only mild to moderate in all cases. Food consumption and body weight gain (Figs. 4 and 5, respectively) were reduced in the high dose group starting at week 4. In contrast, animals in the low-dose (100 mg/kg) and mid-dose groups (300 mg/kg) showed no reduction in activity. Body weight gain and food consumption were not affected in the low and mid dose groups.

Limited hematology and clinical chemistry evaluations at 13 weeks did not reveal any treatment related effects except for a slight reduction in creatinine in the high dose females (Table 5). After 26 weeks, changes in the high dose group were limited to: a decrease in plasma triglycerides in males and females; a reduction in plasma bilirubin, ALT, and creatinine in females. Triglycerides were reduced in the mid dose males after 26 weeks (Table 5). Urinalysis did not show any treatment related effects (data not shown). The heart weight was reduced in the mid- and

Table 3
Absolute and relative (to body weight) organ weights after 4 weeks of oral dosing with AGPC in rats.

Organ (mg) (%)	Males				Females			
	Controls	100 mg/kg	300 mg/kg	1000 mg/kg	Controls	100 mg/kg	300 mg/kg	1000 mg/kg
Heart	783.1 ± 19.3 (0.26%)	788.0 ± 18.2 (0.27%)	810.1 ± 12.4 (0.27%)	774.2 ± 17.2 (0.27%)	632.3 ± 14.8 (0.31%)	639.4 ± 12.7 (0.31%)	640.2 ± 14.2 (0.31%)	640.8 ± 11.5 (0.31%)
Lung	1007 ± 39.6 (0.34%)	1018 ± 43.5 (0.35%)	991.0 ± 37.6 (0.33%)	975.1 ± 39.4 (0.33%)	829.1 ± 23.3 (0.40%)	817.2 ± 19.4 (0.40%)	842.4 ± 19.4 (0.41%)	835.0 ± 17.6 (0.40%)
Liver	7673 ± 178 (2.59%)	7539 ± 165 (2.58%)	7483 ± 170 (2.50%)	7338 ± 180 (2.51%)	6454 ± 165 (3.15%)	6564 ± 173 (3.19%)	6466 ± 144 (3.14%)	6467 ± 155 (3.08%)
Spleen	765.5 ± 22.5 (0.26%)	739.3 ± 18.7 (0.25%)	742.7 ± 22.9 (0.25%)	739.8 ± 21.0 (0.25%)	803.5 ± 20.5 (0.39%)	814.8 ± 18.4 (0.40%)	808.1 ± 19.3 (0.39%)	815.6 ± 20.5 (0.39%)
Kidney	1818 ± 36.1 (0.61%)	1818 ± 38.4 (0.62%)	1800 ± 28.1 (0.60%)	1794 ± 37.9 (0.61%)	1364 ± 31.1 (0.67%)	1378 ± 21.4 (0.67%)	1359 ± 28.3 (0.66%)	1368 ± 22.5 (0.65%)
Adrenal	39.50 ± 1.81 (0.01%)	36.40 ± 2.09 (0.01%)	37.30 ± 1.87 (0.01%)	36.00 ± 2.29 (0.01%)	47.50 ± 1.74 (0.02%)	47.80 ± 1.58 (0.02%)	49.30 ± 2.18 (0.02%)	47.80 ± 1.96 (0.02%)
Testes/ ovaries	2978 ± 114 (1.01%)	2970 ± 52.0 (1.02%)	2948 ± 71.1 (0.99%)	2859 ± 43.7 (0.98%)	60.80 ± 2.31 (0.03%)	59.60 ± 2.02 (0.03%)	58.00 ± 1.60 (0.03%)	61.20 ± 2.82 (0.03%)

Table 4
Histopathological results after 4 weeks of oral dosing with AGPC in rats[a].

Organ	Findings	Controls	100 mg/kg	300 mg/kg	1000 mg/kg
Brain	Glial proliferation	1/20			1/19
	Hemosiderin pigmentation	1/20			
Lungs	Perialveolar inflammation infiltration	2/20		1	1/19
	Perivascular edema	1/20		1	1/19
	Cyst			1	1/19
Liver	Inflammatory infiltration	1/20		1	1/19
	Vacuolar degeneration of liver cells	1/20			
Spleen	Perivascular edema		1		
	Hemosiderin pigmentation		1		1/19
Kidneys	Interstitial nephritis		1		
	Interstitial nephritis and tubular degeneration		1		1/19
Stomach	Inflammatory infiltration	1/20		1	1/19
	Disepithelization			1	
Intestine	Presence of parasites		1		2/19
	Inflammatory infiltration		1		1/19
	Mucosal congestion				1/19
Testes	Atrophy and failing spermatogenesis	1/10			
Thymus	Hemosiderin pigmentation	1/20		1	1/19
	Macrophage infiltration			1	

[a] Tissues from all animals in the high dose and controls were evaluated. Tissues from animals in the mid and low dose groups showing gross changes were also evaluated.

high-dose females (Table 6) but the relative weight (to body-weight) was unchanged. Necropsy (data not shown) and histopathological evaluations (Table 7) did not reveal any treatment related effects. The nature and frequency of the observed pathology was essentially similar in all experimental groups. By the end of the 4 week recovery period, the high dose animals' body weights were the same as the controls and reduction of spontaneous motor activity resolved.

Dosing of rats for 26 week with 300 mg/kg AGPC did not produce any toxic effects. The high dose (1000 mg/kg) induced post-dosing reduced activity lasting for 3–5 h. Food consumption and body weight gains were reduced. Changes in clinical chemistry were limited to reductions which may be related to inactivity and reduced body weight. There were no histopathological correlates. Reduced activity and body weight gain returned to normal during the recovery period.

3.3.2. 26-Week oral dog

A four-week oral range finding study was conducted in 1 male and 1 female dog at doses of 75, 150, or 300 mg/kg. The only effect observed was mild reduced activity at 300 mg/kg (data not shown). Doses of 75,150, and 300 mg/kg were chosen for the 26-week oral study in groups of 3 male and 3 female beagle dogs. There were no deaths during the 26-week study. Administration of 75 and 150 mg/kg for 26 weeks had no effect on behavior or body weight gain. Mild reduced activity lasting 2 to 5 h after dosing began the second week of the study in the high-dose animal group (300 mg/kg daily). Body weight gain was reduced at 13 weeks but not at 26 weeks (Fig. 6). No hematological changes were observed during the 26-week treatment period (Table 8). Clinical chemistry evaluations performed at week 13 showed a significant increase in plasma cholesterol and decreased alkaline phosphatase levels in the mid-dose group. These changes were absent at week 26 (Table 8). In the high-dose group significant changes were observed in plasma bilirubin, plasma triglycerides, and alkaline phosphatase, which were reduced 34%, 56%, and 9%, respectively, relative to controls. No significant urinalysis abnormalities were observed (data not shown). The weights of the liver and of the heart showed a dose-related decrease that did not achieve statistical significance (Table 9). Histopathological evaluation of the

tissues did not reveal any treatment related effects (Table 10). There were no changes associated with the decrease in liver enzymes and liver and heart weight.

Treatment of dogs for 26 weeks with 300 mg/kg AGPC resulted in reduced activity after dosing lasting up to 5 h. Body weight gain was reduced in the males at 13 weeks. Clinical chemistry

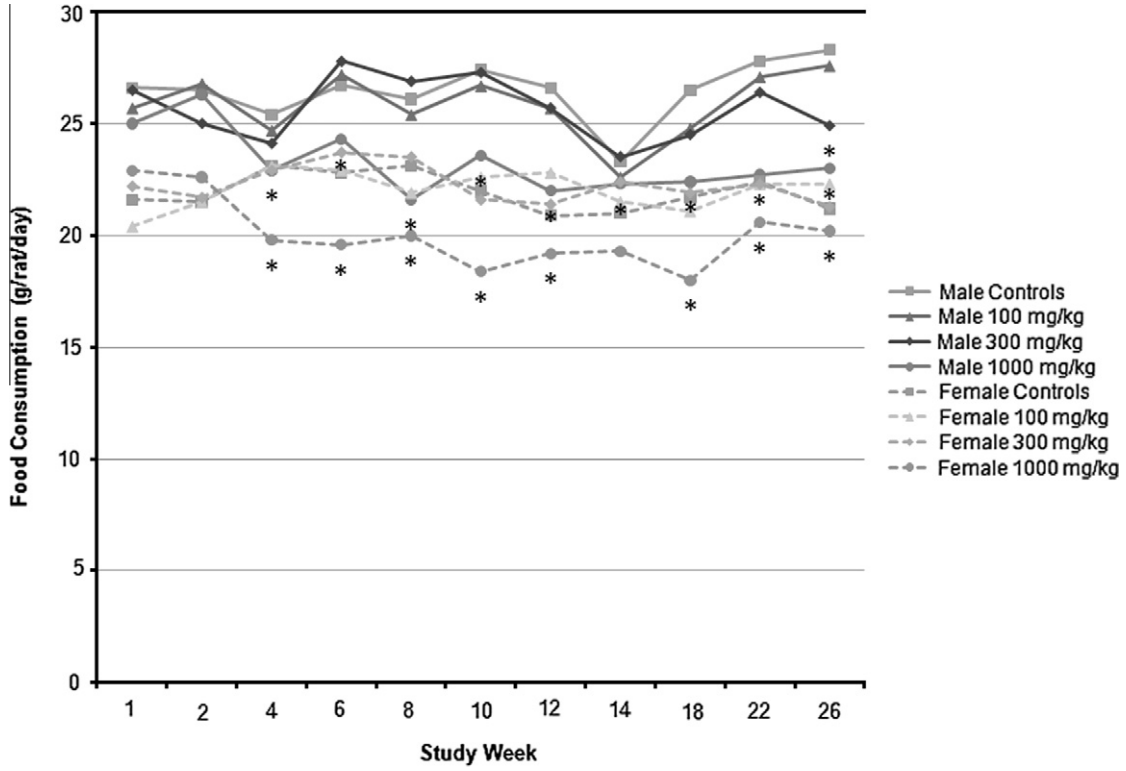

Fig. 4. Chronic oral toxicity of AGPC in male and female rats at 26 weeks: Food consumption. $^{*}P \leqslant 0.05$.

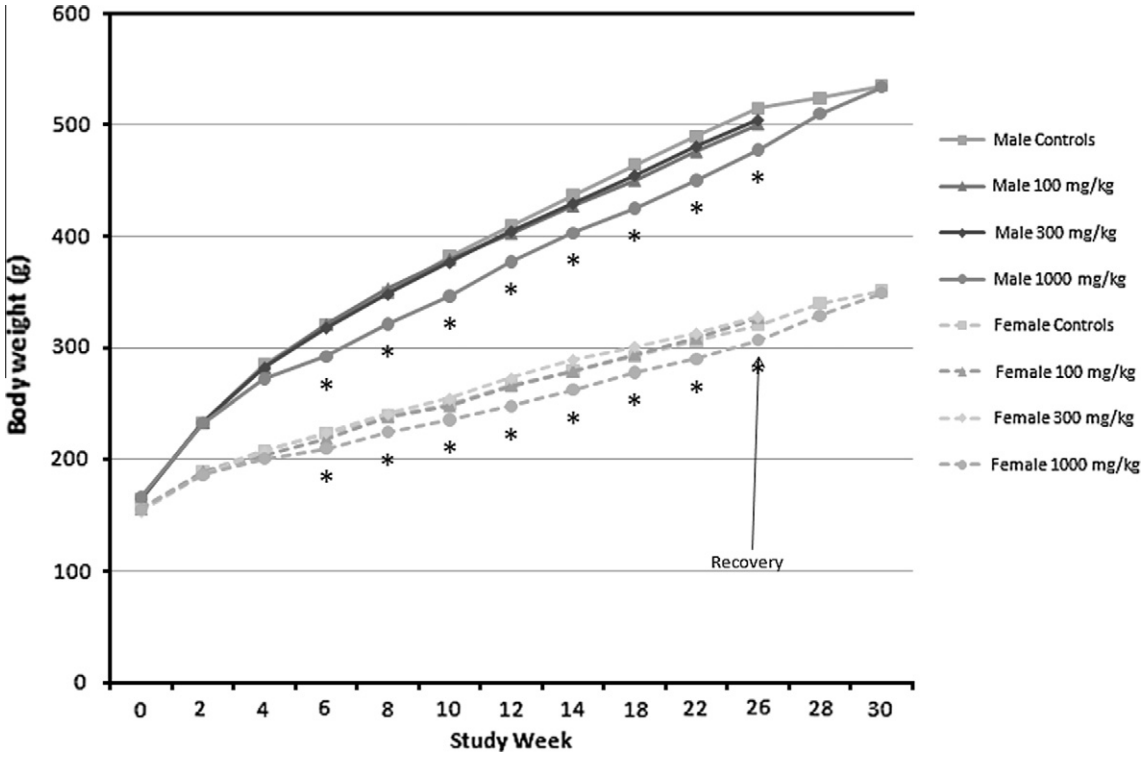

Fig. 5. Chronic oral toxicity of AGPC in male and female rats at 26 weeks: Body weight. $^{*}P \leqslant 0.05$.

evaluations suggested a reduced liver function. Liver and heart weights were reduced. There were no histopathological correlates. These changes may be the result of reduced body weight and activity. Treatment with 75 or 150 mg/kg AGPC had no untoward effects.

3.4. Mutagenicity

3.4.1. Bacterial reverse mutation

There were no significant increases in the number of revertant colonies or cytotoxicity in any *S. typhimurium* strain exposed to

Table 5
Hematology and clinical chemistry results after 13 and 26 weeks of oral dosing with AGPC in rats.

Examination	Males				Females			
	Controls	100 mg/kg	300 mg/kg	1000 mg/kg	Controls	100 mg/kg	300 mg/kg	1000 mg/kg
13 Weeks								
Hemoglobin (g%)	14.57 ± 0.18	14.32 ± 0.18	14.59 ± 0.22	14.76 ± 0.22	14.58 ± 0.16	15.05 ± 0.26	14.58 ± 0.22	15.08 ± 0.18
Hematocrit (%)	47.33 ± 0.65	47.00 ± 0.73	47.80 ± 0.73	48.67 ± 0.94	47.60 ± 0.54	49.75 ± 0.96	47.67 ± 1.04	49.10 ± 0.77
RBC (10^6/cu mm)	7.44 ± 0.17	7.21 ± 0.17	7.49 ± 0.19	7.67 ± 0.22	7.02 ± 0.08	7.80 ± 0.32*	7.06 ± 0.11	7.39 ± 0.13
WBC (10^3/cu mm)	9.57 ± 0.30	9.59 ± 0.42	9.79 ± 0.35	9.31 ± 0.38	7.31 ± 0.18	7.60 ± 0.24	7.40 ± 0.17	7.24 ± 0.25
Lymphocytes (%)	75.11 ± 0.79	71.89 ± 1.64*	73.10 ± 0.84	76.56 ± 0.58	75.30 ± 0.56	72.88 ± 1.14	74.56 ± 0.91	73.60 ± 0.86
Neutrophils (%)	21.22 ± 0.68	24.33 ± 1.28*	23.00 ± 0.87	20.56 ± 0.60	20.40 ± 0.54	22.38 ± 0.82*	20.78 ± 0.72	22.60 ± 0.50
Monocytes (%)	1.11 ± 0.42	1.22 ± 0.32	1.80 ± 0.33	0.89 ± 0.26	1.80 ± 0.33	1.50 ± 0.42	1.56 ± 0.41	1.40 ± 0.31
Eosinophils (%)	1.67 ± 0.29	1.56 ± 0.29	1.10 ± 0.28	1.44 ± 0.24	1.30 ± 0.33	1.88 ± 0.40	1.67 ± 0.29	1.10 ± 0.28
Basophils (%)	0.89 ± 0.26	1.00 ± 0.33	1.00 ± 0.21	0.56 ± 0.18	1.20 ± 0.25	1.38 ± 0.18	1.44 ± 0.24	1.30 ± 0.21
Urea (mg%)	25.98 ± 1.43	26.06 ± 1.65	31.73 ± 1.40*	29.81 ± 2.09	26.71 ± 1.19	28.04 ± 2.44	27.22 ± 1.53	28.01 ± 2.26
Creatinine (mg%)	0.712 ± 0.04	0.787 ± 0.06	0.867 ± 0.08	0.850 ± 0.06	0.829 ± 0.07	0.840 ± 0.07	0.691 ± 0.06	0.607 ± 0.04*
AST (I.U./L)	35.99 ± 1.09	39.03 ± 1.04	37.66 ± 1.27	39.96 ± 1.09	36.78 ± 1.63	34.80 ± 0.86	36.71 ± 1.39	36.89 ± 1.27
ALT (I.U./L)	26.44 ± 1.82	29.29 ± 1.56	28.73 ± 1.21	28.10 ± 1.27	28.92 ± 1.17	27.00 ± 1.35	25.70 ± 1.56	29.31 ± 1.15
26 Weeks								
Hemoglobin (g%)	14.76 ± 0.29	15.01 ± 0.21	15.31 ± 0.21	14.36 ± 0.19	14.61 ± 0.21	14.38 ± 0.21	14.59 ± 0.20	14.23 ± 0.17
Hematocrit (%)	48.22 ± 1.01	49.11 ± 0.77	50.10 ± 0.75	47.11 ± 0.81	47.50 ± 0.91	47.25 ± 0.80	47.89 ± 0.75	48.80 ± 0.26
RBC (10^6/cu mm)	7.63 ± 0.27	7.87 ± 0.18	8.40 ± 0.24*	7.28 ± 0.23	7.03 ± 0.11	6.94 ± 0.12	7.04 ± 0.11	6.82 ± 0.08
WBC (10^3/cu mm)	9.81 ± 0.33	9.52 ± 0.28	9.87 ± 0.27	9.49 ± 0.28	7.18 ± 0.15	7.46 ± 0.17	7.23 ± 0.20	7.41 ± 0.19
Lymphocytes (%)	72.67 ± 1.43	71.22 ± 1.82	74.80 ± 0.95	69.89 ± 1.29	67.60 ± 1.26	69.25 ± 1.63	71.33 ± 1.42	71.20 ± 1.68
Neutrophils (%)	23.56 ± 1.41	25.11 ± 1.37	21.30 ± 0.75	26.67 ± 1.25	28.80 ± 1.21	26.25 ± 1.74	25.11 ± 1.22	24.90 ± 1.55
Monocytes (%)	1.22 ± 0.43	1.44 ± 0.24	0.90 ± 0.23	1.33 ± 0.29	1.20 ± 0.20	2.13 ± 0.30	1.22 ± 0.36	1.10 ± 0.35
Eosinophils (%)	1.78 ± 0.32	1.56 ± 0.29	2.00 ± 0.30	1.33 ± 0.24	1.40 ± 0.34	1.50 ± 0.33	1.44 ± 0.38	1.40 ± 0.27
Basophils (%)	0.78 ± 0.28	0.67 ± 0.24	1.00 ± 0.30	0.78 ± 0.28	1.00 ± 0.26	0.88 ± 0.23	0.89 ± 0.26	1.40 ± 0.22
Platelets (10^3/cu mm)	798.8 ± 15.1	859.2 ± 18.7 *	834.3 ± 11.1	849.6 ± 16.4	861.6 ± 16.9	805.1 ± 17.5	874.1 ± 18.8	842.2 ± 16.6
Prothrombin Time (sec)	14.06 ± 0.11	14.20 ± 0.24	14.30 ± 0.20	14.52 ± 0.19	13.04 ± 0.23	13.39 ± 0.33	13.69 ± 0.22	12.79 ± 0.24
Glucose (mg%)	97.93 ± 2.75	99.52 ± 1.73	102.5 ± 3.58	93.48 ± 1.98	97.06 ± 2.81	97.34 ± 2.51	101.8 ± 3.90	100.7 ± 3.17
Urea (mg%)	28.66 ± 1.40	28.53 ± 2.09	29.18 ± 1.85	29.11 ± 1.51	26.95 ± 1.52	29.60 ± 2.28	31.64 ± 1.68	32.02 ± 1.94
Creatinine (mg%)	0.851 ± 0.07	0.867 ± 0.08	0.990 ± 0.07	0.776 ± 0.08	0.852 ± 0.06	0.954 ± 0.08	0.702 ± 0.05	0.856 ± 0.05
Protein (g%)	6.71 ± 0.07	7.12 ± 0.19	6.93 ± 0.11	6.91 ± 0.05	6.79 ± 0.08	6.61 ± 0.09	6.98 ± 0.15	6.61 ± 0.02
Cholesterol (mg%)	49.74 ± 0.83	52.76 ± 1.92	47.31 ± 1.47	47.57 ± 1.76	50.62 ± 1.87	48.36 ± 1.89	53.94 ± 1.80	47.73 ± 1.46
Triglycerides (mg%)	46.27 ± 1.95	47.71 ± 2.32	39.45 ± 2.25*	38.79 ± 2.13*	44.76 ± 2.30	45.03 ± 2.60	47.27 ± 2.40	36.11 ± 1.87*
Bilirubin (mg%)	0.439 ± 0.03	0.460 ± 0.03	0.385 ± 0.03	0.350 ± 0.03	0.449 ± 0.04	0.475 ± 0.04	0.382 ± 0.03	0.318 ± 0.02*
AST (I.U./L)	35.59 ± 1.57	35.92 ± 1.71	38.33 ± 0.90	37.84 ± 1.67	37.66 ± 1.52	35.91 ± 1.53	38.62 ± 1.58	33.63 ± 0.89
ALT (I.U./L)	28.23 ± 1.59	27.74 ± 1.95	26.03 ± 1.48	26.21 ± 0.90	29.26 ± 1.21	29.83 ± 1.27	28.21 ± 1.19	24.38 ± 1.00**
Alkaline phosphatase (I.U./L)	28.74 ± 1.11	30.09 ± 0.74	29.53 ± 0.94	26.59 ± 0.79	37.44 ± 0.89	36.60 ± 1.57	34.04 ± 1.35	29.67 ± 1.10*

* $P < 0.05$.
** $P < 0.01$.

Table 6
Absolute (g or mg) and relative (to body weight) organ weights in rats after 26 weeks of oral dosing with AGPC.

Organ	Controls	100 mg/kg	300 mg/kg	1000 mg/kg
Males				
Brain	2.05 ± 0.03 (0.40%)	2.02 ± 0.04 (0.40%)	2.02 ± 0.02 (0.40%)	2.06 ± 0.03 (0.42%)
Heart	1.12 ± 0.03 (0.22%)	1.14 ± 0.03 (0.22%)	0.13 ± 0.03 (0.20%)	1.04 ± 0.03 (0.21%)
Lung	1.58 ± 0.03 (0.31%)	1.62 ± 0.04 (0.32%)	1.58 ± 0.02 (0.30%)	1.61 ± 0.02 (0.34%)
Liver	13.22 ± 0.22 (2.53%)	13.52 ± 0.31 (2.56%)	12.74 ± 0.22 (2.38%)	12.96 ± 0.23 (2.52%)
Spleen	653.2 ± 10.3 (0.13%)	659.3 ± 8.87 (0.13%)	643.8 ± 9.70 (0.13%)	656.6 ± 12.0 (0.14%)
Kidney	3.08 ± 0.04 (0.58%)	3.00 ± 0.05 (0.60%)	2.95 ± 0.03 (0.57%)	3.03 ± 0.04 (0.63%)
Adrenal	47.44 ± 0.90 (0.009%)	46.00 ± 1.03 (0.009%)	44.90 ± 1.00 (0.009%)	43.67 ± 0.99 (0.009%)
Testes	5.02 ± 0.07 (0.97%)	5.05 ± 0.09 (1.00%)	5.09 ± 0.08 (0.99%)	5.02 ± 0.10 (1.05%)
Pituitary	18.44 ± 1.45 (0.003%)	19.78 ± 1.64 (0.004%)	19.50 ± 1.28 (0.004%)	18.67 ± 0.93 (0.004%)
Females				
Brain	1.98 ± 0.03 (0.60%)	1.95 ± 0.03 (0.58%)	1.94 ± 0.03 (0.58%)	1.91 ± 0.02 (0.62%)
Heart	850.6 ± 27.5 (0.25%)	866.1 ± 26.7 (0.27%)	818.3 ± 23.1* (0.25%)	774.1 ± 24.0* (0.25%)
Lung	1.20 ± 0.02 (0.37%)	1.17 ± 0.02 (0.34%)	1.20 ± 0.02 (0.37%)	1.15 ± 0.01 (0.36%)
Liver	8.69 ± 0.16 (2.69%)	8.27 ± 0.12 (2.51%)	7.82 ± 0.10 (2.39%)	8.14 ± 0.15 (2.64%)
Spleen	447.7 ± 12.3 (0.14%)	456.1 ± 12.7 (0.14%)	457.7 ± 10.5 (0.14%)	445.0 ± 8.99 (0.15%)
Kidney	1.85 ± 0.05 (0.56%)	1.85 ± 0.04 (0.55%)	1.86 ± 0.03 (0.55%)	1.90 ± 0.03 (0.62%)
Adrenal	55.80 ± 0.98 (0.017%)	55.75 ± 1.25 (0.017%)	54.44 ± 1.23 (0.017%)	55.80 ± 1.12 (0.018%)
Ovaries	99.50 ± 2.27 (0.031%)	96.25 ± 2.79 (0.029%)	100.2 ± 3.92 (0.031%)	101.0 ± 2.56 (0.033%)
Pituitary	13.20 ± 0.51 (0.004%)	14.88 ± 0.69 (0.004%)	12.78 ± 0.64 (0.004%)	14.10 ± 0.75 (0.005%)

* $P < 0.01$.

AGPC, with or without metabolic activation, up to 10,000 μg/plate (Table 11). Under the conditions of this study, AGPC was non-mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 or TA1538.

Table 7
Histopathological results in rats after 26 weeks of oral dosing with AGPC.

Organ	Findings	Controls	100 mg/kg	300 mg/kg	1000 mg/kg
Heart	Inflammatory infiltration of pericardium		1		
	Inflammatory infiltration of ventricular myocardium			1	
	Focal myocardial hemorrhage				
Lungs	Perivascular edema and round cell infiltration	3/19	1		4/19
	Inflammatory thickening of alveolar walls	3/19	1	1	2/19
	Pleural fibrosis		1	1	2/19
	Focal histiocytosis of lung alveoli	3/19		2	3/19
Liver	Diffuse portobillary round cell infiltration	1/19		1	
	Irregular parenchymal cells with enlarged nuclei and hydropic cytoplasm	1/19	2		
	Fat deposits from steatosis and vacuolar degeneration	1/19	1		
Spleen	Blood stasis and hemosiderin pigment deposits	2/19	2	2	2/19
	Congestion of red pulp				
	Round-cell infiltration		2		3/19
Stomach	Round-cell infiltration of stomach mucosa	2/19	2	2	1/19
	Hemosiderin pigment deposits	1/19			3/19
Small intestine	Diffuse round-cell infiltration	1/19		2	2/19
	Eosinophil enteritis with hyperplasia of Peyer's patches	1/19	1		2/19
Kidneys	Mild round-cell interstitial infiltration of renal cortex	2/19	2		3/19
	Hyaline casts in medullary renal tubules	2/19			
	Areas of interstitial nephritis w/mild tubular hydrops (nephrosis)	1/19			2/19
Bladder	Round-cell infiltration of vesical wall	3/19	3		3/19
	Hemosiderin pigment deposits	1/19	2		1/19
Uterus	Eosinophil infiltration of lamina propria	1/10			
Thymus	Hemosiderin pigment deposits	1/19	1/17		1/19
Adrenals	Hemosiderin pigment deposits	1/19	1/17		
Pituitary	Round-cell infiltration	1/19			
Ovaries	Mild multifocal atrophy	1/19			

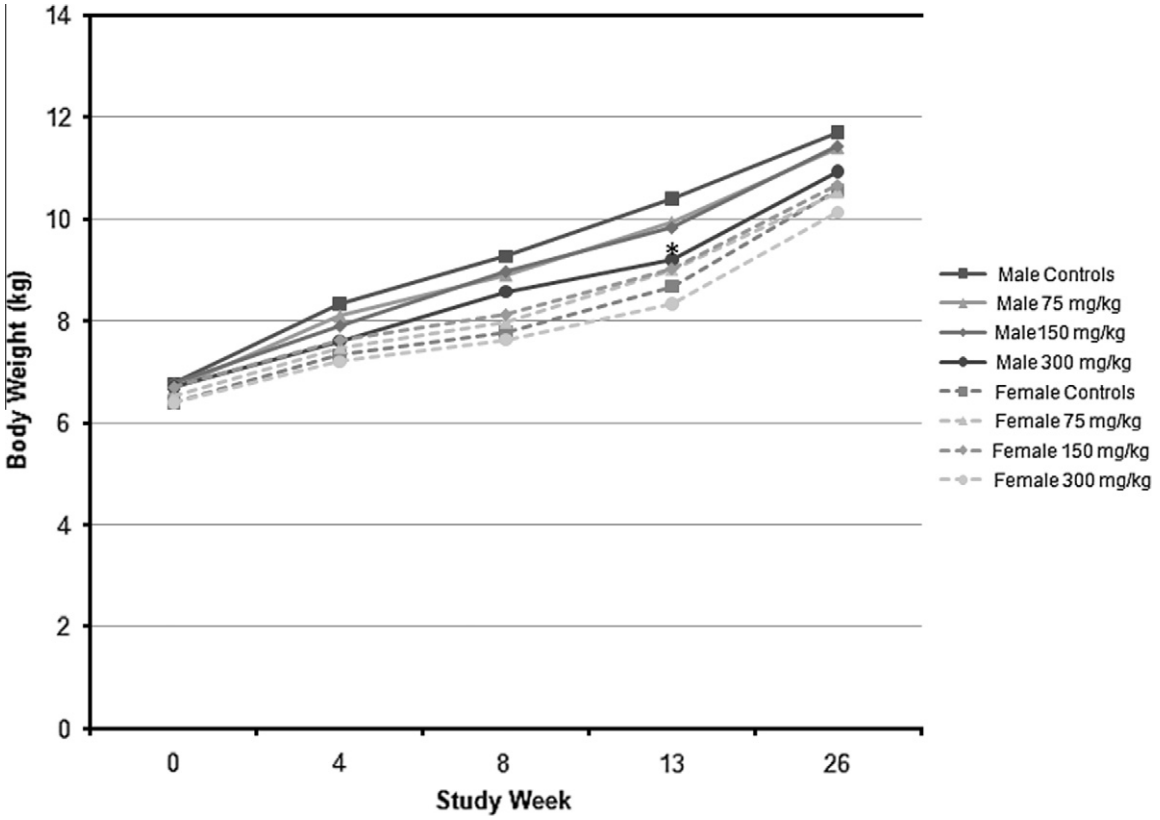

Fig. 6. Chronic oral toxicity of AGPC in male and female dogs at 26 weeks: Body weight. $^{*}P \leqslant 0.05$.

Table 8
Hematology and clinical chemistry results in dogs after 13 or 26 weeks of oral dosing with AGPC.

Parameters	Controls	75 mg/kg	150 mg/kg	300 mg/kg
13 Weeks				
Hemoglobin	14.10 ± 0.59	14.05 ± 0.93	13.53 ± 0.47	14.67 ± 0.47
Hematocrit	44.83 ± 1.70	44.0 ± 2.27	43.00 ± 1.15	45.67 ± 1.09
RBC (10^6/mm^3)	6.42 ± 0.28	6.45 ± 0.47	6.18 ± 0.24	6.75 ± 0.24
WBC (10^3/mm^3)	11.12 ± 0.58	10.17 ± 0.41	10.55 ± 0.78	10.80 ± 0.83
Lymphocytes (%)	29.83 ± 4.36	27.83 ± 3.82	32.17 ± 3.00	30.50 ± 3.34
Neutrophils (%)	63.83 ± 4.09	66.50 ± 3.97	62.33 ± 3.01	65.33 ± 3.91
Monocytes (%)	3.17 ± 0.75	2.50 ± 0.76	2.83 ± 0.83	2.00 ± 0.58
Eosinophils (%)	2.67 ± 0.56	2.50 ± 0.99	1.83 ± 0.48	1.67 ± 0.71
Basophils (%)	0.50 ± 0.22	0.67 ± 0.21	0.83 ± 0.17	0.50 ± 0.22
Platelets (10^3/mm^3)	239.0 ± 20.9	247.3 ± 23.1	260.7 ± 17.3	274.3 ± 11.2
Prothrombin time (sec)	9.35 ± 0.38	9.68 ± 0.23	9.65 ± 0.38	9.60 ± 0.30
Glucose (mg%)	93.40 ± 4.54	109.9 ± 4.85	98.18 ± 4.71	98.03 ± 6.13
Urea (mg%)	24.83 ± 1.95	26.42 ± 4.08	29.35 ± 3.11	28.40 ± 3.24
Creatinine (mg%)	0.957 ± 0.06	0.913 ± 0.07	0.783 ± 0.08	0.796 ± 0.09
Protein (g%)	6.28 ± 0.05	5.98 ± 0.09	5.95 ± 0.18	5.85 ± 0.16
Cholesterol (mg%)	127.8 ± 6.66	144.8 ± 4.93	155.5 ± 5.44*	132.8 ± 9.19
Triglycerides (mg%)	51.37 ± 4.19	53.30 ± 2.54	42.87 ± 4.75	43.68 ± 2.58
Bilirubin (mg%)	0.432 ± 0.04	0.495 ± 0.04	0.438 ± 0.04	0.396 ± 0.06
AST (I.U./L)	29.41 ± 2.57	26.38 ± 3.45	29.73 ± 1.26	22.05 ± 2.54
ALT (I.U./L)	25.35 ± 2.4	28.82 ± 2.33	24.30 ± 2.86	21.53 ± 1.45
Alkaline phosphatase (I.U./L)	40.68 ± 0.66	38.78 ± 0.98	34.07 ± 0.86*	38.02 ± 1.69
Sodium (mEq/L)	149.3 ± 2.38	150.5 ± 1.84	145.2 ± 2.04	149.3 ± 2.75
Potassium (mEq/L)	4.90 ± 0.09	4.91 ± 0.07	4.87 ± 0.06	4.68 ± 0.03
26 Weeks				
Hemoglobin	13.80 ± 0.63	14.65 ± 0.41	13.75 ± 0.70	14.22 ± 0.34
Hematocrit	43.17 ± 1.64	45.17 ± 1.01	43.33 ± 1.71	43.83 ± 1.25
RBC (10^6/mm^3)	6.33 ± 0.31	6.75 ± 0.21	6.32 ± 0.34	6.45 ± 0.22
WBC (10^3/mm^3)	11.50 ± 0.70	11.22 ± 0.75	11.77 ± 0.64	11.46 ± 0.37
Lymphocytes (%)	30.33 ± 4.82	33.33 ± 4.08	27.67 ± 4.26	34.67 ± 1.98
Neutrophils (%)	64.17 ± 3.46	61.33 ± 4.18	65.67 ± 4.20	59.67 ± 2.28
Monocytes (%)	2.50 ± 0.76	2.67 ± 0.84	2.17 ± 0.79	2.35 ± 0.56
Eosinophils (%)	2.50 ± 0.72	2.33 ± 0.76	4.00 ± 0.68	2.63 ± 0.79
Basophils (%)	0.50 ± 0.22	0.33 ± 0.21	0.50 ± 0.22	0.50 ± 0.22
Platelets (10^3/mm^3)	234.7 ± 19.8	207.8 ± 15.8	205.2 ± 12.7	240.0 ± 21.6
Prothrombin time (sec)	9.33 ± 0.19	9.17 ± 0.33	9.33 ± 0.21	9.08 ± 0.34
Glucose (mg%)	103.1 ± 3.25	94.10 ± 3.72	98.80 ± 2.33	91.77 ± 3.13
Urea (mg%)	21.67 ± 1.57	25.40 ± 3.33	23.03 ± 2.96	26.85 ± 2.96
Creatinine (mg%)	0.862 ± 0.05	0.960 ± 0.05	0.890 ± 0.09	0.780 ± 0.09
Protein (g%)	6.13 ± 0.15	6.33 ± 0.24	5.92 ± 0.14	6.13 ± 0.08
Cholesterol (mg%)	125.8 ± 5.18	133.0 ± 8.29	132.0 ± 5.64	119.8 ± 6.54
Triglycerides (mg%)	57.55 ± 5.24	43.58 ± 4.76*	46.57 ± 2.66	37.90 ± 2.86**
Bilirubin (mg%)	0.472 ± 0.06	0.450 ± 0.04	0.427 ± 0.04	0.210 ± 0.05**
AST (I.U./L)	20.08 ± 2.31	23.03 ± 3.02	17.88 ± 1.88	25.92 ± 3.53
ALT (I.U./L)	25.63 ± 1.33	21.38 ± 1.10	28.43 ± 1.73	20.00 ± 1.62
Alkaline phosphatase (I.U.//L)	31.33 ± 1.39	30.90 ± 1.11	32.16 ± 1.02	28.58 ± 0.53**
Sodium (mEq/L)	146.0 ± 1.91	148.2 ± 1.47	146.7 ± 1.15	146.2 ± 2.24
Potassium (mEq/L)	4.81 ± 0.08	4.77 ± 0.08	4.74 ± 0.07	4.79 ± 0.10

* $P < 0.05$.
** $P < 0.01$.

3.4.2. Yeast forward mutation

AGPC did not alter the frequency of spontaneous forward mutations of *S. pombe* P1 at concentrations up to 3000 μg/mL with or without microsomal activation (Table 12).

3.4.3. Yeast gene conversion

AGPC did not alter the mitotic gene conversion frequency of *S. cerevisiae* at concentrations up to 10,000 μg/mL (Table 13). In fact, no concentration of AGPC with or without microsomal activation increased the frequency of Ade2 or Trp5 gene conversion above controls.

3.4.4. Host mediated gene conversion in yeast

AGPC administered s.c. at doses up to 300 mg/kg to rats did not alter the mitotic gene conversion of *S. cerevisiae at the* Ade2 or Trp5 genes (Table 14).

3.4.5. Micronucleus test in mice

There was no mortality. AGPC did not induce any significant increases in the incidence of micronucleated immature erythrocytes

Table 9
Absolute organ weights (g) in dogs after 26 weeks of oral dosing with AGPC.

Organ	Controls	75 mg/kg	150 mg/kg	300 mg/kg
Brain	80.05 ± 3.05	83.42 ± 4.02	80.97 ± 2.23	81.22 ± 1.65
Heart	92.03 ± 2.07	91.45 ± 2.24	88.32 ± 1.75	84.83 ± 1.37
Lung	88.28 ± 1.22	91.80 ± 2.48	90.27 ± 2.89	92.18 ± 1.56
Liver	308.3 ± 10.5	284.0 ± 13.3	298.3 ± 15.9	276.3 ± 9.02
Spleen	59.83 ± 3.95	55.68 ± 5.58	50.88 ± 7.23	62.63 ± 5.68
Kidney	55.57 ± 1.40	53.38 ± 0.82	55.72 ± 1.38	54.17 ± 1.47
Adrenals	1.823 ± 0.0948	1.676 ± 0.0836	1.669 ± 0.0995	1.728 ± 0.0755
Testes	22.57 ± 0.88	23.77 ± 0.38	23.17 ± 0.33	23.00 ± 0.92
Ovaries	1.436 ± 0.165	1.342 ± 0.153	1.382 ± 0.157	1.536 ± 0.163
Thymus	12.65 ± 0.95	11.00 ± 0.18	14.43 ± 0.87	13.15 ± 0.92

Table 10
Histopathological results in dogs after 26 weeks of oral dosing with AGPC.

Organ	Findings	Controls	75 mg/kg	150 mg/kg	300 mg/kg
Heart	Inflammatory infiltration of pericardium		1/6		
	Inflammatory infiltration of ventricular myocardium				1/6
	Focal myocardial hemorrhage				
Lungs	Perivascular edema and round cell infiltration		1/6		1/6
	Inflammatory thickening of alveolar walls	1/6		1/6	
	Pleural fibrosis	1/6			1/6
	Focal areas of alveolar histiocytosis		1/6	1/6	1/6
Liver	Diffuse portobillary round cell infiltration		1/6	1/6	
	Irregular liver cells with enlarged nuclei and hydropic cytoplasm			1/6	
	Fat deposits from steatosis and vacuolar degeneration	1/6			
Spleen	Blood stasis and hemosiderin pigment deposits	1/6	2/6	1/6	1/6
	Congestion of red pulp				1/6
	Round-cell infiltration				1/6
Stomach	Round-cell infiltration of stomach mucosa	1/6			2/6
	Hemosiderin pigment deposits			1/6	1/6
Intestine	Diffuse round-cell infiltration		1/6	1/6	2/6
	Eosinophil enteritis with hyperplasia of Peyer's patches	2/6			2/6
Kidneys	Mild round-cell interstitial infiltration of renal cortex		1/6		2/6
	Hyaline casts in medullary renal tubules				2/6
	Areas of interstitial nephritis and mild tubular hydropic nephrosis	1/6		1/6	
Bladder	Round-cell infiltration				
	Hemosiderin pigment deposits	1/6		1/6	1/6
Uterus	Eosinophil infiltration of lamina propria				1/3
Thymus	Hemosiderin pigment deposits	1/6			
Adrenals	Hemosiderin pigment deposits	1/6			
Mesenteric lymph nodes	Hemosiderin pigment deposits	1/6	1/6		
	Round-cell infiltration				1/6

Table 11
Reverse mutation assay in *S. typhimurium* with AGPC in the absence or presence of S9 mix.

AGPC concentration (μg/plate)	TA 98		TA100		TA1535		TA1537		TA 1538	
	−S9[a]	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
0	22.6 ± 1.7	23.3 ± 3.9	136.6 ± 16.9	132.0 ± 17.0	17.3 ± 4.0	16.0 ± 2.6	9.3 ± 2.3	9.0 ± 2.0	14.0 ± 2.3	13.0 ± 1.7
100	22.0 ± 2.0	22.0 ± 3.2	127.3 ± 9.1	128.3 ± 6.0	19.6 ± 2.4	17.6 ± 1.2	9.6 ± 2.0	9.6 ± 2.3	13.3 ± 2.6	13.3 ± 2.8
300	22.3 ± 2.7	21.3 ± 1.7	133.0 ± 4.3	133.0 ± 4.5	17.3 ± 1.4	18.6 ± 2.4	9.0 ± 2.0	9.6 ± 1.8	13.3 ± 1.4	13.3 ± 0.8
1000	22.0 ± 3.7	22.6 ± 2.6	128.0 ± 6.0	132.6 ± 7.6	17.3 ± 2.1	18.3 ± 1.8	9.0 ± 2.5	9.3 ± 0.8	12.6 ± 2.7	13.0 ± 2.0
3000	22.6 ± 0.8	20.6 ± 2.3	129.6 ± 8.1	132.3 ± 2.3	16.3 ± 1.6	17.0 ± 2.6	9.6 ± 1.7	8.6 ± 1.7	14.0 ± 1.7	14.3 ± 2.6
10000	23.3 ± 1.4	21.0 ± 3.5	134.3 ± 4.6	132.6 ± 10.1	15.6 ± 1.4	16.0 ± 2.0	9.0 ± 3.5	10.0 ± 1.7	14.3 ± 0.8	14.3 ± 1.4
Positive controls										
100 (AAF[b])		>1000		>1000		17.3 ± 1.7		9.3 ± 0.8	13.3 ± 2.7	
20 (MNNG[c])	25.0 ± 4.6		>1000		>1000		9.3 ± 2.3			>1000

[a] Mean number of revertants per plate ($n = 3$).
[b] AAF = 2-acetylaminofluorene.
[c] MNNG = N-methyl-N′-nitro-N-nitrosoguanidine.

Table 12
Forward mutation assay of AGPC in *Schizosacchromyces pombe* P1.

Concentration (μg/plate)	Mutation frequency (10^{-4})	
AGPC	−S9	+S9
0	0	0.98
30	0	1.32
100	0	1.02
300	1.32	0
1000	1.03	1.03
3000	0	1.24
Positive controls		
15 (MMS[a])	21.5	
1000 (DMN[b])		48.5

[a] MMS = methyl methanesulphonate.
[b] DMN = dimethylnitrosamine.

Table 13
Gene conversion assay of AGPC in *S. cervisiae* D4.

Concentration (μg/plate)	Selection for the TRP 5 gene		Selection for the ADE 2 gene	
AGPC	−S9[a]	+S9	−S9	+S9
0	2.7 ± 0.4	3.0 ± 0.3	2.3 ± 0.3	2.4 ± 0.3
100	2.9 ± 0.2	3.0 ± 0.1	2.4 ± 0.4	2.3 ± 0.2
300	2.5 ± 0.2	2.7 ± 0.5	2.3 ± 0.2	2.3 ± 0.4
1000	2.6 ± 0.5	2.7 ± 0.2	2.3 ± 0.2	2.3 ± 0.1
3000	2.8 ± 0.2	2.6 ± 0.4	2.1 ± 0.2	2.1 ± 0.3
Positive controls				
1000 (CP[b])		19.2 ± 3.3		18.6 ± 3.9
20 (MNNG[c])	12.9 ± 2.5		11.8 ± 1.9	

[a] Mean mutation frequency × 10^{-6} ($n = 3$).
[b] CP = Cyclophosphamide.
[c] MNNG = N-methyl-N′-nitro-N-nitrosoguanidine.

Table 14
Host mediated gene conversion assay of AGPC in *S. cervisiae* D4 in rats.

Dose (mg/kg)	Selection for the TRP 5 gene[a] (M ± SE)	Selection for the ADE 2 gene (M ± SE)
AGPC		
0	2.8 ± 0.2	2.5 ± 0.5
3	2.8 ± 0.5	2.3 ± 0.2
30	2.5 ± 0.2	2.6 ± 0.5
300	2.7 ± 0.4	2.4 ± 0.2
Positive control		
500 (CP[b])	16.2 ± 3.0	18.5 ± 4.4

[a] Selection for the mutation/animal/10^{-6} ($n = 3$).
[b] CP = Cyclophosamide.

Table 15
Micronucleus assay of AGPC in swiss mice.

Test material	Dose (mg/kg)	Route	Micronucleated cells/1000 polychromatic erythrocytes (Mean ± SE)[a]	
			Males	Female
Control	0	–	4.6 ± 1.2	3.6 ± 1.2
AGPC	3	sc	3.3 ± 0.3	3.0 ± 0.5
AGPC	30	sc	4.0 ± 1.1	2.6 ± 1.2
AGPC	300	sc	3.3 ± 1.4	4.0 ± 1.0
Mitomycin C	7	ip	42.0 ± 11.5	38.3 ± 5.9

[a] $n = 3$ Males and 3 females per group.

and was not cytotoxic in Swiss mice administered AGPC at doses of 3, 30 or 300 mg/kg bw, compared to the vehicle control (Table 15). Under the conditions of this study, AGPC is not genotoxic in the micronucleus assay.

4. Discussion and conclusion

AGPC is a semi-synthetic derivative of lecithin. Following ingestion, it is converted to phosphorylcholine, a metabolically active form of choline (Abbiati et al., 1993). The phosphorylcholine crosses the blood brain barrier and then may be utilized for acetylcholine and phosphatidylcholine biosynthesis in the brain (Gatti et al., 1992). AGPC is able to increase the amount of acetylcholine released in the brain (Lopez et al., 1991) and reverses scopolamine induced amnesia indicating that AGPC has cholinergic activity (Schettini et al., 1992; Sigala et al., 1992). The major effects of single high doses of AGPC in these studies were motor and respiratory depression. These effects are consistent with central cholinergic stimulation. In young animals AGPC has the effect of stimulating parasympathetic activity (Ferraro et al., 1996). In aged animals it is capable of reversing memory loss (Drago et al., 1992, 1993). AGPC has been investigated extensively in humans for its potential to reduce the symptoms of Alzheimer's disease (Amenta et al., 2001; Ban et al., 1991; De Jesus Moreno Moreno, 2003; Parnetti et al., 1993). Mechanistic studies suggest that it can increase the amount of choline in the brain available for production of acetylcholine.

AGPC displayed a low order of acute toxicity both by oral and parenteral routes. The oral LD_{50} in rodents was equal to or greater than 10 g/kg. The oral LD_{50} in dogs was estimated to be greater than 3 g/kg. Toxic symptoms at lethal doses after parenteral and oral administration consisted of motor and respiratory depression. Depressed animal activity was also observed in the high dose groups after sub-chronic and chronic oral dosing in both rats and dogs. There are no published data to indicate that the reduced activity was species specific. The severity of symptoms was usually limited and stabilization tended to occur within a few weeks of continuous treatment. In some cases this reduced activity was associated with reduced food consumption and failure to gain weight.

In the rat oral sub-chronic (4 weeks) toxicity study, hematology, clinical chemistry, and urinalysis as well as gross autopsy and histological examinations consistently failed to reveal any evidence of a specific toxic effect of AGPC on the principal organs or their function. In chronic toxicity studies (26 weeks), high dose rats and dogs displayed reduced activity after dosing and reduced body weight gain. The reduced activity abated during a 4 week recovery period (rat study only) and the body weights caught up to the controls. Some clinical chemistry parameters did show significant reductions versus control values in the high dose dogs and rats, including plasma triglycerides, plasma bilirubin, and alkaline phosphatase. The toxicologic significance of these changes is unknown. The reduced triglycerides may have been due to reduced food consumption. There were no histopathological correlates to the clinical chemistry changes.

Citicoline (choline cytidine 5′-pyrophosphate), is a precursor in the synthesis of phosphatidylcholine (Cho and Kim, 2009) similar to AGPC (see Fig. 7 for structure). It is used for Alzheimer's disease and other types of dementia (Secades and Lorenzo, 2006). Citicoline is metabolized to choline and cytidine. In a 90-day rat study (Schauss et al., 2009), no toxicity was observed except for renal tubular degeneration at 1000 mg/kg. A high level of Citicoline consumption resulted in increased phosphorus intake in the rats, explaining this result. Citicoline has two phosphorus atoms compared to one in AGPC (Fig. 7). The maximum level of AGPC tested in rats was 300 mg/kg. The kidney effect observed with Citicoline was not observed with AGPC.

AGPC has been investigated in a number of human trials. Ziegenfuss et al. (2008) administered a single oral dose of 600 mg to seven males and did not observe any adverse affects. Barbagallo Sangiorgi et al. (1994) investigated the clinical efficacy and the tolerability in a open multicenter trial on 2044 patients suffering from recent stroke or transient ischemic attacks. AGPC was administered after the attack at the daily dose of 1000 mg i.m. for 28 days and orally at the dose of 400 mg three times a day during the following 5 months after the first phase. Forty-four patients (2.14%) complained of adverse effects. The most frequent complaints were heartburn (0.7%), nausea–vomiting (0.5%), insomnia–excitation (0.4%), and headache (0.2%). Parnetti et al. (1993) orally administered 1200 mg AGPC (800 mg in the morning and 400 mg in the afternoon) to 65 individuals for 6 months in a multicentre, randomised, controlled efficacy study in patients with probable senile dementia of Alzheimer's type of mild to moderate degree. AGPC was well tolerated with only one person experiencing insomnia, gastralgia, and restlessness. Ban et al. (1991) orally administered AGPC to 817 individuals for 6 months at a dose of 1200 mg (400 mg 3 times/day). There were 14 different side effects observed in 34 individuals, including agitation (in 13), heartburn (in 7), nausea (in 5), headache and insomnia (in 3) and orthostatic hypotension (in 2). De Jesus Moreno Moreno (2003) administered AGPC orally (1200 mg/day in 400 mg doses three times per day) for 6 months in a multicenter, double-blind, randomized,

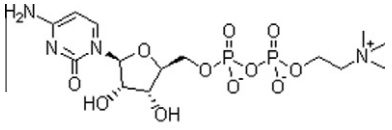

Fig. 7. Structure of citicoline (from www.chemBlink.com).

placebo-controlled trial to patients affected by mild to moderate dementia of the Alzheimer type. AGPC was well tolerated with side effects of 10 episodes of constipation and 5 episodes of nervousness. Parnetti et al. (2001) summarized the clinical studies with AGPC. Thirteen published clinical trials, examining in total 4054 patients, evaluated the use of AGPC in various forms of dementia disorders of degenerative, vascular or combined origin, such as senile dementia of the Alzheimer's type or vascular dementia and in acute cerebrovascular diseases, such as transitory ischemic attack and stroke. Administration of AGPC significantly improved patient clinical condition. Clinical results obtained with AGPC were superior or equivalent to those observed in control groups under active treatment and superior to the results observed in placebo groups. No serious side effects or toxicities were observed in the extensive human trials. Chronic treatment of animals resulted in reduced activity at the highest doses tested. Some clinical chemistry parameters did show reductions (triglycerides, bilirubin, and alkaline phosphatase) but there were no organ weight changes or histopathological correlates and the effects were not seen in the human studies.

AGPC is metabolized to choline. In 1998 choline was classified as an essential nutrient by the Institute of Medicine (1998). Choline intake in the adult human (as free choline and the choline in phosphatidylcholine and other choline esters) is greater than 700–1000 mg/day (Federation of American Societies for Experimental Biology,1975; Zeisel, 1981). Choline is a common component of many foods with the best sources being whole eggs (250 mg/100 g food), meats and fish, followed by whole grains and breakfast cereals (Patterson et al., 2008). The recommended adequate intake (AI) for choline has been set at 425 mg/day for women, 450 mg/day for pregnant women, 550 mg/day for lactating women and 550 mg/day for men (Institute of Medicine, 1998). 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/day, respectively) and the observed levels are similar to the adequate intake (AI) for choline. The Tolerable Upper Limit is 3500 mg/day for adults and is 1000 mg/day for children between age 1 and 8 and 2000 for children between age 9 and 13. Table 16 shows the adequate intake and tolerable upper limit for choline for different age groups and life stages. Choline is GRAS. There are no limits on its use in the US (21CFR182.8252) (US Code of Federal Regulations, 2010). One gram of AGPC will yield 0.40 g of choline, thus it takes 2.5 times as much AGPC to yield an equivalent amount of choline.

Chronic (26 week) oral toxicity studies of AGPC in Sprague–Dawley rats (up to 1000 mg/kg/day) and beagles (up to 300 mg/kg/day) produced symptomology primarily consisting of reduced activity; high dose exposed animals had reduced body weight gains. There was no clear pattern of toxicity in any organs. The NOAEL was 150 mg/kg/day based on the dog study. Extensive human studies at levels up to 1.2 g/day (20 mg/kg/day for a 60 kg individual) for 6 months indicated that AGPC exhibits low oral toxicity and is well-tolerated. AGPC is metabolized to choline, an essential nutrient. It is also an endogenous chemical. It is a component of breast milk and is an important source of choline required by infants for organ growth and membrane biosynthesis (Holmes-McNary et al., 1996; Holmes et al., 2000; Ilcol et al., 2005). It is also present in brain tissue as a product of phospholipid metabolism. In light of the results presented in this report, AGPC should be safe when used as a food ingredient up to a level of 1.2 g/day.

Table 16
Dietary reference intake values for choline.

Population	Age	Adequate intake	Tolerable upper limit
AI for children	1–3 years	200 mg/day	1000 mg/day
	4–8 years	250 mg/day	1000 mg/day
	9–13 years	375 mg/day	2000 mg/day
AI for males	14–18 years	550 mg/day	3000 mg/day
	19 years and older	550 mg/day	3500 mg/day
AI for females	14–18 years	400 mg/day	3000 mg/day
	19 years and older	425 mg/day	3500 mg/day
AI for pregnancy	All ages	450 mg/day	Age appropriate UL
AI for lactation	All ages	550 mg/day	Age appropriate UL

Adapted from (Institute of Medicine, 1998).

Conflict of Interest

The authors declare that there are no conflicts of interest.

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