

Safety Assessment of Citicoline

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Novel Food Classification: 1.1.

Introduction

An application for the authorisation of citicoline (trade name Cognizin[®]) was submitted to the Food Safety Authority of Ireland (FSAI) by Kyowa Hakko Europe GmbH in accordance with *Article 4* of the novel food Regulation (EC) No. 258/97. The application was accepted by the FSAI on March 29th, 2012.

Citicoline (choline cytidine 5'-pyrophosphate) is produced endogenously and dissociates in the body to form choline and cytidine 5'-pyrophosphate. Choline is a water-soluble essential nutrient grouped within the B-complex vitamins and in the human body is involved with a range of structural, metabolic, physiological and neurological functions. Choline is found naturally as free choline and phosphatidylcholine in food such as meat and eggs, while various forms are already approved for addition to infant and follow-on formula in the EU. Cytidine is a constituent of RNA (ribonucleic acid) and therefore found naturally in various foods such as offal and brewer's yeast. The addition of certain forms of cytidine to infant and follow-on formula is permitted in the EU. The applicant intends to market citicoline as a combined source of choline and cytidine in a range of foodstuffs and beverages at a maximum level of 250mg per serving, and in food supplements at a recommended intake of up to 500mg per day.

The applicant categorises citicoline as a novel ingredient within the category of "foods and food ingredients with a new or intentionally modified primary molecular structure" as per *Article 1.2(c)* of the novel food Regulation. The application dossier was prepared pursuant to Commission Recommendation 97/618/EC and in order to assess wholesomeness, citicoline was considered in Class 1 "pure chemicals or simple mixtures from non-genetically modified sources" and sub-class (1) "the source of the novel food has a history of food use in the Community".

I. Specification of the novel food

Citicoline is a white crystalline powder with a specified purity of at least 98.0%, though the actual purity of three consecutive lots tested was closer to 100% in each case. Citicholine is the product of an enzymatic process derived from a combination

of two microorganisms. However, the applicant has demonstrated that protein is not detectable in the final product, an important feature in terms of potential allergenicity. Citicoline in powder form was found to be stable for at least three years and in solution for at least six months. Levels of heavy metals, microbiological contaminants and solvents such as methanol and ethanol are maintained at acceptable levels via product specifications and therefore are not a toxicological concern.

II. Effect of the production process applied to the novel food

Citicoline is produced from orotic acid and choline chloride in an enzymatic process facilitated by inactivated *Corynebacterium ammoniagenes* and *Escherichia coli*. Cultured bacteria are inactivated by xylene before the enzymatic reaction is initiated with the addition of orotic acid and choline chloride. The enzymatic process is terminated by the addition of sulphuric acid (H₂SO₄) and heating to 60°C. Citicoline is isolated from the growth media in a series of extraction and purification steps resulting in a highly purified product.

III. History of the organism used as the source of the novel food

Orotic acid and choline chloride comprise the actual source of citicoline, with *C. ammoniagenes* and *E. coli* being the sources of the enzymatic process. Orotic acid is found in the milk of ruminants including cows, goats and sheep. Choline chloride is a member of the water-soluble B-vitamin family and is approved in the EU for use as a source of choline in dietetic foods (PARNUTS) including infant and follow-on formula.

IV. – VIII. GM Aspects

Though part of the enzymatic process originates in genetically modified (GM) *E. coli*, the final product does not fall within the scope of the GM Food and Feed Regulation EC No. 1829/2003 for a number of reasons. Firstly, xylene is added to the bacterial mix before the production of citicoline begins and, therefore, a viable GMO does not form part of the production process. Secondly, citicoline is purified from the growth media and not isolated or extracted from bacterial cells, while DNA from the GM *E. coli* is not detected in the final ingredient. This means the GM *E. coli* is considered to function as a processing aid in the production of citicoline. Finally, the *E. coli* enzymes are considered a processing aid as they are demonstrably absent from the final product.

IX. Anticipated intake/extent of use of the novel food

The applicant intends to market citicoline in a range of food matrices including cereal and dairy products, confectionaries, beverages including bottled water and vegetable and fruit juices and also in food supplements. Foods and beverages will contain citicoline at 250 mg per serving while the recommended intake from food supplements will be up to 500 mg per day.

The applicant has estimated possible intake scenarios based on data from UK nutrition and diet surveys. At the levels proposed (“worst case” scenario), moderate exposure would be anticipated in the general population with no adverse health risks. Of the individual population groups, male teenagers were identified with the greatest mean intake and 95th percentile all-user intakes at 497 and 1,310 mg/person/day (9.6 and 29.2 mg/kg body weight/day), respectively. Female adults had the lowest mean and 95th percentile all-user intakes of citicoline, at 296 and 847 mg/person/day (4.4 and 16.2 mg/kg body weight/day) respectively. However, on a body-weight basis, children were identified as having the highest mean and 95th percentile all-user intakes of any population group, at 23.5 and 64.1 mg/kg body weight/day, respectively. Male adults were found to have the lowest mean and 95th percentile all-user intakes of citicoline at 3.8 and 10.5 mg/kg body weight/day.

X. Information from previous human exposure to the novel food or its source

Citicoline is produced endogenously from choline and cytidine tri-phosphate (CTP) through a well established pathway. Dietary citicoline is rapidly absorbed following ingestion and is hydrolysed to cytidine and choline either in the intestinal wall, the blood stream or the liver. Choline is an essential nutrient already consumed as phosphatidylcholine or free choline in a variety of foods in the EU. Citicoline is available in the USA in food supplements at a recommended intake of 200 to 1,000 mg/day.

XI. Nutritional information on the novel food

The novel ingredient is intended to supplement dietary or endogenous choline and cytidine levels in the body. Citicoline is a complex organic molecule that functions as an intermediate in the biosynthesis of cell membrane phospholipids. Citicoline and its constituents, choline and cytidine are associated with various metabolic activities as well as maintaining the structural integrity and functionality of cellular membranes.

XII. Microbiological information on the novel food

The microbiological status of the novel ingredient is controlled via product specifications and supported by batch test results in which *E. coli* is not detected at any level.

XIII. Toxicological information on the novel food

A range of pharmacological, biochemical and toxicological studies on citicoline are provided by the applicant, although the endpoints of chronic toxicity and carcinogenicity are not covered, and data on reproductive toxicity are limited. However, considering the fact that citicoline is produced endogenously and its constituent molecules are authorised for use in particular foodstuffs in the EU, the toxicological data is sufficient.

Pharmacological/biochemical effects of citicoline

Citicoline has pharmacological effects on the dopaminergic system. It can also modulate brain biochemistry which may underlie its reported effects on certain brain functions including learning, and its proposed role in the treatment of cerebral vascular deficiencies. Citicoline is involved in the maintenance of the structural integrity of cell membranes, in methyl-metabolism, in cholinergic neurotransmission (as acetylcholine), in trans-membrane signalling and in lipid-cholesterol transport and metabolism. Such pharmacological effects are not unique to citicoline and are also anticipated and reported with other choline salts currently approved for use in PARNUTS including infant and follow-on formula. They do not, therefore, impact on the safety assessment of citicoline.

Absorption, Distribution, Metabolism and Excretion (ADME)

The ADME of citicoline is well established and has been investigated both in animal models and in humans. Citicoline is more than 90% bioavailable and is rapidly absorbed upon ingestion, after which component molecules are found to locate to the cerebral tissues in animal models. Once absorbed it is hydrolysed to cytidine and choline either in the intestinal wall, the blood stream or the liver. Choline enters one of three major metabolic pathways while cytidine is broken down to uridine, the component that crosses the blood brain-barrier. Uridine is phosphorylated to uridine triphosphate (UTP) and converted to cytidine triphosphate (CTP) which is used to resynthesise citicoline. Differences in the metabolism of citicoline have been observed between rodents and humans. Oral administration of citicoline resulted in raised plasma levels of cytidine and choline in rodents, but increased plasma levels of uridine and choline in humans. While the reason for this difference is unclear, it is not considered to have any safety implications for the consumption of citicoline by humans. Very little ingested citicoline is excreted in the faeces of animals or humans. Instead, the elimination of citicoline is found to occur via urinary or CO₂ expiration routes.

Toxicity

Citicoline is of low acute toxicity with an oral LD₅₀ > 2,000 mg/kg. Evidence of genotoxicity was not detected in a bacterial mutagenicity assay at citicoline levels up to 5,000 µg/plate, or a chromosome aberration study in Chinese Hamster Ovary Cells at dose levels of up to 10 µM/ml. Both studies were carried out in the presence or absence of metabolic activation. Genotoxicity was also not detected using the *in vivo* micronucleus test in mice with single doses of 500, 1,000, or 2,000 mg/kg body weight of citicoline being administered *via* intraperitoneal injection.

Administration of citicoline by gavage at levels of 100, 350 or 1,000 mg/kg body weight/day in a 90-day study with Sprague Dawley rats had no effect on mortality, general condition, body weight, food and water intake, ophthalmology, gross necropsy or organ weight. Minor dose-related effects were reported on some haematological and biochemical parameters. All male treatment groups showed a significant decrease in urine volume compared to controls, accompanied by brownish

discoloration of the urine, while a significant, but slight, increase in serum creatinine levels was reported in mid- and high-dose males compared to controls. Similar changes were not reported in female rats. Females in the highest dose group showed a small but significant increase in total white blood cell (WBC) and absolute lymphocyte counts, while low- and mid-dose females were reported to exhibit significant increases in blood urea nitrogen (BUN) compared to their respective controls. This finding was not considered to be toxicologically significant given the lack of a dose-response relationship and absence of similar changes in males. All female dose groups showed a dose-related increase in the incidence and severity of renal tubular mineralisation. This latter finding is common in female rats, and was attributed to the increased intake of phosphate associated with the administration of citicoline; therefore, it is considered to be of no toxicological relevance. The applicant noted that the addition of phosphorus from the proposed uses of citicoline in conventional foods and food supplements would only increase the daily exposure of phosphorus by a relatively small amount compared to the Reference Daily Allowance (RDA) of phosphorus (*i.e.*, 0.7 g/day). Citicoline was also administered orally to male and female beagle dogs at levels of 0 or 1,500 mg/kg body weight/day for a period of six months, without any evidence of treatment-related effects.

The only information available on the reproductive toxicity of citicoline was an unpublished study in which albino rabbits were administered 0 or 800 mg citicoline/kg body weight/day (route of administration not specified) on gestation days 7 to 18. The animals were then killed on gestation day 29 and the foetuses were removed for examination. There were no signs of maternal or foetal toxicity. Approximately 10% of the foetuses exposed to citicoline were reported to display a slight delay in cranial osteogenesis. However, this was not considered by the authors of the study to be representative of an adverse effect due to citicoline.

A number of clinical studies have been identified in the literature where humans who were either healthy, had acute ischemic stroke or dependent on cocaine or methamphetamine were administered a maximum of 2,000 mg/day of citicoline for up to 12 weeks. The majority of these studies were not undertaken to assess the safety of citicoline, however, no treatment-related adverse effects were reported. A meta-analysis involving 1,372 subjects (789 in citicoline group, 583 in placebo group) investigated the safety and efficacy of citicoline as an adjunctive treatment for acute ischemic stroke. There were no significant differences in the incidence of mortality reported between citicoline-treated individuals and controls, with the frequency of adverse events reported being comparable between groups. The authors concluded that a dose of 2,000 mg citicoline/day represented the dose providing the highest beneficial effect, and that the overall safety profile for citicoline was similar to that of a placebo. In a surveillance study involving 2,817 Spanish individuals consuming citicoline for various indications including senility, chronic cerebral vascular insufficiencies, cerebral vascular accident sequelae, and sequelae of cerebral transmission, the author reported that citicoline had a good safety profile, with no subjects discontinuing treatment as a result of adverse side-effects. Citicoline

consumption was reported to be associated with a significant decrease in the assessment scores for memory deficits, headaches, dizziness, and walk instability.

Choline and Cytidine

The applicant provided an overview of the safety of choline and cytidine, the constituent molecules of citicoline. The review included toxicological reference values and the results of some clinical studies on choline with no specific toxicological concerns identified.

Allergenicity

Citicoline will have a purity of at least 98% according to the specifications and protein is an unlikely constituent given the isolation and purification process. In addition, protein has not been detected in batch analysis and therefore allergenicity is not a concern for this novel ingredient.

Conclusions

Though citicoline is considered a novel ingredient under EU legislation, various forms of its constituent molecules are already consumed within the EU in particular foods, while citicoline itself is already available in pharmaceutical form. This application intends to expand the range of foods and beverages through which citicoline will be available to the average consumer. Citicoline is produced endogenously and its absorption, distribution, metabolism and elimination, along with that of its constituents (choline and cytidine) are well characterised. No nutritional concerns have been identified arising from the intended use and use levels in the foods, beverages and food supplements listed in this application.

Toxicological studies in animals along with human studies have not highlighted any particular areas of concern regarding the safety of citicoline as a novel ingredient in general foodstuffs or food supplements at the proposed use levels. No treatment-related adverse effects were reported in humans consuming 500 to 2,000 mg/day of citicoline for periods of 4 to 12 weeks, intakes comparable to or below the maximum estimated intake of citicoline.

Recommendation

The Food Safety Authority of Ireland has not identified any safety concerns with the consumption of foods or food supplements containing citicoline at the proposed use levels and therefore considers that this novel ingredient meets the criteria for novel food set out in *Article 3.1.* of the novel food Regulation.