SCIENTIFIC OPINION

Scientific Opinion on the safety of "citicoline" as a Novel Food ingredient[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on citicoline as a novel food ingredient in the context of Regulation (EC) No 258/97. The novel food ingredient (NFI), citicoline, is choline cytidine 5'-pyrophosphate ($C_{14}H_{26}N_4O_{11}P_2$) with a minimum purity of 98.0 %. The stability, specification and production process of the NFI do not raise safety concerns. Citicoline is intended to be used in food supplements aimed at a target population of middle-aged to elderly adults, at a maximum level of 500 mg/day, and in foods for particular nutritional uses, specifically foods for special medical purposes, at a maximum level of 250 mg/serving, and with a maximum daily intake from these types of foods of 1 000 mg/day. Citicoline is readily hydrolysed on ingestion, breaking down to choline and cytidine, which are normal body constituents that then undergo further metabolism and incorporation into normal pathways of metabolism. The Panel considers that consumption of the NFI is not nutritionally disadvantageous. Available human studies do not raise safety concerns under the proposed conditions of use. The additional data presented by the applicant on safety in laboratory animals, although incomplete by modern standards, provides further reassurance on the safety of the NFI. The Panel concludes that the NFI, citicoline, is safe under the proposed uses and use levels.

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KEY WORDS

citicoline, CDP-choline, novel food, ingredient

SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on citicoline as a novel food ingredient in the context of Regulation (EC) No 258/97, taking into account the comments and objections of a scientific nature raised by Member States.

The novel food ingredient (NFI), citicoline, is choline cytidine 5'-pyrophosphate ($C_{14}H_{26}N_4O_{11}P_2$) with a minimum purity of 98.0 %. The Panel considers that the information provided on the stability, specification and data from batch testing do not raise safety concerns. The production process is sufficiently described and does not raise safety concerns.

Citicoline is intended to be used in food supplements aimed at a target population of middle-aged to elderly adults, at a maximum level of 500 mg/day, and in foods for particular nutritional uses (PARNUTS), specifically foods for special medical purposes, at a maximum level of 250 mg/serving, and would not envisage a daily consumption level above 1 000 mg/day from these types of foods. The NFI is not intended to be consumed by children.

Citicoline is readily hydrolysed upon ingestion, breaking down to choline and cytidine which are normal body constituents, which then undergo further metabolism and incorporation into normal pathways of metabolism.

The Panel considers that consumption of the NFI is not nutritionally disadvantageous. Available human studies do not raise safety concerns under the proposed conditions of use.

The applicant has provided a set of *in vitro* and *in vivo* genotoxicity studies, and based upon the results the Panel concluded that there are no safety concerns related to genotoxicity.

A report was provided by the applicant on a 90-day study in Sprague Dawley rats given 0, 100, 350 or 1 000 mg/kg bw per day citicoline by gavage. The test substance was stated to be 99.8 % pure and was dissolved in distilled water prior to administration. No adverse effects were seen in the study, although in certain respects the study did not meet the current standards defined by OECD guidelines. A published study in which dogs received citicoline by gavage at 0 or 1.5 g/kg bw/day was also considered deficient, but again showed no adverse effects attributable to citicoline treatment. The Panel concluded that the animal data provide some reassurance on the safety of citicoline, but that on their own are not sufficient to assess the safety of the NFI.

On the basis of all of the information provided, the Panel concludes that the novel food ingredient, citicoline, is safe under the proposed uses and use levels.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 29 March 2012, the company Kyowa Hakko Europe GmbH submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to place on the market "citicoline" as a novel food ingredient.

On 02 June 2012, the competent authorities of Ireland forwarded to the Commission their initial assessment report, which came to the conclusion that citicoline may be placed on the market.

On 10 July 2012, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted comments or raised objections.

The concerns of a scientific nature raised by the Member States can be summarised as follows:

- The UV absorbance assay used to measure citicoline is not sufficiently accurate or specific for quality control purposes and should be replaced by an alternative analytical method.

- Citicoline is present at a minimum level of 98 % and the risk assessment does not consider the safety of the other unnamed components which will be present. Additional information about the composition of the product and the safety of secondary components should be provided.

- An analysis for bacteria DNA is not reported. *Corynebacterium ammoniagenes* has not been researched.

- Analysis of xylene in the final product has not been carried out.

- Specifications of the new ingredient could include individual levels of cadmium, lead, mercury, orotic acid, the latter considered as a "compound that gives cause for concern in terms of its action as a promoter of tumours" (EFSA, 2009), xylene, ethanol and methanol.

- Description of the analytical methods used and their validation are missing for some compounds.

- Accreditation of the laboratories which performed the analyses should be provided.

- The production process should be better described, including information about the properties of the microorganism strains used, which enzymes are active, and details about the reactions which take place. The meaning of "inactivated cultures" is unclear.

- The experimental design used for stability testing differs from the process commonly applied making interpretation of the results difficult. The analytical method used to measure citicoline may not be sufficiently specific. There is no stability testing of citicoline during food processing (e.g. heating), over the long term, and/or addressing possible interactions with food matrices.

- The accumulated exposure originating from foods and food supplements to which citicoline has been added should be taken into account. No information about background consumption from the normal diet is presented. The consumption estimates must also take into account the natural presence of orotic acid and choline chloride in certain foods.

- The nutritional consequences of citicoline consumption have not been assessed, particularly in terms of impact on the choline status of consumers.

- Full reports of the toxicity studies should be provided.

- Toxicity studies do not provide safety data on the use of citicoline by children. The safety of intake of citicoline by young children should be considered more carefully.

- In the 90 day study in rats (Schauss et al., 2009), renal tubular degeneration was observed at doses of 1 000 mg/kg.

- The available data on developmental and reproductive toxicity is insufficient. Comparing the intake estimates for citicoline with the highest dose level investigated in the published 90-day toxicological study in rats, the margin of safety is low, especially for young children.

- Reversible phenomena of agitation, a potential secondary effect of the pharmaceutical form of citicoline, has not been taken into account. More information on the use of citicoline as a medicine should be provided, in particular with respect to its interaction with the dopaminergic system. At concentrations of 500 or 1000 mg (pharmaceutical use), citicoline interacts with other medicines, enhancing the effects of medicaments with L-Dopa. It is also recommended not to administer it together with medicaments that contain meclofenoxate. Labelling of the supplements should warn the consumer of this possible interaction, in addition to the importance of not exceeding the daily dose of 500 mg of citicoline.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for "citicoline" as a novel food ingredient in the context of Regulation (EC) No 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of a scientific nature in the comments raised by the other Member States.

ASSESSMENT

In accordance with Commission Recommendation 97/618/EC[4], citicoline is allocated to Class 1.1, i.e. foods or food ingredients that are 'pure chemicals or simple mixtures which are not obtained from plants, animals or microorganisms that have been genetically modified. The source of the NF has a history of food use in the Community'. The assessment of the safety of this novel food ingredient (NFI) is based on data supplied in the original application, the initial assessment by the competent authority of Ireland, the concerns and objections of the other Member States, and the responses of the applicant. The data are required to comply with the information required for novel foods of Class 1.1, i.e. structured schemes I, II, III, IX, XI, XII and XIII of Commission Recommendation 97/618/EC. In the text these structured schemes are listed 1 to 7. This assessment only concerns risk that might be associated with consumption, and is not an assessment of the efficacy of citicoline with regard to any claimed benefit.

1. Specification of the Novel Food Ingredient (NFI)

Citicoline novel food ingredient (also called CDP-Choline) is a white crystalline powder with a minimum purity of 98.0 %. Its chemical name is cytidine 5'-diphosphocholine ($C_{14}H_{26}N_4O_{11}P_2$) and its molecular weight is 488.32 Da. Its CAS Number is 987-78-0. Citicoline is composed of cytosine (a nitrogenous base), ribose, pyrophosphate, and choline (Secades and Frontera, 1995). The chemical structure of citicoline is shown in Figure 1.

Figure 1: Chemical Structure of Citicoline
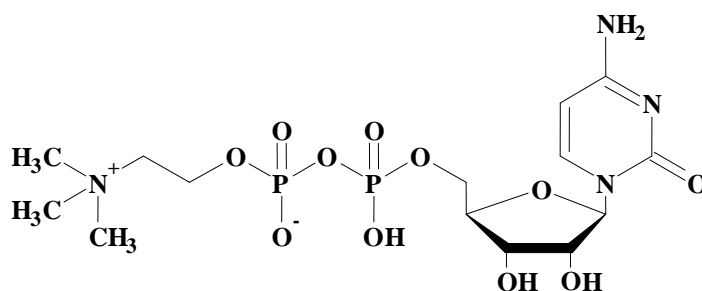
The specifications for the novel food ingredient (NI) are shown in Table 1.

The applicant provided the analytical results of three consecutive batches of citicoline, which were compliant with the specifications (Table 2). The applicant indicates that all analytical methods were performed according to those described in the official monograph for citicoline in the Japanese Pharmaceutical Codex (JPC, 1997), including the analysis of citicoline content by UV absorbance assay at 280 nm. Only determination of 5'-cytidilic acid is performed by an in-house method using high-performance liquid chromatography (HPLC), which has been validated.

According to the applicant, the combination of citicoline assay and impurity determination (i.e. free phosphoric acid, 5'-cytidilic acid and ammonium) provide sufficient evidence for the high purity of the final product.

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[4] Commission Recommendation 97/618/EC: Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 253, 16.9.1997, p. 1–36

Table 1: Specifications for citicoline, as proposed by the applicant

	Specification	Method
Identity		
Identification	Pass	Infrared absorption spectrum
State of Solution	Colourless and clear	Visual test, 12.5 %, H_2O
Appearance	White crystalline powder	Visual Test
pH	2.5 to 3.5	JP <2.54>, Sample solution 1 %
Purity		
Citicoline assay	Not less than 98.0 % [a]	Internal Method [b], [c]
Loss on Drying	Not more than 5.0 %	JP <2.41>, Sample 0.5 g, 100 °C, 4 hr, P_2O_5 (reduced pressure) [c]
Ammonium	Not more than 0.05 %	Atmospheric distillation [c]
Heavy Metals (as Pb)	Not more than 10 ppm	JP <1.07>, Method 1, Sample: 2.0 g [c]
Arsenic	Not more than 2 ppm	JP <1.11>, Method 4, Sample: 1.0 g, Standard solution: 1.0 ml [c]
Free Phosphoric Acids	Not more than 0.1 %	Internal Method [c]
5'-Cytidylic acid	Not more than 1.0 %	Internal Method [d]
Microbial Specifications		
Total Plate Count	Not more than 1 000 CFU/g	USP 32 <61>
Yeast and molds	Not more than 100 CFU/g	USP 32 <61>
Escherichia coli	Absent in 1 g	USP 32 <62>

CFU = Colony Forming Units; HPLC = High Performance Liquid Chromatography; JP = Japanese Pharmacopeia; JPC = Japanese Pharmaceutical Codex; Pb = lead; USP = United States Pharmacopeia

(a) of dry matter
(b) by UV absorbance at 280 nm
(c) as per procedure described in the Japanese official monograph for citicoline (JPC, 1997)
(d) by HPLC

Table 2: Analytical data for 3 batches of citicoline

	Specification	Lot No 080178	Lot No 080177	Lot No 080179
Identity				
Identification	Pass	Pass	Pass	Pass
State of Solution	Colourless and clear	Conforms	Conforms	Conforms
Appearance	White crystalline powder	Conforms	Conforms	Conforms
pH	2.5 to 3.5	3.1	3.1	3.1
Purity				
Citicoline assay	Not less than 98.0 %	100.0	100.3	100.1
Loss on Drying	Not more than 5.0 %	3.70	3.51	3.51
Ammonium	Not more than 0.05 %	< 0.05	< 0.05	< 0.05
Heavy Metals (as Pb)	Not more than 10 ppm	< 10	< 10	< 10
Arsenic	Not more than 2 ppm	< 2	< 2	< 2
Free Phosphoric Acids	Not more than 0.1 %	< 0.1	< 0.1	< 0.1
5'-Cytidylic acid	Not more than 1.0 %	< 1.0	< 1.0	< 1.0
Microbial Specifications				
Total Plate Count	Not more than 1,000 CFU/g	< 1 000	< 1 000	< 1 000
Yeast and molds	Not more than 100 CFU/g	< 100	< 100	< 100
Escherichia coli	Absent in 1 g	Absent	Absent	Absent

Heavy metals

The specifications set a maximum limit for heavy metals (as Pb) at not more than 10 ppm. The applicant indicates that the product will comply with the maximum limits for heavy metals as laid down in the EU legislation for food supplements[5]: not more than 3.0 ppm for lead, not more than 1.0 ppm for cadmium, not more than 0.1 ppm for mercury. Analytical results of 3 batches were provided which were in compliance with these limits.

Solvent Residues

The manufacture of citicoline involves the use of methanol, ethanol and xylene.

The applicant has set in-house specification limits for methanol and ethanol at 100 ppm and 500 ppm, respectively. The NFI is routinely tested for methanol and ethanol residues. The applicant provided results of analysis by gas chromatography for 3 consecutive batches of citicoline, which were compliant with the specified limits.

The applicant indicates that xylene is used at a low level (i.e. 0.69 % of the fermentation broth) and is removed in the evaporation step of the manufacturing process. Xylene was not detectable in 3 batches of citicoline (analysed by Headspace GC-MS; limit of detection (LOD) of 0.02 and 0.01 mg/kg for m/p-xylol and o-xylol, respectively). Given the manufacturing process and analytical results, the Panel considers that there is no need to include a limit for xylene in the specifications.

Residual Enzymes

Citicoline is manufactured from orotic acid and choline chloride through an enzymatic reaction (Section 2). The enzymes used are inactivated by the addition of sulphuric acid and heating, and removed by filtration, resin purification, and decolourisation. The applicant provided results of 3 batches of citicoline where the protein content was below the limit of detection analysed by the Bio-Rad Protein Assay.

The enzymes involved in the production of the NI are partly derived from cultures of genetically modified *E. coli*. The applicant provided analytical results of 3 batches of citicoline showing the absence of recombinant DNA in the final product (analysed by a validated PCR assay; LOD = 10^{-5} ppm).

Orotic acid

The applicant indicates that orotic acid will be removed by the resin purification step in the manufacturing process. The absence of detectable residues of orotic acid has been confirmed in 3 batches of citicoline (analysed by HPLC). Given the manufacturing process and analytical results, the Panel considers that there is no need to include a limit for orotic acid in the specifications.

The Panel considers that the information provided on the composition, specification and data from batch testing do not raise safety concerns.

Stability

The stability of citicoline has been examined as a powder and in solution.

Three lots of citicoline in powder form were stored at a temperature of $25 \pm 2\,°C$ and a relative humidity of $60 \pm 5\,\%$ for three years, and under accelerated storage conditions at a temperature of $40 \pm 2\,°C$ and relative humidity $75 \pm 5\,\%$ for six months. In both tests, analyses for state of solution,

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[5] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, OJ L 364, 20.12.2006, p. 5.

loss on drying, 5'-cytidylic acid and citicoline contents indicated that all three lots complied with the product specifications.

In a six-month stability study, citicoline (0.1 % solution) was generally stable at pH levels of 3.5, 7.0 and 9.3, and at temperatures of 5, 20 or 40 °C.

A forced degradation study was also conducted under acidic, alkaline, oxidative, or high temperature (105 °C) conditions, as well as in aqueous solutions stored at room temperature or at 60 °C. After 24 hours, the degradation of citicoline was analysed by HPLC analysis for 5`-cytidilic acid (CMP), cytidine diphosphate ethanolamine (CDP ethanolamine) and uridine diphosphate choline (UDP Choline). Citicoline was determined to be slightly unstable (i.e. impurities by degradation from 1 to 10 %) under acidic, alkaline, and oxidative conditions, as well as in solutions stored at 60 °C.

The Panel considers that the data provided sufficient information with respect to the stability of the NI.

2. Effect of the production process applied to the NFI

Citicoline is manufactured from orotic acid and choline chloride via reactions catalysed by enzymes present in cultures of *Corynebacterium ammoniagenes* and genetically modified *E. coli*. The strains and characteristics of both bacteria, as well as the reactions involved, were described in the application dossier and are considered confidential by the applicant. Xylene is added to both the cultures, resulting in the inactivation of the microorganisms before their combination and addition to the mixture of orotic acid and choline chloride.

The enzymatic reaction is terminated by adding sulphuric acid and heating. The manufacturing process includes several purification steps, including filtration, resin purification, and decolourisation. Methanol is then added to the concentrated solution to induce crystallisation. Crystals are washed with ethanol, vacuum-dried, and placed into fibre drums for storage.

The enzymes and the microorganisms are not present in the final product (see Section 1).

According to the applicant, the production of citicoline is conducted in accordance with GMP.

The Panel concludes that the production process is sufficiently described and does not raise safety concerns.

3. History of the organism used as a source

Two specific strains of *C. ammoniagenes* and *E. coli* are used as sources of enzymes in the manufacturing process of the NFI (see Section 2). The specific strain of *C. ammoniagenes* and the isogenic strain of *E. coli* have a history of use in industrial enzymatic reactions.

4. Anticipated intake/extent of use of the NFI

The applicant proposed to limit the use of citicoline to food supplements aimed at a target population of middle-aged to elderly adults, at a maximum level of 500 mg/day, and to foods for particular nutritional uses (PARNUTS), specifically foods for special medical purposes, at a maximum level of 250 mg/serving, and would not envisage a daily consumption level above 1 000 mg/day from these types of foods. The applicant states that the amount of citicoline added to foods for special medical purposes will be determined on a case-by-case basis in accordance with the conditions laid down under Commission Directive 1999/21/EC on dietary foods for special medical purposes[6]. The applicant considers it unlikely that both PARNUTS and food supplement products containing citicoline will be consumed together. The applicant indicates that the NFI is not intended for consumption by children.

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[6] Commission Directive 1999/21/EC of 25 March 1999 on dietary foods for special medical purposes (text with EEA relevance). OJ L 91, 7.4.1999, p. 29.

5. Information from previous exposure to the NFI or its source

Citicoline is available in Japan, Spain, France and Italy as a pharmaceutical (Adibhatla and Hatcher, 2005). In the United States, citicoline is available as a component of supplement products at doses ranging between 200 and 1 000 mg/day.

The applicant also provided information on previous exposure to orotic acid and choline.

Orotic acid is an intermediate of pyrimidine biosynthesis. Orotic acid is present in cow's milk at levels ranging from 20 to 100 mg/L, with somewhat higher concentrations reported in goat's and sheep's milk (EFSA, 2009). Orotic acid has been detected in infant formula at concentrations ranging from 15 to 118 mg/L (EFSA, 2009).

Choline is present in foods mainly as phosphatidylcholine and free choline (Zeisel et al., 2003). The dietary intake of choline has been estimated to range between 300 and 1 000 mg/day. Foods with the highest concentration of choline include eggs, liver, soybeans, and pork meat (Zeisel et al., 2003; Bidulescu et al., 2007). Certain forms of choline (i.e. choline, choline chloride, choline citrate, and choline bitartrate) are approved for use in dietetic (PARNUTS) foods (Commission Regulation (EC) No 953/2009[7]), in processed cereal-based foods and baby foods for infants and young children (Commission Directive 2006/125/EC[8]), and in infant and follow-on formulae (Commission Directive 2006/141/EC[9]).

6. Nutritional information on the NFI

The applicant indicates that citicoline is intended for use as an ingredient to "supplement natural levels of choline and cytidine in the body. These natural levels may be endogenous or consumed as part of the background diet".

In response to a Member State request, the applicant indicates that a 500 mg serving of citicoline will deliver approximately 106 mg of choline, which is equivalent to ca. 100 g of meat or fish. The US IOM has established an adequate intake for choline of 425 mg/day for adult women and 550 mg/day for adult men (IOM, 1998). IOM proposed a tolerable upper intake level (UL) of 3 500 mg/day for adults including pregnant and lactating women.

The applicant states that the addition of phosphorus from the proposed uses of citicoline would increase the daily exposure to phosphorus by a small fraction (500 mg citicoline contain 63.45 mg (12.69 %) phosphorus) compared to the recommended daily allowance (RDA) for phosphorus of 700 mg/day as per Commission Directive 2008/100/EC[10]. The average dietary intakes of phosphorus in European countries are of 1 000 to 1 500 mg/person per day, ranging up to about 2 600 mg/day (EFSA, 2005). The Panel notes that the anticipated intake of phosphorus from the NFI is low in comparison to the average daily intake.

The Panel considers that consumption of the NFI is not nutritionally disadvantageous.

7. Microbiological information on the NFI

The citicoline manufacturing process requires enzymes produced by cultures of specific strains of *C. ammoniagenes* and *E. coli* (see Section 2). Both microorganisms are non-pathogenic, non-toxigenic

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[7] Category 6 of the Annex of Commission Regulation (EC) No 953/2009 of 13 October 2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses.

[8] Annex IV of Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children and in infant and follow-on formula.

[9] Annex 1 and Annex 2 of Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC.

[10] Commission Directive 2008/100/EC of 28 October 2008 amending Council directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions (text with EEA relevance). OJ L 285, 29.10.2008, 9-12.

organisms. The applicant indicates that the microorganisms are inactivated during the process by the addition of xylene.

The taxonomic classification of the specific strain of *C. ammoniagenes* has been provided by the applicant. *C. ammoniagenes* has been used in the production of nucleotides, amino acids, and the vitamin riboflavin (Liebl, 2005).

Analytical data on the levels of micro-organisms in 3 batches of NFI showed that the levels of microbiological contamination were either below detection limits or below the limits set in the product specifications.

The Panel considers that the microbiological information provided does not raise safety concern.

8. Toxicological information on the NFI

8.1. Endogenous Production of Citicoline

Citicoline is an intermediate in one of the biosynthetic pathways of membrane phospholipids (Kennedy pathway). In this pathway, choline is phosphorylated by the enzyme choline kinase to produce phosphorylcholine, which then combines with cytidine triphosphate (CTP) to form citicoline (D'Orlando and Sandage, 1995). Citicoline then combines with diacylglycerol (DAG) to form phosphatidylcholine, with choline phosphotransferase catalysing this reaction (D'Orlando and Sandage, 1995).

8.2. Absorption, Distribution, Metabolism, and Excretion

The absorption, distribution and elimination of an oral dose of 4 mg/kg body weight of methyl-^{14}C labelled citicoline was examined in an unspecified number of male Sprague-Dawley rats (Agut et al., 1983). Within the first hour of administration, the maximum percentage of the orally administered dose observed in the blood stream was 1.30 % of radioactivity per 10 mL of blood increasing to 4.20 % 5-6 hours after administration. Low urinary and faecal elimination were reported. After 24 hours, the uptake of radioactivity was reported to be over 90 %. The distribution of orally administered citicoline into cerebral tissues has been examined in various laboratory animal species (Aguilar et al., 1983; Romero et al., 1983a, 1983c, 1983b). However, all these studies used methyl-^{14}C labelled citicoline which tends to only show the fate of the labelled methyl group and not of the whole molecule, hence demonstration of radioactivity in brain tissues provides little evidence for the fate or distribution of citicoline *per se*.

Six healthy adults aged 20-32 yrs were given a single oral dose of 300 mg citicoline (Dinsdale et al., 1983b) consisting of a mixture of the compound labelled at two different sites (i.e. ring of the cytidine residue and methyl position of the choline moiety) and the unmodified compound and followed for five days. During the five-day collection period, < 1 % of the dose was found in the faeces, indicating a high absorption. Elimination primarily occurred via respiratory CO_2 and urinary excretion. Both routes exhibited biphasic patterns probably related to the two labelling locations. During the five days after administration, about 16 % of the dose was excreted, indicating that most of the citicoline and its metabolites was incorporated into the tissues where they enter metabolic pathways.

Lopez et al. (1987) examined the effects of oral citicoline on the plasma concentrations of cytidine, choline and citicoline in four healthy volunteers consuming a single dose of 2 000 mg citicoline. Plasma concentrations of choline and cytidine were reported to increase by 48 % and 136 %, respectively, after two hours. A further four subjects given three doses of 2 000 mg citicoline at two-hour intervals were reported to exhibit peak plasma concentrations of choline approximately four hours after consumption of the initial dose. The maximum achieved concentration of choline in the plasma was 30 % higher than that measured at baseline. The plasma concentrations of cytidine peaked approximately six hours after administration, and the concentrations were five times higher than basal concentrations. The authors also administered citicoline intravenously to rats and four healthy human

volunteers. Citicoline was observed to be rapidly hydrolysed, in both the humans and the rats. In humans, plasma citicoline concentrations were no longer elevated 30 min after the end of the infusion period. Plasma choline and cytidine peaked at the end of the infusion period and their concentrations remained elevated for at least six hours. In rats, citicoline was undetectable in plasma five minutes after bolus injection, while plasma cytidine concentrations increased markedly and remained elevated for at least 60 min.

In a more recent study, 12 mildly hypertensive subjects ingested a single dose of 0 (placebo), 500, 2 000, or 4 000 mg of citicoline following an overnight fast (Wurtman et al., 2000). A significant, dose-dependent increase in plasma choline concentration was observed, with peak plasma levels attained two to three hours after the 500 mg dose, and five hours after the remaining doses. Plasma uridine concentrations significantly increased at all doses, with peak plasma concentrations observed 1.5 hours after the 500 mg dose, and three hours after all the other doses. Plasma uridine concentrations were observed to remain significantly elevated for five to six hours after ingestion and returned to baseline levels within 8-10 hours of citicoline ingestion. In contrast to the findings reported by Lopez et al. (1987), significant quantities of cytidine were not detected in the plasma before or after citicoline ingestion. The authors attributed the increase in plasma uridine levels to cytidine deaminase activity in the gastrointestinal tract and liver, which transforms cytidine to uridine. The results observed in the study by Lopez et al. (1987) were attributed to the low specificity of the assay used to measure cytidine. The authors also note that the proportion of cytidine and uridine in human plasma differ markedly from those in laboratory rodents, where basal levels of cytidine have been shown to be significantly higher than uridine.

Choline functions as a precursor of phospholipids, acetylcholine and betaine. Uridine may enter pyrimidine metabolism, which involves multiple sites of uptake, use and degradation. Cytidine and uridine are components of ribonucleic acid (RNA). Human milk also contains large quantities of nucleosides, in particular cytidine and uridine (Liao et al., 2011). The intake of nucleic acids (DNA and RNA) from the diet has been estimated to range between 100 and 1 000 mg/day (Doerfler and Schubbert, 1998). Cytidine 5'-monophosphate and its sodium salt are permitted as an added nucleotide in PARNUTS foods (Commission Regulation (EC) 953/2009[5]), and for infant and follow-on formulae (Commission Directive 2006/141/EC[7]).

Overall, the Panel notes that data in rodents show that the absorption of citicoline is high, and that its metabolites have been shown to be distributed to various tissues, including the brain. Data available in humans showed that citicoline is well absorbed. Citicoline is rapidly broken down into choline and cytidine, and the latter is readily transformed to uridine. Choline, cytidine and uridine are common constituents of the body. After absorption, citicoline and its metabolites are involved in choline and pyrimidine metabolic pathways and incorporated into tissues. The main route of excretion is respiratory CO_2, although significant amounts are also eliminated via urine. Rat and human metabolism differ in that the predominant circulating pyrimidine in humans is uridine, while it is cytidine in rats.

8.3. Genotoxicity

In a bacterial reverse mutation assay (Ames test) conducted at concentrations of 50, 150, 500, 1 500 and 5 000 µg/plate, in accordance with OECD test guideline No 471, citicoline (sodium salt) was reported to be non-mutagenic in *Salmonella typhimurium* strains TA100, TA98, TA1535 and TA1537, and in *E. coli* WP2uvrA, in the presence and absence of metabolic activation (unpublished study report by Kyowa Safety Research Laboratories (1993)).

In an *in vitro* chromosome aberration test conducted in accordance with OECD test guideline No 473, Chinese hamster ovary cells were exposed to citicoline and its sodium salt at concentrations of 2.5, 5 and 10 mM in the presence and absence of metabolic activation (unpublished study report by Kyowa Safety Research Laboratories (1993)). The NI was not genotoxic in this assay.

In a mammalian erythrocyte micronucleus test conducted in accordance with OECD test guideline No 474, negative results were reported in mice administered single doses of citicoline and its sodium salt of 500, 1 000 or 2 000 mg/kg body weight via intraperitoneal injection (unpublished study report by Kyowa Safety Research Laboratories (1993)).

Citicoline and its sodium salt showed no evidence of genotoxicity in any of these tests.

The Panel concludes that there are no safety concerns related to genotoxicity.

8.4. Acute Toxicity Studies

For oral administration, the LD_{50} has been established to be 27.14 g/kg bw for mice and 18.5 g/kg bw for rats (Kanabayashi et al., 1980).

A study conducted with 99.9 % pure citicoline base, according to GLP and OECD test guideline No 423, showed no adverse effects following a single gavage dose of 2 g/kg bw to groups of 10 male and female Crl:CD BR Sprague Dawley rats[11] (Schauss et al., 2009).

8.5. Subchronic/Chronic Toxicity Studies

A 90-day study on citicoline was performed in compliance with GLP, and claimed to be conducted according to OECD test guideline No 408 (Schauss et al., 2004; Schauss et al., 2009). Groups of 20 Crl:CD:BR Sprague Dawley rats of each sex were given 0, 100, 350 or 1 000 mg/kg bw per day citicoline by gavage. The test substance was stated to be 99.8 % pure and was dissolved in distilled water prior to administration. Rats were caged in pairs during the study and were allowed *ad libitum* access to feed and drinking water. The clinical condition of all animals was checked twice daily and each was given a detailed examination once each week. Body weight, feed and water intake were monitored throughout the study. Ophthalmoscopy was performed on the animals from the control group and the high-dose group prior to treatment, and on the same animals during the last three weeks of the study. Blood and serum analyses were performed on samples obtained from all animals immediately prior to necropsy. Urine was examined after a single collection from 10 rats of each sex per group, during the last week of treatment. At necropsy, a gross examination was made and a range of organs weighed (liver, heart, kidneys, spleen, brain, testes, epididymides, uterus, ovaries, thymus, adrenals) with a wide range of tissues being preserved for histological examination. Slides were prepared and examined for all tissues from the control and high dose groups; examination of kidneys was extended to all groups following the initial examination.

No effects of treatment were reported on mortality or general condition, appearance, body weight, food and water intake, ophthalmology, gross necropsy, or organ weights. A significant, but slight, increase in serum creatinine levels was reported in mid- and high-dose males compared to the control group, whereas no significant effects on serum creatinine levels were observed in females. All male dose groups had a significantly lower urine volume in the urinalysis study, compared to controls. The specific gravity was similar to that of controls. Brownish discoloration of the urine occurred more frequently in all male dose groups but was not individually correlated with urine volume or specific gravity, thus the cause is unknown. The urinalysis did not include any examination of sediment. Similar differences in urinalysis parameters were not found in females administered citicoline compared to their respective controls. A small but significant increase in total white blood cell (WBC) and absolute lymphocyte counts was reported in high-dose females compared to the control, while comparable differences were not found in males.

A dose-related increase in the incidence and severity of renal tubular mineralisation was seen in all female groups, while a similar finding was only observed in two male rats in the high-dose group. Mineralisation in female rats is a common finding that is influenced by the ratio of Ca:P in the diet.

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[11] Study code PCDL-0305

The study authors concluded that the excess of renal mineralisation in treated female rats was likely to be due to the increased dietary intake of phosphorus (citicoline has a 12.69 % phosphorus content).

Upon request from the Panel with respect to the absence of use of a functional observational battery, despite the potential neurological effects of the test substance, the applicant answered that careful clinical examination of the rats was carried out once before the first exposure and weekly thereafter for physical signs of toxicity, including changes in general state, external appearance and behaviour. The Panel notes that such assessment is incomplete and non-compliant with OECD test guideline No 408.

The Panel notes that the limitations of the study (absence of a functional observational battery; histopathology reports that do not detail all tissues examined for each animal; no urine sediment assessment; failure to compensate for the obvious P:Ca imbalance) and unexplained other results (changed urine colour and decreased urine volume in treated males) leave some uncertainty, and that the data are not sufficient to provide a suitable NOAEL for safety assessment.

A published study (Romero et al., 1983b) describes the effects of administration of citicoline to six Beagle dogs by gavage for six months at a dose of 1.5 g/kg bw per day; two additional dogs served as a control group and received the vehicle. The animals were weighed weekly, and blood and urine samples were collected from all animals prior to the start of treatment and at the end of the experimental period. Clinical chemistry and haematology parameters examined were haematocrit, haemoglobin, red blood cell, WBC, leukocyte formula, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), BUN, chloride, protein, globulin, lipid, cholesterol, and bilirubin levels. Urine samples were collected at the same time-points and urobilinogen, blood, bilirubin, ketone, and glucose levels were measured semi-quantitatively, as well as urinary pH, protein content, and density. At the end of the experimental period all animals were subject to necropsy and a gross examination of tissues was made. Liver, kidney, heart, spleen, lung, ovary or testes were weighed, and samples of these plus the mesenteric lymphatic ganglia were preserved for histological examination. Statistical analyses were not conducted by the authors due to the limited number of animals in each group. No deaths were recorded during the experimental period.

No differences were observed in the haematology/clinical chemistry results or in relative or absolute organ weights of animals administered citicoline compared with those in the control group, but the small number of animals and variability in the data makes comparisons very difficult. No histological evidence of citicoline toxicity was found in any of the organs examined. Some individual histological abnormalities were observed, which are considered to be incidental to treatment; these included but were not limited to hepatic granulomas and inflammatory infiltration, renal cortical granulomas and evidence of septic nephritis, myocardial necrosis and pulmonary granulomatous foci. Based on the reported findings, the authors concluded that citicoline was not toxic to dogs under the conditions of the study.

The Panel notes that the full study report is not available for a full assessment. The incidental pathology suggests that the dogs used in the study were not as healthy as might be expected for experimental animals. The Panel considers that the study is not adequate for establishing a NOAEL for consumer safety assessment.

8.6. Developmental and Reproductive Toxicity Studies

No published studies examining the possible reproductive toxicity of citicoline were identified by the applicant. Secades and Frontera (1995) cited unpublished data pertaining to an assessment of the potential teratogenic effects of citicoline. In this assessment, albino rabbits (strain and number of animals not reported) were administered 0 (control) or 800 mg citicoline/kg bw per day (route of administration not specified) on Gestation Days 7 to 18. The animals were then killed on gestation day 29, and the fetuses were removed for examination. No signs of maternal or foetal toxicity were reported. Approximately 10 % of the fetuses exposed to citicoline were reported to display a slight delay in cranial osteogenesis. This difference was not considered by the authors to represent an

adverse effect of citicoline on organogenesis since such delays in ossification can occur through dietary imbalances such as altered Ca:P ratio.

The applicant indicates that there have been no adverse effects on male and female reproductive organs reported in repeated-dose oral toxicity studies conducted in rats and dogs (Section 8.5).

The Panel considers that the subchronic and developmental studies provide some evidence on the safety of citicoline, but that on their own are not sufficient to assess the safety of the NFI.

8.7. Human Studies

8.7.1. Clinical Studies on Citicoline

Two studies reported in three publications used the NFI produced by the applicant (Silveri et al., 2008; Killgore et al., 2010; McGlade et al., 2012), and no adverse events were either reported or addressed.

The applicant identified human studies investigating the effects of other sources of citicoline. Upon request of the Panel regarding the approach taken for selecting the human studies relevant to the safety of citicoline, the applicant clarified that published scientific literature was systematically searched, and studies were included if citicoline was orally administered, safety-related parameters were assessed, and the study population was either healthy subjects, or elderly subjects who have suffered an acute ischemic stroke or have mild-to-moderate cognitive impairments.

These studies were conducted in healthy subjects (Dinsdale et al., 1983a; Spiers et al., 1996; Silveri et al., 2008; Killgore et al., 2010; McGlade et al., 2012), individuals with acute ischemic stroke (Clark et al., 1997; Clark et al., 1999; Warach et al., 2000; Clark et al., 2001; Davalos et al., 2012) or cognitive impairments (Alvarez et al., 1997; Alvarez et al., 1999; Cotroneo et al., 2013).

In a randomised, double-blind, placebo controlled, crossover study by Spiers et al. (1996), 0 (placebo) or 2 000 mg/day citicoline was given for two months to 32 healthy subjects (mean age of 73.1 years) with "relatively inefficient memory". Reported adverse events included insomnia, stomach distress, headache, rash, and cardiac abnormalities but were not related to the administration of citicoline. Overall incidence of reported adverse effects was higher in the placebo group, although statistical significance was not presented.

In a tolerance study by Dinsdale et al. (1983a), oral doses of 600 or 1 000 mg/day citicoline or placebo were administered to 12 healthy volunteers for five days. All subjects received the three tested regimens. Transient headaches were reported by four and five subjects, respectively, in the lower and higher dose regimens, and by one subject in the placebo regimen. Subjects did not show any side effects in terms of haematological or clinical analysis. No clinically significant ECG and EEG abnormalities were observed. Neurological tests, tendon reflexes, mean systemic blood pressure and heart rate were not affected by any dose of citicoline or placebo.

In a cross-over RCT in subjects with memory deficits (n = 8 per group), citicoline in doses of up to 1 000 mg/day or a placebo were provided for four weeks. A significant decrease in systolic blood pressure scores, but not diastolic blood pressure, was found during the treatment period in the whole sample (systolic blood pressure (mean ± SD) in placebo: day 1: 139.6 ± 22.2 - day 28: 141.1 ± 23.7 vs. treatment groups: day 1: 144.0 ± 21.8 - day 28: 133.0 ± 15.3, p < 0.02), and the same tendency was observed in all treatment subgroups; a slight reduction in lymphocyte cell counting was also reported in the groups which received treatment (Alvarez et al., 1997). In a subsequent RCT in 30 subjects with mild to moderate dementia, who received 1 000 mg citicoline or a placebo for 12 weeks, no difference in the tolerability of citicoline compared to placebo, as measured by physical examination, vital signs, haematology and biochemistry tests, ECG and recording of adverse events, were observed (Alvarez et al., 1999).

A meta-analysis investigating the safety and efficacy of citicoline as an adjunctive treatment for acute ischemic stroke was identified by the applicant (Davalos et al., 2002). The following inclusion criteria were used for study selection: 1) placebo-controlled, double-blind, randomised clinical trials with an accurate randomisation process carried out with oral citicoline in acute stroke; 2) > 10 patients in every group; 3) a minimum treatment period of six weeks; 4) efficacy endpoints measured three months after the initiation of treatment using the Barthel index, the National Institutes of Health Stroke Scale (NIHSS), magnetic resonance spectroscopy assessment tools; 5) use of good clinical practices. The authors identified four placebo-controlled, double-blind, randomised, human studies (Clark et al., 1997; Clark et al., 1999; Warach et al., 2000; Clark et al., 2001). A common core of individual patient data was extracted from each study file and pooled in a common data file. A total of 1 372 subjects (789 in the citicoline group, 583 in the placebo group) meeting all inclusion and exclusion criteria for patient selection were identified. The safety of citicoline was evaluated based on adverse events reported for each patient as well as individual patient electrocardiograms, vital signs, biochemistry, and haematology. There were no significant differences in the incidence of mortality reported between groups. Subjects in the citicoline treatment group were further stratified according to dose: 500 mg/day (n = 264), 1 000 mg/day (n = 40), and 2 000 mg/day (n = 485). The mortality rates remained similar between the 500 and 2 000 mg/day dose groups, with rates of 19.7 and 17.1 %, respectively, and the placebo group (i.e. 18.8 %). In the 1 000 mg/day dose group, the mortality rate increased significantly to 32.5 % (13 of 40 subjects); the authors attributed this to the small number of subjects in this group combined with a greater stroke severity, and did not consider this result to be related to the administration of citicoline. The frequency of overall adverse events was reported to be comparable between groups. Significant differences were reported in anxiety (citicoline, 13.7 %; placebo, 9.9 %; p = 0.036), leg oedema (citicoline, 9.7 %; placebo, 6.5 %; p = 0.032), depression (citicoline, 22.5 %, placebo, 27.4 %, p = 0.038); falling down (citicoline, 12.6 %, placebo, 18.7 %, p = 0.002), and urinary incontinence (citicoline, 10.5 %, placebo, 14.0 %, p = 0.047). The authors concluded that the overall safety profile for citicoline was similar to that of a placebo.

The Panel notes that in the meta-analysis by Davalos et al. (2002), results as regards adverse events are not presented in consideration of the citicoline dosage. When looking at the individual studies included in this meta-analysis, the study by Clark et al. (1997) reported the highest incidence of dizziness and accidental injuries in the group receiving 2 000 mg citicoline (n = 66) compared to the groups receiving the placebo (n = 65), 500 mg (n = 62) or 1 000 mg (n = 66) citicoline for six weeks. However, in the study by Clark et al. (2001), where 2 000 mg citicoline was given for six weeks to 453 patients with acute ischemic stroke, adverse events were no more frequently seen in this group than with the placebo group (n = 446). Furthermore, although Warach et al. (2000) reported a greater incidence of oedema of extremities and back pain in subjects treated with 500 mg citicoline (n = 41) for six weeks when compared to a placebo group (n = 40), none of these (or other) side effects were observed in the study by Clark et al. (1999) in which 267 patients were treated with 500 mg citicoline for six weeks in comparison to a placebo group (n = 127).

In a multi-centre, randomised, placebo-controlled, sequential trial in patients with moderate-to-severe acute ischaemic stroke, patients received citicoline or placebo within 24 hours of the onset of symptoms (1 000 mg every 12 hours intravenously during the first 3 days and orally thereafter for a total of 6 weeks) (Davalos et al., 2012). The primary outcome was recovery at 90 days. Safety endpoints included symptomatic neurological deterioration, and mortality. Also assessed were safety and tolerability on the basis of blood pressure and adverse events reported by investigators. Of the 2 298 patients, 1 148 were assigned to citicoline and 1 150 to placebo. The safety analysis was based on data for 2 288 patients: 1 140 in the citicoline group and 1 148 in the placebo group as eight subjects in the citicoline group and two subjects in the placebo group did not start the treatment. No significant differences were reported in the safety variables nor in the rate of adverse events.

In a recent open label multi-centre study, the effectiveness and safety of citicoline, given to 265 older subjects with mild vascular cognitive impairment in a dosage of 500 mg twice per day for nine months, was studied in comparison to a control group of 84 patients (Cotroneo et al., 2013). No significant adverse events were reported during the study. Occasional excitability or restlessness were

found in 5.6 % of the subjects, and digestive intolerance and headaches in 4.5 % and 3.6 % of the subjects, respectively, although it was not specified whether these were in untreated controls or those receiving citicoline.

8.7.2. Epidemiological Studies on Citicoline

Lozano (1983) conducted a drug surveillance study in 2 817 subjects, the majority aged between 51 and 90 years, to assess the efficacy and safety of citicoline. The subjects had been previously diagnosed with various conditions, including senility, chronic cerebral vascular insufficiencies, cerebral vascular accident sequelae and sequelae of cerebral transmission. Each subject was given a citicoline solution orally that provided approximately 600 mg citicoline/day for up to 60 days; however, 169 and 308 subjects could only be followed for 15 and 30 days, respectively. The author noted that no subjects discontinued treatment as a result of experienced side effects. A total of 151 side effects were reported by approximately 5 % of the patient population. Stomach pain and diarrhoea represented the most common side effects, accounting for 102 of the reports. Vascular symptoms of hypotension, tachycardia, and bradycardia represented a further 16 cases, while the remainder consisted of an assortment of side effects. None of the side effects were classified as serious and related to the administration of citicoline.

Cho and Kim (2009) conducted a prospective, monitored, drug surveillance study to examine the safety and efficacy of citicoline for the treatment of acute ischemic stroke. A total of 4 191 patients with a diagnosis of acute ischemic stroke were provided with 500 to 4 000 mg/day citicoline within 24 hours of acute ischemic stroke (n = 3 736) or 24 hours after acute ischemic stroke (n = 455) for a period of 6 weeks. A total of 37 adverse reactions were reported in 31 subjects (0.73 %), with the most frequent findings being nervous system-related symptoms (21 %) and GI symptoms (13.5 %). According to the authors, all adverse events were unrelated to citicoline treatment, with the exception of a case of elevated serum liver enzymes (dose not specified) which returned to normal levels four days after cessation of citicoline. Whether the coincidence of this adverse event with the treatment was causal or incidental has not been clarified. The authors noted that the prevalence of adverse reactions was not related to the dose, however the number of subjects in each dose group was not reported.

The Panel notes that both the studies by Lozano (1983) and by Cho and Kim (2009) did not include a comparative evaluation of a control group; thus no conclusion can be drawn on whether, or the extent to which, the observed adverse events were causally related to the citicoline treatment. Given the age and pre-existing clinical conditions of the subjects, as well as the nature of the reported side effects, these may have been unrelated to citicoline intake.

8.7.3. Conclusions on human studies

The Panel notes that limited information is available from the papers on the methods and protocols used to assess safety endpoints. The Panel notes that numerous human studies providing up to 2 000 mg per day citicoline for up to 12 weeks or up to doses of 1 000 mg for nine months to both healthy subjects and patients were conducted from 1997 onwards. In none of these studies were adverse events reported by the subjects attributed to citicoline intake, and it was concluded that citicoline is well tolerated. Overall, the adverse events noted are heterogeneous as to their nature within and between respective studies, and also inconsistent as to the results. Therefore, and especially as such adverse events have not been confirmed in other studies using similar dosages of citicoline, it seems unlikely that the reported adverse events were related to the citicoline treatment.

A Member State noted that the possibility of reversible phenomenon of agitation is mentioned as a potential secondary effect of the pharmaceutical form of citicoline. The applicant replied that "agitation" is not reported in any of the human studies conducted with citicoline. A Member State requested additional information regarding the interaction of citicoline with the dopaminergic system. The applicant replied that while citicoline has been shown to increase the release of dopamine in the striatum of rats (Agut et al., 2000) and the retina of rabbit (Rejdak et al., 2002), as well as increase

dopamine receptor densities in aging mice (Gimenez et al., 1991), the available clinical data do not indicate any adverse effects of citicoline on the human dopaminergic system.

The Panel notes that the summary of product characteristics for the Spanish pharmaceutical product citicoline (Somazina, Ferrer Internacional, S.A.) indicates that citicoline may enhance the effects of L-Dopa and must not be administered together with medicines that contain L-Dopa without medical consultation. Furthermore, citicoline must not be administered together with medicines that contain meclophenoxate, which is a cerebral stimulant medicine, or by patients suffering from hypertonia of the parasympathetic nervous system. The Panel considers that an interaction with these medicines cannot be excluded.

The Panel considers that available human studies on citicoline do not raise safety concerns under the proposed conditions of use.

9.   Allergenicity

The Panel notes that no information concerning potential food allergenic properties of the NFI was provided. Given the nature of citicoline, the Panel considers it unlikely that the NFI will induce food allergic reactions in the population.

DISCUSSION

Adequate information has been provided on the purity, specification, stability and production process of the novel ingredient, as well as on the potential presence of residues of the manufacturing process in the final product. The Panel has no concerns regarding these aspects of the novel ingredient.

Citicoline is a highly purified substance which is readily hydrolysed upon ingestion, breaking down to choline and cytidine which are normal body constituents, and which then undergo further metabolism and incorporation into normal pathways of metabolism.

The available human studies on citicoline do not raise safety concerns under the proposed conditions of use.

Due to the nature of the product, further safety data are not considered necessary, and thus the additional data presented by the applicant on safety in laboratory animals, although incomplete by modern standards, provides further reassurance on the safety of the NFI.

CONCLUSIONS

The Panel concludes that the novel food ingredient, citicoline, is safe under the proposed uses and use levels.

DOCUMENTATION PROVIDED TO EFSA

1.   Dossier "Application for the Approval of Citicoline for Use in Foods for the General Population and Food Supplements Under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 Concerning Novel Foods and Novel Food Ingredients" received on 15 January 2013. Submitted by Kyowa Hakko Europe GmbH on 29 March 2012. Additional data were provided on 04 July 2013.

2.   Letter from the European Commission to the European Food Safety Authority with the request for an opinion on the safety of 'Citicoline'. SANCO/E6/AK/ks D(2013) 37735, dated 15 January 2013.

3.   Initial assessment report carried out by the Food Safety Authority of Ireland: 'Safety Assessment of Citicoline'.

4. Member States' comments and objections.

5. Response by the applicant to the initial assessment report and the Member States' comments and objections.

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ABBREVIATIONS

ALT	alanine aminotransferase
AST	aspartate aminotransferase
BUN	blood urea nitrogen
cGMP	current good manufacturing practices
CDP	cytidine diphosphate
CFU	colony forming unit
CTP	cytidine triphosphate
DAG	diacylglycerol
DNA	deoxyribonucleic acid
ECG	electrocardiography
GLP	good laboratory practices
GM	genetic modification
GC-MS	gas chromatography - mass spectrometry
HPLC	high performance liquid chromatography
IOM	Institute of Medicine
JPC	Japanese Pharmaceutical Codex
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
NF(I)	Novel Food (Ingredient)
NIHSS	National Institutes of Health Stroke Scale
NOAEL	no observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
PARNUTS	Foods for Particular Nutritional Use
PC	phosphatidylcholine
PCR	polymerase chain reaction
RDA	reference daily allowance
RNA	ribonucleic acid
SCF	Scientific Committee on Food
SD	standard deviation
UL	Tolerable Upper Limit
UDP	uridine diphosphate choline
UTP	uridine 5'-triphosphate
UV	ultraviolet
WBC	white blood cell