SCIENTIFIC OPINION

Safety of synthetic L-ergothioneine (Ergoneine®) as a novel food pursuant to Regulation (EC) No 258/97

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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 synthetic L-ergothioneine, marketed as Ergoneine®, as a novel food submitted pursuant to Regulation (EC) No 258/97 of the European Parliament and of the Council. The novel food, synthetic L-ergothioneine, is produced by a one-pot patented manufacturing process. Chemically, L-ergothioneine is a derivative of thiolhistidine, and it is naturally present in a number of foodstuffs such as mushrooms, some varieties of black and red beans, offal and cereals. The production process for the novel food is sufficiently described and does not raise concerns about the safety of the novel food. The information on the composition, specifications, batch-to-batch variability and stability of the novel food is sufficient and does not raise safety concerns. The applicant intends to use the novel food in quantities of up to 5 mg per serving in alcohol-free beverages, cereal bars, milk, fresh dairy products and chocolate. The applicant also proposes to provide the novel food as a food supplement, with a daily dose of up to 30 mg/day for adults and 20 mg/day for children. The target population is children above 3 years of age and the general adult population, except pregnant and breastfeeding women. Considering the NOAEL of 800 mg/kg bw per day, which was based on two subchronic toxicity studies in rats, and the maximum estimated intake levels for L-ergothioneine from all sources, the Panel concludes that the margins of safety of 470 for adults (except pregnant and breastfeeding women) and of 216 for children above 3 years of age are sufficient. The Panel concludes that the novel food, synthetic L-ergothioneine (marketed as Ergoneine®), is safe under the intended conditions of use as specified by the applicant.

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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 synthetic L-ergothioneine, marketed as Ergoneine®, as a novel food (NF) submitted pursuant to Regulation (EC) No 258/97 of the European Parliament and of the Council. The assessment follows the methodology set out in Commission Recommendation 97/618/EC of 29 July 1997. The assessment is based on the data supplied in the original application, the initial assessment by the competent authority of France, the concerns and objections of a scientific nature raised by the other Member States and the responses of the applicant.

The NF that is the subject of the application is synthetic L-ergothioneine (ET; marketed as Ergoneine®), which is produced by a one-pot patented manufacturing process. Chemically, ET is a derivative of thiolhistidine, i.e. 2-thio-L-histidine-betaine. ET is naturally present in a number of foodstuffs such as mushrooms, some varieties of black and red beans, offal and cereals.

The production process applied in order to obtain the NF, i.e. synthetic ET, is sufficiently described and does not raise concerns about the safety of the NF. The information provided on the composition, specifications, batch-to-batch variability and stability of the NF is sufficient and does not raise safety concerns.

The applicant intends to use the NF in quantities of up to 5 mg per serving in alcohol-free beverages, cereal bars, milk, fresh dairy products and chocolate. The applicant also proposes to provide the NF as a food supplement, with a recommended daily dose of up to 30 mg/day for adults and 20 mg/day for children. The target population proposed by the applicant is children above 3 years of age and the general adult population, with the exception of pregnant and breastfeeding women.

The combined intake of ET from all sources (including the background diet) is unlikely to exceed 1.7 mg/kg body weight (bw) per day for adults and 3.7 mg/kg bw per day for children. Taking into account the intended use levels, the Panel considers that the consumption of the NF is not nutritionally disadvantageous.

ET is absorbed from food in the gastrointestinal tract via a specific transporter, the organic cation transporter novel type 1 (OCTN1), also named ergothioneine transporter (ETT). Tissue concentrations are tightly regulated by this transporter, which is reflected by control of uptake in the small intestine, selective uptake into tissues and control of re-uptake in the kidney.

Based on the genotoxicity tests provided, the Panel concludes that there are no concerns regarding genotoxicity of the NF. Two subchronic toxicity studies in Sprague–Dawley rats were provided, which were carried out with the NF that is the subject of the application and with another synthetic ET (purity > 99%), respectively. Based on the observations from both these studies, the Panel considers that the no observed adverse effect level (NOAEL) of the NF is 800 mg/kg bw per day.

Concerns had been raised by the Member States on the possible role of ET in relation to several health outcomes, in particular diabetes mellitus and inflammatory diseases such as Crohn's disease (CD) and rheumatoid arthritis (RA). The Panel considers that from the available human studies no relationships can be inferred with regard to dietary or supplemental ET and the susceptibility to or development of diabetes mellitus, CD or RA.

With regard to allergenicity, the Panel considers that the likelihood of adverse reactions to the NF is low.

Considering the NOAEL of 800 mg/kg bw per day, and the maximum estimated intake levels of ET from all sources (i.e. fortified foods, food supplements, background diet) of 1.7 mg/kg bw per day for adults and of 3.7 mg/kg bw per day for children, the Panel concludes that the margins of safety of 470 for adults (except pregnant and breastfeeding women) and of 216 for children above 3 years of age are sufficient.

The Panel concludes that the NF, synthetic ET (marketed as Ergoneine®), is safe under the intended conditions of use as specified by the applicant.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

On 25 July 2013, the company Tetrahedron submitted a request under Article 4 of the Novel Food Regulation (EC) No 258/97[1] to place on the market synthetic ʟ-ergothioneine (ET) as a novel food.

On 19 February 2015, the competent authority of France forwarded to the Commission its initial assessment report, which came to the conclusion that synthetic ET meets the criteria for acceptance of a novel food defined in Article 3(1) of Regulation (EC) No 258/97.

On 9 March 2015, 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 (MS) can be summarised as follows:

- The maximum amounts indicated in the specifications for the solvent residues methanol, ethyl acetate, isopropanol and ethanol comply with the European Pharmacopoeia. However, the specifications should be derived from the actual concentrations of the solvents in the product in question, which are considerably lower than the parameters as stated in the specifications.
- One MS considered that ET, when used as a food ingredient, might be a possible contributing factor to inflammatory diseases. In particular in view of the fact that some European countries have seen an increase in the incidence of inflammatory bowel diseases (IBDs) (Vind et al., 2006).
- Some MS criticised the combined subchronic/reproductive and developmental toxicity study, in particular the fact that only five male and five female animals (instead of 10 of each) were tested in the haematological, clinical-chemical and urine analyses, which may have reduced the likelihood of identifying toxicologically relevant effects.
- More information was requested on the pharmacokinetics (absorption distribution metabolism and excretion (ADME)) of the NF, in particular on the mechanism leading to the plateau level in the body.
- More information was requested on the nutritional safety of the novel food in order to assess whether the novel ingredient creates a nutritional disadvantage, in particular as regards the effects of the novel food on the bioavailability of various nutrients owing to its capacity to form complexes with divalent metal cations. It was commented that neither short- nor long-term effects of the NF have been assessed in humans.
- One MS commented that foodstuffs enriched with ET should not be consumed by babies, infants or children, as metabolic side-effects from using the product cannot be ruled out, yet. More information and experience from post-marketing monitoring is needed in respect of these vulnerable target groups.
- It is unclear whether the testing facilities, which were involved in the chemical and microbiological analyses of the batches, were accredited to carry out the analysis according to an internationally recognised system.

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[2], the European Food Safety Authority (EFSA) is asked to carry out the additional assessment for synthetic ET as a novel food 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.

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[1] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.

[2] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

2.   Data and methodologies

2.1.   Data

The assessment of the safety of this novel food (NF) is based on data supplied in the original application, the initial assessment by the competent authority of France, the concerns and objections of a scientific nature of the other MS and the responses of the applicant.

In accordance with Commission Recommendation 97/618/EC[3], synthetic L-ergothioneine (ET) is allocated to Class 1.2, i.e. 'foods and food components that are single chemically defined substances or mixtures of these which are not obtained from plants, animals or microorganisms that have been genetically modified and whose source has no history of food use in the Community'. The data are required to comply with the information required for novel foods of Class 1.2, i.e. structured schemes I, II, III, IX, XI, XII and XIII of Commission Recommendation 97/618/EC. In the current scientific opinion, these structured schemes are listed in Sections 3.1–3.8. The intention is to add the NF to alcohol-free beverages, cereal bars, milk, dairy products, chocolate, and to market the NF as a food supplement. This assessment concerns only the risk that might be associated with consumption of the NF under the proposed conditions of use, and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

2.2.   Methodologies

The assessment follows the methodology set out in Commission Recommendation 97/618/EC 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.

3.   Assessment

3.1.   Specification of the NF

The NF which is the subject of the application is synthetic L(+)-ergothioneine (marketed as Ergoneine®).

ET is a natural compound which was first isolated in 1909 from rye ergot (*Claviceps purpurea*). It is present in a number of foodstuffs such as mushrooms, some varieties of black and red beans (*Phaseolus vulgaris*), offal and cereals.

Chemically, ET (CAS No: 497-30-3; formula: $C_9H_{15}N_3O_2S$) is a derivative of thiolhistidine, 2-thio-L-histidine-betaine, with a molecular mass of 229.3 Da. Its International Union for Pure and Applied Chemistry (IUPAC) name is (2S)-3-(2-thioxo-2,3-dihydro-1*H*-imidazol-4-yl)-2-(trimethylammonio)-propanoate. The chemical structure of ET is indicated in Figure 1.

Figure 1: Chemical structure of L(+)-ergothioneine

The specifications for the NF are indicated in Table 1. They include physical and chemical parameters as well as specifications for residual solvents, contaminants such as heavy metals, microbiological specifications and impurities.

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[3] 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.
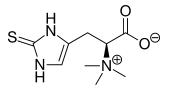
Table 1: Specifications of the NF

Parameter	Specification	Method
Appearance	White powder	Visual
Optical rotation	$[\alpha]_D \geq (+)\ 122°$ (c = 1, H_2O)[a]	Polarimetry
Chemical purity	$\geq 99.5\%$	HPLC [Eur. Ph. 2.2.29]
	$\geq 99\%$	1H-NMR
Identification	Compliant with the structure C: 47.14 ± 0.4% H: 6.59 ± 0.4% N: 18.32 ± 0.4% Elemental analysis	1H-NMR
Total residual solvents (methanol, ethyl acetate, isopropanol, ethanol)	[Eur. Ph. 01/2008:50400] < 1,000 ppm	Gas chromatography [Eur. Ph. 01/2008:20424]
Loss on drying	Internal standard < 0.5%	[Eur. Ph. 01/2008:20232]
Impurities	< 0.8%	HPLC/GPC or 1H-NMR
Heavy metals[b]		
Lead	< 3 ppm	ICP/AES (Pb, Cd)
Cadmium	< 1 ppm	
Mercury	< 0.1 ppm	Atomic fluorescence (Hg) [Reg. No. 1881/2006/EC][c]
Microbiological specifications[b]		
Total viable aerobic count (TVAC)	$\leq 1 \times 10^3$ CFU/g	[Eur. Ph. 01/2011:50104]
Total yeast and mould count (TYMC)	$\leq 1 \times 10^2$ CFU/g	
Escherichia coli	Absent in 1 g	

Eur. Ph.: European Pharmacopoeia; 1H-NMR: proton nuclear magnetic resonance; HPLC: high-performance liquid chromatography; GPC: gel permeation chromatography; ICP/AES: Inductively coupled plasma atomic emission spectroscopy; CFU: colony-forming units.
(a): Lit. $[\alpha]_D = (+)\ 126.6°$ (c = 1, H_2O).
(b): Analyses conducted on each batch.
(c): Amended by Reg. No. 629/2008/EC.

In order to confirm that the manufacturing process is reproducible and adequate to produce a product that is within the specifications as set above, the applicant provided batch-to-batch analyses (Table 2) of four batches produced on different scales. Batch Nos. 12779 and 12799 were obtained by pooling and blending 80 g batches manufactured by Tetrahedron. Batch No. 2299 was a pilot batch manufactured by a provider on an industrial scale in June 2011 and batch No. 2319 was a good manufacturing practice (GMP) industrial scale batch manufactured by a provider in December 2012.

Table 2: Batch-to-batch analyses of the NF

Parameter	Specification	Batch results			
		No. 12779	No. 12799	No. 2299	No. 2319
Appearance	White powder	Compliant	Compliant	Compliant	Compliant
Optical rotation	$[\alpha]_D \geq (+)\ 122°$ (c = 1, H_2O)	+ 122.2	+ 123.5	+ 124.4	+ 126.0
Chemical purity	HPLC $\geq 99.5\%$	99.9%	99.9%	99.6%	99.9%
	1H-NMR $\geq 99\%$	$\geq 99\%$	$\geq 99\%$	$\geq 99\%$	$\geq 99\%$
	Impurities < 0.8%	Compliant	Compliant	Compliant	Compliant
Identification	1H-NMR	Compliant	Compliant	Compliant	Compliant
	Elemental analysis				
	C: 47.14 ± 0.4%	47.00	47.20	47.24	46.99
	H: 6.59 ± 0.4%	6.55	6.39	6.50	6.46
	N: 18.32 ± 0.4%	18.20	18.52	18.32	18.20
Total residual solvents	< 1,000 ppm	Compliant	Compliant	Compliant	Compliant
Loss on drying	< 0.5%	–	–	0.1%	0.1%

Parameter	Specification	Batch results			
		No. 12779	No. 12799	No. 2299	No. 2319
Heavy metals					
Lead	< 3 ppm	Compliant	Compliant	Compliant	Compliant
Cadmium	< 1 ppm	Compliant	Compliant	Compliant	Compliant
Mercury	< 0.1 ppm	Compliant	Compliant	Compliant	Compliant
Microbiological specifications					
Total viable aerobic count (TVAC)	$\leq 1 \times 10^3$ CFU/g	≤ 10 CFU/g	≤ 10 CFU/g	–	≤ 10 CFU/g
Total yeast and mould count (TYMC)	$\leq 1 \times 10^2$ CFU/g	≤ 10 CFU/g	≤ 10 CFU/g	–	≤ 10 CFU/g
Escherichia coli	Absent in 1 g	Compliant	Compliant	–	Compliant

HPLC: high-performance liquid chromatography; 1H-NMR: proton nuclear magnetic resonance; CFU: colony-forming units.

The Panel considers that the information provided on the composition, the specifications and the batch-to-batch variability of the NF is sufficient and does not raise safety concerns.

3.1.1. Stability of the NF

The applicant provided the results of a stability study performed with three batches under intermediate testing conditions (30 ± 2°C, 65 ± 5% relative humidity (RH)) up to 12 months and accelerated testing conditions (40 ± 2°C, 75 ± 5% RH) up to 6 months. The analyses submitted indicate that the NF remains within specifications and is expected to remain stable under normal storage conditions for at least 1 year. The recommended storage temperature is 2–8°C.

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

3.2. Effect of the production process applied to the NF

The synthesis of the NF is a one-pot reaction and is based on a patent (FR0956962), which is described in the publication by Erdelmeier et al. (2012). The production process complies with the standards for GMP. Comprehensive information on the manufacturing process (confidential) was provided by the applicant.

The synthesis of ET is performed in water and includes the following steps. First, L-hercynine is reacted with bromine and then with cysteine. The intermediate obtained is transformed into ET by heating in the presence of mercaptopropionic acid. Finally, the raw product is purified by crystallisation.

The Panel considers that the production process is sufficiently described and does not raise concerns about the safety of the NF.

3.3. History of the organism used as the source of the NF

The NF which is the subject of the application is synthetically produced ET and it is thus not obtained from a biological source. Therefore, this section is not applicable for the NF under evaluation.

3.4. Anticipated intake/extent of use of the NF

The applicant intends to use the NF in quantities of up to 5 mg per serving in the following food groups: alcohol-free beverages 25 mg/kg (200 mL serving); cereal bars 200 mg/kg (25 g serving); milk 25 mg/kg (200 mL serving); fresh dairy products, such as cream, cream cheese and yoghurt 40 mg/kg (125 g serving); chocolate 250 mg/kg (20 g serving). These values represent the maximum use levels in the specified food groups.

The applicant also proposes to provide the NF as a food supplement, with a recommended daily dose of up to 30 mg/day for adults and 20 mg/day for children.

The target population proposed by the applicant is children above 3 years of age and the general adult population, with the exception of pregnant and breastfeeding women.

3.4.1. Intake from fortified foods

The applicant provided intake estimates for the French population (for adults and children) based on the INCA 2 consumption survey (AFSSA, 2009) as well as estimates for the European population (for adults) using the EFSA Concise Food Consumption Database.

When using summary statistics from the EFSA Concise Food Consumption Database, the estimated mean intakes (for adults) from fortified foods ranged from 0.23 (Sweden) to 1.26 (Iceland) mg/kg body weight (bw) per day, and the high (95th percentile) intakes ranged from 0.43 (Sweden) to 3.31 (Iceland) mg/kg bw per day. The Panel notes that in these intake estimates the food group 'cereals' was the greatest contributing food group, and that cereals as such are not proposed to be fortified but only 'cereal bars', which might have resulted in a gross overestimation of intake.

The applicant also submitted an intake estimate based on data from the INCA 2 consumption survey from France (AFSSA, 2009). In order to estimate high level intakes, the applicant considered the 95th percentile of consumption of the two main food categories (adults: alcohol-free beverages and milk; children: alcohol-free beverages and chocolate) for the population of consumers, plus the mean consumption of the other food categories considering the total population. This intake estimate resulted in mean and high level intakes of 0.204 and 0.545 mg/kg bw per day, respectively, for adults, and 0.600 and 1.179 mg/kg bw per day, respectively, for children (3–17 years).

3.4.2. Intake from the background diet

The main dietary sources of ET are mushrooms, certain varieties of black and red beans, offal and cereals (Dubost et al., 2007a; Dubost et al., 2007b; Ey et al., 2007). The foods with the highest ET content are mushrooms, and in particular *Boletus edulis* (528 mg ET/kg wet weight) and *Pleurotus ostreatus* (119 mg ET/kg wet weight) (Ey et al., 2007), which are widely consumed in Europe. Mushrooms account for about 95% of ET intake via the diet. For this reason (and owing to the lack of detailed intake data for mushrooms throughout Europe), the intake estimate provided by the applicant was mostly based on data from an EFSA evaluation on the presence of nicotine in wild mushrooms (EFSA, 2009).

The highest chronic exposure among adults was found for Italy, with a mean and high (95th percentile) consumption of 0.06 and 0.48 mg/kg bw per day, respectively, for the total population, and a mean and high consumption of 0.22 and 0.70 mg/kg bw per day, respectively, for consumers. Also for the population of children, the highest chronic exposure was found for Italy, with a mean and high consumption of 0.06 and 0.64 mg/kg bw per day, respectively, for the total population, and a mean and high consumption of 0.41 and 1.110 mg/kg bw per day, respectively, for consumers.

These values are in line with a recent publication by Ramirez-Martinez et al. (2016) who assessed the intake of ET in a number of European countries (adults: Belgium, Finland, France, Ireland, Italy; children: Belgium and Italy). According to this study, the mean chronic dietary intake of ET in the investigated countries ranges from 0.051 to 0.255 mg/kg bw per day for adults (for consumers), and from 0.306 to 0.409 mg/kg bw per day for children (for consumers). The high (95th percentile) intake of ET in consumers ranges from 0.203 (FR) to 0.660 (IT) mg/kg bw per day for adults, and from 1.017 (BE) to 1.110 (IT) mg/kg bw per day for children.

3.4.3. Combined intake from all sources

Considering all sources, in adults, the combined intake of high consumption of the NF from fortified foods (0.545 mg/kg bw per day) plus high consumption of natural ET from the background diet (0.70 mg/kg bw per day) plus intake via food supplements (0.43 mg/kg bw per day (i.e. 30 mg/70 kg)) results in a maximum ET intake of 1.68 mg/kg bw per day.

In children, the combined intake of high consumption of the NF from fortified foods (1.179 mg/kg bw per day) plus high consumption of natural ET from the background diet (1.110 mg/kg bw per day) plus intake via food supplements (1.429 mg/kg bw per day (20 mg/14 kg – 5th percentile bw for the age group of children from 3 to 10 years (EFSA Scientific Committee, 2012))) amounts to a maximal ET intake of 3.72 mg/kg bw per day.

The Panel notes that the above values represent conservative estimates, which likely overestimate the actually expected intakes of ET from all sources.

3.5. Nutritional information on the NF

Concerns were raised by some MS in particular as regards potential effects of the NF on the bioavailability of various nutrients owing to the capacity of ET to form complexes with divalent metal cations.

The applicant was requested to provide more information/studies on the chelating ability of the NF, and to which extent and in which direction putatively formed chelates might influence absorption and bioavailability of nutrients. In reply, the applicant acknowledged the ability of ET to form complexes *in vitro* with divalent metal cations, such as Zn^{2+} and Cu^{2+}, respectively, and that this ability was found comparable to that of glycine and histamine, a compound structurally similar to ergothioneine (Hanlon, 1971). However, this chelating ability was not associated with an inhibition of zinc or copper enzymes from mammals (Hanlon, 1971). The applicant also informed that no data were available regarding the significance of metal chelates of ET and the relevance of their effects *in vivo* that could in particular influence absorption and bioavailability of these metals or other nutrients. Finally, the applicant pointed out that, with regard to zinc, in the submitted reproductive toxicity study (CiToxLAB France, 2013b; Forster et al., 2015) no significant effect was observed on mating, reproductive performance, fertility and offspring development, biological parameters that could be altered by zinc deficiency.

Taking into account the intended use levels, the Panel considers that the consumption of the NF is not nutritionally disadvantageous.

3.6. Microbiological information on the NF

The applicant indicated that the risk of microbiological contamination of the NF during production or storage is low. Nonetheless, regular controls are carried out to verify the microbiological quality of the NF in compliance with the specifications (see Section 3.1) and in compliance with the standards of the European Pharmacopoeia (Eur. Ph. 01/2011:50104). The analytical results of such testing were provided for three batches (Table 2), which showed compliance with the specifications.

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

3.7. Toxicological information on the NF

3.7.1. Absorption, distribution, metabolism and excretion (ADME)

3.7.1.1. Absorption

ET is absorbed from food in the gastrointestinal tract via a specific transporter, the organic cation transporter novel type 1 (OCTN1) (Gründemann et al., 2005; Kato et al., 2010). Due to its specificity for ET, it is also called ʟ-ergothioneine transporter (i.e. ETT).

After a single oral administration of 330 μg ³H-ET/kg bw to mice, levels of radioactivity in plasma rapidly increased and then stayed constant for about 10 days. Levels of radioactivity in whole blood increased more slowly and then stayed constant as well (Kato et al., 2010).

In the 90-day feeding study with Sprague–Dawley rats described under 3.7.4 (CiToxLAB France, 2012; Forster et al., 2015), plasma concentrations were measured. Samples from control animals were below the limit of quantification for ET (1,000 ng/mL). Plasma levels increased with dose level, but were not proportional to the administered doses, suggesting saturation of uptake mechanisms of the absorbed ET. Mean plasma concentrations in weeks 12/13 were lower than those in week 2.

In the subchronic study by Marone et al. (2016, see Section 3.7.4) in Sprague–Dawley rats with administration via gavage, plasma values of controls were 1,602 ng/mL for males and 2,484 ng/mL for females, i.e. higher than in the study by CiToxLAB (2012). There was also a different pattern concerning dose; after 90 days, there was a proportional increase in plasma levels of ET with dose and the levels were significantly higher than baseline levels of ET. Compared with the study by CiToxLAB (2012), ET levels were somewhat higher (6,090 and 7,997 ng/mL for males and females dosed 400 mg/kg bw per day compared to approximately 5,500 and 3,500 ng/mL for males and females dosed 615 and 725 mg/kg bw per day, respectively).

In a human study (Toh et al., 2014) in 10 healthy adult Chinese men, three meals with or without 250 g shiitake mushrooms containing 264 mg ET/kg mushrooms (198 mg ET/day) were consumed and the following day (in the morning) ET levels in plasma were measured. The median plasma concentrations were 172 ng/mL without mushroom meal and 425 ng/mL with mushroom meal.

In another human study (Weigand-Heller et al., 2012), the bioavailability of ET from mushrooms after consumption of a meal containing 0, 4.4 or 8.8 mg ET in dried mushroom powder (0, 8 and 16 g *Agaricus bisporus*) was measured in erythrocytes from 10 healthy men. ET was increased in erythrocytes within 1–6 h from 3.4 mg/100 mL (34 µg/mL) slightly by 0.1–0.2 mg/100 mL blood (1–2 µg/mL). The difference to the control was only statistically significant (p < 0.05) for 8.8 mg ET after 2 h. The difference between the two dose levels was small. The mushroom powder with 4.4 mg ET corresponded, according to the authors, to a normal mushroom serving (however without specifying the serving size).

3.7.1.2. Distribution

According to several animal studies, highest ET concentrations occur in the liver, red blood cells and kidney. Kato et al. (2010) examined the distribution of radioactivity after oral administration of ^{3}H-ET at a concentration of 330 µg/kg bw in mice. Highest concentrations of radioactivity were observed in the upper and middle parts of the small intestine at 4 h after oral administration. After 14 days, the pattern had changed and high levels of radioactivity were found in the liver > kidney > erythrocytes > intestine. Compared to plasma, much higher concentrations of radioactivity were detected in erythrocytes.

In studies with rats (Wolf et al., 1961), a similar tissue distribution was observed, i.e. 4–5 h after intraperitoneal (i.p.) injection of ^{14}C-ET (dose only provided in µCi) 20–30% of the radioactivity was detected in the liver. The majority was in the non-protein fraction. Similarly, 24 h after intravenous injection of ^{3}H-ET (dose only provided in µCi) to rats highest levels of radioactivity were found in the liver > kidney > spleen > lung > blood > testis > plasma (Mayumi et al., 1978). Ten to 19 days after oral application of ^{35}S-ET (250 mg within 21 day via drinking water) to rats, highest levels of radioactivity were found in the liver, followed by the blood cells and kidney (Heath, 1953).

The OCTN1/ETT transporter is responsible for the selective uptake of ET into different tissues. Its role has been demonstrated with OCTN1 knockout mice compared to wild-type mice (Kato et al., 2010), by analysing OCTN1 transporter mRNA levels in rats (Wu et al., 2000) and in different human tissues (Gründemann et al., 2005; Taubert et al., 2009; Gründemann, 2012). The transporter is highly specific for ET, and its amino acid sequence is well preserved between different species (Gründemann, 2012), but there are species differences in expression profiles within different tissues (Nikodemus et al., 2011; Gründemann, 2012).

The metabolic turnover rate of ET in various organs is low. Mayumi et al. (1978) found that ET concentrations in blood and in the liver increased in rats linearly during daily i.p. injection of 16 mg ET/kg bw for 1 week. In addition, ET was not or only to a minor degree eliminated from the liver or erythrocytes during a fasting period of 1 week (Mayumi et al., 1978) or 2 weeks (Kawano et al., 1982). Similarly in 10 Chinese male subjects plasma levels increased with dose and duration of ET administration (0, 5, and 25 mg/day for 7 days). The levels slowly decreased in the subsequent 4 weeks, while ET concentrations in whole blood continued to increase and plateaued at about 4 weeks (Cheah et al., 2016a).

In rats, ET concentrations in the liver increased with age with a maximum at 11 weeks (Kawano et al., 1982). Similarly ET increases with age in erythrocytes, both in rats (Mackenzie and Mackenzie, 1957) and humans (Kumosani, 2001). In female rats, it increased twofold between the first and third month of life and then remained constant, while in male rats levels increased during, and up to, 18 months (Mackenzie and Mackenzie, 1957). In a cohort of 205 men, the maximum ET level in erythrocytes was at 18 years, then it stayed constant, although somewhat higher levels were reported for the age group > 51 years (Kumosani, 2001). A decline with age (from < 65 to > 75 years), was found by Cheah et al. (2016b) in an elderly (> 55 years of age) cohort (n = 136, 68% female), both for levels in whole blood and in plasma. Accordingly, in the investigation by Sotgia et al. (2014) in a cohort of 439 subjects (age 55–85 years), plasma levels decreased with age.

The ET content in human erythrocytes determined by high-performance liquid chromatography (HPLC) ranges from about 1.5–4 mg/100 mL (corresponding to 15–40 µg/mL) (Kumosani, 2001; Kato et al., 2010; Weigand-Heller et al., 2012). There are also several earlier measurements available (summarised by Cheah and Halliwell, 2012), which are quite similar to those described above. As in the animal experiments described above (Mayumi et al., 1978; Kato et al., 2010), ET concentrations in human erythrocytes are about 100-fold higher than in plasma (Chaleckis et al., 2014; Sotgia et al., 2014; Cheah et al., 2016a). The median ET plasma concentration in the study by Sotgia et al. (2014) was 1.01 µmol/L (corresponding to 23.2 µg/100 mL or 232 ng/mL). The concentration was not affected by gender or by the presence of chronic medical conditions.

3.7.1.3. Metabolism

After i.p. injection in rats, Wolf et al. (1961) identified herzynine (*N,N,N*-trimethylhistidine) as main metabolite of ET in the liver, plasma and urine (80% in the liver) indicating a loss of the thiol group during metabolism.

In a human metabolome analysis (Chaleckis et al., 2014), besides ET, herzynine and *S*-methyl-ET were detected in plasma and in red blood cells. These metabolites were also detected in a recent human study in whole blood and plasma after 0, 5, and 25 mg ET/day for 7 days (Cheah et al., 2016a). In addition, low amounts of ET sulfonate were found by Chaleckis et al. (2014).

Servillo et al. (2015) investigated the products of ET oxidation produced by neutrophils (from six healthy volunteers) during oxidative burst and in addition, the oxidation products of the reaction of ET with hypochlorite in cell-free solutions. Based on the metabolites identified, the following reactions were proposed: first, the ET disulfide is formed. ET disulfide then hydrolyses to ET sulfenic acid and ET. ET sulfenic acid then disproportionates into ET sulfinic acid and ET. ET sulfinic acid finally decomposes into herzynine and sulfurous acid, which is further oxidised to sulfate.

3.7.1.4. Excretion

After i.p. injection to rats, within 4.5 h 51% of the ^{14}C-ET dose was excreted in the urine, 1/3 as ET, 1/3 as herzynine and 1/3 as unknown metabolites. Only 0.14% of the dose was detected as CO_2 in the breath (Wolf et al., 1961). Heath (1953) detected in addition 35% free sulfate in the urine after oral administration of ^{35}S-ET to rats. In faeces, only traces of radioactivity were found.

Kato et al. (2010) detected about 10% of the dose as ET in the urine of mice within 14 days after oral administration. According to *ex vivo* experiments with kidney cells, ET may be reabsorbed in the kidney via the OCTN1 transporter (Kato et al., 2010), thus reducing excretion of ET. Accordingly, with OCTN1 knockout mice, excretion of ET in the urine was considerably enhanced.

In a human study (Cheah et al., 2016a), during 7 days of a daily dose of 0, 5 or 25 mg ET and in 28 days post-exposure, ET, *S*-methyl-ergothioneine and herzynine were detected in the urine. Levels of ET and *S*-methyl-ergothioneine dependently increased during exposure and then gradually declined. Levels of herzynine were neither dose nor duration dependent, indicating that there are other sources of herzynine than the actual exposure to ET.

Overall, the Panel notes that tissue concentrations are tightly regulated by the ET transporter, which is reflected by control of uptake in the small intestine, selective uptake into tissues and finally control of re-uptake in the kidney. There is no proportional increase with dose or duration in several studies. The Panel notes that there is no information on the overall percentage of the dose absorbed and excreted.

3.7.2. Genotoxicity

A bacterial reverse mutation test (Vivotecnia, 2013), which was in compliance with the OECD Test Guideline (TG) 471, was provided by the applicant. This study was conducted with four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537) and one strain of *Escherichia coli* (WP2 (pKM101)) in the presence or absence of a metabolic activation system (S9 mix). The ET concentrations (batch No. 2299, > 99% purity) used in this Ames test were determined by a preliminary toxicity study conducted on *S.* Typhimurium strain TA100. Under the experimental conditions (0.06–5.0 mg/well), no significant increase in the number of mutant colonies was observed for any strain (in the presence or absence of the metabolic activation system).

A further Ames test (Schauss et al., 2010) was carried out with a commercial synthetic ET, EGT™ (obtained from Oxis International; > 99% pure as assessed by HPLC and chiral HPLC). The test (in compliance with the OECD TG 471) with the *S.* Typhimurium strains TA98, TA100, TA1535 and TA1537 as well as *E. coli* WP2 uvrA was negative with and without S9 mix.

Furthermore, another Ames test (Marone et al., 2016) carried out with another commercial synthetic ET, i.e. Mironova EGT+ (manufactured by Mironova Labs Inc., purity > 99%), performed with the same tester strains as mentioned above, was also negative with and without S9 mix.

In an *in vitro* chromosomal aberration test with V79 cells (in compliance with the OECD TG 473; Schauss et al., 2011) in the presence and absence of S9 mix no increases in the number of cells with structural chromosome aberrations were observed up to a concentration of 5,000 μg/mL ET, which was slightly cytotoxic. This test as well as the following one were performed with synthetic ET (EGT™, > 99% pure) from Oxis International.

In an *in vivo* micronucleus test (in compliance with the OECD TG 478; Schauss et al., 2011), with up to 1,500 mg/kg bw ET (i.e. EGT™) no increases in the number of micronuclei was observed 24 and 48 h after application of the test substance. The proportion of polychromatic to normochromatic erythrocytes was not decreased. As several studies showed the presence of ET in plasma and erythrocytes, it can be considered that the bone marrow is reached by the test substance.

Based on the information provided, the Panel concludes that there are no concerns regarding genotoxicity of the NF.

3.7.3. Acute toxicity studies

An acute oral toxicity study (CiToxLAB France, 2013a; Forster et al., 2015) was conducted (in compliance with the good laboratory practice (GLP) and OECD TG 423) in six nulliparous female Sprague–Dawley rats. The test substance (batch No. 2299, > 98% purity) was administered by gavage at a dose level of 2,000 mg/kg bw (the test dose was selected on the basis of the toxicological data available). During the 14-day observation period, no death occurred and the animals did not present with any clinical sign. One animal out of the six tested presented with weight gain that was less than normal over the second week of observation. In contrast, one animal presented with weight gain that was greater than normal during the same period. However, the relationship between animal body weight and the test substance was not established. Two animals presented with red discoloration of the thymus during the gross autopsy examination. These findings were not considered as related to the test substance.

Under the above experimental conditions, the median lethal dose (LD_{50}) of synthetic ET was greater than 2,000 mg/kg bw.

3.7.4. Subacute and subchronic toxicity studies

A 2-week subacute toxicity study (CiToxLAB France, 2012; Forster et al., 2015) was conducted in six male and six female rats, which were administered feed containing ET (batch No. 2299, > 99% purity) in various amounts, i.e. 0% (control), 0.3%, 0.5% or 0.9%. The plasma concentrations of ET were found to be increased in all the animals which were given ET. On the basis of the findings of this investigation, the highest administered concentration of 0.9% was chosen as a suitable maximum concentration for a study with longer exposure.

A subchronic toxicity study (CiToxLAB France, 2013b; Forster et al., 2015) was submitted that was combined with a screening test for reproductive and developmental toxicity (based on the OECD TG 422 and 408). Groups of Sprague–Dawley rats, 10 males and 10 females in each, were given feed with ET (batch 2299, > 99% purity, from Tetrahedron, France). ET was administered via feed in concentrations of 0% (control), 0.3%, 0.5% or 0.9% to the male animals 10 weeks prior to mating and during mating (up to 3 weeks), for a total of at least 13 weeks. The female animals were treated 13 weeks prior to mating, during mating (up to 3 weeks) and during the gestation and lactation periods up until the fifth day post-partum. Five male and five female animals per dose group were examined for the endpoints of the repeated dose toxicity study. No substance related findings were observed concerning any of the parameters tested. The maximum concentration that was tested in the feed, equivalent to a dose of 614.9 mg/kg bw per day for male animals and 725 mg/kg bw per day for female animals, can therefore be considered as the NOAEL.

Another synthetic ET, i.e. Mironova EGT+ (purity > 99%), was tested in a preliminary 28 day study and in a subchronic toxicity study (Marone et al., 2016), according to the OECD TG 407 and TG 408. In the subchronic study, Sprague–Dawley rats (10 per sex and dose group) were daily exposed to 0, 400, 800 and 1,600 mg/kg bw per day ET via gavage in distilled water. Only at the highest dose level of 1,600 mg/kg bw per day the following statistically significant findings were reported: (i) changes in haematology and coagulation parameters for both males (absolute monocytes counts and prothrombin time) and females (haemoglobin, haematocrit, mean corpuscular volume, red cell width, absolute eosinophil and reticulocyte count and prothrombin time); (ii) serum chemistry changes in males (alkaline phosphatase, glucose, sodium and chloride) and females (alkaline phosphatase, creatinine, albumin, total protein and triglycerides) (with the exception of the absolute eosinophilic levels in high-dose females, all values were within historical control values); (iii) decreased absolute and relative thymus weights, increased relative kidney and liver weights only in females. The Panel considers that the number of effects observed at the highest dose may indicate first signs of toxicity in the haematopoietic system, liver and kidney, where the highest ET concentrations are found. The Panel therefore considers as the NOAEL the dose level of 800 mg/kg bw per day, which is supported

by the absence of effects at a similar dose level in the study provided by the applicant (CitoxLAB France, 2013b; Forster et al., 2015).

3.7.5.   Reproductive toxicity

As described under Section 3.7.4, a combined repeated dose reproductive toxicity study with rats was provided by the applicant (CiToxLAB France, 2013b; Forster et al., 2015), which was based on the OECD TG 422 and TG 408. There were no effects of ET treatment on any parameter of reproductive or developmental toxicity. The NOAEL therefore is the maximum concentration that was tested in the feed, equivalent to a dose of 614.9 mg/kg bw per day for male animals and 725 mg/kg bw per day for female animals.

3.7.6.   Human studies

The applicant provided one placebo-controlled, double-blind study (Cheah et al., 2016a), in which 47 healthy Chinese males were randomised to consume a daily dose of 0, 5 or 25 mg ET for 7 days and were followed up to 28 days post-exposure. Apart from the uptake of ET and its pharmacokinetics (described in Section 3.7.1), putative effects of ET on some biomarkers of oxidative damage (protein carbonyls, F2 isoprostanes), allantoin, 8OH-deoxyguanosin (8OHdG) and inflammation (C-reactive protein) were studied. No significant differences were found for these parameters between the study groups. No adverse effects or apparent side effects were noted throughout the study. No significant differences were observed in both liver function tests and lipid profiles for the duration of the study. The Panel notes that although results from this study do not raise safety concerns, no conclusion on the safety of long-term intake of ET can be drawn.

Apart from the studies in healthy subjects by Kumosani (2001) and Cheah et al. (2016b), which are described in the ADME Section (3.7.1), the applicant provided studies reporting on ET concentrations in blood and/or plasma in patients with leukaemia (McMenamy et al., 1960), pre-eclamptic pregnant women (Turner et al., 2009) and in cataractous lenses (Shukla et al., 1981). The Panel notes that in these studies ET concentrations in red blood cells were decreased in patients with chronic granulocytic leukaemia but not in other leukaemic patients compared to normal subjects, were increased in pre-eclamptic pregnant women compared to normotensive women, and were decreased in cataractous lenses. The Panel considers that these studies do not inform on the safety of ET and do not allow inferring conclusions on relationships between ET concentrations and the disease states studied.

Concerns have been raised by the MS as regards a possible effect of ET on glucose homoeostasis. The Panel notes four studies (Salt, 1931; Fraser and Jegard, 1950; Epand, 1982; Epand et al., 1988) which reported on ET in diabetic patients.

Salt (1931) determined ET content in blood from healthy subjects (n = 11), diabetics (n = 17), nephritic patients (n = 14) and patients with miscellaneous diseases (n = 13). A large range of ET values (given as mg per 100 mL blood corpuscles) was found in all groups. Mean values (ranges) from healthy, nephritic patients and patients with various pathological conditions were 7.3 (3.1–12), 7.8 (4.9–14.6) and 7.8 (4–14.7) mg/100 mL, respectively. In the diabetics, the ET content was slightly higher than in healthy subjects (mean 10.2, range 7–15 mg per 100 mL). There was no correlation between ET and blood glucose values and neither were ET values related to any stage of the disease or to the treatment.

Fraser and Jegard (1950) determined the ET content in blood from 94 healthy persons (age 29.5 years) and 107 diabetic patients (age 52.1 years). No ET was found in plasma. Mean (SD) ET level (given as mg/100 mL blood corpuscles) for female and male subjects combined was significantly higher in subjects with diabetes 12.7 (8.3) than in normal subjects 9.6 (3.1). No relationship was found between the ET level and the diabetic state of control, duration of diabetes, age, sex, blood cholesterol, uric acid, and non-protein nitrogen.

Based on the observation that potent chelators of divalent metal ions such as diphenylthiocarbazone and quinaldic acid are known to be diabetogenic agents, Epand (1982) hypothesised that ET, which is known to chelate divalent metal ions, may be a contributing factor leading to the development of diabetes mellitus in some individuals through its chelation of zinc, which is important for the storage of insulin and glucagon.

Epand et al. (1988) measured ET concentrations in the blood of 113 patients with diabetes mellitus and 22 non-diabetic individuals. There was no significant difference between the mean ET concentrations of the diabetic and non-diabetic populations. Within the diabetic population, males had a higher ET concentration than females; type II diabetics had a higher ET concentration than type I diabetics; diabetics receiving a higher insulin dose had higher ET concentrations. There was no

correlation of ET concentration with the concentration of haemoglobin A1 or with the number of years diabetic. Neither zinc status nor zinc binding of ET was evaluated.

The Panel notes that two studies found enhanced ET concentrations in blood of diabetic patients compared to non-diabetic controls, whereas another study did not observe such a difference. The Panel considers that a relationship between ET concentrations in blood and the development of diabetes cannot be inferred from these studies.

Concerns have been raised by MS in view of the fact that the incidence of inflammatory bowel diseases has increased in industrialised countries (Vind et al., 2006). One MS considered that there is insufficient evidence for the safety of ET, which might be a possible contributing factor to inflammatory diseases, when used as a food ingredient.

The applicant provided several studies that investigated ET and its possible role in relation to Crohn's disease (CD) and rheumatoid arthritis (RA), of which six studies (Peltekova et al., 2004; Taubert et al., 2005, 2009; Leung et al., 2006; Kato et al., 2010; Huff et al., 2012) reported on possible associations of variants in OCTN1/ETT with the susceptibility to CD.

The Panel notes that mutations in the ET transporter locus, and especially the 503F variant, have been associated with the susceptibility to CD in Caucasian populations (Peltekova et al., 2004; Taubert et al., 2005, 2009; Leung et al., 2006; Huff et al., 2012).

In studies with HEK293 fibroblasts transfected with solute carrier family 22 member 4 gene (*SCL22A4*, encoding OCTN1/ETT), the 503F variant resulted in a 50% higher initial transport capacity at low ET levels (Taubert et al., 2005). The Panel notes that based on these results, it was speculated that carriers of the 503F allele accumulate higher ET concentrations in *SCL22A4* expressing cells compared with carriers of the wild-type 503L allele, and therefore high tissue levels may constitute a possible risk factor for CD. The Panel notes that no data were provided to prove this hypothesis.

The Panel notes enhanced cell proliferation in the colon cancer epithelial cell line Caco-2 that was shown to be homozygous for the 503F allele and to express high levels of OCTN1 mRNA in a dose-dependent manner after exposure to ET concentrations above 20 μmol/L for 24 h (Taubert et al., 2005). From this result and from the previous finding that levels of *SLC22A4* mRNA were upregulated by pro-inflammatory cytokines, it was suggested that ET may accelerate the inflammatory process by transcriptional activation of fibroblast repair proliferation, thereby also conferring susceptibility of CD patients to develop colorectal cancer. The Panel notes that it is unclear how these results in Caco-2 cells translate into *in vivo* conditions and that no data were provided to prove this hypothesis.

The Panel also notes one study in which mutations reported to be associated with CD patients in Caucasians (C1672T/L503F in *SLC224*) were completely absent in Japanese CD patients (Kato et al., 2010).

The Panel notes that ET levels in peripheral blood mononuclear cells and in intact mucosa were significantly higher in 503F than in 503L carriers without differences between patients and controls, and that ET levels were elevated in mucosal biopsies from inflamed segments of CD patients by twofold compared with adjacent normal mucosa, which correlated with increases in OCTN1 mRNA (Taubert et al., 2009).

The Panel notes a study (Huff et al., 2012) indicating an increase in frequency of the 503F variant due to recent positive selection, that disease-causing variants in linkage disequilibrium with 503F have hitchhiked to relatively high frequency, thus forming the inflammatory bowel disease 5 gene (*IBD5*) risk haplotype, and that the CD association at *IBD5* does not result from 503F itself, but from a nearby hitchhiking variant in the interferon regulatory factor 1 gene (*IRF1*).

The Panel considers that no relationship can be inferred from the above described studies with regard to dietary or supplemental ET and the susceptibility for CD.

Three studies (Reglinski et al., 1991; Tokuhiro et al., 2003; Taubert et al., 2006) report on a potential relationship between ET and RA.

The Panel notes that one study (Reglinski et al., 1991) found lower ET concentrations in red blood cells in RA compared to healthy volunteers, whereas another study (Taubert et al., 2006) reported increased ET concentrations in RA patients when compared to patients with coronary heart disease or osteoarthritis. Whether the discrepancy in results might be influenced by the different control groups used in these studies remains unknown.

The Panel also notes one study (Tokuhiro et al., 2003) indicating that genetic modifications in both *SLC22A4* (the expression of which is usually increased in inflammatory condition) and runt-related transcription factor 1 gene (*RUNX1,* encoding a transcription factor that is involved in the regulation of *SLC22A* expression and was strongly associated with RA) leading to suppression of the expression of *SLC22A4* may affect susceptibility to RA.

The Panel considers that no relationship can be inferred from these studies with regard to dietary or supplemental ET and the susceptibility to RA.

3.8.   Allergenicity

The applicant stated that the NF, as a molecule of low molecular weight, would only be allergenic after binding to a macromolecule (basically protein in nature) or because of proteinaceous contaminants. However, according to the applicant, the NF has no chemical group capable of reacting with proteins under physiological conditions. The manufacturing process for the NF does not involve any raw material that may contain proteinaceous contaminants.

The Panel considers that the likelihood of adverse reactions to the NF is low.

4.   Discussion

The NF which is the subject of the application is synthetic ET (marketed as Ergoneine®). Natural ET is present in a number of foodstuffs such as mushrooms, some varieties of black and red beans, offal and cereals. Chemically, ET is a derivative of thiolhistidine, i.e. 2-thio-L-histidine-betaine.

The information provided on the composition, the specifications, batch-to-batch variability and the stability of the NF is sufficient and does not raise safety concerns. The production process is sufficiently described and does not raise concerns about the safety of the NF.

The applicant intends to use the NF in quantities of up to 5 mg per serving in alcohol-free beverages, cereal bars, milk, fresh dairy products and chocolate. The applicant also proposes to provide the NF as a food supplement, with a recommended daily dose of up to 30 mg/day for adults and 20 mg/day for children. The target population proposed by the applicant is children above 3 years of age and the general adult population, with the exception of pregnant and breastfeeding women.

The combined intake of ET from all sources (including the background diet) is unlikely to exceed 1.7 mg/kg bw per day for adults and 3.7 mg/kg bw per day for children.

Taking into account the intended use levels, the Panel considers that the consumption of the NF is not nutritionally disadvantageous.

ET is absorbed from food in the gastrointestinal tract via a specific transporter, the OCTN1/ETT. Tissue concentrations are tightly regulated by this transporter, which is reflected by control of uptake in the small intestine, selective uptake into tissues and control of re-uptake in the kidney.

Based on the genotoxicity tests provided, the Panel concludes that there are no concerns regarding genotoxicity of the NF.

Two subchronic toxicity studies in Sprague–Dawley rats were provided, which were carried out with the NF that is the subject of the application and with another synthetic ET of high purity, respectively. Based on the observations from both these studies, the Panel considers that the NOAEL of the NF is 800 mg/kg bw per day.

Concerns had been raised by the MS on the possible role of ET in relation to several health outcomes, in particular diabetes mellitus and inflammatory diseases such as CD and RA. The Panel considers that from the available human studies no relationships can be inferred with regard to dietary or supplemental ET and the susceptibility to or development of diabetes mellitus, CD or RA.

Considering the NOAEL of 800 mg/kg bw per day, and the maximum estimated intake levels for ET from all sources (i.e. fortified foods, food supplements, background diet) of 1.7 mg/kg bw per day for adults and of 3.7 mg/kg bw per day for children, the Panel concludes that the margins of safety of 470 (800/1.7) for adults (excluding pregnant and breastfeeding women) and of 216 (800/3.7) for children above 3 years of age are sufficient.

5.   Conclusions

The Panel concludes that the NF, synthetic ET (marketed as Ergoneine®), is safe under the intended conditions of use as specified by the applicant.

Steps taken by EFSA

1) Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on the safety of synthetic L-ergothioneine. Ref. Ares(2015) 4280093, dated 14 October 2015.

2) On 22 October 2015, EFSA received the following documentation: dossier 'Tetrahedron Ergoneine Documentation February confidential', submitted by Tetrahedron; initial assessment report carried out by the Food Safety Authority of France: 'OPINION of the Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (Anses, Agency for Food, Environmental and Occupational Health and Safety) on an application for authorisation to place on the market a novel food ingredient: synthetic L-ergothioneiné'. Referral No 2013-SA-0220; the Member States' comments and objections; response by the applicant to the initial assessment report and the Member States' comments and objections.

3) On 4 December 2015, EFSA sent a request to the applicant to provide missing information to accompany the application.

4) On 14 December 2015, EFSA received the missing information as submitted by the applicant. After checking the content of the full dossier, including the missing information, EFSA considered the application valid as of 11 January 2016.

5) On 14 June 2016, EFSA sent a request to the applicant to provide additional information to accompany the application.

6) Additional data were provided by the applicant on 26 July 2016.

7) During its meeting on 26 October 2016, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of synthetic L-ergothioneine (Ergoneine[®]) as a novel food pursuant to Regulation (EC) No 258/97.

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Abbreviations

8OHdG	8OH-deoxyguanosin
ADME	absorption distribution metabolism excretion
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony-forming units
CD	Crohn's disease
DNA	deoxyribonucleic acid
ET	L(+)-ergothioneine
ETT	L(+)-ergothioneine transporter
Eur. Ph.	European Pharmacopoeia
GLP	good laboratory practice
GMP	good manufacturing practice
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
IBD	inflammatory bowel disease
ICP/AES	inductively coupled plasma/atomic emission spectroscopy;
IRF1	interferon regulatory factor 1
IUPAC	International Union for Pure and Applied Chemistry
i.p.	intraperitoneal
LD_{50}	median lethal dose
mRNA	messenger RNA
MS	Member State
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NF	novel food
NOAEL	no observed adverse effect level
OCTN1	organic cation transporter novel type 1
OECD	Organisation for Economic Co-operation and Development
RA	rheumatoid arthritis

RH	relative humidity
RNA	ribonucleic acid
RUNX1	runt-related transcription factor 1
SD	standard deviation
SLC22A4	solute carrier family 22 member 4
TG	Test Guideline
TVAC	total viable aerobic count
TYMC	total yeast and mould count