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Safety of freeze-dried mycelia of *Antrodia camphorata* as a novel food pursuant to regulation (EU) 2015/2283

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on freeze-dried mycelia of *Antrodia camphorata* as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is produced by solid-state cultivation from tissue cultures derived from the fungus *Antrodia camphorata.* The applicant intends to market the NF in food supplements at a maximum dose of 990 mg per day. The target population is the general population. The NF mainly consists of carbohydrates, proteins and fats, and it contains numerous constituents, such as β-glucans, antroquinonol and triterpenoids. Taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. There are no concerns regarding genotoxicity of the NF. Based on a 90-day repeated dose toxicity study and a prenatal developmental toxicity study performed with the NF, the Panel derives a safe level of 16.5 mg/kg body weight per day. The Panel concludes that the NF, freeze-dried mycelia of *Antrodia camphorata*, is safe at the proposed use level for individuals aged 14 years and above.

Keywords: Antrodia camphorata, food supplement, novel foods, safety, Taiwanofungus camphoratus

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1. Introduction

1.1. Background and terms of reference as provided by the requestor

On 5 November 2108, the company 'Golden Biotechnology Corp' (Taiwan) submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to authorise the placing on the market of *Antrodia camphorata* mycelia powder as a Novel Food (NF).

The application requests to authorise the use of *Antrodia camphorata* mycelia powder in food supplements. The target population is the general population.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on the safety of *Antrodia camphorata* mycelia powder as a NF.

2. Data and methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA's requests for supplementary information.

During the assessment, the Panel identified additional data that were not included in the application.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[1].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of an NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application does not include a request for the protection of proprietary data.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

Additional information which was not included in the application was retrieved by literature search following a search strategy and standard operating procedure as described by UCT Prague (2020).

This assessment concerns only the risks that might be associated with the 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 health benefit.

3. Assessment

3.1. Introduction

The NF which is the subject of the application is the freeze-dried mycelia of *Antrodia camphorata*.

The NF falls under category (vi), i.e. food consisting of, isolated from or produced from cell culture or tissue culture derived from animals, plants, microorganisms, fungi or algae, according to Article 3(2) (a) of Regulation (EU) No 2015/2283.

The NF is produced from tissue cultures derived from the fungus *Antrodia camphorata* which is grown in solid culture medium and subsequently subjected to freeze-drying and milling. The NF is proposed to be used in food supplements as defined in Directive 2002/46/EC[2]. The target population proposed by the applicant is the general population.

[1] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

[2] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements.

3.2. Identity of the NF

The NF comprises freeze-dried mycelia produced by solid-state cultivation from tissue cultures derived from the fungus *Antrodia camphorata*.

Antrodia camphorata is a synonym of *Taiwanofungus camphoratus* (family: Fomitopsidaceae).[3] Other synonyms are *Antrodia cinnamomea* and *Ganoderma camphoratum*. In this opinion, the Panel has decided to use the synonym name *Antrodia camphorata,* which is commonly used in the scientific literature.

The identity of *Antrodia camphorata* was confirmed by BLAST-searching the 400 bp ITS (Internal Transcriber Spacer) sequence fragment against the NCBI database, after DNA extraction, amplification by PCR (polymerase chain reaction) and sequencing of the fragment. The sequence showed a 100% identity with ITS fragments of several *Antrodia camphorata* strains.

Seed cultures of *Antrodia camphorata* are deposited at the Food Industry Research and Development Institute Bioresource Collection and Research Center (Taiwan) (*Antrodia camphorata* Ac-S1; deposited strain BCRC 39106).

3.3. Production process

According to the information provided, the NF is produced in line with good manufacturing practice (GMP) and hazard analysis critical control points (HACCP) principles.

Seed cultures of *Antrodia camphorata* are stored frozen. To start the cultivation, seed culture is thawed and streaked on agar plates where it grows, and then, it is transferred to a liquid broth where it is incubated for several days. The culture is then transferred to a solid medium where it grows. This step is called 'solid-state cultivation'. The mycelia together with a fraction of the solid medium are collected and lyophilised. The final material is milled to form a powder, which is subject to quality control testing. Considering the production process, the NF includes both *Antrodia camphorata* mycelia and solid medium, the latter being a minor part of the final product.

For encapsulation, the NF is mixed with magnesium stearate at a ratio of 99:1. Capsules are packed in high-density polyethylene bottles.

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

3.4. Compositional data

Publications on the chemical content of biologically active substances in *Antrodia camphorata* were either provided by the applicant or retrieved by EFSA (Chen et al., 2007a,b, 2016; Huang et al., 2014). Terpenoids and benzenoids are mainly present in fruiting bodies, whereas antroquinonol B is mainly present in mycelia (Geethangili and Zeng, 2011). Other compounds identified in the mycelia are adenosine, antrosterol and gamma-aminobutyric acid (Chen et al., 2016), benzoquinone derivatives, beta-glucans (Geethangili and Zeng, 2011; Chen et al., 2018). Polysaccharides and glycoproteins (Chen et al., 2007a,b), succinic and maleic acid derivatives have been identified in both fruiting body and mycelia of *Antrodia camphorata* (Geethangili and Zeng, 2011; Lu et al., 2013).

The applicant provided analytical information for five batches of the NF (Table 1a).

Upon EFSA's request for additional information, the applicant tested additional batches of the NF for the presence of antroquinonol, triterpenoids and β-glucans (Tables 1b and 1c) and for the presence of three other compounds (i.e. pyrroledione, ergostatrien-3ß-ol and 4-acetylanthroquinonol B), which were identified by Chen et al. (2011a) and Huang et al. (2014). The concentrations of these compounds in one batch of the NF were pyrroledione 73.4 mg/kg, ergostatrien-3ß-ol 1,343.6 mg/kg and 4-acetylanthroquinonol B 233.96 mg/kg. Regarding triterpenoids, the discrepancy in the results obtained for triterpenoids (Tables 1b and 1c) in the NF is due to different methods of extraction and of analysis applied.

The applicant tested five additional batches of the NF for aflatoxins B1, B2, G1, G2 (plus the sum) and ochratoxin A which were below their limits of quantification (LOQ for ochratoxin A = 0.3 μg/kg; LOQ for aflatoxin B1 and G1 = 0.2 μg/kg; LOQ for aflatoxin B2 and G2 = 0.1 μg/kg).

Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

[3] https://www.indexfungorum.org/Names/Names.asp

The Panel considers that the information provided on the composition of the NF is sufficient and does not raise safety concerns.

Table 1a: Batch to batch analysis of the NF

Parameter	Batch number					Method of analysis
	17C007	17C008	17A0013	17A0015	17A0020	
Composition (per 100 g)						
Energy (kcal)	384	383	379	382	388	Calculation
Moisture (g)	6.8	7.2	5.8	5.6	6.3	Not reported
Protein (g)	24.0	23.2	13.0	14.8	15.2	CNS (Chinese National Standard) 5,035 (4 August 1986) micro-Kjeldahl
Fat (g)	4.5	5.1	2.1	2.6	4.5	CNS (Chinese National Standard) 5,036 (14 January 1984) Extraction/gravimetry
Saturated fat (g)	0.61	0.61	0.13	0.26	0.31	MOHW (Taiwanese Ministry of Health and Welfare) Food No. 1021950978 Announcement (28 November 2013) GC-FID after extraction, saponification, methylation
Carbohydrate (g)	61.9	61.1	77.1	74.9	71.6	Calculation
Ash (g)	2.8	3.4	2.0	2.1	2.4	Not reported
Sugars (g)	9.0	9.0	18.2	17.0	19.4	CNS (Chinese National Standard) 12,634 (19 April 2006) and CNS 3445 (19 January 2007)/TFDA (12 December 2015) – HPAEC-PAD
Sodium (mg)	16.1	17.6	14.8	15.8	18.3	AOAC (Association of Official Agricultural Chemists) - 984.27 – ICP-AES
Microbiological parameters						
TAMC (CFU/g)	< 10	< 10	< 10	< 10	< 10	Ph. Eur. 8.0–2.6.12
TYMC (CFU/g)	< 10	< 10	< 10	< 10	< 10	
E. coli (per 1 g)	Not detected	Not detected	Not detected	Not detected	Not detected	Ph. Eur. 8.0–2.6.13
Salmonella[(a)] (per 1 g)	Not detected	Not detected	Not detected	Not detected	Not detected	
P. aeruginosa (per 1 g)	Not detected	Not detected	Not detected	Not detected	Not detected	
S. aureus (per 1 g)	Not detected	Not detected	Not detected	Not detected	Not detected	
Heavy metals						
Lead (mg/kg)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	MOHW (Taiwanese Ministry of Health and Welfare) Food No. 1031901169 Announcement (25 August 2014) ICP/MS
Cadmium (mg/kg)	0.12	0.06	0.06	0.05	0.06	
Mercury (mg/kg)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	
Arsenic (mg/kg)	0.41	0.50	0.18	0.14	0.24	

GC-FID: gas chromatography-flame-ionisation detection; HPAEC-PAD: high-performance anion-exchange chromatography - pulsed amperometric detection; ICP-AES: inductively coupled plasma-atomic emission spectroscopy; ICP-MS: inductively coupled plasma-mass spectrometry; TAMC: total aerobic microbial count; TYMC: total combined yeast and mould count; CFU: colony forming units.
(a): Upon EFSA's request, the applicant tested five additional batches of the NF for *Salmonella*, which was not detected in 25 g of the NF.

Table 1b: Batch-to-batch analysis of the NF for additional parameters

Parameter	Batch n. 18C0010	Batch n. 18C0011	Batch n. 18C0014	Batch n. 18C0015	Batch n. 18C0016
β-glucans (g/100 g) by enzymatic spectrophotometry; AOAC 995.16	12	11	11	13	14
Antroquinonol (mg/g) by UPLC-PAD	11.69	14.64	9.58	11.00	6.83
Triterpenoids (g/100 g) by UPLC-PAD	6.90	7.86	6.71	6.54	6.00

AOAC: Association of Official Analytical Chemists; UPLC-PAD: ultra-high-performance liquid chromatography-photodiode array detector.

Table 1c: Batch-to-batch analysis of additional batches of the NF for triterpenoids

Parameter	Batch n. 16C0009	Batch n. 16C0010	Batch n. 16C0011	Batch n. 17C0007	Batch n. 17C0008	Batch n. 17A0013	Batch n. 17A0015	Batch n. 17A0015
Total triterpenoids (g/100 g)(*)	4.19	3.91	4.75	5.17	4.80	4.91	5.02	4.98

(*): via colorimetric method (Wang et al. 2006).

3.4.1. Stability

The applicant performed stability tests with three batches of the NF, which were packed with two different sets of materials [package A: polyethylene (PE) bag, desiccant, foil bag, carton box; package B: PE bag, carton box]. The tests were carried out at 25 ± 2°C/60 ± 5% relative humidity (RH) for 36 months (long-term test) and at 40 ± 2°C/75 ± 5% RH for 6 months (accelerated test). The batches were analysed for appearance, loss on drying, total triterpenes and microorganisms (total aerobic microbial count, total yeast and mould count, *S. aureus, E. coli, Salmonella*). Both in the long-term and in the accelerated stability test, the parameters met the specifications at each time point tested. However, the loss on drying was more pronounced in the batches with packaging B both in the long-term and in the accelerated stability tests (from 2% at t = 0 up to 8–9% at the end of the testing period). No other relevant differences between the two packaging methods were noted in the stability tests.

Based on the results of the long-term stability test, the applicant proposed a shelf-life for the NF of 3 years stored below 25°C.

The Panel considers that the data provided sufficient information with respect to the stability of the NF for 3 years stored below 25°C.

3.5. Specifications

The specifications of the NF are presented in Table 2.

Table 2: Specifications of the NF

Description: brown powder of freeze-dried mycelia produced by solid-state cultivation from tissue cultures derived from the fungus *Antrodia camphorata*	
Parameter	**Limits or range**
Loss on drying	< 10%
Total triterpenoids[a]	1.0–10.0 g/100 g
Antroquinonol	1.0–20.0 mg/g
Total carbohydrates	≤ 80 g/100 g
Protein	≤ 20 g/100 g
Ash	≤ 15 g/100 g
Fat	≤ 6 g/100 g
TAMC	≤ 10^3 CFU/g
TYMC	≤ 10^2 CFU/g
E. coli	Not detected/10 g
Salmonella	Not detected/25 g

S. aureus	Not detected/10 g
Arsenic	< 0.5 mg/kg

TAMC: total aerobic microbial count; TYMC: total yeast and mould count; CFU: colony forming units.
(a): Colorimetric method in Wang et al. 2006.

The Panel notes that *E. coli* and *S. aureus* should not be detected in 10 g of the NF (instead of 1 g as proposed by the applicant).

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

3.6. History of use of the NF and/or of its source

3.6.1. History of use of the source

Antrodia camphorata has a long history of use as a traditional medicine in Taiwan, which can be traced back to the 1700s (Tsai, undated; Chen et al., 2000; Su, 2002; Chen, 2005; Geethangili and Zeng, 2011). Currently, *Antrodia camphorata* (both mycelia and fruiting bodies) are commercially available in Taiwan and are added to foods (e.g. biscuits, sweets) or used to prepare beverages, teas or wines. Both mycelia and fruiting bodies of *A. camphorata* are also available in powdered forms as tablets or capsules in Taiwan.

3.6.2. History of use of the NF

The NF has been on the market in Taiwan in the form of capsules, to be consumed at a maximum daily dose of 3 g NF since 2008. In 2012, the NF was classified as a food in Japan.

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The target population proposed by the applicant is the general population.

3.7.2. Proposed uses and use levels

The applicant intends to market the NF in food supplements, in the form of capsules, at a maximum daily dose of 990 mg.

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

No ADME data have been provided for the NF.

3.9. Nutritional information

The NF mainly consists of carbohydrates (61–77%), proteins (13–24%) and fats (2–5%) and contains numerous constituents, such as β-glucans, antroquinonol and triterpenoids.

The Panel considers that, taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous.

3.10. Toxicological information

In the initial application, the applicant provided four references on toxicological studies performed with the NF (Table 3).

Table 3: List of toxicological studies with the NF

Reference	Type of study
Unpublished study report (dated 2010a)	Ames test
Unpublished study report (dated 2010b)	*In vitro* chromosomal aberration assay
Unpublished study report (dated 2010c)	*In vivo* erythrocyte micronucleus assay

Reference	Type of study
Unpublished study report (dated 2014)	Ames test *In vivo* bone marrow polychromatic erythrocyte micronucleus test *In vivo* sperm abnormality test 90-day repeated dose oral toxicity study Teratogenicity study

In addition, publications on toxicological studies that were conducted with powders of mycelia of *Antrodia camphorata* were either provided by the applicant (Chen et al., 2011a,b; Lin et al., 2015) or retrieved by EFSA (Huang et al., 2014; Lin et al., 2016). The publications by Chen et al. (2011a, 2011b) and Huang et al. (2014) relate to powders of mycelia of *Antrodia camphorata* that were produced with different production processes and conditions (i.e. not solid-state cultivation). Thus, the Panel considers that these studies do not provide support to the assessment of the NF. The publications by Lin et al. (2015, 2016) relate to powders of mycelia of *Antrodia camphorata* that were produced with solid-state cultivation processes (even though not explicitly detailed). Although the test materials may not be fully representative of the NF, they may have similarities to the NF, and therefore, the Panel considers that the studies by Lin et al. (2015, 2016) provide support to the assessment of the NF.

3.10.1. Genotoxicity

The applicant provided four different references covering genotoxicity studies that had been conducted with the NF (Table 3). The Panel notes that in the Ames test reported in the unpublished study report dated (2010a) and in the *in vitro* chromosomal aberration test (unpublished study report dated 2010b), aqueous extracts of the test materials (supernatants that were obtained after centrifugation of suspensions of test materials) were used and that the insoluble fractions were not tested. A further Ames test (unpublished study report dated 2014) displayed no statistically significant increase in revertants in any of the tester strains. However, it is unclear whether the test material was fully soluble in the stock preparation that had also been prepared with water. In addition, the Panel notes that not all relevant parameters were assessed or reported in the *in vitro* chromosomal aberration test (i.e. there is no direct information on the extent of polyploidy) (unpublished study report dated 2010b). Regarding the *in vivo* micronucleus tests, the Panel notes that there was no indication of the exposure of the bone marrow to the test material [i.e. no decrease in the PCE (polychromatic erythrocytes)/NCE (normochromatic erythrocytes) ratios]. Thus, taking into account the above-mentioned limitations in the genotoxicity tests, the Panel considers that these studies are not sufficient to draw conclusions on the genotoxicity of the NF.

The Panel notes a publication provided by the applicant on an Ames test and an *in vitro* chromosomal aberration assay that were performed with DMSO (dimethyl sulfoxide) extracts of *Antrodia camphorata* (parts of the fungi used not specified) (Wu et al., 2011). The Ames test showed an increase in revertants by 61% for TA98 in the presence of metabolic activation (S-9 mix) at the highest concentration tested (5,000 μg/plate). The *in vitro* chromosomal aberration assay showed a dose-dependent increase in chromosomal aberrations (up to 50 mg/mL, the highest concentration tested). Despite the limitations of this publication (test material not clearly specified and high concentrations tested in the chromosome aberration test), the Panel considered the findings reported in this publication relevant for the assessment of the NF.

Thus, EFSA requested the applicant to perform an additional Ames test and an *in vitro* mammalian cell micronucleus test with the NF dissolved in DMSO, which were provided by the applicant. These tests were performed in accordance with the OECD Test Guideline 471 and 487 (2016, 2020), respectively.

The Ames test was performed with concentrations of the NF (dissolved in DMSO) of up to 5,000 μg/plate using Salmonella Typhimurium tester strains TA98, TA100, TA102, TA1535 and TA1537 (unpublished study report, dated July 2021). The plate incorporation method in the presence or absence of S-9 mix was applied. The highest dose of 5,000 μg/plate was chosen based on a dose range finding test in TA100 (absence of S-9 mix). There were no increases in the number of revertant colonies, neither in the presence nor absence of S-9 mix, in both trials up to the highest concentration tested. The Panel notes that despite the fact that the test material was dissolved in DMSO, the study report referred to precipitation of the test material, without indicating at which concentration(s) precipitation occurred. However, taking into account that the concentrations tested were the same as

in the publication by Wu et al. (2011), the Panel considers that the Ames test was relevant for the assessment of the NF. The Panel concluded that the NF did not induce mutagenicity under the testing conditions.

The *in vitro* mammalian cell micronucleus test was performed in human lymphocytes with the NF dissolved in DMSO (unpublished study report, dated January 2022a). In the cytotoxicity tests, in both experimental conditions (3 h, with and without S-9 mix; 24 h, without S-9 mix), the highest concentration applied was 150 μg/mL, owing to the solubility of the test material. Based on the observed cytotoxicity i.e. a fluctuating low cytotoxicity (20–34%) not concentration-dependent between 40 and 150 μg/mL and with no cytotoxicity from 10 to 20 μg/mL (13%), the concentrations selected were 100, 125 and 150 μg/mL for the main micronucleus experiment under the 3 h treatment with S-9 mix conditions. Owing to the marked cytotoxicity in the 3 h treatment and the 24 h treatment without S-9 experimental conditions, the concentrations inducing a reduction of the replication index to 45 ± 5% as recommended by the OECD TG 487 (2016) were not obtained. Thus, two additional experimental tests were performed for cytotoxicity for the 3 h and 24 h treatment without S-9 mix experimental conditions. For the short-term treatment (without S-9 mix), concentrations from 30 to 90 μg/mL were applied. Based on the observed toxicity (from 32% to 86%; with cytotoxicity of 53% at 50 μg/mL), concentrations of 30, 40 and 50 μg/mL were selected for the 3 h treatment (without S-9 mix). For the long-treatment condition (without S-9 mix), concentrations ranging from 1 to 40 μg/mL were selected. Based on the observed toxicity [i.e. around 50% cytotoxicity at 10 μg/mL (46%), 15 μg/mL (47%) and 20 μg/mL (59%)], concentrations of 2.5, 7.5, 15 and 20 μg/mL were selected for the 24-h treatment without S-9 mix. There was no increase in the percentage of micronuclei at any of the tested concentrations compared to the vehicle control, both in the short-term and in the long-term experimental conditions (with and without S-9 mix).

Taking into account the test results provided, the Panel considers that there are no concerns regarding the genotoxicity of the NF.

3.10.2. Subchronic toxicity studies

The applicant provided a 90-day study with the NF (unpublished study report dated 2014). The test material was provided with feed to groups of 10 male and 10 female Sprague Dawley (SD) rats at dose levels corresponding approximately to 0, 1, 2.3 and 3.3 g/kg bw daily for 90 days. No mortality occurred during the study. Some statistically significant increases and decreases in feed intake were observed between groups in some weeks. However, since these changes were not associated with statistically significant changes in body weights, the Panel considers the changes in feed intake as not adverse. Regarding haematological parameters, at day 45, red blood cell (RBC) counts of male rats in the low- and high-dose groups were significantly higher (5%) as compared to the control group; at day 90, RBC counts of male rats in the mid- and high-dose groups, as well as of female rats in the mid-dose group, were significantly higher (3%) than in the control groups. The Panel considers the increases observed in RBC counts as not adverse. No statistically significant differences were observed in the remaining haematological parameters investigated (haemoglobin, white blood cells, granulocytes and lymphocytes), either at day 45 or at day 90. There were no statistically significant differences between groups in the clinical biochemistry parameters investigated (alanine aminotransferase, aspartate aminotransaminase, total protein, albumin, blood urea nitrogen, creatinine, blood glucose, triglyceride, total cholesterol) and in the organs weighed (liver, kidneys, spleen, testicles).

There were no findings in histopathological examinations in the liver, stomach, jejunum, spleen, testicles and ovaries in the high-dose group. Histopathological changes were observed in one animal in the high-dose group in the kidneys (calcium deposits), which was considered by the Panel a possible incidental finding. The no observed adverse effect level (NOAEL) from this study was the highest dose tested of 3.3 g/kg bw per day. The Panel notes that the list of haematological and clinical biochemistry parameters tested was not in accordance with the requirements set in the OECD Guideline 408 (1998), as some parameters were missing. Additionally, only a limited number of organs were weighed and sampled for histopathological examination as compared to the requirements set in the OECD Guideline 408 (1998).

The Panel notes that the publications by Lin et al. (2015, 2016) reported 90-day repeated dose toxicity studies with powders of mycelia of *Antrodia camphorata,* which were performed according to OECD Guideline 408 (1998) and at similar dose levels as in the study with the NF (Table 4). These studies investigated parameters that were not tested in the study with the NF (e.g. haematocrit, platelet counts, organ weights and histopathology of additional organs such as brain, heart, adrenals).

Even though the test materials may not be fully representative of the NF, the Panel assessed whether these studies may provide indications on hazards that were not investigated in the study with the NF. The findings in parameters assessed in these studies did not raise safety concerns. The increase in total cholesterol observed with the test material used by Lin et al. (2015, 2016) was not considered relevant, given the difference in cholesterol metabolism in humans and rodents and the fact that such an effect was not observed in the study with the NF. Therefore, the Panel considers that these studies support the NOAEL obtained in the study with the NF.

Table 4: Publications on 90-day studies with powders of mycelia of *Antrodia camphorata*

Type of study	Reference	Test system	Test material and doses	Relevant findings considered as treatment related, remarks
90-day repeated dose oral toxicity study OECD 408, including urine analysis	Lin et al. (2015)	Sprague–Dawley rats, m + f 12 per group	Powder of *Antrodia camphorata* 94% mycelium from high-efficient solid-state cultivation, 5% extract of fruiting body from cut-log cultivation and 1% magnesium stearate 0, 0.7, 1.4, 2.8 g/kg bw per day in water	m: dose dependent ↑ in liver weight, stat. Sign. at 2.8 g/kg bw (21% increase), glucose stat sign. ↑ (22%) at 2.8 g/kg bw; f: cholesterol dose dependent ↑, stat. Sign. at 1.4 g/kg bw (34%) and 2.8 g/kg bw (36%), haematocrit ↓ (3%) at 2.8 g/kg bw m + f: urine pH ↓ (4% in m; 7% in f) at 2.8 g/kg bw
90-day repeated dose oral toxicity study OECD 408, including urine analysis, oestrous cycle	Lin et al. (2016) (reference retrieved by EFSA)	Sprague–Dawley rats, m + f 12 per group	Powder of *Antrodia camphorata* mycelium from high-efficient solid-state cultivation 0, 0.5, 1.5, 2.5 g/kg bw per day in water	f: 2.5 g/kg bw erythrocytes ↓ (5%), haemoglobin ↓ (5%) and haematocrit ↓ (6%); cholesterol dose dependent ↑ stat. Sign. at 2.5 g/kg bw (20%) m: 2.5 g/kg bw albumin ↓ (24%) m 2.5 g/kg bw urine pH ↓ (4%)

The lack of raw data and of historical control data. Overall, the Panel considers that these publications provide supporting evidence to the NOAEL from the prenatal developmental study carried out with the NF.

3.10.3. Reproductive and developmental toxicity

The applicant provided an *in vivo* sperm abnormality test that was conducted in adult mice that received doses of up to 5 g/kg bw per day of a suspension of the NF in water during 5 consecutive days. This study did not show a statistically significant increase in abnormal sperm on the 35th day from the initiation of treatment.

The applicant provided a teratogenicity study with the NF (unpublished study report, dated 2014). The test material was administered to groups of 12–14 pregnant SD rats daily orally by gavage at dose levels of 0, 1, 2.33 and 3.33 g/kg bw on days 7–16 of gestation. The rats were sacrificed on day 20 of gestation and the uterus was excised and weighed. The numbers of corpora lutea, implantations and resorptions, live and dead fetuses and placental weight, and fetal body weight and size were recorded. The fetuses were examined for gross, skeletal and visceral malformations. In the high-dose group, maternal body weights and body weight gains were slightly lower than in controls (4% and 12%, respectively), even though not statistically significantly different. No treatment-related effects were observed on the number of corpora lutea, implantations and the number of live and dead foetuses. The numbers of resorbed foetuses were higher in the middle- and high-dose groups as compared to control, although not clearly dose-related and not reaching statistically significant difference (control group: 0.29 ± 0.47; low dose: 0.33 ± 0.49; middle dose: 0.86 ± 2.66; high dose: 0.69 ± 0.85). In the high-dose group, the number of pregnant rats with resorption and fetus resorption ratio in litters was slightly higher than in the control, even though not statistically significantly different. No clear treatment-related effect on placental weight, fetal weight and fetal length was observed. Several gross, visceral and skeletal malformations were observed (e.g. chest bone missing, renal ectopia and hydronephrosis). Upon EFSA's request for information, the applicant replied that both historical control and individual data on 'malformations' and 'resorptions' were not available.

During the assessment, the applicant provided an additional prenatal developmental toxicity study with the NF (unpublished study report, dated 2022b) that was conducted in accordance with the OECD Test Guideline 414 (2018). The test material was administered to groups of 20–21 pregnant SD rats daily orally by gavage at dose levels of 0, 1, 2.33 and 3.33 g/kg bw on days 6–19 of gestation. The rats were sacrificed on day 20 of gestation and the uterus was excised and weighed. In pregnant females, there were no deaths, premature births or abortions in any group. No statistical differences in pre-implantation or post-implantation losses were observed. However, the Panel considered that it was difficult to conclude on the results pertaining to the absence of an increase in preimplantation losses, as implantation would be expected to take place in the rat from day 4 to day 6 of gestation. Thus, implantation would have largely already occurred before maternal animals had been exposed to the test substance (initiation of treatment on day 6 of gestation). There were no significant maternal toxicity evidences noted in mortality/moribundity and clinical observation. Also, no statistically significant changes were noted in hormones (thyroxine, triiodothyronine and thyroid stimulating hormone) analyses, thyroid/parathyroid organ weights, gross necropsy, reproductive parameters and histopathological evaluation. In fetuses, there was no developmental toxicity noted in external, visceral and skeletal evaluation. The Panel notes that this study, which was performed with the same rat species and the same doses of the initial study, was designed to overcome the shortcomings of the study initially submitted (i.e. more animals per groups, longer exposure during pregnancy, in accordance with the OECD Test Guideline 414 (2018)). The Panel considers that the NOAEL of this study is the highest dose tested, i.e. 3.33 g/kg bw per day both for maternal and fetal toxicity.

The Panel notes that the publications by Lin et al. (2015, 2016) also reported on prenatal developmental toxicity studies at similar dose levels as in the study with the NF without adverse effects (Table 5).

Individual malformations occurred with low frequency and were not statistically significant. In many cases, only one fetus carried a specific malformation and there was no clear dose response. The Panel notes that the relevance of these effects was difficult to interpret owing to the lack of raw data and of historical control data. Overall, the Panel considers that these publications provide supporting evidence to the NOAEL from the prenatal developmental study carried out with the NF.

Table 5: Publications on prenatal developmental studies with powders of mycelia of *Antrodia camphorata*

Type of study	Reference	Test system	Test material Doses exposure duration	Relevant findings considered as treatment related, remarks
Prenatal developmental toxicity study According to the 'Safety Evaluation Method for Health Food' by Department of Health (Taiwan)	Lin et al. (2015)	Sprague–Dawley rats, 20 per group	Powder of *Antrodia camphorata* 94% mycelium from high-efficient solid-state cultivation, 5% extract of fruiting body from cut-log cultivation and 1% magnesium stearate. 0, 0.7, 1.4, 2.8 g/kg bw per day by water, GD 6–15.	No relevant findings No historical control data
Prenatal developmental toxicity study According to the 'Safety Evaluation Method for Health Food' by Department of Health (Taiwan)	Lin et al. (2016) (reference retrieved by EFSA)	Sprague–Dawley rats, 22-25/group	Powder of *Antrodia camphorata* mycelium from high-efficient solid-state cultivation 0, 0.5, 1.5, 2.5 g/kg bw per day by water, GD 6–15	Fertility index dose related ↑ (not adverse) ≥ 1.5 g/kg: fetal bw stat. Sign. ↑ (6%) (not adverse) 2.5 g/kg bw: preimplantation loss ↑ (from 0% in the control to 5% in the high-dose group) (stat. Sign.) (this parameter is not considered treatment related since implantation predominately occurs before the starting of the dosing) Historical control data from another laboratory

bw: body weight; GD: gestation day.

3.10.4. Human data

No human studies with the NF have been provided by the applicant.

EFSA retrieved a publication of a randomised, double-bilnded, placebo-controlled human intervention study on a powder of freeze-dried mycelia of *Antrodia camphorata,* which was obtained by fermenting the mycelia in a liquid medium (Chen et al., 2016). Participants with mild hypertension were randomised to consume either 1.26 g per day of *Antrodia camphorata* powder (n = 21) or placebo (starch) (n = 20) for 8 weeks. No adverse events were reported during the study. No statistically significant differences between the intervention and the placebo group were reported in the parameters investigated, except for decreases in diastolic and systolic blood pressure and in plasma renin activity in the intervention group, which are not considered as adverse.

The applicant referred to the safety surveillance system which is in place in Taiwan, where the NF has been on the market since 2008. The applicant indicated that no complaints from customers or letters on adverse reactions associated with the NF were received through this system up to 2014.

3.11. Allergenicity

The Panel notes that the protein content in the NF ranges from 13 to 24 g/100 g NF (Table 1a) and that glycoproteins, considered as potential allergens, were reported to be present in *Antrodia camphorata* (Chen et al. 2007a,b; Ker et al., 2014).

Soy proteins and wheat protein (gluten) present in the cultivation solid medium were analysed in the NF and not detected by ELISA at their respective LOQs (0.3 mg/kg for soy proteins and 5 mg/kg for wheat protein).

The applicant was requested to perform a literature search to retrieve publications concerning the allergenic potential of *Antrodia camphorata*. No data concerning cases of sensitisation to, or allergic reactions following oral consumption of, *Antrodia camphorata* were retrieved by either the applicant or EFSA.

No other information or data have been provided by the applicant to assess the allergenic potential of *Antrodia camphorata*.

The Panel considers that there is no sufficient basis to conclude on the risk of allergenicity for the NF and, given the protein content, some risk is present.

4. Discussion

The NF which is the subject of this application is freeze-dried mycelia produced by solid-state cultivation from tissue cultures from the fungus *Antrodia camphorata*.

The applicant intends to market the NF in food supplements (in the form of capsules) at a maximum daily dose of 990 mg. The target population is the general population.

Taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous.

The Panel considers that the NOAEL of the provided 90-day repeated dose toxicity study with the NF is the highest dose tested, i.e. 3.3 g/kg bw per day.

By applying an uncertainty factor of 200 [10 (interspecies variability) × 10 (intraspecies variability) × 2 (subchronic to chronic study duration)][4] to the NOAEL of 3.3 g/kg bw per day, the Panel derives a safe level of 16.5 mg/kg bw per day. The Panel notes that the safe level derived from the 90-day study is also protective against developmental toxicity.

Considering the safe level of 16.5 mg/kg bw per day and the default body weights for the target population (i.e. the general population), the Panel notes that the proposed use level (990 mg per day) is safe for individuals aged 14 years and above (Table 6).

Table 6: Safe intake levels of the NF

Population group (age in years)	Default body weights[(a)] (kg)	Safe intake levels of the NF (mg/day) based on the safe level of 16.5 mg/kg bw per day
Infants (< 1)	5	82.5
Young children (1 to < 3)	12	198

[4] EFSA Scientific Committee, 2012.

Population group (age in years)	Default body weights[(a)] (kg)	Safe intake levels of the NF (mg/day) based on the safe level of 16.5 mg/kg bw per day
Other children (3 to < 10)	23.1	381
Young adolescents (10 to < 14)	43.4	716
Old adolescents (14 to < 18)	61.3	1,011
Adults (≥ 18)	70	1,155

(a): EFSA Scientific Committee, 2012.

5. Conclusions

The Panel concludes that the NF, freeze-dried mycelia of *Antrodia camphorata*, is safe at the proposed use level for individuals aged 14 years and above.

6. Steps taken by EFSA

1) On 5 August 2020 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of *Antrodia camphorata* mycelia powder as novel food. Ref. Ares(2020)2507455–5 December 2020.
2) On 5 August 2020, a valid application on *Antrodia camphorata* (freeze-dried mycelia), which was submitted by Golden Biotechnology Corp, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2018/0329) and the scientific evaluation procedure was initiated.
3) On 17/07/2020 and 17/03/2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 21/12/2020 and on 2 November 2022, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) During its meeting on 18/05/2022, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of freeze-dried mycelia of *Antrodia camphorata* as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
bw	body weight
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
ITS	internal transcriber spacer
LOQ	limit of quantification

NCBI	National Center for Biotechnology Information
NCE	normochromatic erythrocytes
NDA	Panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCE	polychromatic erythrocytes
PCR	polymerase chain reaction
PE	polyethylene
RBC	red blood cell
RH	relative humidity
S-9 mix	metabolic activation
SD	Sprague Dawley