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Safety of Vitamin D2 Mushroom Powder as a Novel Food Pursuant to Regulation (EU) 2015/2283

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Safety of vitamin D_2 mushroom powder 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 a scientific opinion on vitamin D_2 mushroom powder as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is an ingredient produced from *Agaricus bisporus* mushrooms that have been exposed to ultraviolet (UV) light to induce the conversion of provitamin D_2 (ergosterol) to vitamin D_2 (ergocalciferol). The NF contains concentrations of vitamin D provided by vitamin D_2 in the ranges of 1,000–1,300 μg/g. The information provided on the manufacturing process, composition and specifications of the NF does not raise safety concerns. The applicant intends to add the NF in a variety of foods and beverages, including food for special medical purposes and food supplements. The target population is the general population except for food supplements, for which the target population is individuals above seven months of age. The Panel concludes that the NF, used as an ingredient, is safe for the general population at the proposed condition of use in foods and beverages and that the NF used as a food supplement, is safe for individuals above 1 year. The Panel, however, notes that the UL for infants aged 0–6 months may be exceeded in high consumers of infant formula (IF) and/or follow-on formula (FoF) that may also be high consumers of foods fortified with the NF and for infants aged 7–12 months consuming a daily vitamin D oral supplementation of 10 μg. However, the Panel considers this scenario unlikely as complementary feeding in high consumers of IF and/or FoF may be limited. Furthermore, the combined consumption of vitamin D via fortified foods and supplements does not specifically concern this NF application. The Panel concludes that the NF is safe under the proposed conditions of use for the proposed target populations.

Keywords: Novel food, ingredient, safety, mushroom powder, UV treatment, vitamin D

Requestor: European Commission following an application by Oakshire Naturals, LP

Question number: EFSA-Q-2018-00601

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Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 17 July 2018, the company Oakshire Naturals, LP submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) No 2015/2283[1] to place vitamin D_2 mushroom powder on the Union market as a novel food.

The novel food (vitamin D_2 mushroom powder) is intended for use in foods, beverages and food supplements. The target population is the general population with the exception of food supplements, which are intended for the general population upwards of 7 months of age.

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 vitamin D_2 mushroom powder as a novel food.

1.2. Additional information on existing evaluations and authorisations

Controlled ultraviolet (UV) irradiation is used to enhance the concentration of vitamin D_2 in mushrooms. Commercially grown UV-treated *Agaricus bisporus* mushrooms are approved in the European Union (EU) market as a novel food (NF) since 2016 (FSAI, 2017). No safety concerns associated with the consumption of commercially cultivated mushrooms subjected to controlled UV light were raised by the competent authorities of EU Member States.

UV irradiation technique to enhance the content of vitamin D has been used in other approved novel foods evaluated by EFSA: UV-treated baker's yeast (*Saccharomyces cerevisiae*) (EFSA NDA Panel, 2014), UV-treated bread (EFSA NDA Panel, 2015) and UV-treated milk (EFSA NDA Panel, 2016c). All of them are currently under the Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods.

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 requests for supplementary information.

During the assessment, the Panel identified additional data which were not included in the application (Gordon et al., 2008; Gallo et al., 2013; Cashman et al., 2016).

The applicant also provided a comprehensive literature search conducted in several databases to identify issues of potential safety relevance for vitamin D_2 mushroom powder (Annex V of the dossier).

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[2].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016a). 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 to supporting the safety of the proposed NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. Data claimed to be proprietary by the applicant include: Raw materials and processing aids (Annex I to the dossier), Certificates of Analysis and batch data (Annex II to the dossier) and Stability reports (Annex III to the dossier).

[1] Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, p. 1.

[2] 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.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications 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.

This assessment concerns only risks that might be associated with consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of vitamin D_2 mushroom powder with regard to any claimed benefit.

3. Assessment

3.1. Introduction

The NF which is the subject of the application is vitamin D_2 mushroom powder as a novel food as defined in article 3 of the NF Regulation 2015/2283. The NF falls under the category (ii) '*food consisting of, isolated from or produced from microorganisms, fungi or algae*'.

The NF contains concentrations of vitamin D as vitamin D_2 in the range of 1,000–1,300 μg/g.

The applicant intends to market the NF as an ingredient in foods and beverages for consumption by the general population. Vitamin D_2 mushroom powder food supplements are intended for individuals above 7 months of age.

3.2. Identity of the NF

The NF is a whole mushroom powder containing vitamin D_2 (ergocalciferol) induced by UV treatment.

According to the applicant, this vitamin D_2 whole mushroom powder is a slightly brown or tan granular powder made from homogenised *Agaricus bisporus* mushrooms that have been exposed to UV light to induce the conversion of provitamin D_2 (ergosterol) to vitamin D_2 (ergocalciferol).

The product contains vitamin D_2 with registered CAS number 50-14-6 and its IUPAC 5*Z*,7*E*,22*E*)-(3*S*)-9,10-seco-5,7,10(19),22-ergostatetraen-3-ol. The molecular formula of vitamin D_2 is $C_{28}H_{44}O$.

The source of the NF is the fungus *Agaricus bisporus* as listed in the Index fungorum (http://www.indexfungorum.org/names/names.asp). Various common synonyms for this mushroom are closed cap, large – flat, common, cremini, button, cultivated, chestnut or portobello mushroom, depending on size, shape, and colour of the mushroom.

3.3. Production process

The mushrooms used in the production of vitamin D_2 mushroom powder are cultivated *Agaricus bisporus*. The cultivation is in accordance with current Good Agricultural Practices (GAP) and all facilities comply with Food Safety Modernization Act (FSMA) produce rules. The producer has a Hazard Analysis Critical Control Points (HACCP) plan in place.

As a first step, the mushrooms are washed and homogenised in a mixing kettle to produce a mushroom puree. The mushroom puree is transferred onto a shaker tray that passes under a UV lamp. The slurry is then filtered, dried and ground, producing vitamin D_2 mushroom powder.

The process conditions of the UV treatment, i.e. energy input and range of wavelength, have been provided by the applicant, after request for additional information from EFSA.

Based on previous EFSA assessments on UV-treated novel foods (EFSA NDA Panel, 2014, 2015, 2016c) that are currently authorised, the Panel considers the specified parameters, including the range of wavelength for the UV treatment, as being similar.

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

3.4. Compositional data

The applicant provided product analysis of six independent batches of the NF (Table 1). The applicant provided the certificate of analysis for each batch.

Table 1: Batch-to-batch analysis of six batches of the NF

Parameter	Batch number and date of manufacture						Method of analysis
	Batch #1	Batch #2	Batch #3	Batch #4	Batch #5	Batch #6	
Vitamin D_2 (μg/g)	1,276.9	1,129.2	1,066.6	1,257.3	1,172.1	1,169.5	HPLC
Moisture (%)	4.39	3.24	5.07	4.77	3.39	4.87	AOAC 964.22
Ash (%)	6.12	4.87	4.80	6.40	6.28	5.71	AOAC 945.46

AOAC: Association of Official Analytical Chemists; HPLC: high-performance liquid chromatography.

The analytical method used by the applicant for vitamin D_2 is validated in-house as well as by sample exchange with two reference laboratories. Vitamin D_2 is quantified using high-performance liquid chromatography (HPLC) with UV detection and [^{3}H]-vitamin D_3 as the internal standard as described in Phillips et al. (2011).

Another form of vitamin D described as vitamin D_4 (22-dihydroergocalciferol) has been observed and confirmed by HPLC in edible mushrooms in a compositional analysis by Phillips et al. (2012). To account for the separation of vitamin D_4 from the D_3 internal standard, modified HPLC conditions as described by Phillips et al. (2012) are applied by the applicant.

Upon an EFSA request, the applicant provided the chromatograms of 6 reported batches of the NF showing the peaks for the different forms of vitamin D (vitamin D_2, vitamin D_3 internal standard and vitamin D_4). The analyses indicated that the concentrations of vitamin D_4 in the NF are at approximately 1/10th the concentration of vitamin D_2.

The physical parameters particle size and appearance indicated that the six analysed batches of the NF are a slightly brown or tan, granular powder that passes through an 80-mesh sieve.

Upon request of EFSA, the applicant provided full compositional information, including nutritional composition, on 5 batches.

The applicant also provided analyses of chemical and microbiological contaminants of 6 independent batches of the NF (Tables 2 and 3). All investigated batches met the specifications (see Section 3.5) regarding microbiological and chemical contaminants.

The chemical contaminants within the investigated batches of the NF, where applicable, were below the limits specified in Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels (MLs) for certain contaminants in foodstuffs.[3] For contaminants with maximum levels in mushrooms (i.e. the source of the NF), specific MLs from the legislation were used (i.e. lead).

The results for arsenic, lead, mercury, and cadmium have been converted by the applicant to equivalent wet weight values based on the moisture content of vitamin D_2 mushroom powder (10%) and of *Agaricus bisporus* mushrooms (approximately 90% (OECD, 2007)).

Table 2: Analysis of chemical contaminants of 6 batches of the NF

Potential chemical contaminants	Batch number and date of manufacture						Method of analysis
	Batch #1	Batch #2	Batch #3	Batch #4	Batch #5	Batch #6	
Arsenic (mg/kg)	0.011*	0.011*	0.011*	0.011*	0.011*	0.011*	USP 730
Lead (mg/kg)	0.006*	0.006*	0.006*	0.006*	0.006*	0.006*	
Mercury (mg/kg)	0.004*	0.004*	0.004*	0.004*	0.004*	0.004*	
Cadmium (mg/kg)	0.003*	0.003*	0.003*	0.003*	0.003*	0.003*	
Aflatoxins (sum of B1, B2, G1 and G2) (μg/kg)	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	AOAC 994.08

AOAC: Association of Official Analytical Chemists; USP: United States Pharmacopeia.

*: Equivalent wet weight values calculated based on a 10% moisture content of vitamin D2 mushroom powder and a moisture content of approximately 90% (OECD, 2007)] for *Agaricus bisporus* mushrooms Calculation = reported value × (1/9).

[3] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006R1881-20180319&from=EN [Consolidated]

The applicant states that screenings for the presence of pesticides in batches of the NF are conducted using internationally accepted methods (EN 15662/Canadian Food Inspection Agency (CFIA) PMR-006).

The applicant conducted a multi-residue pesticide screen on a representative batch of vitamin D2 mushroom powder, and the full results were provided in the certificates of analysis in Annex II of the application. None of the pesticides were detected (detection limit of < 0.005 mg/kg for all compounds, with the exception of 2-(thiocyanomethylthio) benzothiazole (< 0.006 mg/kg), folpet (< 0.012 mg/kg) and fenamiphos sulfone (< 0.013 mg/kg)). The Panel notes that those levels are below the EU maximum residue levels for pesticides.[4]

Microbiological analyses for vitamin D_2 mushroom powder are presented in Table 3.

Table 3: Microbiological analysis of six batches of the NF

Microbiological contaminants	Batch number and date of manufacture						Method of analysis
	Batch #1	Batch #2	Batch #3	Batch #4	Batch #5	Batch #6	
Standard plate count (CFU/g)	100	< 100	600	< 100	< 100	< 100	FDA (BAM) CH. 3
Yeast (CFU/g)	< 10	< 10	< 10	< 10	< 10	10	FDA (BAM) CH. 18
Mould (CFU/g)	40	30	50	20	20	60	FDA (BAM) CH. 18
Salmonella spp.	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	FDA (BAM) CH. 5
Staphylococcus aureus (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	FDA (BAM) CH. 12
Escherichia coli (CFU/g)	< 10	< 3	< 3	< 3	< 3	< 3	FDA (BAM) CH. 4
Coliforms (CFU/g)	< 10	< 3	< 3	< 3	< 3	< 3	FDA (BAM) CH. 4
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	FDA (BAM) CH. 29
Listeria monocytogenes	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	AOAC 2004.06

AOAC: Association of Official Analytical Chemists; CFU: colony forming units; NT: not tested; FDA (BAM): United States Food and Drug Administration (Bacteriological Analytical Manual).

The conversion of ergosterol into vitamin D_2 with UV exposure is accompanied by photochemical isomerisations resulting in photoisomers such as lumisterol and tachysterol (Havinga et al., 1960). Both lumisterol and tachysterol are biologically inactive and known to be formed in the course of the UV-induced conversion of epidermal 7-dehydrocholesterol into vitamin D_3 (Holick et al., 1981).

The applicant, therefore, performed analyses to investigate the potential formation of these sterols under the conditions of the UV treatment of the mushroom powder. The results of HPLC showed that, apart from the intended vitamin D_2, both tachysterol and lumisterol were detected. The applicant provided information on three representative batches of the NF (UV-treated batches) and three representative batches of the NF not exposed to UV treatment (untreated batches). The parameters analysed were vitamin D_2, ergosterol and lumisterol at a limit of detection of 0.5 μg/g and tachysterol at a limit of detection of 1 μg/g.

In the UV-treated batches, the mean vitamin D_2 content was 1,500 μg/g and the mean ergosterol content was 8,747 μg/g. The untreated batches reported a mean vitamin D_2 content of 0.72 μg/g and a mean ergosterol content of 9,223 μg/g. The mean contents of the photoisomers in UV-treated batches were lumisterol at concentrations of 206 μg/g of NF and tachysterol at 111 μg/g of NF.

The applicant indicated a content of vitamin D from the NF of 2.25 μg/100 g in food products other than beverages that would result in intakes of lumisterol and tachysterol of 0.0005 μg/100 g and 0.0003 μg/100 g, respectively.

The NDA Panel noted that the tachysterol concentrations are lower than those reported in previous EFSA Scientific Opinions on UV-treated novel foods (EFSA NDA Panel, 2014, 2015, 2016c). The exposure to these compounds from the NF can therefore be considered not to be of toxicological relevance.

[4] Regulation (EC) No 396/2005 of the European Parliament and of the Council of February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1.

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

3.4.1. Stability

The applicant performed stability tests with five batches of the NF (stability of the bulk powder). The tests were carried out at representative storage conditions (room temperature in sealed polypropylene bags). The batches were analysed for vitamin D_2 content. The results demonstrated that the vitamin D_2 content of the mushroom powder is stable for at least 3 years (intended shelf-life of 2 years).

The applicant also provided stability studies of vitamin D_2 mushroom powder as an ingredient in foods. The NF was used in the production process of granola bars. Granola bars with and without the NF were stored at room temperature for up to 3 months. The batches were analysed for vitamin D_2 content. A minor reduction of vitamin D_2 was identified over time. The applicant noted that vitamin D is a liposoluble vitamin and degradation over time due to oxidation is reported in the literature (EVM, 2003). The granola bar samples with the added NF showed no difference in taste in comparison with the controls.

The applicant also tested the stability of the NF as an ingredient in soya drink. Results showed variability (increase and decrease in vitamin D_2 concentrations) and were therefore considered as not suitable for the risk assessment.

The Panel considers that vitamin D_2 from the NF is not expected to behave differently from vitamin D_2 from other production process.

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

3.5. Specifications

The applicant describes the NF ingredient, i.e. vitamin D_2 mushroom powder, as a slightly brown or tan granular powder made from homogenised *Agaricus bisporus* mushrooms that have been exposed to UV light.

The specifications for vitamin D_2 mushroom powder as proposed by the applicant are reported in Table 4. These specifications include physical and chemical parameters as well as chemical and biological contaminants.

Upon request of EFSA, the applicant provided a maximum specification limit for vitamin D_2 and clarified the non-inclusion of the photoisomers tachysterol and lumisterol in the specifications (based on the very low concentrations in the final product and low exposure through the NF, see Section 3.4).

Table 4: Specifications for vitamin D_2 mushroom powder

	Specification limit	Method
Chemical parameters		
Vitamin D_2	1,000–1,300 μg/g[(a)]	HPLC
Moisture	≤ 10.0%	AOAC 964.22
Ash	≤ 13.5%	AOAC 945.46
Physical parameters		
Particle size	100% through 80 mesh sieve	–
Appearance	Slightly brown or tan, granular powder	–
Microbiological parameters		
Standard plate count	5,000 CFU/g	FDA (BAM) CH. 3
Yeast	100 CFU/g	FDA (BAM) CH. 18
Mould	100 CFU/g	FDA (BAM) CH. 18
Salmonella	Absent in 25 g	FDA (BAM) CH. 5
Staphylococcus aureus	< 10 CFU/g	FDA (BAM) CH. 12
Escherichia coli	< 10 CFU/g	FDA (BAM) CH. 4
Coliform	< 10 CFU/g	FDA (BAM) CH. 4
Enterobacteriaceae	< 10 CFU/g	FDA (BAM) CH. 29
Listeria monocytogenes	Absent in 25 g	AOAC 2004.06

	Specification limit	Method
Mycotoxins		
Aflatoxins (sum of B1+B2+G1+G2)	< 4 μg/kg	AOAC 991.08
Heavy metals		
Arsenic	≤ 0.3 mg/kg	USP 730
Lead (as Pb)	≤ 0.5 mg/kg	USP 730
Mercury	≤ 0.1 mg/kg	USP 730
Cadmium	< 0.5 mg/kg	USP 730
Arsenic	≤ 0.3 mg/kg	USP 730

AOAC: Association of Official Analytical Chemists; CFU: colony forming units; FDA (BAM): United States Food and Drug Administration (Bacteriological Analytical Manual); HPLC : high-performance liquid chromatography; USP: United States Pharmacopeia; –: no information available.
(a): Converted from International Units (IU) using the conversion factor of 0.025 μg = 1 IU stated in the EFSA Technical Report on Dietary Reference Values for nutrients (EFSA, 2017).

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

The source of the NF is the mushroom *Agaricus bisporus.* The applicant listed several publications describing the history of the consumption, cultivation and production for human consumption of these mushrooms within and outside the EU (FAO, 2004; OECD, 2007; FSAI, 2017).

In addition, according to the applicant, UV-treated *Agaricus bisporus* mushrooms have a history of use in the EU (as they have been approved as a novel food ingredient since 2016) and in several non-EU countries, including the United States, Canada and Australia (FSAI, 2017).

3.6.2. History of use of the NF

The NF, i.e. the powder from UV-treated *Agaricus bisporus* mushrooms, has no history of use in the EU.

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The target population for the consumption of the NF added to foods and beverages is the general population.

The target population for the consumption of the NF added to foods for special medical purposes (FSMPs) as defined in Regulation (EU) No 609/2013[5] is individuals above 1 year of age.

The target population proposed by the applicant for the consumption of the NF added to food supplements is individuals above 7 months of age.

3.7.2. Proposed uses and use levels

The applicant intends to use the NF as an ingredient in a variety of foods and beverages as indicated in Tables 5 and 6, in foods for special medical purposes as defined in Regulation (EU) No 609/2013 (excluding those intended for infants) and in food supplements. The use level of the NF in food or beverages is determined by the applicant based on the vitamin D_2 content per gram of NF (1,000–1,300 μg vitamin D_2/gram NF). The proposed levels of vitamin D_2 from the NF are 2.25 μg/100 g for products other than beverages (excluding food supplements) and 1.125 μg/100 mL for beverages (i.e. using the minimum specification for vitamin D content in the NF of 1,000 μg vitamin D2/gram NF).

[5] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009 Text with EEA relevance, OJ L 181, 29.6.2013, p. 35–56.

The maximum proposed level of vitamin D_2 as consumed using the maximum specification for vitamin D content in the NF (i.e. 1,300 μg vitamin D_2/gram NF) would result in an intake of 2.93 μg of vitamin D_2/100 g in foods and 1.46 μg of vitamin D_2/100 mL for beverages.

The applicant also intends to market the NF as an ingredient in food supplement. In their initial proposal, the applicant proposed to market the NF as food supplement at the maximum daily intake of vitamin D_2 of 15 μg for the general population excluding infants and children. Upon question from EFSA, the applicant modified the use levels and proposed 15 μg/day for individuals above 1 year and 10 μg/day in food supplements for infants from 7 to 11 months. The applicant based those use levels on the adequate intakes (AIs) for vitamin D specified by EFSA (EFSA, 2017). According to the applicant, the NF used in food supplement would typically be taken as an alternative supplement replacing other vitamin D_2-containing supplements on the market.

The applicant also intends to market the NF as an ingredient in FSMPs (excluding those intended for infants). For the adult population, the maximum intended use level in FSMPs is 15 μg vitamin D_2/day.

Table 5: Proposed uses and use levels for the NF in the EU

Food groups	Proposed food category name	Proposed maximum use level of the NF (mg/100 g or 100 mL), as consumed[(a)]
Breakfast cereals	Breakfast cereals	2.25
Bakery products	Yeast-leavened bread and pastries	2.25
Processed cereal food	Grain products and pastas	2.25
Fruit juice and fruit/vegetable blend beverages	Fruit and vegetable juices (incl. powders and concentrates)	1.125
Dairy products	Milk and dairy products (excl. fluid milks)	2.25/1.125 (beverages)
Cheese and cheese products	Cheese (excl. cottage cheese, ricotta cheese, and hard-grating cheeses)	2.25
Meal replacement	Meal replacement bars and beverages, not intended for weight control	2.25/1.125 (beverages)
Dairy analogues	Dairy Imitates	2.25/1.125 (beverages)
Meat imitates	Meat imitates	2.25
Soups and broths	Soups (RTE and dry mixtures)	2.25
Vegetable snacks	Extruded vegetable snacks	2.25

RTE: ready to eat.
(a): Proposed maximum use level of the NF per 100 g or 100 mL of each food category, considering the minimum specification for vitamin D content in the NF of 1,000 μg vitamin D2/gram of NF.

Table 6: Proposed food categories based on the FoodEx2 classification system and use levels for the NF in the EU

FoodEx2 code	FoodEx2 level	FoodEx2 name	Proposed maximum use level of the NF (mg/100 g or 100 mL), as consumed[(a)]
A00CV	2	Breakfast cereals	2.25
A0BY0	3	Leavened bread and similar	2.25
A00BK	3	Yeast leavened pastry	2.25
A000K	2	Cereals and cereal primary derivatives	2.25
A007D	3	Pasta and similar products	2.25
A040M	3	Pastas and rice (or other cereal) – based dishes	2.25
A0BX9	2	Fruit/vegetable juices and nectars	1.125
A03BM	2	Concentrated or dehydrated fruit/vegetables juices	7.875[(b)]
A02LR	2	Milk and dairy products	2.25
A02MZ	3	Fermented milk or cream	2.25
A02NA	3	Sour cream products	2.25

FoodEx2 code	FoodEx2 level	FoodEx2 name	Proposed maximum use level of the NF (mg/100 g or 100 mL), as consumed[(a)]
A0C69	4	Fermented milk products	2.25
A02NE	4	Yoghurt	2.25
A02NQ	4	Yoghurt drinks	1.125
A02NR	4	Probiotic milk-like drinks	1.125
A0C69	4	Fermented milk products	2.25
A02NT	5	Traditional sour milk products	2.25
A02NV	5	Kefir	1.125
A02NY	5	Traditional Nordic fermented milks	1.125
A02PC	5	Flavoured traditional sour milk products	2.25
A02PD	3	Milk and dairy powders and concentrates	2.25
A02PE	3	Milk and dairy concentrate	2.25
A02PH	4	Milk and dairy powders	22.5[(c)]
A02PJ	4	Milk powder	22.5[(c)]
A02PM	4	Cream powder	92.25[(d)]
A02PN	4	Whey powder	22.5[(c)]
A02MP	4	Flavoured milks	1.125
A02MK	3	Cream and cream products	2.25
A0EZB	3	Whey	2.25
A02MV	3	Buttermilk	2.25
A02PT	2	Dairy dessert and similar	2.25
A02QE	3	Cheese	2.25
A02QF	4	Fresh uncured cheese	2.25
A02QH	4	Mascarpone	2.25
A02QJ	4	Mozzarella	2.25
A02QK	4	Quark	2.25
AS04NV	4	Miscellaneous fresh uncured cheeses	2.25
A0CRN	4	Cheese curd	2.25
A02RA	4	Brined cheese (feta-type and similar)	2.25
A02RB	4	Soft brined cheese (feta-type)	2.25
A02RG	4	Ripened cheese	2.25
A02RH	4	Soft – ripened cheese	2.25
A031A	3	Processed cheese and spreads	2.25
A03RS	4	Food for weight reduction[(e)]	2.25[(f)]
A03RV	4	Single meal replacement for weight reduction	2.25
A03TH	4	Milk imitates	1.125
A03TQ	4	Dairy imitates other than milks	2.25
A0BXC	4	Dairy imitates	2.25
A03TE	2	Meat imitates	2.25
A0B9J	3	Soups, dry mixture, uncooked	20.25[(g)]
A041L	3	Soups, ready-to-eat	2.25
A0EQV	4	Vegetable snacks, puffs/curls-type extruded snack	2.25
A011L	5	Potato crisps or sticks	2.25

NF: novel food.

(a): The use level of vitamin D_2 mushroom powder is determined based on maximum 1,000 μg vitamin D2/gram of mushroom powder.

(b): Adjusted for being present in concentrated or dehydrated form; reconstitution factor of 7 (based on available products on the market).

(c): Adjusted for being present in powder form; reconstitution factor of 10 (based on available products on the market).

(d): Adjusted for being present in powder form – assumed to be used as coffee whitener; reconstitution factor of 41 (based on available products on the market).
(e): While the food use name includes the term 'for weight reduction', these are representative of use in the general population and not intended to be used for special dietary purposes.
(f): Unable to distinguish between food products and beverages. Therefore, the higher use level (i.e., 2.25 for food products) was applied in this category.
(g): Adjusted for being present in powder form; reconstitution factor of 9 (based on available products on the market).

3.7.3. Anticipated intake of the NF

Anticipated intake of the NF from the proposed food uses, as an ingredient in foods and beverages

The applicant provided estimates of the anticipated daily intake of the NF for each food category indicated in Table 6, based on the summary statistics from the EFSA Comprehensive European Food Consumption Database.[6]

EFSA performed an additional intake assessment of the NF based on the individual consumption data from the EFSA Comprehensive Food Consumption Database (EFSA, 2011). For this estimation, EFSA considered the food categories and the maximum use levels proposed by the applicant in Table 6.

The ranges of the estimated daily intake of the NF (i.e. lowest and highest means and 95th percentiles among surveys covered by the EFSA Database), from foods to which the NF is added, are presented in Table 7 (on a mg/kg body weight (bw) per day basis). The estimated daily intake of the NF for each population group from each EU dietary survey is available in the excel file annexed to this scientific opinion (under supporting information).

Table 7: Anticipated daily intake of the NF on a **mg/kg bw per day basis**: lowest and highest means and 95th percentiles anticipated daily intake of the NF among the EU surveys in the EFSA Comprehensive European Food Consumption Database (calculated by EFSA)

Population group	Number of EU surveys	Estimated intake of the NF as food/beverage ingredient (mg/kg bw per day)	
		Range of means (minimum and maximum) across EU dietary surveys	Range of 95th percentile (minimum and maximum) across EU dietary surveys
Infants (≤ 11 months)	11	0.06–0.52	0.29–1.87
Young children[7] (12–35 months)	14	0.34–0.67	0.65–1.28
Children (3–9 years)	19	0.26–0.50	0.49–0.97
Adolescents (10–17 years)	18	0.13–0.22	0.26–0.45
Adults (18–64 years)	19	0.08–0.15	0.15–0.31
Pregnant women	2	0.10–0.12	0.18–0.21
Lactating women	2	0.12–0.14	0.22–0.24
Elderly (65–74 years)	18	0.07–0.14	0.13–0.32
Very elderly (≥ 75 years)	14	0.07–0.14	0.13–0.27

bw: body weight; NF: novel food.

As stated in Section 3.5 on Specifications, the NF contains 1,000–1,300 μg vitamin D_2/g NF. Therefore, based on estimated daily intakes of the NF and using a maximum content of 1,300 μg vitamin D_2/g NF, estimated daily intakes of vitamin D_2 calculated both in absolute values (μg/day) and on a per body weight basis (μg/kg bw per day) are reported in Table 8.

[6] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database
[7] Referred as toddlers in the EFSA food consumption comprehensive database (EFSA, 2011).

Table 8: Anticipated P95 of daily intake of the vitamin D_2 from the NF as ingredient in foods and beverages considering a vitamin D_2 concentration in the NF of 1,300 μg/g (calculated by EFSA)

Population class	Anticipated P95 daily intake of vitamin D_2 from the NF as ingredient in foods and beverages	
	(μg/kg bw per day)	(μg/day)[a]
Infants (≤ 11 months)	2.43	12.2
Young children (12– 35 months)	1.66	19.9
Other children (3–9 years)	1.26	28.9
Adolescents (10–17 years)	0.59	25.3
Adults (18–64 years)	0.40	28.1
Pregnant women	0.27	19.2
Lactating women	0.31	21.9
Elderly (65–74 years)	0.42	29.2
Very elderly (≥ 75 years)	0.36	25.0

bw: body weight; NF: novel food.
(a): Based on default body weights as given for the different age groups as described in (EFSA Scientific Committee, 2012).

The Panel notes that the intake estimates for vitamin D_2 from the foods added with the NF are based on conservative assumptions, which are that the NF always contains the upper specification limit of vitamin D (i.e. 1,300 μg/g NF) and that all proposed food items consumed by an individual actually contain the NF at the maximum proposed use levels.

3.7.4. Combined vitamin D intake from the NF and other sources

The potential combined intake of vitamin D from the NF (vitamin D_2) and other sources (vitamin D_2 or D_3) is estimated by summing the contribution to vitamin D intake from the NF as estimated by EFSA (Table 8) and the high vitamin D intakes from other food sources as reported by the EFSA NDA Panel in 2012 based on a literature review (EFSA NDA Panel, 2012).

In this Opinion from 2012, the highest 95th percentile (P95) dietary intake across surveys in adults was 16 μg vitamin D/day. The P95 exposure from the background diet alone was not available for all children in the EFSA opinion from 2012, and as a substitute, the highest mean intakes across the covered surveys for each age category of children were used. The highest mean intakes were 5.6 μg/day in younger children (1–5 years), 2.7 μg/day in older children (4–13 years) and 4.0 μg/day in adolescents (11–18 years). The P95 intakes from the sum of food and supplements were however available for children (up to 15 μg/day) and adolescents (up to 8 μg/day) in this previous opinion of the EFSA NDA Panel (2012) and were also used in the present calculations, but without it to the intake of vitamin D_2 from the NF as food supplement.

Table 9 provides an overview of the exposure to vitamin D from different sources separately and combined, and a comparison of the total estimated intake with the tolerable upper intake levels (ULs) established for young children, children, adolescents and adults.

Table 9: Estimated daily intakes of vitamin D (μg/day) from the different sources and combined intake of vitamin D compared with ULs per population group > 1 year

Population group	1) Intake of vitamin D from foods in the background diet (P95 intake for adults and highest mean intakes for children and adolescents) EFSA NDA Panel (2012)	2) Highest P95 estimated intake of vitamin D_2 from the NF as an ingredient in foods and beverage (from Table 8)	3) Intake of vitamin D_2 from the NF as food supplements	4) Combined intake of vitamin D from 1+2+3 (background diet, NF as ingredient, NF as supplement if applicable)	UL (μg/day) EFSA NDA Panel (2012)
Young children (12–35 months)	5.6[(a)] 15[(b)]	19.9 19.9	15 –	40.5 34.9[(c)]	50
Other children (3–9 years)	2.7[(a)] 15[(b)]	28.9 28.9	15 –	46.6 43.9[(c)]	50
Adolescents (10–17 years)	4[(a)] 8[(b)]	25.3 25.3	15 –	44.3 33.3[(c)]	100
Adults (18–64 years)	16	28.1	15	59.1	100
Pregnant women	16	19.2	15	50.2	100
Lactating women	16	21.9	15	52.9	100
Elderly (65–74 years)	16	29.2	15	60.2	100
Very elderly (≥ 75 years)	16	25.0	15	56.0	100

UL: tolerable upper intake level; NF: novel food.

(a): Maximum mean/median intake of vitamin D from foods only. Data collected from different surveys/studies (EFSA NDA Panel, 2012).

(b): Combined vitamin D intake from foods and supplements; vitamin D intake from high consumers (90th or 95th percentile depending on surveys) in infants, children and adolescents (EFSA NDA Panel, 2012).

(c): Dietary intake of vitamin D included in foods and food supplements (EFSA NDA Panel, 2012). In order to avoid overestimation of vitamin D intake, the maximum intake of vitamin D from the total diet (combined intake, column 5) does not include the contribution of the vitamin D from the NF used as ingredient in food supplements.

The Panel notes that estimates for combined intake of vitamin D_2 from the NF (added to the foods, beverages and food supplements) plus estimated intake of vitamin D from the background diet result in overall vitamin D intakes of 44, 59, 60 and 56 μg/day for adolescents, adults, elderly and very elderly, respectively.

For pregnant and lactating women, such combined intake estimates result in values of 50 and 53 μg/day, respectively.

Those intake estimates are below the UL of 100 μg/day (EFSA NDA Panel, 2012) for each of these population groups.

In children, estimating the combined intake of vitamin D_2 from the NF (19.9 and 28.9 μg/day for young children and other children, respectively) plus intake of vitamin D from other dietary sources (including 15 μg/day from the NF when used in food supplements) results in vitamin D intakes of 40 and 47 μg/day for young children and other children, respectively. The Panel notes that those estimated combined intakes are below the upper level (UL) established by EFSA for children aged 1–10 years (50 μg/day) (EFSA NDA Panel, 2012).

For infants aged from 4 to 12 months, data on vitamin D intake were estimated by EFSA using composition data from the EFSA nutrient composition database and individual consumption data from national surveys from 6 European countries (EFSA NDA Panel, 2018). In addition to the vitamin D intake provided by infant formula (IF) or follow-on formula (FoF), the vitamin D intake from

complementary feeding was considered, including foods naturally containing vitamin D and foods fortified with vitamin D, but intake of vitamin D via supplements was not considered.

For this age group, P95 intakes for vitamin D ranged across the surveys from 13.2 to 16.9 μg/day in formula consumers[8] not consuming (voluntarily) fortified foods. For non-formula consumers that were also not consuming (voluntarily) fortified foods, the P95 vitamin D intake from the diet ranged between 0.7 and 2.8 μg/day (EFSA NDA Panel, 2018).

For formula consumers[8] consuming also fortified foods, the P95 vitamin D intake ranged from 15.2 to 22.2 μg/day. For non-formula consumers, the P95 intake from diet including fortified foods ranged from 1.6 to 10 μg/day (based on scenario 6 from Annex B of EFSA NDA Panel, 2018).

For infants, the estimated P95 intake of vitamin D_2 from the NF as an ingredient in foods is 12.2 μg/day (see Table 8). The addition of this amount to the highest P95 vitamin D intake of formula consumers not consuming fortified foods (16.9 μg/day) results in a combined intake of 29.1 μg/day (for comparison, the highest P95 intake of vitamin D in formula consumers consuming also fortified foods was 22.2 μg/day, according to EFSA, 2018). This estimated combined intake of 29.1 μg/day can be considered an overestimation as highest formula consumers can be assumed not to be also highest consumers of fortified foods including foods with the added NF. The Panel notes that this estimated combined intake of 29.1 μg/day is below the UL of 35 μg/day for infants aged 6 to less than 12 months established by EFSA (EFSA NDA Panel, 2018).

The addition of 10 μg/day of vitamin D_2 from the NF used as an ingredient in supplement (which the applicant intends to market for infants aged from 7 to 12 months) to the (most likely overestimated) intake of 29.1 μg/day would result in an intake of 39.1 μg/day and thus would exceed the UL.

When adding 10 μg/day to the previously estimated intake of 22.2 μg/day (as calculated by EFSA NDA Panel, 2018), the combined estimated intake of vitamin D from formula, fortified foods and supplements containing the NF amounts to 32.2 μg vitamin D/day and thus would be below the UL for vitamin D of 35 μg/day established by EFSA in 2018.

3.7.5. Estimate of exposure to undesirable substances in mushrooms

The undesirable toxicological substances in mushrooms have been described by the Consensus document on compositional considerations for new varieties of the cultivated mushrooms *Agaricus bisporus* (OECD, 2007). Agaritine (β-*N*-[γ-L-(+)-glutamyl]-4-hydroxymethylphenyl-hydrazine) is a natural occurring phenylhydrazine derivative found in mushrooms. Agaritine ranges were reported by OECD between 80 and 1,730 mg/kg fresh weight of mushrooms (OECD, 2007).

As explained in Section 3.3 Production process, the NF is produced from the fresh cultivated *Agaricus bisporus* mushrooms. Based on the NF production yield from fresh mushroom and on the highest estimated intakes provided by the applicant, and considering that a food portion size of mushrooms is approximately 70 g (FSA, 2002), the estimated maximum additional intake due to the NF would correspond to less than 1.3% of a single serving of mushrooms.

As reported in Section 3.4 Compositional data, the UV processing inducing the conversion of ergosterol to vitamin D_2 is accompanied by isomerisations resulting in tachysterol and lumisterol. The applicant indicated a maximum use level of the NF of 2.25 μg/100 g for food products other than beverages that would result in intakes of lumisterol and tachysterol of 0.0005 μg/100 g and 0.0003 μg/100 g, respectively. The Panel also noted that the tachysterol concentrations are lower than those reported in previous EFSA Scientific Opinions on UV-treated novel foods, which did not give rise to safety concerns (EFSA NDA Panel 2014, 2015, 2016c).

The Panel considers that there is no concern with respect to the exposure to undesirable substances from mushrooms (i.e. agaritine) and from the NF production process (lumisterol and tachysterol) from the consumption of NF at the proposed uses.

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

The applicant has not provided specific ADME studies for the NF, but has submitted publicly available animal and human studies that suggest that vitamin D_2 from powder from UV-irradiated mushrooms is bioavailable, as dose-related increase in serum concentrations of 25(OH)D_2 are observed upon its oral consumption. Some of these studies have been carried out with material similar to the NF

[8] Containing maximum regulated vitamin D content (2.5 μg/100 kcal for IF; 3.0 μg/100 kcal for FoF), in accordance with Commission Delegated Regulation (EU)2019/828 amending Delegated Regulation (EU) 2016/127.

(i.e. powder from UV-radiated *Agaricus bisporus* mushrooms). These studies (Bennett et al., 2013; Calvo et al., 2013; Koyyalamudi et al., 2009; Stephensen et al., 2012; Shanley et al., 2014; Stepien et al., 2013) are presented in Appendices A and B.

Results from a meta-analysis on randomised clinical trials (RCTs) with UV-exposed and vitamin D_2-enriched mushrooms suggest an effect of their consumption on serum total 25(OH)D concentration when the mean baseline vitamin D status of the participants is inadequate [serum 25(OH)D < 50 nmol/L], but no effect when vitamin D status is 'high' [mean baseline 25(OH)D: 81.5 nmol/L]], due to a reduction in serum 25(OH)D_3 that accompanies the increase in serum 25(OH)D_2 (Cashman et al., 2016).

Studies comparing the effects of vitamin D_2 and D_3 supplementation on serum 25(OH)D concentrations in a small number of infants, showed no statistically significant differences between the effects of the two vitamin forms (Gordon et al., 2008; Gallo et al., 2013).

3.9. Nutritional information

The applicant refers to a publication by Simon et al. (2011) where the vitamin content of *Agaricus bisporus* mushrooms exposed to UV light was investigated. In this study, the nutritional content of the mushrooms (including macronutrients, fatty acids, amino acids, water-soluble vitamins) remained unchanged by the UV treatment, with the exception of the intended increase in the vitamin D_2 content.

The Panel notes that the intakes of vitamin D_2 in high consumers of foods and beverages fortified with the NF are above the AIs for all age groups set by the NDA Panel of EFSA (EFSA NDA Panel, 2016b). However, together with the intake of vitamin D from background diet and possible intake of the NF supplement (see Section 3.7.4), the total daily vitamin D intakes do not exceed the ULs set for children aged 1–10 years (50 μg/day), adolescents and adults (100 μg/day) (EFSA NDA Panel, 2012).

For infants, the Panel notes that, based on the intake assessment for vitamin D in infants (EFSA NDA Panel, 2018), combined highest P95 intakes of vitamin D from background diet, including IF and/or FoF and fortified foods and foods in which the NF would be added (see Section 3.7.4), do not exceed the ULs set for infants aged 0–6 months at 25 μg/day and for infants 6–12 months at 35 μg/day. With regard to infants aged ≥ 7 to 12 months, the Panel notes that the additional intake of 10 μg/day of vitamin D_2 from the NF used as an ingredient in food supplements, in high consumers of vitamin D (from background diet, including IF and/or FoF and fortified foods), would result in a total vitamin D intake of 32 μg/day, close to, but not exceeding the UL of 35 μg/day.

Given the assumption that infants who are high consumers of IF and/or FoF may also be high consumers of foods fortified with the NF, then some infants aged 0–6 months may be at risk of exceeding the UL for vitamin D (25 μg/day). For infants aged 7–12 months, using the (most likely overestimated) intake of 29.1 μg vitamin D/day, the additional intake of 10 μg/day could result in exceeding the UL of 35 μg/day. However, the Panel considers this scenario unlikely as complementary feeding in high consumers of IF and/or FoF may be limited.

Considering that daily oral supplementation of 10 μg vitamin D is generally recommended for all infants during the first year of life starting from birth onwards (ESPGHAN Committee on Nutrition, Braegger et al., 2013 cited in EFSA NDA Panel, 2016b), there is a potential risk of approaching or exceeding the UL for vitamin D in infants. However, this is a general issue related to the combined consumption of vitamin D via fortified foods and supplements and does not specifically concern the NF of this application.

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

3.10. Toxicological information

No specific toxicity studies on the NF have been submitted by the applicant. For previous scientific opinions on similar NFs (on UV-treated baker's yeast, bread or milk), no toxicological studies were considered necessary (EFSA NDA Panel, 2014, 2015, 2016c).

3.10.1. Genotoxicity

No specific studies on genotoxicity of the NF were provided. The Panel notes that given the source, nature, and the intended use of the NF, genotoxicity studies are not required.

3.10.2. Subacute, subchronic and chronic toxicity

No specific studies with the NF have been carried out, but several publicly available animal studies with material similar to the NF have been submitted by the applicant. The studies in which powder from UV-treated *Agaricus bisporus* was tested are summarised in Appendix A (subacute and chronic toxicity studies).

3.10.3. Carcinogenicity

No studies on carcinogenicity were provided. The Panel notes that given the source, nature, and the intended use of the NF carcinogenicity studies are not required.

3.10.4. Reproductive and developmental toxicity

No studies on reproductive and developmental toxicity were provided. The Panel notes that given the source, nature, and the intended use of the NF reproductive and developmental toxicity studies are not required.

3.10.5. Human data

No specific studies on the NF were provided, but the applicant provided several publicly available human intervention studies where material similar to the NF has been tested. The studies in which powder from UV-treated *Agaricus bisporus* was investigated are summarised in Appendix B.

The studies presented in Annex B show that consumption of powder from UV-treated mushrooms from 4 to 6 weeks, significantly increases serum concentrations of 25(OH)D_2 and total 25(OH)D in humans, while no other changes in blood parameters or adverse effects (see Appendix B for individual parameters) were observed. The doses of vitamin D_2 (contained in the mushroom powders) given ranged between 15 and 17.1 μg/day. The Panel notes that the estimated 'worst case' vitamin D_2 intakes in humans, combining intake of vitamin D_2 from the NF together with other dietary sources (see Section 3.7.4 'Combined vitamin D intake from the NF and other sources' of this opinion), were higher (ranging from 40.5 to 60.2 μg/day in the different age groups) than these doses. Therefore, the human studies presented are of limited value for the assessment of potential effects on human health of the NF.

3.11. Allergenicity

The applicant refers to the conclusion by the Food Safety Authority of Ireland (FSAI) in 2017 regarding UV-treated mushrooms approved as novel food, i.e. that 'allergenicity or other food hypersensitivities associated with *Agaricus bisporus* are rare and there is no reason to believe that the additional UV treatment will alter that risk' (FSAI, 2017). The Panel concurs with this view.

4. Discussion

The NF is produced from *Agaricus bisporus* mushrooms that have been exposed to UV light to induce the conversion of provitamin D_2 (ergosterol) to vitamin D_2 (ergocalciferol). The NF contains levels of vitamin D provided by vitamin D_2 in the range of 1,000–1,300 μg/g.

The information provided on the manufacturing process, composition and specifications of the NF does not raise safety concerns.

The applicant intends to add the NF in a variety of foods and beverages, including food for special medical purposes (excluding those intended for infants) and food supplements. The target population is the general population except for food supplements, for which the target population is individuals above seven months of age.

The conservative highest vitamin D estimates for combined intake of vitamin D_2 from the NF together with intake from other dietary sources were below the upper levels for vitamin D as established previously by the NDA Panel for 'Young children' and 'Other children', 'Adolescents', 'Adults', 'Pregnant women', 'Lactating women', 'Elderly' and 'Very elderly' (NDA Panel, 2012) and 'Infants' (NDA Panel, 2018).

The Panel notes that vitamin D intake in high consumers of IF and or FoF with the maximum regulated content of vitamin D consuming also fortified foods as calculated by EFSA (NDA Panel, 2018) does not result in exceeding the ULs set for infants aged 0–6 months or ≥ 7–12 months.

Given the assumption that infants aged 0–6 months who are high consumers of IF and/or FoF may also be high consumers of foods fortified with the NF, some infants may be at risk of exceeding the UL for vitamin D (25 μg/day). For infants aged 7–12 months, using the (most likely overestimated) intake of

29.1 μg vitamin D/day, the additional intake of 10 μg/day could result in exceeding the UL of 35 μg/day. However, the Panel considers this scenario unlikely as complementary feeding in high consumers of IF and/or FoF may be limited.

Considering that daily oral supplementation of 10 μg vitamin D is generally recommended for all infants during the first year of life starting from birth onwards (ESPGHAN Committee on Nutrition, Braegger et al., 2013 cited in EFSA NDA Panel, 2016b), there is a potential risk of approaching or exceeding the UL for vitamin D in infants. However, this is a general issue related to the combined consumption of vitamin D via fortified foods and supplements and does not specifically concern the NF of this application.

5. Conclusions

The Panel concludes that the NF, vitamin D_2 mushroom powder, is safe under the proposed conditions of use. The target population is the general population, except for food supplements for which the target population is individuals above 7 months of age.

The Panel concludes that the NF, used as an ingredient, is safe for the general population at the proposed condition of use in foods and beverages.

The Panel concludes that the NF is safe for the adult population at intake levels up to 15 μg vitamin D_2/day used in foods for special medical purposes.

The Panel concludes that the NF, used as a food supplement, is safe for individuals above 1 year at a level up to 15 μg vitamin D_2/day.

The Panel, however, notes that the UL for infants may be exceeded in high consumers of IF and/or FoF that may also be high consumers of foods fortified with the NF, and infants aged 7–12 months consuming the food supplement containing 10 μg of vitamin D. However, the Panel considers this scenario unlikely as complementary feeding in high consumers of IF and/or FoF may be limited.

Considering that daily oral supplementation of 10 μg vitamin D is generally recommended for all infants during the first year of life starting from birth onwards (ESPGHAN Committee on Nutrition, Braegger et al., 2013 cited in EFSA NDA Panel, 2016b), there is a potential risk of approaching or exceeding the UL for vitamin D in infants. However, this is a general issue related to the combined consumption of vitamin D via fortified foods and supplements and does not specifically concern the NF of this application.

The Panel could not have reached the conclusions on the safety of the NF under the proposed conditions of use without the following data claimed as proprietary by the applicant:

- Annex I: Raw materials and processing aids
- Annex II : Certificates of Analysis and batch data
- Annex III: Stability reports.

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 vitamin D_2 mushroom powder as a NF. Ref. Ares (2018)5350698, dated 18 October 2018.
2) On 18 October 2018, EFSA received a valid application from the European Commission on vitamin D_2 mushroom powder as NF, which was submitted by Oakshire Naturals, LP, and the scientific evaluation procedure started.
3) On 30 January, 4 June and 28 October 2019, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 4 April, 21 August, 12 and 26 November 2019, additional information was provided by the applicant and the scientific evaluation was restarted.
5) During its meeting on 28 November 2019, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of vitamin D_2 mushroom powder as a novel food pursuant to Regulation (EU) 2015/2283.

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Abbreviations

$24,25(OH)_2D_3$	24,25-hydroxycholecalciferol
$25(OH)D_2$	25-hydroxyvitamin D_2
$25(OH)D_3$	25-hydroxyvitamin D_3
ADME	absorption, distribution, metabolism and excretion
AOAC	Association of Official Analytical Chemists
AI	adequate intake
AST	aspartate aminotransferase
BMI	body mass index
BP	blood pressure
bw	body weight
CAS	Chemicals Abstracts Service
CPK	creatine phosphokinase
FoF	follow-on formula
FSAI	Food safety Authority of Ireland
FSMA	Food Safety Modernization Act
GAP	Good Agricultural Practices
HACCP	Hazard Analysis Critical Control Points
HDL	high-density lipoprotein
HPLC	high-performance liquid chromatography
hsCRP	high-sensitivity C-reactive protein
IF	Infant formula
IL-6	interleukin 6
iPTH	intact parathyroid hormone
IU	International Units
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
MB	myoglobin
ML	maximum levels
ND	not detected
NDA	Nutrition, Novel Foods and Food Allergens
NEFA	non-esterified fatty acids
NF	novel food

NFI	novel food ingredient
OECD	Organisation for Economic Cooperation and Development
PAI-1	plasminogen activator inhibitor-1
PTH	parathyroid hormone
RCT	randomised clinical trial
TAG	triacylglycerol
TNF-α	tumour necrosis factor-α
UL	tolerable upper intake level
USP	United States Pharmacopeia
UV	ultraviolet

Appendix A – Subacute and chronic toxicity animal studies with powder of UV-radiated *Agaricus bisporus*

Species (strain), sex; number of animals	Route of administration/ duration	Test item and dose groups	Parameters evaluated	Results	Reference
Mouse (transgenic APPswe/PS1dE9 and wild-type); M; 10–13 per group	Oral (diet); 7 months	Wild-type groups: control (basal diet); 5% UV-C-irradiated *Agaricus bisporus* mushroom powder (54 IU vitamin D_2/kg or 1.35 μg vitamin D_2/kg feed) Transgenic groups: control (basal diet); 5% UV-C-irradiated *Agaricus bisporus* mushroom powder(54 IU vitamin D_2/kg or 1.35 μg vitamin D_2/kg feed). These feed concentrations correspond to a dose of around 0.18 μg vitamin D_2/kg bw per day[(a)]	Clinical signs, bw, Morris water maze, Barnes maze, Y-maze, liver function biomarkers (plasma total protein, albumin, globulin), cholesterol, calcium, 25(OH)D_2, 25(OH)D_3, β-amyloid load (Aβ40, Aβ42) in plasma and brain, immunohistochemistry (brain)	No reported clinical signs or differences in bw gain between groups. No differences in liver function biomarkers or cholesterol between groups. ↑ 25(OH)D_2 and 25(OH)D_3, ↑learning and memory and ↓ β-amyloid plaque load in brain of treated animals	Bennett et al. (2013)
Rat (SD); F; 6 per group	Oral (diet); 10 weeks	Control (basal diet); control (vitamin D-deficient diet); Control (non-irradiated *Agaricus bisporus* mushroom powder); 2.5 or 5% UV-B-irradiated *Agaricus bisporus* mushroom powder corresponding to 300 or 600 IU or 7.5 or 15 μg vitamin D_2/day. These correspond to doses of around 37.5 or 75 μg vitamin D_2/kg bw per day[(a)]	Bw, femur density, plasma 25(OH)D_2 and histopathology (kidney, liver, and spleen)	No reported clinical signs or differences in bw between groups. ↑ 25(OH)D_2 in treated animals No test item-related histopathological findings	Calvo et al. (2013)
Rat (SD); M; 5 per group	Oral (gavage); 3 weeks	Control (non-irradiated *Agaricus bisporus* mushrooms) 50, 100 or 200 mg/kg bw per day UV-C-irradiated *Agaricus bisporus* mushroom powder. At a concentration of 17.6 μg vitamin D_2/g (as reported in the paper), this corresponds to doses of 0.88, 1.76 and 3.52 μg vitamin D_2/kg bw per day	bw, food intake and plasma 25(OH)D_2	No reported clinical signs or effects on bw or food intake.↑ 25(OH)D_2 in animals given vitamin D_2 mushroom powder (all doses)	Koyyalamudi et al. (2009)

25(OH)D_2: 25-hydroxyvitamin D_2; 25(OH)D_3: 25-hydroxyvitamin D_3; bw: body weight; F: females; IU: International Units; M: males; SD: Sprague–Dawley; UV: ultraviolet.
(a): Because doses were not reported, concentrations in feed were converted to doses following EFSA guidance (EFSA Scientific Committee, 2012).

Appendix B – Human intervention studies with powder of UV-radiated *Agaricus bisporus*

Study design	Duration	Tested material	Dosage	Parameters investigated	Results	Reference
Double-blinded, randomised, placebo-controlled trial; 38 healthy adults (9–10 per group)	6 weeks	UV-B-irradiated *Agaricus bisporus* mushroom powder (10–25 μg/serving)	Placebo control: untreated mushrooms providing 34 IU (or 0.85 μg) vitamin D_2/day; Test group 1: UV-B-irradiated mushroom powder (352 IU (8.8 μg) vitamin D_2/day); Test Group 2: UV-B-irradiated mushroom powder (684 IU (17.1 μg) vitamin D_2/day); Supplement Group: purified ergocalciferol + untreated mushrooms (total of 1,128 IU (28.2 μg) vitamin D_2/day)	Serum 25(OH)D_2, 25(OH)D_3; 24,25(OH)2D_3	No reported adverse effects. ↑ 25(OH)D2 and ↓ 25 (OH)D_3 in Test groups 1, 2 and supplement group	Stephensen et al. (2012)
Double-blinded, randomised, placebo-controlled trial; 33 vitamin D insufficient highschool athletes (16–17 per group)	6 weeks	UV-B-irradiated-*Agaricus bisporus* mushroom powder	Placebo control: Untreated mushroom powder (ND vitamin D_2); Test Group: UV-B-irradiated mushroom powder (600 IU (15 μg) vitamin D_2/day)	Serum total 25(OH)D, 25 (OH)D_2, 25(OH)D_3, LDH, AST, CPK, MB	No reported adverse effects. ↑ 25(OH)D_2, ↓ 25 (OH)D_3 and ↑ total 25(OH)D in test group No effects on the blood parameters investigated	Shanely et al. (2014)
Double-blinded, randomised, placebo-controlled trial 90 healthy adults (22 per group)	4 weeks	UV-B-irradiated *Agaricus bisporus* mushroom powder	Study 1: Placebo control: Untreated mushroom powder (ND vitamin D_2) Test Group: UV-irradiated mushroom powder (600 IU (15 μg) vitamin D_2/day) Study 2: Placebo control: placebo capsule (ND vitamin D_3); Supplement Group: vitamin D_3 capsule (600 IU (15 μg) vitamin D_3/day)	Bw, height, BMI, BP (systolic and diastolic) Serum total 25(OH)D, 25 (OH)D_2, 25(OH)D_3, glucose, insulin, HR, HOMA-IR, C-peptide, TAG, cholesterol (total, HDL, and LDL), NEFA, PTH, calcium, adiponectin, ferritin, leptin, resistin, sCRP, TNF-α, IL-6, PAI-1	No reported adverse effects. ↑ 25(OH)D_2 in test group and ↑ 25 (OH)D3 in vitamin D_3 supplement group No effects on the blood parameters investigated	Stepien et al. (2013)

24,25(OH)$_2$D$_3$: 24,25-hydroxycholecalciferol; 25(OH)D_2: 25-hydroxyvitamin D_2; 25(OH)D_3: 25-hydroxyvitamin D_3; AST: aspartate aminotransferase; BMI: body mass index; BP: blood pressure; bw: body weight; CPK: creatine phosphokinase; HDL: high-density lipoprotein; hsCRP: high-sensitivity C-reactive protein; IL-6: interleukin 6; iPTH: intact parathyroid hormone; IU: International Units; LDH: lactate dehydrogenase; LDL: low-density lipoprotein; MB: myoglobin; ND: not detected; NEFA: non-esterified fatty acids; PAI-1: plasminogen activator inhibitor-1; PTH: parathyroid hormone; TAG: triacylglycerol; TNF-α: tumour necrosis factor-α; UV: ultraviolet.