Safety of vitamin d2 mushroom powder (agaricus bisporus) as a novel food pursuant to regulation (eu) 2015/2283

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Safety of Vitamin D_2 mushroom powder (*Agaricus bisporus*) 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* mushroom powder that has been exposed to ultraviolet (UV) irradiation 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 580–595 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 and Foods for Special Medical Purposes (FSMPs), for which the target population is individuals above 1 year 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.

Keywords: novel food, safety, *Agaricus bisporus* mushroom powder, ingredient, food supplement, UV, vitamin D_2

Requestor: European Commission

Question number: EFSA-Q-2019-00550

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1. Introduction

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

On 29 July 2019, the company MBio, Monaghan Mushrooms submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to authorise placing on the Union market of mushroom powder (*Agaricus bisporus*) as a novel food.

The application requests to authorise use of mushroom powder (*Agaricus bisporus*) in a number of food categories.

The applicant has also requested data protection under Article 26 of Regulation (EU) 2015/2283.

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 mushroom powder (*Agaricus bisporus*) as a novel food.

1.2. Additional information

Ultraviolet (UV) irradiation technique to enhance the content of vitamin D has been used in some foods making the resulting foods as novel. The novel foods (NFs) evaluated by EFSA are: 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, 2016b). All of them are currently under the Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods.

In November 2019, the EFSA NDA Panel adopted an opinion on mushroom powder produced from *Agaricus bisporus* mushroom exposed to UV irradiation as NF (EFSA NDA Panel, 2020). The NF is authorised by Commission Implementing Regulation (EU) 2020/1163[2].

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.

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[3].

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, 2016c). 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 includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise:

- Production Process: 2.3.1 Production Process Confidential_Final
- Compositional Data: Annex 1 Particle Size Report, Annex 3 NIZO Report physico-chemical properties, Annex 4 COA vitamin D analysis, Annex 5 COA nutritional analysis, Annex 7 MBio SOP Vitamin D2 analysis, Annex 8 MBio Vit D analysis validation report, Annex 9 Stability Study Report UCC, Annex 14 COA Vit D stability study, Annex 16 COA Toxicological analysis, Annex 17 Report Tachysterol and lumisterol, Annex 20 COA Ergosterol ratio analysis, Annex 21 COA Vitamin D ratio analysis, Annex 22 MBio Ergosterol, Annex 24 Stability study Report CampdenBRI, Annex 25 Stability study report meat free product, Annex 29 COAs Stability Meat free

[1] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 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) 2020/1163 of 6 August 2020 authorising the placing on the market of vitamin D2 mushroom powder as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470. OJ L 258, 7.8.2020, p. 1–5.

[3] 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.

- Specifications: Annex 13 COA fresh mushrooms analysis
- Allergenicity: Annex 12 MBio Allergen Policy

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016c) 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 the 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 the NF 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 from *Agaricus bisporus*. The NF falls under the category (ii) of article 3 of the NF Regulation 2015/2283, 'food consisting of, isolated from or produced from microorganisms, fungi or algae'.

The NF contains vitamin D_2 in the range of 580–595 μg/g.

The NF is proposed to be used as an ingredient in foods and beverages for consumption by the general population. The NF is also intended to be used in Foods for Special Medical Purposes (FSMPs) and as a food supplement for individuals above 1 year old.

3.2. Identity of the NF

The NF is a whole fruiting body mushroom powder containing vitamin D_2 (ergocalciferol) induced by UV treatment. Vitamin D_2's registered CAS number is 50-14-6 and its IUPAC (3β,5Z,7E,22E)-9,10-secoergosta-5,7,10(19),22-tetraen-3-ol. The molecular formula of vitamin D_2 is $C_{28}H_{44}O$ and the molecular weight is 396.66 g/mol.

According to the applicant, this vitamin D_2 whole mushroom powder is a 100% food grade powder obtained from the dried whole fruiting body of *Agaricus bisporus.* The mushroom powder includes the whole white closed cup mushrooms which contain stalk component or unusually shaped mushrooms that have been sliced, dried, milled and UV irradiated to induce the conversion of provitamin D_2 (ergosterol) to vitamin D_2 (ergocalciferol). Provitamin D_2 is naturally found in fresh and dried mushrooms and the conversion to vitamin D_2 occurs when the mushrooms are exposed to sunlight (Cardwell et al., 2018). However, since the commercial mushrooms are cultivated in the dark, this reaction rarely occurs. UV irradiation converts the provitamin D_2 into vitamin D_2.

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 white button mushroom, champignon de Paris, cremini, chestnut mushrooms and, when fully developed, Portobello mushroom.

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.

The mushrooms used in the production of vitamin D_2 mushroom powder are cultivated *Agaricus bisporus.* The process includes drying, milling and the controlled exposure of the mushroom powder to UV irradiation.

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

3.4. Compositional data

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with the required characteristics, the applicant provided analytical information for five independent batches of the NF (Tables 1 and 2).

The applicant provided a certificate of analysis for each batch.

Table 1 includes information about the vitamin D_2 concentrations, moisture, ash and proximate composition of the NF. Table 2 includes information about the main chemical and microbiological parameters included in the batch to batch analysis of the NF.

Table 1: Batch to batch analysis of the NF

Parameter (unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Vitamin D_2 (μg/g)	591	580	595	593	589	HPLC-PDA[(a)] (In-house method)
Moisture (g/100 g)	4.27	4.19	4.11	4.51	4.25	Gravimetry method
Ash (g/100 g)	9.1	9.2	9.4	9.4	9.2	Gravimetry method
Proximates						
Crude protein (g/100 g)	30.5	30.9	30.5	30.6	30.5	Dumas (N × 6.25)
Total fat (g/100 g)	3.2	3.5	2.8	2.6	3.6	Acid hydrolysis and gravimetry
Carbohydrates (available) g/100 g	34.3	33.3	34.3	33.3	32.3	Calculation
Total dietary fibre g/100 g	18.7	18.8	18.9	19.6	20.2	Enzymatic-gravimetry (AOAC 991.43)

(a): HPLC-PDA: high-performance liquid chromatography-photodiode array.

Table 2: Chemical and microbiological parameters in batch to batch analysis of the NF

Parameter (unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Heavy metals						
Lead (mg/kg)	0.007	0.008	0.008	0.008	0.008	ICP-MS[(b)]
Cadmium (mg/kg)	0.17	0.174	0.186	0.169	0.186	ICP-MS
Arsenic (mg/kg)	0.17	0.175	0.193	0.175	0.191	ICP-MS
Mercury (mg/kg)	0.018	0.017	0.02	0.05	0.02	ICP-MS
Process contaminants						
Acrylamide (μg/kg)	200	240	220	240	230	LC-MS/MS[(c)]
Poly aromatic hydrocarbons (PAH)						
Benzo(a)pyrene (μg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	GC-MS/MS[(d)]
Sum of PAH4 (μg/kg)[(a)]	0.11	0.16	0.12	0.15	0.12	GC-MS/MS
Mycotoxins						
Ochratoxin A (OTA (μg/kg)	< 2	< 2	< 2	< 2	< 2	IAC-LC-FLD[(e)]
Aflatoxin B1 (μg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	IAC-LC-FLD
Aflatoxin B2 (μg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	IAC-LC-FLD
Aflatoxin G1 (μg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	IAC-LC-FLD
Aflatoxin G2 (μg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	IAC-LC-FLD
Sum of aflatoxins (B1,B2,G1,G2) (μg/kg)	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	IAC-LC-FLD
Microbiological parameters						
Total Viable count (TVC) (CFU/g)	20	30	< 10	30	20	In-house method validated based on ISO 4833-1:2013
Escherichia coli (CFU/g)	< 10	< 10	< 10	< 10	< 10	In-house method validated based on ISO 16649-2
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	< 10	In-house method validated based on ISO 21528-2
Salmonella species (in 25 g)	n.d	n.d	n.d	n.d	n.d	In-house method validated based on ISO 6579-1

Parameter (unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Listeria species (in 25 g)	n.d	n.d	n.d	n.d	n.d	In-house method validated based on ISO 11290-1
Yeast and moulds (CFU/g)	< 10	< 10	< 10	< 10	< 10	In-house method validated based on ISO 6611:2004

n.d: not detected; CFU: colony forming units.
(a): benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene (upper bound (UB); LOQ for individual PAHs 0.1 μg/Kg).
(b): ICP-MS: inductively coupled plasma mass spectrometry.
(c): LC-MS/MS: liquid chromatography-tandem mass spectrometry.
(d): GC-MS/MS: Gas Chromatography-tandem mass spectrometry.
(e): IAC-LC-FLD: Immunoaffinity extraction column/clean-up-Liquid chromatography fluorescence detector.

In addition to the chemical contaminants described in Table 2, a multiresidue pesticides analysis was performed by the applicant (results not reported). The Panel notes that the pesticides levels reported are below the EU maximum residue levels for pesticides in fungi.[4]

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 also in humans in the course of the UV-induced conversion of epidermal 7-dehydrocholesterol into vitamin D_3 (Holick et al., 1981). Upon an EFSA request, the applicant provided data on vitamin D photoisomers formed during the production process.

The concentrations of tachysterol and lumisterol are within the range of other UV-treated NF (EFSA NDA Panel, 2014, 2015, 2016b, 2020).

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

The Panel considers that the information provided on the composition is sufficient for characterising the NF.

3.4.1. Stability

The applicant performed stability tests with five independently produced batches of the NF. The tests were carried out at representative storage conditions (20°C) and accelerated conditions at 50°C and at < 60% RH for a period of 6 months.

The batches were analysed for vitamin D_2, microbiological contaminants (yeast and moulds, *E. coli*, *Salmonella*, coliforms and total viable count) and colour changes.

The stability tests indicated an average loss of vitamin D of 18% at 20°C and 30% at 50°C in the five batches of the NF. The microbiological stability was confirmed (all parameters below the microbiological specifications). Regarding colour changes, no significant change in colour was observed at the end of storage.

Upon EFSA's request, the applicant also provided stability studies of the NF as an ingredient in foods. Stability studies were performed on a bread (Pitta bread for 14 days) and a meat replacer (meat-free patty for 7 days). The vitamin D_2 concentration was stable over the storage period.

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

3.5. Specifications

The NF is 100% mushroom powder derived from the processing of fresh whole *Agaricus bisporus* mushrooms.

The specifications of the NF are indicated in Table 3.

[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.

Table 3: Specifications of the NF

Parameter	Specification
Chemical parameters	
Ash	≤ 13.5%
Water activity	< 0.5
Moisture content	≤ 7.5%
Carbohydrate	≤ 35.0%
Total Dietary Fibre	≥ 15%
Crude protein (N × 6.25)	≥ 22%
Fat	≤ 4.5%
Vitamin D_2	580–595 μg/g
Heavy metals	
Lead	< 0.5 mg/kg
Cadmium	< 0.5 mg/kg
Mercury	< 0.1 mg/kg
Arsenic	< 0.3 mg/kg
Mycotoxins	
Aflatoxin B1	≤ 0.10 μg/kg
Aflatoxins (sum of B1 + B2 + G1 + G2)	< 4 μg/kg
Microbiological	
TVC	1×10^4 CFU/g[(a)]
TYMC	< 100 CFU/g
E. coli	< 10 CFU/g
Salmonella spp.	Not detected in 25 g
Listeria spp.	Not detected in 25 g
Enterobacteriaceae	< 10 CFU/g

TVC: Total Viable Count; TYMC: total yeast and mould count; CFU: colony forming units.
(a): The Panel notes that lower specification values can be met for TVC.

The applicant proposes a specification limit for TVC as $< 1 \times 10^4$ CFU/g. The Panel notes that considering the NF production process (thermal drying) and compositional analyses of five batches (showing consistently values < 30 CFU/g) a lower specification limit could be met. A similar NF is authorised by Commission Implementing Regulation (EU) 2020/1163[2], where the microbiological criteria for total plate count are ≤ 5,000 CFU/g.

The Panel notes that TVC is an indicator of hygiene and considers that this quality parameter ultimately also contributes to the safety of an NF.

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 indicated several publications describing the history of the consumption and cultivation and production for human consumption of these mushrooms (Filipov, 1998 as cited in FAO, 2004; Peintner et al., 2013) as well as data for consumption of commercially cultivated *Agaricus bisporus* within and outside the EU (Cardwell et al., 2018).

The history of UV processing to increase vitamin D content has been also addressed by the applicant (OECD, 2007).

3.6.2. History of use of the NF

There is no history of use of the NF.

A vitamin D_2 mushroom powder with a different production process and different specifications is already included in the list of NFs with a maximum concentration of 2.25 μg of vitamin D_2/100 g for several food categories and a maximum of 15 μg vitamin D_2/day for Foods for Special Medical Purposes (FSMPs) as defined under Regulation (EU) No 609/2013 excluding those intended for infants and food supplements as defined in Directive 2002/46/EC intended for the general population excluding infants.

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The target population proposed by the applicant 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 FSMPs as defined in Regulation (EU) No 609/2013 is individuals above 1 year of age.

The target population for the consumption of the NF added to food supplements is individuals above 1 year old.

3.7.2. Proposed uses and use levels

The NF is proposed to be used as an ingredient in several food products. These food products, defined using the FoodEx2 hierarchy, and the maximum use levels are reported in Table 4.

The maximum proposed levels of the NF as an ingredient proposed by the applicant are 3.58 mg/100 g for products other than beverages (excluding food supplements) and 1.79 mg/100 mL for beverages. These conditions of use would correspond to levels of 2.1 μg of vitamin D_2/100 g for products other than beverages and 1.1 μg of vitamin D_2/100 mL for beverages. The applicant also intends to market the NF for use in food supplements and foods for medical special purposes, at a maximum dose of 15 μg of vitamin D_2/day.

Table 4: Food categories and maximum NF use levels intended by the applicant

FoodEx2 level	FoodEx2 code	Food category	Max use level proposed by the applicant (mg NF/100 g)	Corresponding levels of vitamin D_2 (μg/100 g or 100 mL)[a]
2	A00CV	Breakfast cereals	3.58	2.1
3	A0BY0	Leavened bread and similar	3.58	2.1
3	A00BK	Yeast leavened pastry	3.58	2.1
2	A000K	Cereals and cereal primary derivatives	3.58	2.1
3	A007D	Pasta and similar products	3.58	2.1
3	A040M	Pastas and rice (or other cereal) – based dishes	3.58	2.1
2	A0BX9	Fruit/vegetable juices and nectars	1.79	1.1
2	A03BM	Concentrated or dehydrated fruit/vegetable juices	12.52[b]	7.4
2	A02LR	Milk and dairy products	3.58	2.1
3	A02MZ	Fermented milk or cream	3.58	2.1
3	A02NA	Sour cream products	3.58	2.1
4	A02NE	Yoghurt	3.58	2.1
4	A02NQ	Yoghurt drinks	1.79	1.1
4	A02NR	Probiotic milk-like drinks	1.79	1.1
4	A0C69	Fermented milk products	3.58	2.1
5	A02NT	Traditional sour milk products	3.58	2.1
5	A02NV	Kefir	1.79	1.1
5	A02NY	Traditional Nordic fermented milks	1.79	1.1
5	A02PC	Flavoured traditional sour milk products	3.58	2.1
3	A02PD	Milk and dairy powders and concentrates	3.58	2.1

FoodEx2 level	FoodEx2 code	Food category	Max use level proposed by the applicant (mg NF/100 g)	Corresponding levels of vitamin D_2 (µg/100 g or 100 mL)[a]
3	A02PE	Milk and dairy concentrate	3.58	2.1
4	A02PH	Milk and dairy powders	35.78[c]	21.3
4	A02PJ	Milk powder	35.78[c]	21.3
4	A02PM	Cream powder	146.68[d]	87.3
4	A02PN	Whey powder	35.78[c]	21.3
4	A02MP	Flavoured milks	1.79	1.1
3	A02MK	Cream and cream products	3.58	2.1
3	A0EZB	Whey	3.58	2.1
3	A02MV	Buttermilk	3.58	2.1
2	A02PT	Dairy dessert and similar	3.58	2.1
3	A02QE	Cheese	3.58	2.1
4	A02QF	Fresh uncured cheese	3.58	2.1
4	A02QH	Mascarpone	3.58	2.1
4	A02QJ	Mozzarella	3.58	2.1
4	A02QK	Quark	3.58	2.1
4	A04NV	Miscellaneous fresh uncured cheeses	3.58	2.1
4	A0CRN	Cheese curd	3.58	2.1
4	A02RA	Brined cheese (feta-type and similar)	3.58	2.1
4	A02RB	Soft brined cheese (feta-type)	3.58	2.1
4	A02RG	Ripened cheese	3.58	2.1
4	A02RH	Soft-ripened cheese	3.58	2.1
3	A031A	Processed cheese and spreads	3.58	2.1
4	A03RS	Food for weight reduction	3.58	2.1
4	A03RV	Single meal replacement for weight reduction	3.58	2.1
4	A03TH	Milk imitates	1.79	1.1
4	A03TQ	Dairy imitates other than milks	3.58	2.1
4	A0BXC	Dairy imitates	3.58	2.1
2	A03TE	Meat imitates	3.58	2.1
3	A0B9J	Soups, dry mixture, uncooked	32.2[e]	19.2
3	A041L	Soups, ready-to-eat	3.58	2.1
4	A0EQV	Puffs/curls-type extruded snack	3.58	2.1
5	A011L	Potato crisps or sticks	3.58	2.1

(a): The maximum specification for vitamin D content in vitamin D_2 mushroom powder of 595 µg vitamin D_2/gram of mushroom powder is used.
(b): Adjusted for being present in concentrated or dehydrated form; reconstitution factor of 7.
(c): Adjusted for being present in powder form; reconstitution factor of 10.
(d): Adjusted for being present in powder form – assumed to be used as coffee whitener; reconstitution factor of 41.
(e): Adjusted for being present in powder form; reconstitution factor of 9.

3.7.3. Anticipated intake of the NF

EFSA performed an assessment of the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 4), using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The estimated lowest and highest mean and 95th percentile daily intake of the NF among the EU dietary surveys are presented in Tables 5 and 6, expressed as mg/kg body weight (bw) per day and mg per day, respectively.

The estimated daily intake of the NF for each population group from each EU dietary survey is available in the Excel files annexed to this scientific opinion (under supporting information).

Table 5: Estimated intake of the NF based on its use as an ingredient in the intended food categories at the maximum proposed use levels (mg NF/kg bw per day)

Population group	Age (years)	Mean intake (mg/kg bw per day)		P95th intake (mg/kg bw per day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	0.06	0.83	0.34	2.98
Young children[(c)]	1–< 3	0.40	1.07	0.84	1.97
Other children	3–< 10	0.35	0.77	0.67	1.34
Adolescents	10–< 18	0.15	0.34	0.32	0.62
Adults[(d)]	≥ 18	0.20	0.23	0.35	0.51

(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 04/12/2020. The lowest and the highest mean intake observed among all available EU surveys are reported in these columns. The data relate to a period in which UK was still a Union Member State.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 4/12/2020. The lowest and the highest P95th intake observed among all EU surveys are reported in these columns (P95th based on less than 60 individuals are not considered). The data relate to a period in which UK was still a Union Member State.
(c): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant and lactating women.

Table 6: Estimated intake of the NF based on its use as an ingredient in the intended food categories at the maximum proposed use levels (mg NF/day)

Population group	Age (years)	Mean intake (mg/day)		P95th intake (mg/day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	0.56	5.69	3.00	21.36
Young children[(c)]	1–< 3	4.83	14.52	9.89	21.00
Other children	3–< 10	8.47	17.60	14.64	28.66
Adolescents	10–< 18	8.12	15.90	16.47	34.45
Adults[(d)]	≥ 18	12.58	17.24	22.33	34.45

(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 4/12/2020. The lowest and the highest averages observed among all EU surveys are reported in these columns. The data relate to a period in which UK was still a Union Member State.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 4/12/2020. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on less than 60 individuals are not considered). The data relate to a period in which UK was still a Union Member State.
(c): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant and lactating women.

As stated in Section 3.6 on Specifications, the NF contains 580–595 μg vitamin D_2/gram NF. Therefore, based on the highest (P95) estimated daily intakes of the NF and using a maximum content of 595 μg vitamin D_2/g NF, the highest P95 estimated daily intakes of vitamin D_2 calculated both in absolute values per day (μg/day) and on a per body weight basis (μg/kg bw per day) are reported in Table 7.

Table 7: Estimated highest P95 of daily intake of vitamin D_2 from the NF as ingredient in foods and beverages considering a vitamin D_2 concentration in the NF of 595 μg/g (calculated by EFSA)

Population group	Age (years)	Vitamin D_2 P95th intake	
		(μg/kg bw per day)	(μg/day)
Infants	< 1	1.77	12.71
Young children[(a)]	1–< 3	1.17	12.49
Other children	3–< 10	0.80	17.05

Population group	Age (years)	Vitamin D_2 P95th intake	
		(μg/kg bw per day)	(μg/day)
Adolescents	10–< 18	0.37	20.50
Adults[b]	≥ 18	0.30	20.50

(a): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(b): Includes elderly, very elderly, pregnant and lactating women.

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 up the contribution to vitamin D intake from the NF as estimated by EFSA (Table 7) 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 considering the intake of vitamin D_2 from the NF as food supplement.

Table 8 provides an overview of the exposure to vitamin D from different sources separately and combined, and the tolerable upper intake levels (ULs) established for young children, children, adolescents and adults.

Table 8: Total vitamin D intake (μg/day) resulting from the uses of the NF as an ingredient and as a food supplement

Population group	Intake of vitamin D from EFSA NDA Panel (2012) (P95th intake for adults and highest mean intakes for children and adolescents)	Highest P95th vitamin D_2 intake from the NF used as an ingredient	Intake of vitamin D_2 from the NF used as a food supplement	Total intake[d]	UL (μg/day) EFSA NDA Panel (2012)
Young children	5.6[a] 15[b]	12.49	15 –	33.09 27.49[c]	50
Other children	2.7[a] 15[b]	17.05	15 –	34.75 32.05[c]	50
Adolescents[e]	4[a] 8[b]	20.50	15 –	39.50 28.50[c]	100
Adults	16	20.50[f]	15	51.50	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) does not include the contribution of the vitamin D from the NF used as food supplements.
(d): Total intake is the estimate of vitamin D calculated as the sum of vitamin D intake from the background diet (EFSA NDA Panel (2012), from NF ingredient used in food (highest P95th) and from the NF used as a food supplement, for each population group.
(e): If food supplement for all adolescents: Intakes are assessed separately for young [10–14 years] and older adolescents [14–18 years]; the maximum intake among these two subpopulations is reported here.
(f): Intakes are assessed separately for adults [18–65 years], elderly [65–75 years] and very elderly [≥ 75 years]; the maximum intake among these three subpopulations is reported here.

For infants (4–12 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 six 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 not consuming (voluntarily) fortified foods. For non-formula consumers who 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, 2020).

For formula consumers 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 maximum P95 intake of vitamin D_2 from the NF as an ingredient in foods is 12.7 μg/day (see Table 7). The addition of this amount to the highest P95 vitamin D intake of formula consumers not consuming fortified foods (16.9 μg/g per day) results in a combined intake of 29.6 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 NDA Panel, 2018). This estimated combined intake of 29.6 μg/day can be considered an overestimate, as highest formula consumers can be assumed not to be also highest consumers of fortified foods including foods with the added NF (EFSA NDA Panel, 2018, 2020).

3.7.5. Estimate of exposure to undesirable substances

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 (b-N-[c-L-(+)-glutamyl]-4-hydroxymethylphenyl-hydrazine) is a natural occurring phenylhydrazine derivative found in mushrooms, including *Agaricus bisporus*. Agaritine concentrations range in *Agaricus bisporus* between 80 and 1,730 mg/kg fresh weight of mushrooms (OECD, 2007). Mushroom processing at high temperatures was found to reduce the levels of agaritine by 20–75% (Roupas et al., 2010). Urbain et al. (2016) investigated the effects of UV on agaritine content in mushrooms and concluded that it is not influenced by the UV processing.

The applicant did not perform studies to investigate the levels of agaritine in the NF. The applicant referred to the thermal instability of agaritine, the fresh UV-treated *Agaricus bisporus* mushrooms approved as NF under regulation (EC) No 258/97, indicating significantly lower ($p < 0.05$) agaritine levels for UV-treated mushrooms (340.89 mg/kg ± 28.79) than for non-treated mushrooms (373.63 mg/kg ± 20.46) and the low proposed use level of the NF. The Panel noted that based on the highest estimated NF intake (34.45 mg/day), a moisture in fresh mushroom of 92% and a portion size of mushrooms of ~ 70 g (FSA, 2002), the estimated maximum additional mushroom intake from the NF would correspond to ~ 0.6% of a single mushroom serving.

The Panel considers that there is no concern with respect to the exposure to undesirable substances from the consumption of NF at the proposed uses.

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

No specific ADME studies for the NF have been provided. The applicant performed a literature search on mushroom vitamin D_2 and supplemental vitamin D_2 and vitamin D_3.

The publicly available human studies suggest that vitamin D_2 from powder from UV-irradiated mushrooms is bioavailable, and dose-related increases in serum concentrations of 25(OH)D2 were observed upon oral consumption (EFSA NDA Panel, 2020).

A systematic review and meta-analysis showed that bolus doses of vitamin D_3 (> 125 μg/day) were more effective in raising total serum 25(OH)D concentrations in adults compared with equivalent vitamin D_2 doses (Tripkovic et al., 2012). In another study from the same author (Tripkovic et al., 2017), a 12-week randomised controlled trial (RCT) conducted in healthy South Asian and European women, both vitamin D_3 and D_2 at doses of 15 μg/day were effective in raising serum 25(OH)D concentrations during wintertime; however, vitamin D_3 was still more effective than vitamin D_2 (EFSA NDA Panel, 2016a, 2018).

3.9. Nutritional information

The applicant provided a nutritional analysis of the NF. The NF is composed of available carbohydrates (~ 33%), protein (~ 31%), fibre (~ 19%), ash (~ 9%), moisture (~ 4%) and fat (~ 3%) and, in addition, contains vitamins and minerals. Considering the proposed maximum use and intake levels, the Panel considers that the NF contribution of these nutrients to the overall daily intake is low, except for vitamin D.

Effects of the processing on the nutritional content of the NF have been studied by the applicant. The NF manufacturing process of drying, milling and UV exposure did not significantly affect the nutrient content with exception of the final levels of vitamin D_2. These observations are in line with the results provided by Simon et al. (2011) where the vitamin content of *Agaricus bisporus* mushrooms exposed to UV irradiation 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 (EFSA NDA Panel, 2020).

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 maximum vitamin D intakes of 39.5 and 51.5 μg/day for adolescents and adults, respectively. Those intake estimates (as reported in Table 8 of Section 3.7.4 'Combined vitamin D intake from the NF and other sources') are below the UL of 100 μg/day (EFSA NDA Panel, 2012) for each of these population groups.

In children, estimating the combined maximum intake of vitamin D_2 from the NF (12 and 17 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 33 and 35 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).

The Panel notes that the intakes of vitamin D_2 in high consumers of foods and beverages fortified with the NF are above the adequate intakes (AIs) for all age groups set by the EFSA NDA Panel (EFSA NDA Panel, 2016a). 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).

The Panel notes that the combined estimated intake of 29.6 μg/day is below the UL of 35 μg/day for infants aged 6–11 months established by EFSA (EFSA NDA Panel, 2018). However, following the EU supplementation recommendations of 10 μg vitamin D per day in infants (ESPGHAN (European Society for Paediatric Gastroenterology, Hepatology and Nutrition) Committee on Nutrition, Braegger et al., 2013 cited in EFSA NDA Panel, 2016a), there is a potential risk of approaching or exceeding the UL for vitamin D in infants (EFSA NDA Panel, 2018, 2020). This is a general issue related to the combined consumption of vitamin D via fortified foods and supplements and does not specifically relate to this NF application.

Given the fact that the range of foods fortified with vitamin D has increased over the years as well as the marketing of high-dose vitamin D supplements, the Panel also notes uncertainty regarding the calculated combined exposures to vitamin D of the general population (as these were based on data available only up to 2012 for the age groups above 1 year (EFSA NDA Panel, 2012) and up to 2018 for infants (EFSA NDA Panel, 2018)).

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

3.10. Toxicological information

The Panel notes that no toxicological studies with the NF were provided.

Publicly available studies with material similar to the NF (powder of UV-radiated *Agaricus bisporus*) were assessed in the previous NDA Scientific Opinion on vitamin D mushroom powder (EFSA NDA Panel, 2020).

Taking into account the source, nature and the intended use of the NF, the Panel considers that no toxicological studies are required on the NF.

3.10.1. Human data

The applicant did not provide specific human studies on the NF, or similar products, i.e. irradiated powder of *Agaricus bisporus*. Instead, the applicant submitted several publicly available human intervention studies, which investigated the effect on serum 25(OH)D of vitamin D_2 from *Agaricus bisporus*, using either non-irradiated mushrooms and mushroom extract (Bogusz et al., 2013; Keegan et al., 2013), UV-treated mushrooms (Urbain et al., 2011; Stephensen et al., 2012), or lyophilised UV-treated mushrooms (Stepien et al., 2013). In another study, not submitted by the applicant, the test substance was UV-treated powder from *Agaricus bisporus* (Shanely et al., 2014). All studies consistently showed an increase in serum levels of 25(OH)D_2 following the intervention with UV-treated *Agaricus bisporus*, while no adverse effects were reported.

The doses of vitamin D in all the human studies were 15 μg/day. The Panel notes that the estimated combined intake of vitamin D based on the use of the NF in food and supplements is higher (ranging from 27 to 51 μg/day in the different age groups) than the dose tested in these studies. 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 NF has the same composition as dried traditional common button mushrooms (*Agaricus bisporus*) with the exception of the enhanced levels of vitamin D_2. Therefore, the allergenicity risk is not expected to be greater compared to that associated with normal consumption of *Agaricus bisporus* mushrooms.

Additional UV treatment will not alter the risk (EFSA NDA Panel, 2020).

4. Discussion

The NF which is the subject of the application is an *Agaricus bisporus* mushroom powder that has been exposed to UV irradiation to induce the conversion of provitamin D_2 (ergosterol) to vitamin D_2 (ergocalciferol). The NF contains levels of vitamin D in the form of vitamin D_2 in the range of 580–595 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, FSMP and in food supplements. The target population is the general population except for food supplements and FSMP, for which the target population is individuals above 1 year of age.

The conservative highest vitamin D estimates for combined intake of vitamin D_2 from the NF, together with intake of all forms of vitamin D 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', (EFSA NDA Panel, 2012) and 'Infants' (EFSA NDA Panel, 2018).

However, the Panel also notes uncertainty regarding the calculated combined exposures to vitamin D of the general population, given the fact that the range of foods fortified with vitamin D has increased over the years as well as the marketing of high-dose vitamin D supplements.

5. Conclusions

The Panel concludes that the NF, Vitamin D_2 mushroom powder, containing vitamin D_2 in the ranges of 580–595 μg/g, is safe under the proposed conditions of use.

5.1. Protection of Proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant (Production process confidential data and Annexes on Compositional data).

6. Steps taken by EFSA

1) On 24/01/2020 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of mushroom powder (*Agaricus bisporus*) as a novel food. Ref. Ares(2020)464211 – 24/01/2020.

2) On 24/01/2020, a valid application on the safety of vitamin D_2 mushroom powder (*Agaricus bisporus*) as a novel food, which was submitted by MBio, Monaghan Mushrooms, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/1237) and the scientific evaluation procedure was initiated.
3) On 08/05/2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 05/11/2020, additional information was provided by the applicant through the Commission e-submission portal.
5) On 26/11/2020, 23/12/2020 and 15/01/2021, EFSA requested the applicant to provide further clarifications to the additional information provided.
6) On 03/12/2020, 07/01/2021 and 26/01/2021 additional clarifications were provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
7) During its meeting on 24/02/2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of vitamin D_2 mushroom powder (*Agaricus bisporus*) as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
AI	adequate intake
AOAC	Association of Official Analytical Chemists
bw	body weight
CAS	Chemicals Abstracts Service
CFU	colony forming units
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FoF	Follow-on formula
IF	Infant formula
FSMPs	Foods for Special Medical Purposes
GC-MS/MS	Gas chromatography-tandem mass spectrometry
GMP	good manufacturing practice

HACCP	Hazard Analysis Critical Control Points
HPLC	High-performance liquid chromatography
HPLC-PDA	High-performance liquid chromatography-photodiode array
IAC-LC-FLD	Immunoaffinity extraction column/clean-up -Liquid chromatography fluorescence detector
ICP-MS	inductively coupled plasma mass spectrometry
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LOQ	Level of Quantification
NDA	Nutrition, Novel Foods and Food Allergens
NF	Novel Food
OECD	Organization for Economic Cooperation and Development
PAHs	Poly Aromatic Hydrocarbons
RCT	randomised clinical trial
RH	relative humidity
TVC	total viable count
TYMC	total yeast and mould count
UB	Upper Bound, non-detected concentrations are set equal to the LOQ
UL	tolerable upper intake level
UV	ultraviolet

Annex A – Supporting information: estimated daily intake of the NF for each population group from each EU dietary survey

Annex A can be found in the online version of this output ('Supporting information' Section).