Safety of vitamin D-2 mushroom powder as a Novel food pursuant to Regulation (EU) 2015/2283 (NF 2019/1471)

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Safety of vitamin D_2 mushroom powder as a Novel food pursuant to Regulation (EU) 2015/2283 (NF 2019/1471)

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on vitamin D_2 mushroom powder as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is produced from *Agaricus bisporus* mushroom 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 levels of vitamin D in the form of vitamin D_2 in the range of 125–375 μg/g. The information provided on the production process, composition and specifications of the NF does not raise safety concerns. The applicant intends to add the NF as an ingredient in a variety of foods and beverages in amounts that result in either 1.125 or 2.25 μg vitamin D_2 per 100 g or 100 mL of the food as consumed. The applicant also intends to add the NF in food supplements, for infants from 7 to 11 months at a maximum of 10 μg vitamin D_2/day and of 15 μg vitamin D_2/day for individuals aged 1 year or older, as well as in foods for special medical purposes (FSMPs) and total and meal diet replacement for weight control. For the adult population, the maximum intended use level in FSMPs is 15 μg vitamin D_2/day and 5 μg vitamin D_2/meal in total and meal diet replacement for weight control. The Panel concludes that the NF is safe under the proposed conditions of use. The Panel notes uncertainty regarding the calculated combined exposures to vitamin D for 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.

Keywords: Novel Foods, safety, mushroom powder, *Agaricus bisporus*, UV treatment, vitamin D_2, food supplement

Requestor: European Commission

Question number: EFSA-Q-2020-00417

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1. Introduction

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

On 21 February 2020, the company Monterey Mushrooms Inc. submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to authorise the placing on the Union market of vitamin D_2 mushroom powder as a novel food.

The application requests to authorise use of vitamin D_2 mushroom powder in a number of foods.

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 vitamin D_2 mushroom powder as a novel food.

The European Commission asks the European Food Safety Authority to evaluate and inform the Commission as to whether and if so, to what extent, the requirements of Article 26(2)(c) of Regulation (EU) 2015/2283 are fulfilled in elaborating its opinion on vitamin D_2 mushroom powder regarding the proprietary data for which the applicant is requesting data protection.

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 novel. The novel foods evaluated by EFSA are UV-treated baker's yeast (*Saccharomyces cerevisiae*) (EFSA NDA Panel, 2014, 2021b), UV-treated bread (EFSA NDA Panel, 2015) and UV-treated milk (EFSA NDA Panel, 2016c). All of them are currently authorised under Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017, establishing the Union list of novel foods.

In November 2019 and February 2021, the EFSA NDA Panel adopted two opinions on vitamin D mushroom powders produced from *Agaricus bisporus* as novel food (NF) (EFSA NDA Panel, 2020, 2021a). The NFs named 'vitamin D_2 mushroom powder' with different production processes, specifications and conditions of use were authorised and included in the Annex to Implementing Regulation (EU) 2017/2470[2]. Both inclusions were based on proprietary scientific evidence and scientific data protected in accordance with Article 26 of Regulation (EU) 2015/ 2283[1].

2. Data and methodologies

2.1. Data

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

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

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[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, 2016a). As indicated in this guidance, it is the duty of the applicant to provide all 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:

Identity of the NF report, batch analysis information and the respective certificates of analysis; the stability reports and the applicant intake assessment report.

[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 (0.1 L 327. 11.12.2015, p. 1).

[2] Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods.

[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.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016a,b,c) 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.

The legal provisions for the assessment of food for specific groups are laid down in Regulation (EU) 609/2013[4] and, in Commission Delegated Regulation 2017/1798[5] in the case of total diet replacement for weight control, and in Commission Delegated Regulation (EU) 2016/128[6] for food for special medical purposes.

This assessment concerns only the risks that might be associated with the consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed 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 125–375 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 total diet and meal replacement for weight control, Foods for Special Medical Purposes (FSMPs) for individuals above 1 year old and as a food supplement for individuals above 7 months old.

3.2. Identity of the NF

The NF is a whole fruiting body mushroom powder containing vitamin D_2 (ergocalciferol) produced 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.

The source of the NF is the fungus *Agaricus bisporus* as listed in the Index fungorum (https://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. The identity of the NF source has been confirmed by the applicant by comparison to an authentic specimen using high-performance thin-layer chromatography analysis (HPTLC).

3.3. Production process

According to the information provided, the NF is produced in line to good manufacturing practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles.

The raw materials used are *Agaricus bisporus* mushrooms grown and harvested in the US, under controlled environmental conditions.

The NF is produced from the sliced/diced whole fresh *Agaricus bisporus* mushrooms that are transferred into conveyer belts and consequently exposed to controlled UV-B irradiation. After the UV irradiation, the mushrooms are refrigerated and dehydrated. Finally, the dehydrated UV-exposed mushrooms are ground into a fine final mushroom powder. The mushroom powder is afterwards packaged into hermetically sealed bags and stored until use.

The mushroom powder with enhanced vitamin D_2, which is the NF, is a light to dark brown, milled powder with a particle size, shape and distribution of 90% through 80 mesh sieve.

[4] 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. OJ L 181,29.6.2013, p. 35–56.

[5] Commission Delegated Regulation (EU) 2017/1798 of 2 June 2017 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for total diet replacement for weight control OJ L 259, 7.10.2017, p. 2–10.

[6] Commission Delegated Regulation (EU) 2016/128 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for food for special medical purposes. OJ L 25, 2.2.2016, p. 30–43.

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 batches of the NF for vitamin D_2 and moisture concentrations (Table 1).

Table 1: 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)	151.5	169.4	167.0	159.3	182.6	HPLC/DAD
Moisture (%)	< 5	< 5	< 5	< 5	< 5	Gravimetry

HPLC/DAD: high-performance liquid chromatography/diode array detector; NA: not applicable.

The results of the proximate analysis of the NF are presented in Table 2.

Table 2: Proximate batch to batch analysis of the NF

Parameter (Unit)	Batch number					Method of analysis
	#6	#7	#8	#9	#10	
Total fat (g/100 g)	2.92	2.75	2.80	2.80	2.84	GC-FID (AOAC 996.06)
Total carbohydrate (g/100 g)	51.4	52.9	52.2	53.2	47.0	Calculation based on individual sugars measured by internal HPLC/RI
Dietary fibre (g/100 g)	23.4	19.9	20.2	19.9	20.4	Enzymatic-gravimetry (AOAC 991.43)
Protein (F 6.25) (g/100 g)	33.1	32.1	32.4	32.0	36.2	Dumas Combustion method (AOAC 992.23)

AOAC: Association of Official Analytical Chemists; HPLC-RI: High-performance liquid chromatography-refractive index detector; C-FID: Gas chromatography-flame ionisation detector.

The applicant provided analytical information for six independently produced batches of the NF for heavy metals (Table 3) and information for five batches for the microbiological parameters (Table 4).

Table 3: Batch to batch heavy metals analysis of the NF

Parameter (unit)	Batch number						Method of analysis
	#11	#12	#13	#14	#15	#16	
Heavy metals							
Arsenic (mg/kg)	0.23	0.19	0.28	0.29	0.20	0.22	ICP-MS (USP 730)
Cadmium (mg/kg)	0.067	0.061	0.066	0.063	0.050	0.035	
Lead (mg/kg)	0.02	0.02	0.02	0.02	0.04	0.01	
Mercury (mg/kg)	0.027	0.021	0.042	0.035	0.027	0.025	

ICP-MS: Inductively Coupled Plasma-Mass Spectroscopy; USP: United States Pharmacopeia.

Table 4: Batch to batch microbiological analysis of the NF

Parameter (Unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Aerobic plate count (CFU/g)	980	4,700	1,400	400	100	AOAC 966.23
Yeast (CFU/g)	< 10	< 10	< 10	< 10	< 10	Dilution plating technique (FDA-BAM, 7th ed.)
Mould (CFU/g)	30	30	10	10	10	

Parameter (Unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Salmonella (in 25 g)	n.d	n.d	n.d	n.d	n.d	ELFA screening method (AOAC 2004.03)
***Staphylococcus aureus* (in 10 g)**	n.d	N.A	n.d	n.d	n.d	Surface plating (AOAC 975.55)
***Escherichia coli* (in 10 g)**	n.d	< 3/10 g	n.d	n.d	n.d	Enumeration (Current USP/NF, 62)
Coliforms (MPN/g)	43	23	23	93	9.1	Enumeration MPN (AOAC 966.24)
***Listeria* spp. (in 25 g)**	n.d	n.d	n.d	n.d	n.d	ELFA (AOAC 2004.06)

AOAC: Association of Official Analytical Chemists; CFU: colony forming units; MPN = most probable number; n.d: non-detected; NA: not analysed; FDA-BAM: United States Food and Drug Administration Bacteriological Analytical Manual; ELFA: Enzyme-linked fluorescent immunoassay.

Upon an EFSA request, the applicant provided analytical results for six batches for the sum of aflatoxins and aflatoxin B1 (Table 5).

Table 5: Aflatoxins and aflatoxin B1 batch-to-batch analysis of the NF

Parameter (unit)	Batch number						Method of analysis
	#17	#18	#19	#15	#20	#21	
Aflatoxins (sum of B1, B2, G1 and G2) μg/kg (w/w)	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	HPLC/FLD (AOAC 991.31 (Mod.))
Aflatoxin B1 μg/kg (w/w)	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	

HPLC/FLD: high performance liquid chromatography with fluorescence detection.

In addition, a multiresidue pesticide screening was performed by the applicant in one representative batch of the NF. The Panel notes that the pesticide levels reported are below the EU maximum residue levels for pesticides in mushrooms.[7]

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; Wolpowitz and Gilchrest, 2006). The applicant provided data on vitamin D photoisomers formed during the production process in three representative batches of the NF presented in Table 6 below. The results indicate that lumisterol is more abundant than tachysterol. The concentrations of tachysterol and lumisterol are within the range of other UV-treated mushroom powders (EFSA NDA Panel, 2020, 2021a).

Table 6: Lumisterol and tachysterol batch-to-batch analysis of the NF

Parameter (unit)	Batch number			Method of analysis
	#22	#23	#24	
Lumisterol (μg/g)	48.29	32.14	78.55	HPLC- DAD
Tachysterol (μg/g)	4.10	6.93	12.29	

HPLC: High-performance liquid chromatography; DAD: Diode array detector.

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.

[7] 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.

3.4.1. Stability

The applicant performed stability tests with five independently produced batches of the NF. The batches were analysed for vitamin D_2 content and microbiological parameters.

The tests for the vitamin D_2 content were carried out at representative storage conditions: ambient temperature [range = 17–25°C; average = 21°C], low humidity [range = 40–63%; average = 53%], away from sunlight, for up to 4 years (3 years for four batches, 4 years for one batch) (Table 7).

The microbiological stability tests were carried out under the same conditions as for the vitamin D_2 content, with the presence of microorganisms being analysed on day 0 and after 3 years (Table 8). The Panel notes that one batch was above the specifications for aerobic plate counts at time 3 years. The vitamin D_2 concentrations remained within the proposed specification limits and the applicant proposed a shelf-life of at least 3 years.

Table 7: Vitamin D_2 (μg/g) stability in the NF

Batch number	Vitamin D_2 content (μg/g)			Method of analysis
	0 years	3 years	4 years	
#3	167.0	148.6	n.a	HPLC/DAD
#4	159.3	129.6	n.a	
#5	182.6	142.6	n.a	
#25	198.2	n.a	133.6	
#22	190.1	181.7	n.a	

n.a: not analysed; HPLC: High-performance liquid chromatography; DAD: diode array detector.

Table 8: Microbiological stability of the NF

Parameter (unit)	Specifications	Batch number										Method of analysis
		#1		#2		#3		#4		#5		
		Time (years)										
		0	3	0	3	0	3	0	3	0	3	
Aerobic plate count (CFU/g)	< 5,000	980	7,600	4,700	600	1,400	< 100	400	< 100	100	< 100	AOAC 966.23
Yeast (CFU/g)	< 100	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	FDA-BAM, 7th ed.
Mould (CFU/g)	< 100	30	30	30	10	10	10	10	< 10	10	< 10	FDA-BAM, 7th ed.
***Salmonella* (in 25 g)**	Negative in 25 g	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	AOAC 2004.03
***Staphylococcus aureus* (in 10 g)**	Negative in 10 g	n.d	n.d	n.a	n.d	n.d	n.d	n.d	n.d	n.d	n.d	AOAC 975.55
***Escherichia coli* (in 10 g)**	Negative in 10 g	n.d	n.d	< 3 / 10 g	< 3/ 10g	n.d	n.d	n.d	n.d	n.d	n.d	Current USP/NF, 62
Coliforms (MPN/g)	< 100	43	< 3	23	3.6	23	< 3	93	< 3	9.1	< 3	AOAC 966.24

CFU: colony forming units; MPN: most probable number; AOAC: Association of Official Analytical Chemists; FAD-BAM: Food and Drug Administration's Bacteriological Analytical Manual; USP-NF: United States Pharmacopeia-National Formulary; n.d: not detected; n.a: not analysed.

The applicant also provided studies on sensory stability and stability of vitamin D_2 content within the NF used in the intended food products (fruit juice drink and cereal bar) under typical commercial storage conditions.

The stability of the vitamin D_2 content added to a fruit juice drink was assessed at day 0, 7 and 14 days (standard shelf-life for this type of product on the market) after refrigerated storage at 4°C. The level of vitamin D_2 in the beverages was generally stable over the course of 14-day storage period.

Sensory stability tests were performed for assessing changes in taste of the fruit beverage containing the NF compared to control beverages at day 0, 7 and 14 days after refrigerated storage. The results indicate little difference in taste between the control samples and those containing the NF and no notable change in taste after storage for the 14-day shelf-life of the product.

The applicant also performed stability tests for the vitamin D_2 content from the NF added to a cereal bar which was assessed at day 0, and 1 and 3 months after storage at room temperature, as well as sensory stability tests performed in the same period. No significant degradation of the vitamin D_2 was found throughout the shelf-life period and overall stability in a cereal bar for 3 months was demonstrated. Also, sensory tests' results indicated no difference in taste between the control samples and those containing the NF and no notable change in taste of the product after storage for 3 months.

Provided that the specifications are met also at the end of shelf-life and that products containing the NF are compliant with respective legislative limits, the stability data do not raise safety concerns.

3.5. Specifications

The specifications of the NF as proposed by the applicant are indicated in Table 9.

Table 9: Specifications of the NF

Description: light to dark brown milled powder, produced by exposing sliced/diced *Agaricus bisporus* mushrooms to ultraviolet light followed by dehydration and homogenisation to form a powder	
Source: *Agaricus bisporus*	
Parameter	Specification
Vitamin D_2	125–375 µg/g*
Moisture	≤ 7.0%
Ash	≤ 13.5%
Water activity	≤ 0.5
Proximate parameters	
Total fat	≤ 4.5%
Total carbohydrate	≤ 60%
Protein	≤ 40%
Heavy metals	
Lead	≤ 0.5 mg/kg
Cadmium	≤ 0.5 mg/kg
Mercury	≤ 0.1 mg/kg
Arsenic	≤ 0.3 mg/kg
Mycotoxins	
Aflatoxins (sum of B1, B2, G1 and G2)	< 4 µg/kg
Aflatoxin B1	< 2 µg/kg
Microbiological	
TAMC	< 5,000 CFU/g
TYMC	< 100 CFU/g
Coliforms	< 100 MPN/ g
Salmonella	Not detected in 25 g
Staphylococcus aureus	Not detected in 10 g
Escherichia coli	Not detected in 10 g
Listeria monocytogenes	Not detected in 25 g

TAMC: total aerobic microbial count; TYMC: total yeast and mould count; CFU = colony forming units.

*: Converted from International Units (IU) using the conversion factor of 0.025 µg = 1 IU stated in the European Food Safety Authority 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

There is no history of use of the NF.

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

In addition, 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).

3.7. 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 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 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 Table 10 in FSMPs as defined in Regulation (EU) No 609/2013 (excluding those intended for infants), food supplements and total diet replacements for weight control as defined in Regulation (EU) No 609/2013 and meal replacements for weight control.

Table 10: Proposed uses and use levels for the NF

Propose uses	Food category	Corresponding levels of vitamin D_2 (µg/100 g or 100 mL)[a]
Dairy analogues	Milk imitates	1.125
	Dairy imitates other than milks	2.25
Breakfast cereals and bars	Breakfast cereals, plain	2.25
	Muesli and similar mixed breakfast cereals	2.25
	Processed and mixed breakfast cereals	2.25
	Cereal bars	2.25
Soups and broths	Soups, RTE	2.25
	Soups, dry mixture uncooked	22.5[c] (equivalent to 2.25 µg /100 mL RTE)
Protein products[b]	Whey powder	14.0625[d] (equivalent to 1.125 µg/100 mL RTE)
	Soya drink	1.125
	Rice drink	1.125
	Almond drink	1.125
	Oat drink	1.125
Fruit/vegetable juices and nectars	Fruit/vegetable juices and nectars	1.125
	Fruit/vegetable juice powder	12.375[e] (equivalent to 1.125 µg/100 mL RTE)
	Fruit/vegetable juice concentrate	3.375[f] (equivalent to 1.125 µg /100 mL RTE)

Propose uses	Food category	Corresponding levels of vitamin D_2 (μg/100 g or 100 mL)[a]
Flavoured drinks	Functional drinks[g]	1.125
	Fortified bottled water	1.125
Dietary foods for medical purposes	Foods for special medical purposes as defined in Regulation (EU) No 609/2013h (excluding those intended for infants)	15 μg/day
Dietary foods for weight control	Total diet replacement for weight control as defined in Regulation (EU) No 609/2013 and meal replacements for weight control	5 μg/meal
Food supplements	Food supplements as defined in Directive 2002/46/EC, excluding food supplements for infants and young children	15 μg/day
	Food supplements (infants 7 to 11 months)	10 μg/day

(a): Each NF batch should be analysed for final vitamin D_2 content. The NF addition as an ingredient is based on a maximum amount of vitamin D_2 in each food category.
(b): Relevant drinks are also included under 'milk imitates'.
(c): Reconstitution factor of 10 applied in the dietary exposure assessment.
(d): Reconstitution factor of 12.5 applied in the dietary exposure assessment.
(e): Reconstitution factor of 11 applied in the dietary exposure assessment.
(f): Reconstitution factor of 3 applied in the dietary exposure assessment.
(g): This food category includes: energy drinks, Isotonic and sport drinks and fermented functional drinks (i.e. 'fermented non-alcoholic drinks (with exclusion of dairy fermented drinks)'. The use of this code does not indicate a Health claim under Regulation 1924/2006.

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 11.

The applicant intends to add the NF as an ingredient in a variety of foods and beverages in amounts that result in either 1.125 or 2.25 μg vitamin D_2 per 100 g or 100 mL of the food as consumed.

According to the applicant 'the amounts of the NF (vitamin D_2 mushroom powder) added to food are adjusted to provide the desired amount of vitamin D_2 (depending on vitamin D_2 content of the batch being used); therefore, whilst the amounts of the NF in final foods may change, the vitamin D_2 content of final foods will never exceed the maximum use levels that have been proposed'.

The maximum proposed levels of the NF as an ingredient proposed by the applicant are 18 mg/100 g for products other than beverages (excluding food supplements) and 9 mg/100 mL for beverages. These conditions of use would correspond to levels of 2.25 μg of vitamin D_2/100 g for products other than beverages and 1.125 μg of vitamin D_2/100 mL for beverages.

The applicant also intends to market the NF for use in food supplements, FSMPs (excluding those intended for infants), total diet replacements for weight control as defined in Regulation (EU) No 609/2013 and meal replacements for weight control.

The proposed vitamin D_2 concentrations in food supplements are a maximum of 10 μg vitamin D_2/day in food supplements for infants from 7 to 11 months and 15 μg vitamin D_2/day for the general population from 1 year upwards.

For the adult population, the maximum intended use level in FSMPs is 15 μg vitamin D_2/day and 5 μg vitamin D_2/meal in total diet replacement for weight control as defined in Regulation (EU) No 609/2013 and meal replacements for weight control.

Table 11: Food categories based on the FoodEx2 classification system, maximum use levels for the NF and corresponding levels of vitamin D_2 intended by the applicant

FoodEx2 level	FoodEx2 code	Food category	Max use level (mg NF/100 g)	Corresponding levels of vitamin D_2 (µg/100 g or 100 mL)[a]
Conventional foods				
4	A03TH	Milk imitates	9	1.125
4	A03TQ	Dairy imitates other than milks	18	2.25
3	A04LH	Breakfast cereals, plain	18	2.25
3	A00EJ	Muesli and similar mixed breakfast cereals	18	2.25
3	A04LK	Processed and mixed breakfast cereals	18	2.25
3	A00EY	Cereal bars	18	2.25
3	A041L	Soups, RTE	18	2.25
3	A0B9J	Soups, dry mixture uncooked	180.0[c] (equivalent to 18.0 mg/100 mL RTE)	22.5[c] (equivalent to 2.25 µg /100 mL RTE)
4	A02PN	Whey powder	112.5[d] (equivalent to 9.0 mg/100 mL RTE)	14.0625[d] (equivalent to 1.125 µg/100 mL RTE)
5	A03TJ*	Soya drink	9	1.125
5	A03TM*	Rice drink	9	1.125
5	A03TK*	Almond drink	9	1.125
5	A03TL*	Oat drink	9	1.125
2	A0BX9	Fruit/vegetable juices and nectars	9	1.125
3	A0ETX	Fruit/vegetable juice powder	99.0[e] (equivalent to 9.0 mg/100 mL RTE)	12.375[e] (equivalent to 1.125 µg/100 mL RTE)
3	A0ETV	Fruit/vegetable juice concentrate	27.0[f] (equivalent to 9.0 mg/100 mL RTE)	3.375[f] (equivalent to 1.125 µg/100 mL RTE)
3	A03FZ	Functional drinks[g]	9	1.125
4	A03GC	Fortified bottled water	9	1.125

RTE: ready-to-eat.
*: In the hierarchical parent–child relationship, relevant 'Milk imitates' FoodEx subcategories (Level 5/child category) are also included in category 12.9 for completeness; however, only Level 4 of FoodEx 'parent' category 'Milk imitates' is applied in the intakes assessment.
(a): Reconstitution factors were applied to food codes representative of powders and concentrates. These were obtained from EFSA (2018): https://zenodo.org/record/1256085#.XJwuCOSQxMs.
(b): All batches of NF are produced with a vitamin D_2 content within the proposed minimum specification of 125 µg/g. In order to produce a conservative estimate, the highest use level (g powder/100 g food) was utilised.
(c): Reconstitution factor of 10 applied in the dietary exposure assessment.
(d): Reconstitution factor of 12.5 applied in the dietary exposure assessment.
(e): Reconstitution factor of 11 applied in the dietary exposure assessment.
(f): Reconstitution factor of 3 applied in the dietary exposure assessment.
(g): This food category includes energy drinks, isotonic and sport drinks and fermented functional drinks (i.e. 'fermented non-alcoholic drinks (with exclusion of dairy fermented drinks)'. The use of this code does not indicate a Health claim under Regulation 1924/2006.

3.7.3. Anticipated intake of the NF

EFSA performed an intake assessment of the anticipated daily intake of the NF (Tables 12 and 13) and vitamin D_2 intakes from the NF (Table 14), using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest mean and 95th

percentile anticipated daily intake of the NF among the EU dietary surveys are presented in Tables 12 and 13, expressed as mg/kg bw per day and mg per day, respectively.

The estimated daily intake of the NF and vitamin D_2 for each population group from each EU dietary survey is available in the Excel file annexed to this scientific opinion (under supporting information).

Table 12: Estimated intake of the NF as an ingredient in the intended food categories at the maximum proposed use levels (mg/kg bw per day)

Population group	Age (years)	Mean intake (mg/kg bw per day)		P95 intake (mg/kg bw per day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	0.03	0.87	0.09	3.56
Young children[(c)]	1 to < 3	0.26	2.58	1.19	4.03
Other children	3 to < 10	0.34	1.12	1.10	2.64
Adolescents	10 to < 18	0.13	0.65	0.46	1.94
Adults[(d)]	≥ 18	0.17	0.48	0.57	1.45

(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 10/12/2021. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 10/12/2021. The lowest and the highest P95 observed among all EU surveys are reported in these columns (P95 based on less than 60 individuals are not considered).
(c): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant and lactating women.

Table 13: Estimated intake of the NF 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)		P95 intake (mg/day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	0.28	7.78	0.94	34.24
Young children[(c)]	1 to < 3	3.03	34.70	14.87	46.37
Other children	3 to < 10	7.88	26.23	25.08	63.61
Adolescents	10 to < 18	5.85	38.92	22.50	121.50
Adults[(d)]	≥ 18	10.93	32.55	37.05	88.80

(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 10/12/2021. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 10/12/2021. The lowest and the highest P95 observed among all EU surveys are reported in these columns (P95 based on less than 60 individuals are not considered).
(c): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant and lactating women.

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

Considering the proposed conditions of use, based on vitamin D_2 levels of 2.25 μg of vitamin D_2/100 g for products other than beverages and 1.125 μg of vitamin D_2/100 mL for beverages, maximum estimated daily intakes of vitamin D_2 from the NF as an ingredient in foods and beverages calculated both in absolute values (μg/day) and on a per body weight basis (μg/kg bw per day) are reported in Table 14.

Table 14: Anticipated highest P95 of daily intake of vitamin D_2 from the NF as ingredient in foods and beverages

Population group	Age (years)	Vitamin D_2 P95 intake	
		(μg/kg bw per day)	(μg/day)
Infants	< 1	0.44	4.28
Young children[(a)]	1 to < 3	0.50	5.80
Other children	3 to < 10	0.33	7.95
Adolescents	10 to < 18	0.24	15.2
Adults [(b)]	≥ 18	0.18	13.2

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

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 14) 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 the Opinion from 2012, the highest 95th percentile (P95) of 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 categories. 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 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 15 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 15: 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 the background diet EFSA NDA Panel (2012)[(g)]	Highest P95 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)]	5.80	15	26.4	50
	15[(b)]	5.80	–	20.8[(c)]	
Other children	2.7[(a)]	7.95	15 –	25.7	50
	15[(b)]	7.95		23.0[(c)]	
Adolescents[(e)]	4[(a)]	15.2	15	34.2	100
	8[(b)]	15.2	–	23.2[(c)]	
Adults[(f)]	16	13.2	15	44.2	100

UL: tolerable upper intake level; NF: novel food; P95: 95th percentile.
(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 ingredient in food supplements.
(d): Total intake is the sum of the intake from the background diet, from NF ingredient use (highest P95) 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 old adolescent [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.
(g): P95 intake for adults and highest mean intakes for children and adolescents.

In addition, (as stated in Section 3.7.2 Proposed use and use levels) the NF is proposed as an ingredient in FSMPs as defined in Regulation (EU) No 609/2013 (excluding those intended for infants) providing a maximum of 15 μg vitamin D_2/day, in total diet replacement for weight control as defined in Regulation (EU) No 609/2013, and in meal replacements for weight control providing 5 μg vitamin D_2/meal.

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).

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 maximum P95 intake of vitamin D_2 from the NF as an ingredient in foods is 4.28 μg/day (see Table 14). 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 21.2 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 21.2 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.

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

No specific ADME studies for the NF have been provided. The applicant refers to publicly available animal and human studies on vitamin D_2 from UV-irradiated mushrooms and supplemental vitamin D_2 and vitamin D_3 (Jasinghe et al., 2005; Koyyalamudi et al., 2009; Keegan et al., 2013; Stepien et al., 2013; Bennett et al., 2013; Calvo et al., 2013; Shanely et al., 2014; Mehrotra et al., 2014).

The human studies Keegan et al. (2013) and Stepien et al. (2013) and the animal studies Koyyalamudi et al. (2009), Bennett et al., (2013), Calvo et al., (2013) and Shanely et al., (2014) were evaluated by EFSA in the assessment of similar NF ingredients (EFSA NDA Panel, 2020, 2021a).

The Panel concludes that vitamin D_2 from powder from UV-irradiated mushrooms is bioavailable, and dose-related increases in serum concentrations of 25(OH)D_2 are observed upon oral consumption.

3.9. Nutritional information

The applicant provided a nutritional analysis of the NF. The NF is composed of carbohydrates (~ 50%), protein (~ 33%), dietary fibre (~ 20%), ash (~ 9%) moisture (< 5%) and fat (~ 3%) and, contains vitamins and minerals. In addition to the proximate analysis of the NF (see Table 2, Section 3.4 Compositional data), analytical information for five independent batches of the NF for a nutritional analysis was provided by the applicant (Table 16).

[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.

Table 16: Nutritional analysis of the NF

Parameter (unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Calories (kcal/100 g)	364.4	364.8	363.5	366.0	358.3	Calculation
Monounsaturated fat (g/100 g)	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	AOAC 996.06
Polyunsaturated fat (g/100 g)	2.11	1.98	2.02	2.03	2.06	
Saturated fat	0.62	0.58	0.58	0.59	0.58	
Trans fat (g/100 g)	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	
Cholesterol (mg/100 g)	< 0.8	< 1.0	< 1.0	< 1.0	< 1.0	Direct saponification-GC/FID method (AOAC 994.10)
Glucose (g/100 g)	1.30	1.40	0.94	1.90	1.60	LC/RI (AOAC 980.13)
Sodium (mg/100 g)	97.1	108	103	99.5	104	ICP-AES (AOAC 984.27)
Potassium (mg/100 g)	4,310	4,170	3,890	3,910	3,990	
Calcium (mg/100 g)	76.9	72.4	69.1	69.2	73.3	
Iron (mg/100 g)	2.7	5.6	2.9	4.9	3.3	
Moisture (g/100 g)	3.15	2.73	3.35	2.71	4.41	Gravimetry, (AOAC 926.08)
Ash (g/100 g)	9.45	9.47	9.32	9.28	9.59	Gravimetry (AOAC 945.46)
Vitamin D_2 (μg/g)[1]	250	268.05	160.16	128.39	342.22	UHPLC-MS/MS (AOAC 2011.11 Mod)
Vitamin D_3 (μg/g)	N.A	< 0.0055	< 0.0055	< 0.0055	< 0.0055	

AOAC: Association of Official Analytical Chemists; GC/FID: Gas Chromatography/Flame Ionisation Detection; LC/RID: Liquid Chromatography/Refractive Index Detection; ICP-AES: Inductively Coupled Plasma – Atomic Emission Spectroscopy; UHPLC-MS/MS: Ultra high performance liquid chromatography-mass spectrometry tandem mass spectrometry; N.A: not analysed.

(1): The applicant provided 2 sets of batch-to-batch analyses on the vitamin D_2 content in the NF performed by different laboratories using different methods of analysis (HPLC-DAD and UHPLC-MS/MS (AOAC 2011.11 Mod)). Considering that all 10 batches are produced with the same production process and vitamin D_2 content is within the specifications range, all 10 batches are representative of the final ingredient. Therefore, the applicant indicates that all 10 analyses should be considered for the risk assessment.

The nutritional content of mushrooms exposed to UV light remains unchanged, with the exception of the intended increase in vitamin D_2 content (Simon et al., 2011; EFSA NDA Panel, 2020,2021a,b).

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 34.2 and 44.2 μg/day for adolescents and adults, respectively. Those intake estimates (as reported in Table 15 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 young children and other children, the estimated combined maximum intake of vitamin D_2 from the NF plus intake of vitamin D from other dietary sources (including 15 μg/day from the NF when used in food supplements) amounts to vitamin D intakes of 26.4 and 25.7 μg/day, 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 estimated combined vitamin D intake in infants of 21.2 μg/day is below the UL for vitamin D 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 intake of 21.2 μg/day would result in an intake of 31.2 μg/day and thus would be still below the UL for vitamin D of 35 μg/day established by EFSA in 2018. 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 if an additional supplementation is used.

The Panel notes that intake of other sources of vitamin D, e.g. fortified foods and supplements containing vitamin D in addition to the NF might lead to vitamin D intakes above the upper level (UL).

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 (2016b).

The Panel also notes that the stability of vitamin D_2 could be influenced by the food processing of the matrix where the NF is added as an ingredient, and that this depends on the foodstuffs and heating conditions (Jakobsen and Knuthsen, 2014). The Panel notes that the stability of vitamin D_2 in the food matrix after thermal processing conditions has not been taken into account in the calculations of total vitamin D dietary intakes.

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 a previous similar NF ingredient, vitamin D_2 mushroom powder (produced by homogenisation of mushrooms before exposure to UV light) (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 provided six publicly available human studies with the UV-irradiated mushrooms or similar material, two of which were conducted using ingredients supplied by the applicant.

In those studies, the effect on serum 25(OH)D of vitamin D_2 from *Agaricus bisporus*, using either dried non-irradiated *Agaricus bisporus* mushroom extract (test material supplied by the applicant) and vitamin D_2 or D_3 supplements (Keegan et al., 2013[9]), UV-B irradiated mushrooms (Mehrotra et al., 2014; Stephensen et al., 2012[5]; Urbain et al., 2011), or lyophilised UV-treated mushrooms (Stepien et al., 2013), or UV-treated powder from *Agaricus bisporus* (Shanely et al., 2014) were assessed.

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 (EFSA NDA Panel, 2021a).

The Panel notes that these studies are on vitamin D availability and are of limited value for the safety assessment of the NF.

3.11. Allergenicity

The Panel considers that the allergenicity risk is not expected to be greater compared to that associated with normal consumption of *Agaricus bisporus* mushrooms and the additional UV-treatment is not expected to alter the risk (EFSA NDA Panel 2020, 2021a).

4. Discussion

The NF which is the subject of the application is a powder from *Agaricus bisporus* mushrooms that had 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 125–375 μg/g. The applicant intends to add the NF as an ingredient in a variety of foods and beverages at standardised levels of vitamin D_2 of either 1.125 μg per 100 mL or 2.25 μg per 100 g of the food as consumed. The NF is also proposed to be used in FSMP, total diet/meal replacement for weight control and in food supplements.

The target population is the general population except for FSMPs for which the target population is individuals above 1 year of age, and food supplements for which the target population is individuals from 7 months onwards.

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 ULs for vitamin D as established previously by the NDA Panel for infants (EFSA NDA Panel, 2018), and for children, adolescents and adults (EFSA NDA Panel, 2012).

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 notes uncertainty regarding the

[9] (test material supplied by the applicant).

calculated combined exposures to vitamin D for 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, 2021a).

The Panel notes that intake of other sources of vitamin D e.g. fortified foods and supplements containing vitamin D in addition to the NF might lead to vitamin D intakes above the tolerable upper intake level (UL). 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 containing vitamin D_2 in the range of 125–375 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 (Identity of the NF report, batch analysis information, the respective certificates of analysis and the stability studies).

6. Steps taken by EFSA

1) On 05/02/2021 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of vitamin D_2 mushroom powder as a novel food Ares(2021) 1025888- 05/02/2021.
2) On 05/02/2021, a valid application on vitamin D_2 mushroom powder as a novel food, which was submitted by Monterey Mushrooms Inc., was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/1471) and the scientific evaluation procedure was initiated.
3) On 12/05/2021 and 22/02/2022 EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 07/01/2022 and 09/03/2022, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) During its meeting on 26/04/2022, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of vitamin D_2 mushroom powder as a novel food as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADME	Absorption, distribution, metabolism and excretion
AOAC	Association of Official Analytical Chemists
Bw	body weight
CAS	Chemical Abstracts service
CFU	colony-forming units
ELFA	Enzyme-linked fluorescent immunoassay.
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FAO	Food and Agriculture Organization
FDA-BAM	United States Food and Drug Administration Bacteriological Analytical Manual
FoF	Follow-on-Formula
FSAI	Food Standard Authority of Ireland
FSMPs	Foods for medical special purposes
GC-FID	Gas chromatography-flame ionisation detector
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
HPLC/DAD	High-performance liquid chromatography/diode array detector
HPLC/FLD	High-performance liquid chromatography with fluorescence detection
HPLC-RI	High-performance liquid chromatography - refractive index detector
HPTLC	High-performance thin-layer chromatography analysis
ICP-AES	Inductively Coupled Plasma – Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma-Mass Spectroscopy
IF	Infant Formula
IU	International Unit
IUPAC	International Union of Pure and Applied Chemistry
LC/RID	Liquid Chromatography/Refractive Index Detection
MPN	most probable number
NA	Not analysed
NDA	Panel on Nutrition, Novel Foods and Food Allergens
n.d	non detected
NF	novel food
NOAEL	no observed adverse effect level

OECD	Organisation for Co-operation and Economic Development
RTE	Ready to eat
TAMC	total aerobic microbial count
TYMC	total yeast and mould count
UHPLC-MS/MS	Ultra high performance liquid chromatography-mass spectrometry tandem mass spectrometry
ULs	tolerable upper intake levels
USP	United States Pharmacopeia
UV	Ultraviolet
w/w	weight per weight

Annex A – Dietary exposure estimates to the Novel Food for each population group from each EU dietary survey

Information provided in this Annex is shown in an Excel file (downloadable at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2022.7326#support-information-section).