REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment of UV-treated *Agaricus Bisporus* Mushroom Powder With Increased Vitamin D_2 as a Novel Food for Use in Food and Food Supplements (RP1550)

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FSA Research and Evidence

The Food Standards Agency (FSA) and Food Standards Scotland (FSS) received an application from Monterey Mushrooms, LLC ("the applicant") for the authorisation of UV-treated *Agaricus bisporus* Mushroom Powder with increased vitamin D_2 as a novel food in April 2022.

The novel food is an ingredient produced by the exposure of *A. bisporus* mushrooms to ultraviolet light which catalyses the conversion of endogenous ergosterol within the mushrooms to vitamin D_2. The mushrooms are then dehydrated and ground into a powder.

The novel food contains vitamin D_2 content ranging between 125-463 µg/g and is intended to be used as ingredient to achieve a maximum dose of vitamin D_2 of 2.25 µg/100 g for food products, and 1.125 µg/100 mL for beverages. In food supplements the proposed maximum dose of vitamin D_2 is 15 µg/day in food supplements for the general population older than 1 year of age and at 10 µg/day in food supplements for infants from 7 to 11 months.

To support the FSA and FSS in their evaluation of the application, the Advisory Committee on Novel Foods and Processes (ACNFP) were asked to review the safety dossier and supplementary information provided by the applicant. The views of the Committee were taken into account by the FSA and FSS who concluded that the applicant had provided sufficient information to assure the novel food, UV-treated *A. bisporus* Mushroom Powder with increased vitamin D_2, was safe under the proposed conditions of use. The anticipated intake levels and the intended use in food and food supplements was not considered to be nutritionally disadvantageous.

This safety assessment represents the opinion of the FSA and FSS.

This is a joint FSA and FSS publication

1. Introduction

In April 2022, Monterey Mushrooms, LLC ("the applicant") submitted a full novel food application for the authorisation of UV-treated *A. bisporus* Mushroom Powder with increased vitamin D_2. The novel food is produced by the exposure of *Agaricus bisporus* mushrooms to ultraviolet light which catalyses the conversion of endogenous ergosterol within the mushrooms to vitamin D_2. The novel food is intended to be used as an ingredient in various food products, beverages and food supplements.

The FSA and FSS have undertaken a safety assessment for UV-treated *A. bisporus* Mushroom Powder with increased vitamin D_2 under the novel foods legislation, assimilated Regulation (EU) 2015/2283. To support the safety assessment, the ACNFP provided the advice outlined in this opinion to the FSA and FSS.

The evaluation by the ACNFP assessed the food safety risks of the novel food and its production, in line with Article 7 of assimilated Commission Implementing Regulation (EU) 2017/2469. The regulatory framework and the technical guidance put in place by the European Food Safety Agency (EFSA) for full novel food applications is retained as the basis and structure for the assessment (EFSA NDA Panel, 2016).

Following the review by the ACNFP in September 2023, further information was requested from the applicant concerning the identity, the production process, the compositional information, the stability, the nutritional information, and allergenicity information on the novel food, in order to address information gaps in the initial dossier. The final advice from the Committee was agreed at the 167th meeting, allowing the FSA and FSS to complete the risk assessment.

The document outlines the conclusions of the FSA and FSS on the safety of UV-treated *A. bisporus* Mushroom Powder with increased vitamin D_2 as a novel food.

2. Assessment

2.1. Identity of the novel food

The novel food is a mushroom powder containing vitamin D_2 which is produced by subjecting fungi of the species *Agaricus bisporus,* commonly known as cultivated mushrooms, to ultraviolet irradiation. Confirmation that *A. bisporus* mushrooms are used in the production process was demonstrated by high-performance thin-layer chromatography (HPTLC). Figure 1 shows the structural formula for vitamin D_2.

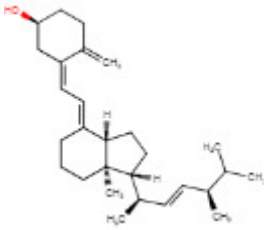

Figure 1. The structural formula of Vitamin D_2

Vitamin D_2 is characterised by the following information:

> IUPAC name
> (3β,5Z,7E,22E)-9,10-secoergosta-5,7,10(19),22-tetraen-3-ol
>
> CAS number 50-14-6
>
> Molecular formula $C_{28}H_{44}O$

2.2. Production Process

The production process for UV-treated *A. bisporus* Mushroom Powder with increased vitamin D_2 involves one raw material; *A. bisporus* mushrooms, which are grown and harvested in the USA under controlled environmental conditions in compliance with Regulation (EC) No. 852/2004 and within the principles of HACCP.

The mushrooms are diced (to increase available surface area) and exposed to UV light under appropriate conditions to achieve targeted levels of vitamin D_2. The diced mushrooms are then dehydrated, ground and packaged in a temperature-controlled environment to control moisture levels in the product.

Prior to packaging, analyses are performed to ensure microbial safety as well as determine that the amount of vitamin D_2 is within the food specification level of 125-463 µg/g.

Variability of the vitamin D_2 content resulting from the variation of the effectiveness of the ultraviolet irradiation process due to factors including, exposure time, lamp properties, as well as variation in the raw material was noted. The conditions are managed to ensure the vitamin D_2 level in the final product falls within the target range. It was also noted that the product may be blended again after final testing if the vitamin D levels do not meet client-specific requirements.

To ensure that the final product is homogeneous and that samples of the novel food tested for safety parameters such as moisture and vitamin D_2 content reflected the composition of the batch tested, details of the blending and sampling processes were provided. A blender validation study involving 3 batches of vitamin D_2 mushroom powder was undertaken to demonstrate the efficiency of the cone blender for creating homogeneous blends with targeted levels of vitamin D_2 according to the specification. Information on how contamination is minimised during these stages of production and controls in the packaging stage to manage moisture levels was also provided.

The production process has characterised the potential hazards and the corresponding control measures are appropriate.

2.3. Compositional information

Results from five independent batches of UV-treated *A. bisporus* Mushroom Powder with increased vitamin D_2 demonstrated that the novel food is produced consistently (Table 1). Vitamin D_2 levels were assayed using high-performance liquid chromatography (HPLC). The analytical method is similar to that described by Phillips *et al*. (2011, 2012).

Certification was provided to demonstrate that the contract laboratories were accredited to perform these analytical studies. Where in-house analysis was utilised, full methodology and supporting validation documentation was provided.

Table 1. Compositional analysis of the novel food

Parameter	Specification	Method of Analysis	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Batch No. 5
Vitamin D_2 (µg/g)	125 to 463*	HPLC-DAD	151.5	169.4	167.0	159.3	182.6
Moisture	≤7.0	O'haus Scale	<5	<5	<5	<5	<5

Physical Parameters

Parameter	Specification	Method of Analysis	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Batch No. 5
Mesh size	90% through 80 mesh sieve	Sieving	Conforms	Conforms	Conforms	Conforms	Conforms
Appearance	Light to dark brown milled powder	NA	Conforms	Conforms	Conforms	Conforms	Conforms
Aroma	Earthy	NA	Conforms	Conforms	Conforms	Conforms	Conforms
Taste	Mushroom savoury, umami flavour	NA	Conforms	Conforms	Conforms	Conforms	Conforms

Microbiological

Parameter	Specification	Method of Analysis	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Batch No. 5
Aerobic plate count (CFU/g)	<5,000	AOAC 966.23	980	4,700	1,400	400	100
Yeast (CFU/g)	<100	FDA-BAM, 7th ed.	<10	<10	<10	<10	<10
Mould (CFU/g)	<100	FDA-BAM, 7th ed.	30	30	10	10	10
Salmonella spp.	Negative in 25 g	AOAC 2004.03	Negative	Negative	Negative	Negative	Negative
S. aureus	Negative in 10 g	AOAC 975.55	Negative	NT	Negative	Negative	Negative
E. coli	Negative in 10 g	Current USP/ NF, 62	Negative	Negative[a]	Negative	Negative	Negative
Coliforms (MPN/g)	<100	AOAC 966.24	43	23	23	93	9.1

Listeria

Parameter	Specification	Method of Analysis	Batch No. 7	Batch No. 8	Batch No. 9	Batch No. 10	Batch No.11
Listeria spp.	Negative in 25 g	AOAC 2004.06	Negative	Negative	Negative	Negative	Negative

HPLC = high-performance liquid chromatography; NA = not applicable. *Converted from 5,000 to 18,500 International Units (IU) using the conversion factor of 0.025 µg = 1 IU stated in the EFSA Technical Report on Dietary Reference Values for nutrients (EFSA, 2017).
AOAC = Association of Official Analytical Chemists; CFU = colony-forming units; MPN = most probable number; NT = not tested; FDA-BAM = United States Food and Drug Administration Bacteriological Analytical Manual
[a]3 tube MPN method (Method Reference: AOAC 966.24). No growth of the target organism at the limit of detection, 3 MPN/g

Table 2. Heavy metals analysis for 6 batches of the novel food

Parameter	EU Limits[a] (wet weight)	Monterey Specification	Method of Analysis	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Batch No. 5	Batch No.6
Arsenic (mg/kg)	0.3	≤0.3	USP 730	0.23	0.19	0.28	0.29	0.20	0.22
Cadmium (mg/kg)	0.2	≤0.5	USP 730	0.067	0.061	0.066	0.063	0.050	0.035
Lead (mg/kg)	0.3	≤0.5	USP 730	0.02	0.02	0.02	0.02	0.04	0.01
Mercury (mg/kg)	0.1	≤0.1	USP 730	0.027	0.021	0.042	0.035	0.027	0.025

EU = European Union; USP = United States Pharmacopeia.

[a] Maximum level according to Commission Regulation (EC) No. 1881/2006 (taken from the closest relevant finished products categories).

It was noted that one batch was analysed using a different method of analysis, MPN-most probable number technique, which estimates the concentration of a microbe in a sample. No microbial growth was seen below the higher limit of detection for this test of 3MPN/g hence, all samples were considered to be within specification. Laboratory analysis showed that microbial contamination was appropriately managed and that controls were working effectively.

A Certificate of Analysis for testing of a wide range of pesticides showed that no residues were detected in the novel food.

Heavy metal levels were analysed and compared to established EU limits for the closest relevant finished product categories (Table 2). All analyses were below EU limits.

Three batches of the novel food were analysed for the presence of biologically inactive photoisomers (lumisterol and tachysterol) which are formed during the conversion of ergosterol to vitamin D_2. These photoisomers are also formed endogenously in humans during cutaneous production of vitamin D following exposure to UV-B radiation from sunlight. The results demonstrated that only negligible amounts of these photoisomers are detected in the final ingredient (Table 3), and the amounts are lower than those in other UV-treated foods such as baker's yeast and bread, which gave rise to no safety concerns in previous EFSA Scientific Opinions (EFSA NDA Panel, 2014, 2015).

Table 3. Concentration of Lumisterol and Tachysterol in the novel food

Parameter	Method of Analysis	Batch No. 12	Batch No. 13	Batch No. 14
Lumisterol (mg/g)	HPLC	0.05	0.03	0.08
Tachysterol (mg/g)	HPLC	0.004	0.006	0.01

The presence of aflatoxins was measured in six independent batches of UV- treated *A. bisporus* Mushroom Powder with increased vitamin D_2. The maximum levels permitted in foods were taken from the closest relevant category (cereals and all products derived from cereals, including processed cereal products) detailed in assimilated Commission Regulation (EC) No 1881/2006, as amended, for aflatoxin B1 and the total sum of B1, B2, G1 and G2. Results demonstrated that the levels to be low and within established limits (Table 4).

Table 4. Aflatoxin analysis for 6 Batches of the novel food

Parameter	Method of Analysis	EU Limit[a] (µg/kg)	Batch No. 15	Batch No. 16	Batch No. 17	Batch No. 18	Batch No. 19	Batch No. 20
Aflatoxins (sum of B1, B2, G1 and G2) µg/kg (w/w)	AOAC 991.31 (Mod.)[b]	4	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7
Aflatoxin B1 µg/kg (w/w)	AOAC 991.31 (Mod.)[b]	2	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6

EU = European Union.

[a] Maximum levels according to assimilated Commission Regulation (EC) No. 1881/2006 (taken from the closest relevant finished products categories).

[b] The modification applies to the initial steps in the assessment of the performance of the immuno affinity columns used for the isolation and purification of the aflatoxin compounds. Whereas the AOAC method uses a manual fluorescence procedure, the modified method uses a fortified sample (spiked sample) of 5 ng of each aflatoxin type (20 ng total) carried through the entire procedure including the full HPLC steps.

The data presented indicate the novel food and any hazards present were appropriately characterised.

2.4. Stability

Stability tests were carried out on four batches for three years and on one batch for four years to test the stability of the vitamin D_2 content under representative storage conditions (17°C-25°C and 40%-63% humidity). The results showed the vitamin D_2 levels remained within the specified range and supported the proposed 3-year shelf life. Microbial analysis was carried out on the samples in the 3-year study at the start and end of the storage period, without any concerns identified. The results of both tests suggests that that the product is stable for up to 3 years under the recommended storage conditions.

The stability of vitamin D_2 content was also tested within some foods that the novel food would be an added ingredient (fruit juice drink and cereal bar) under typical commercial storage conditions for the products. In addition to the analysis of vitamin D_2 content, sensory parameters were

tested. No significant degradation of vitamin D_2 was observed throughout the test period of 14 days (the standard shelf life) for the juice and 3 months for the cereal bar. Sensory results did not indicate notable taste changes for the products.

On this basis, the data provided supports the stability of the novel food within the period of 36 months and storage conditions proposed.

2.5. Specification

The specification parameters for the novel food (Table 5) were assessed using internationally recognised methods or are otherwise determined using internally developed and validated methods.

Table 5. Specification for the novel food

Parameter	Specification	Method
Vitamin D_2	125 to 463 µg/g*	HPLC-DAD
Moisture	≤7%	O'haus Scale
Water activity	<0.5%	AOAC 978.18
Ash	≤13.5%	AOAC 945.46
Physical Parameters		
Appearance	Light to dark brown milled powder	Organoleptic
Aroma	Earthy	Organoleptic
Taste	Mushroom savoury, umami flavour	Organoleptic
Proximate Analysis		
Total Fat	≤4.5%	AOAC 996.06
Total Carbohydrate	≤60%	Calculation
Protein	≤40%	AOAC 992.23
Microbiological Parameters		
Standard plate count	<5,000 CFU/g	AOAC 966.23
Total yeast and mould count	<100 CFU/g	FDA-BAM, 7th ed.
Salmonella	Negative in 25 g	AOAC 2004.03
Staphylococcus aureus	Negative in 10 g	AOAC 975.55
Escherichia coli	Negative in 10 g	Current USP/NF, 62
Coliform	<100 MPN/ g	AOAC 966.24
Listeria spp.	Negative in 25 g	AOAC 2004.06
Aflatoxins (sum of B1, B2, G1 and G2)	<4 µg/kg	AOAC 991.31 (Mod.)
Aflatoxin B1	<2 µg/kg	AOAC 991.31 (Mod.)
Heavy Metals		
Arsenic	≤0.3 mg/kg	USP 730
Lead (as Pb)	≤0.5 mg/kg	USP 730
Mercury	≤0.1 mg/kg	USP 730
Cadmium	≤0.5 mg/kg	USP 730

General Description

UV-treated *A. bisporus* Mushroom Powder with increased vitamin D_2 is a light to dark brown milled powder, produced by exposing sliced/diced *Agaricus bisporus* mushrooms to ultraviolet light followed by

homogenisation to form a powder.
AOAC = Association of Official Analytical Chemists; CFU = colony-forming units FDA-BAM = United States Food and Drug Administration Bacteriological Analytical Manual; HPLC = high-performance liquid chromatography; MPN = most probable number; USP = United States Pharmacopeia.
*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 information provided is sufficient for the specification of the novel food, and appropriately characterises the novel food seeking authorisation.

2.6. History of Use

Wild edible fungi, including macro-fungi such as mushrooms, have been collected and consumed for thousands of years (FAO, 2004). Cultivated and wild grown *A. bisporus* mushrooms (the source of the novel food) have been consumed within and outside of the UK for a long time (FSAI, FSAI (Food Safety Authority of Ireland), 2017).

The novel food, UV-treated *A. bisporus* Mushroom Powder with increased vitamin D_2 has no history of use in the UK. As the UV irradiation process is considered a novel process and the parameters for its operation will be slightly different between operators, each is subject to a separate review and authorisation.

UV-treated *A. bisporus* mushrooms have been consumed in the UK with their approval as a novel food ingredient since 2016 (FSAI, 2017). A similar ingredient produced by a different manufacturer using a different production process, vitamin D_2 mushroom powder produced by homogenisation of mushrooms before exposure to UV light was authorised as a novel food in 2020 (EUR-Lex, 2020). Another form of vitamin D_2 mushroom powder was considered safe under its intended uses (EFSA, 2021) and is authorised for use in the EU. The novel food that is subject to this application, uses a different process whereby mushrooms are first exposed to UV light before being dried and ground into a powder.

The history of use does not indicate any further areas for evaluation.

2.7. Proposed Use and Intake

The target population is the general population. For food supplements, the target population is for those aged 7 months and above.

The vitamin D_2 mushroom powder is to be used in certain foods as well as food supplements as defined in GB food supplements legislation. A summary of the foods proposed to use the novel food as an ingredient and the maximum use levels for the novel ingredient in the form of a powder is provided in Table 6. These include breakfast cereals, dairy analogues, fruits and vegetable juices and non-alcoholic beverages.

Table 6. Proposed Food Uses and Use Levels for UV treated *A. bisporus* Mushroom Powder with increased vitamin D_2

Food Categories [for inclusion in the Union list of novel foods]	Specified Food Categories[a]	Expected level of Vitamin D_2 from proposed use, as consumed (µg/100 g or 100 mL)	Novel Food Use Level (mg/ 100 g or 100 mL)
Conventional Food Categories			
Breakfast cereals (RTE)	Breakfast cereals (RTE)	2.25	4.9 to 18.0
Cereal and nutrition bars	Cereal bars	2.25	4.9 to 18.0
	Energy bars	2.25	4.9 to 18.0
	Protein bars	2.25	4.9 to 18.0
Chocolate confectionery	Superfood chocolate bars[b]	2.25	4.9 to 18.0
Dairy analogues	Milk imitates	1.125	2.4 to 9.0
	Cream imitates, including non-dairy coffee creamer	2.25	4.9 to 18.0
	Non-dairy yogurt	2.25	4.9 to 18.0
	Non-dairy cheese	2.25	4.9 to 18.0
	Non-dairy ice cream	2.25	4.9 to 18.0
Fruit and vegetable juices	Fruit juices and nectars (including powders and concentrates)	1.125	2.4 to 9.0
	Vegetable juices (including powders and concentrates)	1.125	2.4 to 9.0
Hot breakfast cereals (dry and RTE)	Hot breakfast cereals (instant and regular)	2.25	4.9 to 18.0
Non-alcoholic beverages	Energy Drinks	1.125	2.4 to 9.0
	Sports and Isotonic Drinks	1.125	2.4 to 9.0
	Fortified water beverages	1.125	2.4 to 9.0
	Fruit smoothies (RTD)	1.125	2.4 to 9.0
	Fruit juice-based drinks	1.125	2.4 to 9.0
	Protein drinks	2.25	4.9 to 18.0
	Nutritional drinks	2.25	4.9 to 18.0
Soups and broths	Soups (RTE and dry mixtures)	2.25	4.9 to 18.0
Specialised Food Categories			
Food supplements as defined in Directive 2002/46/ EC, excluding food supplements for infants and young children		15 µg/day	32 to 120
Food supplements as defined in The Food Supplements (England) Regulations 2003 and associated provisions in Wales and Scotland		10 µg/day	22 to 80
Foods for special medical purposes as defined in Regulation (EU) No 609/2013[d,e] (excluding those intended for infants)		15 µg/day	32 to 120

Food Categories [for inclusion in the Union list of novel foods]	Specified Food Categories[a]	Expected level of Vitamin D_2 from proposed use, as consumed (µg/100 g or 100 mL)	Novel Food Use Level (mg/ 100 g or 100 mL)
Total diet replacement for weight control as defined in Regulation (EU) No 609/2013[d,e]		5 µg/meal	10.8 to 40

EU = European Union; RTD = ready-to-drink; RTE = ready-to-eat; UK = United Kingdom.

[a] Surrogates were included for this food use.

[b] All batches of UV treated *A. bisporus* Mushroom Powder with increased vitamin D_2 are produced with a vitamin D_2 content within the proposed specifications 125 to 463 µg/g (see Table 2.c.1-1 for further details). Thus, use levels of UV treated *A. bisporus* Mushroom Powder with increased vitamin D_2 are dependent on the vitamin D_2 content of the selected batch/batches used. In order to produce a conservative estimate, the highest use level (g powder/100 g food) was utilised.

[c] EUR-Lex, 2002. Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. Available online : https://www.legislation.gov.uk/eudr/2002/46/contents.

[d] EUR-Lex, 2013. 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. Available online : https://www.legislation.gov.uk/eur/2013/609/contents.

[e] EUR-Lex, 2016. 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. Available online: https://www.legislation.gov.uk/eur/2016/128/contents

The proposed maximum use levels of the novel food provide the following maximum additional intake of vitamin D_2: for food products 2.25 µg/100g (15% of the adequate intake for vitamin D identified by EFSA), 1.125 µg/100ml (7.5% of the adequate intake identified by EFSA), for beverages, 15 µg/day for food supplements for age +1yr. 10µg/day for food supplements for infants 7-11 months, which is intended to provide the full adequate intake for vitamin D for the relevant age groups. This is based on EFSAs recent defined adequate intake (15µg/day for adults including children above the age of 1 and 10 µg/day for ages 7-11 months) as a replacement for nutrient reference value (EFSA, 2016).

Estimated intakes of vitamin D_2 calculated based on the proposed use levels as presented in Table 6 were provided. The estimated total intake of novel food (mg/person/day) from all proposed food uses were calculated using the lowest dose of vitamin D in the powder i.e., 125µg vitamin D_2/g powder, giving a maximum dose of 18µg powder per 100g.

Also provided was a summary of the estimated daily per kilogram body weight intake of the novel food from all proposed food categories in the UK by population group (Table 7). This was calculated with consumption data from the UK National Diet and Nutrition Survey (NDNS).

Table 7. Summary of the Estimated Daily Intake of Vitamin D_2 from the novel food from All Proposed Food Categories in the UK by Population Group (NDNS Data, Years 7 to 9).

Population Group	Age Group (Years)	n	Total Population Intake (µg/ person/day) Mean	Total Population Intake (µg/person/day) 95th Percentile
Toddlers	1.5 to 3	309	0.252	0.691
Children	4 to 10	740	0.175	0.482
Female Teenagers	11 to 18	373	0.066	0.171
Male Teenagers	11 to 18	363	0.081	0.232
Female Adults	19 to 64	833	0.047	0.159
Male Adults	19 to 64	618	0.048	0.173
Elderly	≥65	430	0.034	0.107
Total Population	≥1.5	3,666	0.065	0.235

bw = body weight; n = sample size; NDNS = National Diet and Nutrition Survey; UK = United Kingdom.

The maximum intended use level in Foods for Special Medical Purposes (FSMPs) is 15 µg vitamin D_2/day, excluding those intended for infants. This is with the assumption that the powder would be the only source of vitamin D in the diet as many Foods for Special Medical Purposes replaces significant components in the diet. This is also the same maximum amount intended for total diet replacement for weight control and meal replacements for weight control.

Combined intake from the novel food and other sources was considered. Results from the summary of the estimated daily intake of vitamin D from the total diet (Table 8) is used to calculate the cumulative exposure of vitamin D from the total diet. It is concluded that based on these results, 29 µg/day for infants up to and including 11 months of age, and 25 and 26.3 µg/day for toddlers and other children, respectively, do not exceed the tolerable upper intake level (UL) of 35µg/day established by EFSA for infants aged 6 to 12 months of age and that of 50 µg/day for children aged 1 to 9 years. Similarly, the cumulative intakes of vitamin D from the total diet of 19.9, 30.5 and 23.3 µg/day among adolescents, adults, and elderly subjects, respectively, are well below the UL of 100 µg/day for each of these population groups.

Table 8. Estimated Daily Intake of Vitamin D from the Total Diet

Population Group	Intake of Vitamin D from Foods (µg/day)[a] 95th percentile	Intakes of Vitamin D_2 from the novel food (µg/day) 95th percentile	Intake of Vitamin D_2 from proposed use in Food Supplements (µg/day)	Intake of Vitamin D from the Total Diet (µg/day)[b] 95th percentile	UL (µg/day)
Infants (≤11 months)	19[c]	10.0[d]	10.0 (infants 7 to 11 months)	29[e]	25 (0 to 6 months)[f] 35 (6 to 12 months)[f]
Toddlers (1 to 3 years)	15[c]	10.0[d]	15.0	25[e]	50[g]
Other children (3 to 9 years)	15[c]	11.3	15.0	26.3[e]	50[g]
Adolescents (10 to 17 years)	8[c]	9.8 to 11.9	15.0	17.8 to 19.9[e]	100[g]
Adults (18 to 64 years)	16	10.7 to 14.5	15.0	26.7 to 30.5	100[g]
Elderly (65 to 74 years)	16	7.3	15.0	23.3	100[g]

NDNS = National Diet and Nutrition Survey; UL = tolerable upper intake level.

[a] EFSA NDA Panel (2016): https://www.efsa.europa.eu/en/efsajournal/pub/4547.

[b] Intake of vitamin D from the total diet = (intake of vitamin D from foods) + (intake of vitamin D_2 from UV treated *A. bisphorus* Mushroom Powder with increased vitamin D_2 + (intake of vitamin D_2 from food supplements).

[c] Intake from foods and food supplements.

[d] No data on intakes for infants in the NDNS; as such, the estimated values for toddlers were applied to the cumulative calculation for both infants and toddlers.

[e] As the intake of vitamin D from foods also includes the intake from food supplements for this population group, the cumulative intake of vitamin D_2 from the total diet does not include the intake of vitamin D_2 from food supplements (to avoid duplication).

[f] EFSA NDA Panel (2018): https://www.efsa.europa.eu/en/efsajournal/pub/5365.

[g] EFSA NDA Panel (2012): https://www.efsa.europa.eu/en/efsajournal/pub/2813

Data on the likely impact of vitamin D exposure and the vitamin D_2 vitamin, in particular from consumption of the novel food was noted. The additional consumption is predicted not to lead to increases above safe levels.

2.8. Absorption, Distribution, Metabolism and Excretion (ADME)

Two studies using UV-Irradiated mushroom products supplied by Monterey were undertaken. The first was a human study performed in a randomised, double-blinded, placebo-controlled 6-week study involving 38 healthy adults (14 males and 24 females) allocated 1 of 4 groups.

The groups consisted of; a placebo control group receiving non-exposed mushroom powder, a group receiving UV-B-exposed mushroom powder providing 8.8µg/day, a group receiving 17.1 µg/day of vitamin D_2, plus the placebo control dose and 1 supplement control group receiving vitamin D_2 supplements providing 28.2 µg/day vitamin D_2. All groups consumed similar meals containing 1 serving (87.9 g) of cooked white button mushrooms. The study measured serum 25(OH)D_2, 25(OH)D_3, 24,25-hydroxycholecalciferol [24,25$(OH)_2D_3$] and total 25(OH)D from blood samples taken at baseline, after 3 weeks and after 6 weeks.

The authors of the study concluded that that vitamin D_2 from UV-exposed mushrooms is absorbed and metabolized to 25(OH)D_2. They measured a small but significant decrease in serum 25(OH)D_3. The authors hypothesise that this is a result of the study being undertaken in a sunny climate and so exposure to vitamin D from sunlight was a confounder in interpreting their results due to wider regulation of vitamin D levels by the body (Stephensen et al., 2012).

The second human ADME study involved a 12-week randomised study carried out during winter to minimise influence of environmental UV-B exposure, where 25 healthy adults consumed either UV-exposed mushroom extract (supplied by Monterey), vitamin D_2 supplement or vitamin D_3 supplement (all providing 50µg vitamin D_2/day) in capsules once daily for 12 weeks. The study measured serum 25(OH)D, 25(OH)D_2 and 25(OH)D_3 from blood samples taken weekly throughout the study, to determine the bioavailability of the supplements. The study concluded that the mushroom extract was as effective at increasing and maintaining total serum 25(OH)D levels as supplemental vitamin D_2 (Keegan et al., 2013).

The ADME information does not indicate any further areas of concern.

2.9. Nutritional Information

The novel food is mainly composed of approximately 50% carbohydrates, 33% protein, 20% dietary fibre, 9% ash, 3% fat, and less than 5% moisture. It also contains minerals and vitamins.

The nutritional content of mushrooms exposed to UV light is unchanged, with the exception of the intended increase in vitamin D_2 content (Simon et al., 2011). Vitamin D_2 from UV-irradiated mushrooms has been shown to be bioavailable in several human studies using 25(OH)D as an indicator (Keegan et al., 2013; Mehrotra et al., 2014; Outila et al., 1999; Shanely et al., 2014; Stepien et al., 2013), and that increases in 25(OH)D have also been observed in animal studies (Bennett et al., 2013; Calvo et al., 2013; Jasinghe et al., 2005; Koyyalamudi et al., 2009).

The ADME studies outlined bioavailability, and it was noted that while serum levels increased in some studies this was not necessarily related to changes in vitamin D status. This might be because of a difference in starting vitamin D status or exposure to sunlight. This was explored further in a study undertaken in the winter months.

Consumption of the novel food at the proposed use levels is not expected to be nutritionally disadvantageous for consumers.

2.10. Toxicological Information

2.10.1. Genotoxicity and Sub-chronic toxicity

Genotoxicity and sub-chronic toxicology studies were not provided on the basis that the impact of the novel process is on the vitamin D_2 content of mushrooms. There are not expected to be specific concerns as a result of the conversion of the endogenous precursor to vitamin D_2.

There are a number of authorised foods that are UV-treated; UV-treated baker's yeast, UV-treated bread and UV-treated milk. There are also studies with ingredients similar to the novel food i.e. vitamin D_2 mushroom powder produced by homogenisation of mushrooms before exposure to UV light (EFSA NDA Panel, 2020). No specific toxicological issues beyond vitamin D exposure were highlighted.

2.10.2. Human studies

A review of six human studies (Keegan et al., 2013; Mehrotra et al., 2014; Shanely et al., 2014; Stephensen et al., 2012; Stepien et al., 2013; Urbain et al., 2011) was provided with the conclusion that daily consumption of UV-B-exposed *A. bisporus* mushrooms providing up to 65 µg (2,600 IU) vitamin D_2/day for 16 weeks is safe. There were not any associated reported adverse effects. The studies indicated Vitamin D_2 from UV-B-exposed *A. bisporus* mushroom products was bioavailable, as evidenced by increased serum 25(OH)D_2 (the direct metabolite of vitamin D_2) concentrations.

2.11. Allergenicity

UV-treated *A. bisporus* Mushroom Powder with increased vitamin D_2 is expected to have the same allergenic risk as that associated with consumption of *A. bisporus* mushrooms, as the ultraviolet treatment did not alter the composition of the mushrooms apart from the content of vitamin D_2.

2.12. Discussion

The novel food is UV-treated *A. bisporus* Mushroom Powder with increased vitamin D_2, produced by the exposure of *Agaricus bisporus* mushrooms to ultraviolet light which catalyses the conversion of endogenous ergosterol within the mushrooms to vitamin D_2. The mushrooms are then dehydrated and ground into a powder.

The target population is the general population. UV-treated *A. bisporus* Mushroom Powder with increased vitamin D_2 in food supplements is not intended to be consumed by those under the age of 7 months.

The novel food contains vitamin D_2 content ranging between 125-463 µg/g and is intended to be used as ingredient to achieve a maximum dose of vitamin D_2 of 2.25 µg/100 g for food products, and 1.125 µg/100 mL for beverages. In food supplements the proposed maximum dose of vitamin D_2 is 15 µg/day in food supplements for the general population older than 1 year of age and at 10 µg/day in food supplements for infants from 7 to 11 months.

The proposed maximum vitamin D_2 levels meet the Acceptable Intake level set by EFSA for these population groups. These use levels ensure that the required amount to be deemed as a source of vitamin D [as stipulated in Regulation (EC) No 1924/2006] would be met.

Combined intake of vitamin D_2 from the novel food with that of the other dietary sources was considered to not exceed the upper limits for vitamin D previously established by the NDA Panel for infants (EFSA NDA Panel, 2018), and for children, adolescents and adults (EFSA NDA Panel, 2012).

3. Conclusions

The FSA and FSS have undertaken the assessment of the novel food, UV-treated mushrooms from Monterey Mushrooms Inc, and concluded that the novel food is safe under the proposed conditions of use and does not pose a safety risk to human health. The anticipated intake level and the proposed use in food and food supplements was not considered to be nutritionally disadvantageous.

These conclusions based on the information in the novel food dossier submitted by the applicant plus the supplementary information and could not have been reached without the following data claimed as proprietary by the applicant:

- annexes to the dossier which relate to the identity of the novel food, the production process, composition, stability, and the intakes assessment report.

Abbreviations

ACNFP	Advisory Committee on Novel Foods and Processes
ADME	Absorption, Distribution, Metabolism and Excretion
AOCS	Association of Official Analytical Chemists
BW	body weight
CAS	Chemical Abstracts Service
CFU	Colony Forming Unit
cGMP	Current Good Manufacturing Practice
EC	European Commission
EFSA	European Food Safety Agency
EU	European Union
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration
FSA	Food Standards Agency
FSAI	Food Safety Authority Ireland
FSS	Food Standards Scotland
GLP	Good Laboratory Practice
HACCP	Hazards Analysis and Critical Control Points
HPLC	High Performance Liquid Chromatography
HMBC	Heteronuclear multiple bond correlation
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin-Layer Chromatography
ISO	International Organisation for Standardization
IU	International Unit
IUPAC	International Union of Pure and Applied Chemistry
LOD	Limit of detection
LOQ	Limit of quantification
ND	not determined
NDA	Dietetic products, Nutrition and Allergies
NLT	less than not
NMT	not more than
NMR	Nuclear Magnetic Resonance
NOAEL	No Observable Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
USP	United States Pharmacopoeia
UV	Ultra-Violet
25(OH)D	25-hydroxy vitamin D

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