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SCIENTIFIC OPINION

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# Safety of vitamin $D_2$ mushroom powder as a Novel food pursuant to Regulation (EU) 2015/2283 (NF 2020/2226)

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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 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 in the form of vitamin $D_2$ in the range of 245–460 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 1.2 or 2.4 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 at a maximum of 15 μg vitamin $D_2$/day for individuals above 1 year of age, as well as in foods for special medical purposes (FSMPs). The estimates for combined intake of vitamin D from the NF, the background diet and fortified foods, were below the ULs for vitamin D as established previously by the NDA Panel for children, adolescents and adults, i.e. 50 and 100 μg/day. The estimated combined vitamin D intake in infants (6–12 months) is also below the UL for vitamin D of 35 μg/day. The Panel considers that taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous for the proposed target population. The Panel concludes that the NF is safe under the proposed conditions of use.

**KEYWORDS**

*Agaricus bisporus*, food supplement, mushroom powder, novel food, safety, UV treatment, vitamin $D_2$

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# 1 | INTRODUCTION

## 1.1 | Background and Terms of Reference as provided by the requestor

On 14 December 2020, the company Luxidum GmbH 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 applicant 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 has been used to enhance the content of vitamin D in some foods, making the resulting foods novel. The EFSA NDA Panel had previously adopted opinions on vitamin D mushroom powders produced from *Agaricus bisporus* as novel food (NF) (EFSA NDA Panel, 2020, 2021, 2022). 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 Commission Implementing Regulation (EU) 2017/2470.[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.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in 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 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 the production process and the stability tests including the respective certificate of analysis.

## 2.2 | Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016a) 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 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 and in Commission Delegated Regulation (EU) 2016/128 for food for special medical purposes.

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.

[1]Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods, amending Regulation (EU) No 1169/ 2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (2013/0435 (COD). OJ L 327, 11.12.2015, p. 1–22.

[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

# 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 245–460 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 in individuals above 1 year of age.

## 3.2 | Identity of the NF

The NF is a powder of the fruiting bodies of the mushroom *Agaricus bisporus* containing vitamin $D_2$ (ergocalciferol) induced by UV treatment.

Vitamin $D_2$ has a registered CAS number 50-14-6 and its IUPAC name is (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 (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, is also known as the Portobello mushroom.

## 3.3 | Production process

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

The mushrooms used in the production of the NF are cultivated *Agaricus bisporus*. The applicant is not growing or processing the mushroom but is buying the mushroom powder from a producer. The NF production process includes a controlled exposure of the mushroom powder to UV irradiation (UV-LED (light-emitting diode) irradiation developed by the applicant). According to the applicant, once the mushroom powder is received, it is conveyed on a tray by a vibrating plate powered by an electromagnetic vibration drive. On its way to the collection tank, the mushroom powder passes a UV light unit irradiating the product with UV light (the UV light unit LED compact Vitamin D-Booster, which is equipped with a patented LED2D® technology using a wavelength combination). The UV light causes a photochemical process by converting its precursor ergosterol to vitamin $D_2$ in the mushroom (*Agaricus bisporus)* powder.

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 certain characteristics, the applicant provided analytical information for independently produced batches of the NF. Information about the vitamin $D_2$ concentrations, moisture, ash and proximate characteristics are included in Table 1.

**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) | 337 | 303 | 297 | 334 | 278 | LC–MS/MS |
| Moisture (g/100 g) | 7.7 | 6.3 | 6.4 | 6.4 | 6.3 | Calculated from dry matter analysis |
| Ash (g/100 g) | 9.6 | 9.8 | 9.9 | 9.9 | 9.9 | Gravimetry ASU L 06.00–4 |
| Proximates | | | | | | |
| Crude protein (g/100 g) | 33.9 | 31.2 | 33.7 | 31.8 | 35.4 | Titrimetry based on Kjeldahl |
| Total fat (g/100 g) | 3.3 | 3.6 | 3.8 | 3.4 | 3.8 | Gravimetry based on Weibull–Stoldt |
| Carbohydrates (g/100 g) | 43.8 | 42.1 | 42.4 | 44.0 | 41.2 | Calculation |

| Parameter (unit) | Batch number #1 | #2 | #3 | #4 | #5 | Method of analysis |
|---|---|---|---|---|---|---|
| Total dietary fibre[4] (g/100 g) | 18.5 | 18.4 | 18.8 | 19.0 | 19.7 | Enzymatic gravimetry ASU L 00.00–18, mod. |
| Energy value (kcal/100 g) | 340 | 326 | 339 | 334 | 341 | Calculation |
| Water activity (at 25°C) | 0.397 | 0.422 | 0.413 | 0.388 | 0.405 | Dew point measurement (ISO 18787) |

Abbreviations: ISO, International Organization for Standardization; LC–MS/MS, liquid chromatography–tandem mass spectrometry; LED, Light-emitting diode.

The applicant also provided detailed analyses of heavy metals, mycotoxins and microbiological quality (Table 2).

**TABLE 2** Chemical and microbiological parameters in batch-to-batch analysis of the NF.

| Parameter (unit) | Batch number #1 | #2 | #3 | #4 | #5 | Method of analysis |
|---|---|---|---|---|---|---|
| Heavy metals | | | | | | |
| Lead (mg/kg) | 0.04 | 0.05 | 0.04 | 0.05 | 0.05 | ICP-MS DIN EN 15763, mod |
| Cadmium (mg/kg) | 0.04 | 0.05 | 0.04 | 0.04 | 0.04 | ICP-MS DIN EN 15763, mod |
| Arsenic (mg/kg) | 0.14 | 0.13 | 0.13 | 0.14 | 0.14 | ICP-MS DIN EN 15763, mod |
| Mercury (mg/kg) | 0.011 | 0.024 | 0.028 | 0.031 | 0.02 | ICP-MS DIN EN 15763, mod |
| Mycotoxins | | | | | | |
| Aflatoxin B1 (µg/kg) | 0.6 | < 0.2 | < 0.2 | < 0.2 | < 0.2 | LC–MS/MS |
| Sum of aflatoxins (B1, B2, G1, G2) (µg/kg) | 0.6 | < 0.2 | < 0.2 | < 0.2 | < 0.2 | LC–MS/MS |
| Microbiological parameters | | | | | | |
| Total viable count (CFU/g) | 3400 | 4300 | 2400 | 3200 | 4700 | Enumeration. In-house method validated based on ISO 4833-1 |
| *Escherichia coli* (in 1 g) | n.d | n.d | n.d | n.d | n.d | Detection (DIN EN ISO 16649-3) |
| Coliforms (CFU/g) | < 100 | < 10 | < 10 | < 10 | < 10 | Enumeration (ISO 4832) |
| Coagulase positive staphylococci (in 1 g) | n.d | n.d | n.d | n.d | n.d | Detection (DIN EN ISO 6888-3) |
| Enterobacteriaceae CFU/g) | < 100 | < 10 | < 10 | < 10 | < 10 | Enumeration. In-house method validated based on DIN EN ISO 21528-2 |
| *Salmonella* species (in 25 g) | n.d | n.d | n.d | n.d | n.d | In-house method validated based on DIN 10135 – PCR |
| *Listeria monocytogenes* (CFU/g) | < 100 | < 10 | < 10 | < 10 | < 10 | Enumeration. In-house method validated based on DIN EN ISO 11290-2 |
| Yeast (CFU/g) | < 100 | < 100 | < 100 | < 100 | < 100 | Enumeration. In-house method validated based on ISO 21527-2 |
| Mould (CFU/g) | < 100 | < 100 | < 100 | < 100 | < 100 | Enumeration. In-house method validated based on ISO 21527-2 |

Abbreviations: CFU, colony forming units; ICP-MS, inductively coupled plasma mass spectrometry; ISO, International Organization for Standardization; LC–MS/MS, Liquid chromatography tandem mass spectrometry; mod, modified; n.d, not detected; PCR, polymerase chain reaction.

The Panel notes that the applicant did not report the detection values for *L. monocytogenes* and used an enumeration method. Provided that the specifications are met, the microbiological compositional data do not raise safety concerns.

In addition to the chemical contaminants described in Table 2, a multiresidue pesticides analysis was performed by the applicant. The Panel notes that the pesticide levels reported are below the EU maximum residue levels for pesticides.[5]

The conversion of ergosterol into vitamin $D_2$ with UV exposure is accompanied by photochemical isomerisation resulting in photoisomers such as vitamin $D_4$, lumisterol and tachysterol (Havinga et al., 1960). Both lumisterol and tachysterol are biologically inactive (Holick et al., 1981), while vitamin $D_4$ has approximately 60% of the vitamin $D_3$ activity (Schümmer et al., 2021). The applicant provided data on the vitamin D photoisomers in five representative batches of the NF (Appendix A). The mean concentrations of vitamin $D_4$ and tachysterol, measured by GC–MS (Sommer et al., 2023), were 5.5 µg/g NF and 21.2 µg/g NF, respectively The concentration of lumisterol was below the LOD (4.6 µg/g NF). The concentrations of the

[4]The term is used as synonymous of non-digestible carbohydrates and does not reflect the additional requirement of having a beneficial physiological effect demonstrated by generally accepted scientific evidence laid down in Annex I of Regulation (EC) 1169/2011.

[5]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.

photoisomers in the NF are lower than those reported in previous EFSA opinions on UV-treated NFs (EFSA NDA Panel, 2014, 2015, 2020, 2021, 2022). The exposure to these compounds from the NF is not considered of toxicological relevance. 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 three independently produced batches of the NF. The batches were analysed for the vitamin $D_2$ content. The tests were carried out at normal storage conditions at 21 ± 2°C and at 62 ± 10% RH for 37 months. A statistically significant decrease of 31% (ANOVA, LSD post hoc test) of the vitamin $D_2$ content was found at month 37 (312 μg/g) compared to the initial value on day 0 (451 μg/g). The applicant also performed microbiological stability tests of the NF after storage time of 51 months and the results were below the limit of detections and within the specifications.

The applicant proposed a shelf-life of minimum 24 months. The Panel considers that the applicant provided sufficient information with respect to the stability of the NF for 24 months.

The applicant also performed stability tests of the NF used in two different food supplements developed for the tests. The tests were carried out at representative storage conditions (room temperature) and the test material (2 types of food supplements in capsule form containing the NF) was kept in screw-cap brown glasses. The applicant provided data on the stability of the food supplements containing the NF on the initial, the 4th and 15th month. According to the stability tests provided, there was no statistically significant difference (*t*-test) between the vitamin $D_2$ contents on the initial, the 4th and 15th month in the two different supplements analysed.

For the use of the NF in food matrices, the applicant performed a stability test of the NF used in a representative processed food with short shelf-life, i.e. bread, with vitamin $D_2$ content of 2.5 and 5 μg/100 g dough, with no special packaging of the baked bread which was stored for 5 days at 19.8°C and 65.2% RH. The final vitamin $D_2$ content in the baked bread was higher (3.3 and 6.6 μg/100 g) than the initial content (2.5 and 5 μg/100 g, respectively), possibly due to weight loss by water evaporation during baking. Vitamin $D_2$ content was considered to be stable over the 5-day shelf-life period.

Additionally, the applicant provided a stability test for the NF used in three batches of a processed beverage (dairy imitate – hemp seed drink) containing 4.34 mg NF/100 mL and providing approximately 2 μg/g vitamin $D_2$. Vitamin $D_2$ content was measured using LC/MS–MS in the unopened hemp drink containing the NF after 1-, 2-, 4- and 6-month storage at room temperature of 18–20°C and relative air humidity below 40% as well as after opening and storage at 6°C for 0, 24, 48, 72 and 96 h. The results of these stability studies indicate a stable vitamin $D_2$ content both in unopened and in opened, refrigerated hemp seed drink containing the NF.

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

## 3.5 | Specifications

The specifications of the NF are indicated in Table 3.

**TABLE 3** Specifications of the NF.

| **Description:** Slightly brown, granular powder made from UV-irradiated Agaricus bisporus mushroom powder | |
|---|---|
| **Parameter** | **Specification** |
| Vitamin $D_2$ | 245–460 μg/g |
| Moisture | ≤ 10% |
| Ash | ≤ 12% |
| Water activity | ≤ 0.5 |
| Proximate parameters | |
| Total carbohydrates | 40%–45% |
| Protein | 30%–36% |
| Fat | 3.0%–4.5% |
| Heavy metals | |
| Arsenic | ≤ 0.3 mg/kg |
| Lead | ≤ 0.3 mg/kg |
| Cadmium | ≤ 0.05 mg/kg |
| Mercury | ≤ 0.1 mg/kg |
| Mycotoxins | |
| Aflatoxin B1 | ≤ 2 μg/kg |
| Aflatoxins (sum of B1 + B2 + G1 + G2) | < 4 μg/kg |

**TABLE 3** (Continued)

| **Description:** Slightly brown, granular powder made from UV-irradiated Agaricus bisporus mushroom powder | |
|---|---|
| **Parameter** | **Specification** |
| Microbiological | |
| TAMC | ≤ 5000 CFU/g |
| TYMC | ≤ 100 CFU/g |
| *Salmonella* spp. | Not detected in 25 g |
| *Escherichia coli* | Not detected in 10 g |
| *Staphylococcus aureus* | Not detected in 10 g |
| *Listeria monocytogenes* | Not detected in 25 g |

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

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*. *Agaricus bisporus* mushrooms have a long history of use and consumption in the EU.

Similar NFs, 'vitamin $D_2$ mushroom powders' with different production processes, specifications or proposed uses are already authorised and included in the Annex to Implementing Regulation (EU) 2017/2470 (see Section 1.2 Additional information).

## 3.7 | Proposed uses and use levels and anticipated intake

### 3.7.1 | Target population

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

The target population proposed by the applicant for the consumption of the NF added to food supplements is individuals above 1 year of age.

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.

### 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 are defined using the FoodEx2 hierarchy, and the maximum use levels are reported in Table 4.

The use level of the NF in foods or beverages is determined by the applicant based on the vitamin $D_2$ content per gram of NF (245–460 µg vitamin $D_2$/g NF).

The proposed levels of the NF are 5.22 mg/100 g for products other than beverages (excluding food supplements) and 2.61 mg/100 mL for beverages. The maximum proposed level of vitamin $D_2$ as consumed using the maximum specification for vitamin $D_2$ content in the NF (i.e. 460 µg vitamin $D_2$/g NF) would result in an intake of 2.4 µg of vitamin D2/100 g in foods and 1.2 µg of vitamin $D_2$/100 mL for beverages.

The applicant also intends to market the NF for use in food supplements and FSMPs, at a maximum dose of 15 µg of vitamin $D_2$/day, for individuals above 1 year of age.

**TABLE 4** Food categories and maximum use levels intended by the applicant and corresponding vitamin $D_2$ levels with maximum specifications for vitamin D content.

| FoodEx2 level | FoodEx2 code | Food category | Max use level (mg NF/100 g) | Corresponding concentrations of vitamin $D_2$ (μg/100 g or 100 mL)[1] |
|---|---|---|---|---|
| 2 | A00CV | Breakfast cereals | 5.22 | 2.4 |
| 3 | A0BY0 | Leavened bread and similar | 5.22 | 2.4 |
| 3 | A00BK | Yeast leavened pastry | 5.22 | 2.4 |
| 3 | A007D | Pasta and similar products | 5.22 | 2.4 |
| 2 | A0BX9 | Fruit/vegetable juices and nectars | 2.61 | 1.2 |
| 2 | A03BM | Concentrated or dehydrated fruit/vegetables juices | 18.3[2] | 8.4 |
| 4 | A02NQ | Yoghurt drinks | 2.61 | 1.2 |
| 4 | A02NR | Probiotic milk-like drinks | 2.61 | 1.2 |
| 3 | A02PE | Milk and dairy concentrate | 5.22 | 2.4 |
| 4 | A02PJ | Milk powder | 52.2[3] | 24.0 |
| 4 | A02PM | Cream powder | 214.0[4] | 98.4 |
| 4 | A02PN | Whey powder | 52.2[3] | 24.0 |
| 4 | A02MP | Flavoured milks | 2.61 | 1.2 |
| 3 | A0EZB | Whey | 5.22 | 2.4 |
| 2 | A02PT | Dairy dessert and similar | 5.22 | 2.4 |
| 3 | A02QE | Cheese | 5.22 | 2.4 |
| 4 | A03RS | Food for weight reduction | 5.22 | 2.4 |
| 2 | A03TD | Meat and dairy imitates | 5.22 | 2.4 |
| 3 | A0B9J | Soups, dry mixture, uncooked | 47.0[5] | 21.6 |
| 3 | A041L | Soups, ready-to-eat | 5.22 | 2.4 |
| 4 | A0EQV | Puffs/curls-type extruded snack | 5.22 | 2.4 |
| 5 | A011L | Potato crisps or sticks | 5.22 | 2.4 |

Abbreviations: NF, Novel Food.

[1]The corresponding concentrations of vitamin $D_2$ indicated for the maximum specification of the NF for vitamin $D_2$ (460 μg/g).

[2]Adjusted for being present in concentrated or dehydrated form; reconstitution factor of 7.

[3]Adjusted for being present in powder form; reconstitution factor of 10.

[4]Adjusted for being present in powder form – assumed to be used as coffee whitener; reconstitution factor of 41.

[5]Adjusted for being present in powder form; reconstitution factor of 9.

### 3.7.3 | Anticipated intake of the NF

EFSA performed an intake assessment of the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 4), using the EFSA Dietary Exposure (DietEx) Tool,[6] which is based on 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 is presented in Table 5, expressed in mg per day.

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

[6]https://www.efsa.europa.eu/en/science/tools-and-resources/dietex

**TABLE 5** Estimated intake of the NF as an ingredient in the intended food categories at the maximum proposed use levels (mg/day).

| Population group | Age (years) | Mean intake (mg/day) | | P95 intake (mg/day) | |
|---|---|---|---|---|---|
| | | Lowest[a] | Highest[a] | Lowest[b] | Highest[b] |
| Infants | < 1 | 0.38 | 5.72 | 2.18 | 25.87 |
| Young children[c] | 1 to < 3 | 3.95 | 11.51 | 10.32 | 23.16 |
| Other children | 3 to < 10 | 9.22 | 23.01 | 17.98 | 38.91 |
| Adolescents | 10 to < 18 | 9.67 | 21.98 | 20.94 | 47.75 |
| Adults[d] | ≥ 18 | 12.5 | 20.31 | 20.90 | 44.75 |

Abbreviation: P95, 95th percentile.

[a]Intakes are estimated for all EU dietary surveys available in the food comprehensive database on 21 March 2024. 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 21 March 2024. 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]It includes elderly, very elderly, pregnant and lactating women.

## 3.8 | Absorption, distribution, metabolism and excretion (ADME)

No specific ADME studies for the NF have been provided.

In a meta-analysis on randomised clinical trials performed by the applicant and collaborators (Cashman et al., 2016), UV-irradiated vitamin D mushrooms consumption was associated with a significant increase in serum concentrations of total 25(OH)D when the mean baseline vitamin D status of the participants is inadequate [serum 25(OH)D < 50 nmol/L]. The applicant also performed a literature search on UV-irradiated mushroom powder absorption and metabolism in animals and humans. The publicly available studies suggest that vitamin $D_2$ from powder from UV-irradiated mushrooms is bioavailable, as dose-related increase in serum concentrations of 25(OH)$D_2$ is observed upon its consumption (EFSA NDA Panel, 2020, 2021).

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 consumption (EFSA NDA Panel, 2022).

## 3.9 | Nutritional information

The applicant provided a nutritional analysis of five batches of the NF. The NF is composed of digestible carbohydrates (~ 40%–45%), protein (30%–36%), non-digestible carbohydrates (~ 19%), ash (~ 10%) moisture (~ 6%), fat (~ 3%) and in addition, contains vitamins and minerals. A detailed analysis of the mineral and vitamin B content of the NF in five independent batches was provided by the applicant (Appendix B). 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.

### *Combined vitamin D intake from the NF and other sources*

Considering the maximum proposed conditions of use based on maximum vitamin $D_2$ levels of 2.4 μg/100 g for products other than beverages and 1.2 μg/100 mL for beverages, maximum estimated P95 daily intakes of vitamin $D_2$ from the NF as an ingredient in foods and beverages calculated in absolute values (μg/day) are reported in Table 6 below.

**TABLE 6** Anticipated highest P95 of vitamin $D_2$ daily intake from the NF as an ingredient in foods and beverages considering the maximum specification of vitamin $D_2$ (460 μg/g NF).

| Population group | Age (years) | Vitamin $D_2$ P95 intake (μg/day) |
|---|---|---|
| Infants | < 1 | 11.9 |
| Young children[a] | 1 to < 3 | 10.6 |
| Other children | 3 to < 10 | 17.9 |
| Adolescents | 10 to < 18 | 22.0 |
| Adults[b] | ≥ 18 | 20.6 |

[a]Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

[b]It 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 6) and the high vitamin D intakes from other food sources as reported by the EFSA NDA Panel in the recent opinion on the tolerable upper intake

level for vitamin D (EFSA NDA Panel, 2023). In this Opinion, the highest 95th percentile (P95) background dietary vitamin D intakes (i.e. excluding food fortification and food supplements) across national food consumption surveys was 9.1 μg/day in toddlers (1 to < 3 years), up to 10.1 μg/day in children (3 to < 10 years) up to 11.9 in adolescents (10 to < 18 years), and up to 16.1 μg/day in adults (≥ 18 years) (EFSA NDA Panel, 2023).

Table 7 below provides an overview of the exposure to vitamin D from different sources separately and combined based on the maximum specification range for vitamin $D_2$ in the NF, and the tolerable upper intake levels (ULs) established for young children, children, adolescents and adults.

**TABLE 7** Total vitamin D intake (μg/day) resulting from the uses of the NF as an ingredient and as a food supplement based on its maximum specification range in comparison to the ULs for vitamin D.

| Population group | Highest P95 vitamin D intake from the background diet[a] (EFSA NDA Panel, 2023) | Highest intake of vitamin D from the background diet + fortified foods[b] (EFSA NDA Panel, 2023) | 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 vitamin D intake[g] | Total vitamin D intake[h] | Vitamin D UL (EFSA NDA Panel, 2023) |
|---|---|---|---|---|---|---|---|
| Young children (1 to < 3 years)[c] | 9.1 | 11.7 | 10.6 | 15 | 34.7 | 37.3 | 50 |
| Other children (3 to < 10 years)[d] | 10.1 | 11.7 | 17.9 | 15 | 43.0 | 44.6 | 50 |
| Adolescents (10 to < 18 years)[e] | 11.9 | 13.1 | 22.0 | 15 | 48.9 | 50.1 | 100 |
| Adults (≥ 18 years)[f] | 16.1 | 19.5 | 20.6 | 15 | 51.7 | 55.1 | 100 |

Abbreviations: NF, novel food; P95, 95th percentile; UL: tolerable upper intake level.

[a]Excluding food fortification and food supplements (EFSA NDA Panel, 2023).

[b]Combined intakes of vitamin $D_2$ and vitamin $D_3$ from the background diet and fortified foods based on published data from national food consumption surveys. Figures correspond to the highest reported P95 for the relative age category. The highest P95 reported for toddlers is used for children 3–10 years, as intake estimates for this specific age category were not available.

[c]Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

[d]Intakes are assessed separately for young [(3 to < 7 years] and older children (7 to < 10 years]; the maximum intake among these subpopulations is reported here.

[e]Intakes are assessed separately for young [10–14 years] and old adolescent [14–18 years]; the maximum intake among these 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 subpopulations is reported here.

[g]Resulting from the sum of the combined intake of vitamin D from the NF as an ingredient, as food supplement, and the highest intake of vitamin D from the background diet (excluding fortified foods).

[h]Resulting from the sum of the combined intake of vitamin D from the NF as an ingredient, as food supplement, and the highest intake of vitamin D from the background diet and fortified foods.

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 48.9 and 51.7 μg/day for adolescents and adults, respectively. Those intake estimates (as reported in Table 9) are below the UL of 100 μg/day (EFSA NDA Panel, 2023) 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 34.7 and 43.0 μg/day, respectively. Those intake estimates (as reported in Table 7) are below the UL of 50 μg/day (EFSA NDA Panel, 2023) for each of these population groups.

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 that were also not consuming (voluntarily) fortified foods, the P95 vitamin D intake from the diet ranged between 0.7 and 2.8 μg/day (EFSA NDA Panel, 2018, 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 11.9 μg/day considering the maximum specifications (see Table 6). The addition of this amount to the highest P95 vitamin D intake of formula consumers not consuming fortified foods (16.9 μg/day from EFSA NDA Panel, 2018) results in a combined intake of 28.8 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). The Panel notes that the estimated combined maximum vitamin D intake (from the NF as food ingredient and from formula consumption) in infants of 28.8 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). However, 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 exceeding the UL for vitamin D in infants if additional supplementation is used.

In the recent UL vitamin D Opinion from EFSA, combined intakes of vitamin D from food, including fortified foods but excluding food supplements, are available from national food consumption surveys (EFSA NDA Panel, 2023). However, considering the combined intakes of vitamin D from food (including fortified foods), the intakes of vitamin D from the NF as an ingredient and the NF as food supplement, the total vitamin D combined intakes are still below the ULs for vitamin D for all population groups (as reported in Table 7).

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

### 3.10 | Toxicological information

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 human studies with the NF.

The applicant performed a literature search on UV-irradiated *Agaricus bisporus* mushroom and specific similar products to the NF, i.e. irradiated powder of *Agaricus bisporus*. Most of these studies (Keegan et al., 2013; Shanely et al., 2014; Stephensen et al., 2012; Stepien et al., 2013; Urbain et al., 2011) were assessed in previous NDA Scientific Opinions on vitamin D mushroom powder (EFSA NDA Panel, 2020, 2021). All studies consistently showed an increase in 25(OH)$D_2$ following the intervention with *Agaricus bisporus,* while no adverse effects were reported. Two additional recent publications provided by the applicant were in agreement with those findings (Pinto et al., 2020; Zajac et al., 2020).

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 than that associated with normal consumption of *Agaricus bisporus* mushrooms and that the additional UV treatment is not expected to alter the risk (EFSA NDA Panel, 2020, 2021, 2022).

## 4 | DISCUSSION

The NF which is the subject of the application is a powder from *Agaricus bisporus* mushrooms exposed to UV irradiation to induce the conversion of provitamin $D_2$ (ergosterol) to vitamin $D_2$ (ergocalciferol). The NF contains vitamin $D_2$ concentrations in the range 245–460 µg/g. The target population is the general population, except for FSMPs and food supplements, for which the target population is individuals above 1 year of age.

The conservative vitamin D estimates for combined intake of highest P95 vitamin $D_2$ from the NF when the maximum specifications for vitamin D in the NF are used, together with the highest P95 intake of all forms of vitamin D from the background diet and fortified foods, were below the ULs for vitamin D as established by the NDA Panel for children, adolescents and adults (EFSA NDA Panel, 2023). The Panel also notes that the estimated combined vitamin D intake in infants is also below the UL for vitamin D for infants aged 6 to less than 12 months established by EFSA (EFSA NDA Panel, 2018).

The Panel considers that taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF as a food ingredient and as a food supplement is not nutritionally disadvantageous for the proposed target population. Sources of uncertainty in the intake estimates for vitamin D from the background diet and fortified foods are discussed in the scientific opinion on the UL for vitamin D, where the Panel concluded that it is unlikely that the UL for vitamin D is exceeded in European populations, except for regular users of food supplements containing high doses of vitamin D (EFSA NDA Panel, 2023).

## 5 | CONCLUSIONS

The Panel concludes that the NF, vitamin $D_2$ mushroom powder containing vitamin $D_2$ in the range of 245–460 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 the production process and the stability tests including the respective certificate of analysis.

## STEPS TAKEN BY EFSA

1. On 28/05/2021 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. Ref. Ares(2021)3541234–28/05/2021.
2. On 28/05/2021, a valid application on vitamin $D_2$ mushroom powder as a novel food, which was submitted by Luxidum GmbH, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/2226) and the scientific evaluation procedure was initiated.
3. On 16/07/2021, 16/05/2022 and 21/07/2023 EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4. On 11/04/2022, 10/05/2023, 16/04/2024, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5. During its meeting on 30/04/2024, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of vitamin $D_2$ mushroom powder as a NF pursuant to Regulation (EU) 2015/2283.

### ABBREVIATIONS

| | |
|---|---|
| ADME | absorption, distribution, metabolism and excretion |
| ANOVA | Analysis of variance |
| 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 |
| GM | good manufacturing practice |
| HACCP | Hazard Analysis Critical Control Points |
| HPLC | High-performance liquid chromatography |
| 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 |
| LED | Light Emitting Diode |
| LOD | Limit of Detection |
| n.d | Not detected |
| NDA | EFSA Panel on Nutrition, Novel Foods and Food Allergens |
| NF | Novel Food |
| PCR | Polymerase Chain Reaction |
| RH | relative humidity |
| TYMC | total yeast and mould count |
| UL | tolerable upper intake level |
| UV | ultraviolet |

### CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

### REQUESTOR

European Commission

### QUESTION NUMBER

EFSA-Q-2020-00849

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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## APPENDIX A

### Batch-to-batch analysis of vitamin $D_2$ photoisomers in the NF

| | Batch number | | | | | |
|---|---|---|---|---|---|---|
| **Parameter (unit)** | **#1** | **#2** | **#3** | **#4** | **#5** | **Method of analysis** |
| Vitamin $D_2$ (μg/g)[1] | 337 | 303 | 297 | 334 | 278 | LC–MS/MS |
| Ergosterol (μg/g) | 2500 | 2500 | 2400 | 2000 | 2300 | GC–MS |
| Vitamin $D_2$ (μg/g)[2] | 310 | 304 | 304 | 311 | 299 | GC–MS |
| Vitamin $D_4$ (μg/g) | 4.0 | 4.7 | 3.4 | 6.0 | 9.4 | GC–MS |
| Tachysterol (μg/g) | 20 | 22 | 24 | 19 | ND | GC–MS |
| Lumisterol (μg/g) | < 4.6[3] | < 4.6 | < 4.6 | < 4.6 | < 4.6 | GC–MS |

Abbreviations: GC/MS, gas chromatography mass spectrometry; LC–MS/MS, liquid chromatography tandem mass spectrometry; ND, not detected.

[1]Vitamin $D_2$ batch-to-batch analysis with LC–MS/MS.

[2]Additional vitamin $D_2$ analysis performed with GC–MS (as part of photoisomers analysis).

[3]4.6 μg/g is the limit of detection (LOD) for the analysis of lumisterol.

## APPENDIX B

### Batch-to-batch analysis of mineral and vitamin B content of the NF

| Parameter | Batch number #1 | #2 | #3 | #4 | #5 | Method of analysis |
|---|---|---|---|---|---|---|
| Minerals (mg/100 g) | | | | | | |
| Sodium | 52.9 | 50.7 | 53.4 | 54.1 | 51.1 | DIN EN 15621, mod |
| Calcium | 41.1 | 39.8 | 39.9 | 41.8 | 43.6 | |
| Potassium | 4067 | 4121 | 4230. | 4112 | 4341 | |
| Phosphorus | 1267 | 1334 | 1312 | 1291 | 1352 | |
| Magnesium | 131 | 128 | 128.9 | 136 | 129 | |
| Copper | 2.85 | 3.21 | 2.60 | 3.01 | 2.83 | |
| Iron | 4.60 | 4.80 | 4.75 | 4.69 | 5.15 | ICP-MS DIN EN 15763, mod |
| Vitamins | | | | | | |
| Vitamin B1 (mg/100 g) | 0.402 | 0.392 | 0.492 | 0.412 | 0.433 | DIN EN 14122, HPLC/FI |
| Vitamin B2 (mg/100 g) | 3.76 | 3.44 | 4.00 | 3.75 | 4.01 | DIN EN 14152, HPLC/FI |
| Total vitamin B6* (mg/100 g) | 0.769 | 0.757 | 0.763 | 0.774 | 0.833 | Calculation (based on DIN EN 14663, HPLC/FI) |
| Niacin (mg/100 g) | 46.6 | 44.2 | 45.0 | 46.9 | 48.4 | AOAC 944.13, Microbiology |
| Pantothenic acid (mg/100 g) | 11.2 | 12.2 | 13.3 | 10.9 | 11.9 | AOAC 945.74, Microbiology |
| Biotin (µg/100 g) | 39.0 | 39.4 | 38.7 | 39.5 | 42.5 | LC–MS/MS |
| Vitamin B12 (µg/100 g) | 0.144 | 0.131 | 0.161 | 0.154 | 0.169 | AOAC 952.20/986.23, Microbiology |

Abbreviations: AOAC, Association of Official Analytical Chemists; HPLC/FI, High-performance liquid chromatography/flame ionisation; ICP-MS: inductively coupled plasma mass spectrometry; LC–MS/MS, Liquid chromatography tandem mass spectrometry.

*According to the applicant, the total vitamin B6 is calculated as a sum of individual concentrations of pyridoxal, pyridoxamine and pyridoxine.

## Annexes

### 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 under supporting information).