SCIENTIFIC OPINION

Scientific Opinion on the safety of "*Lentinus edodes* extract" (Lentinex®) as a Novel Food ingredient[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies was asked to carry out the additional safety assessment for Lentinex®, an aqueous mycelial extract of *Lentinula edodes* (Shiitake mushroom), as a novel food ingredient in the context of Regulation (EC) No 258/97. Lentinex® consists of approximately 98 % water and 2 % dry matter (β-glucan lentinan, free glucose and N-containing constituents). The mushroom *Lentinula edodes* has an established history of consumption throughout the world. The applicant intends to market the ingredient in a wide range of products: dietary supplements, yoghurts, soft drinks, cooked and processed foods, and baked goods. The proposed intake of 2.5 mL Lentinex® containing 1 mg lentinan (β-glucan)/mL corresponds to 41.7 µg/kg body weight for a 60 kg person. The intake of β-glucan resulting from the proposed use is low compared to the intake estimated from the consumption of the mushroom *Lentinula edodes* and of other β-glucan sources. The animal and the human studies provided were primarily carried out to determine the efficacy of the novel food ingredient; they are supporting but of limited value regarding a safety assessment. Owing to the fermentative production of the novel food ingredient from the mycelium and the final application of a heat-induced sterilisation step, adverse effects reported after the consumption of the fruiting body of the Shiitake mushroom are not considered relevant. Although an allergenic risk cannot be excluded for sensitive subjects, this risk is expected not to be higher than that resulting from the normal consumption of the fruiting body of *Lentinula* edodes. The Panel notes the presence of soy peptides in the culture medium. The safety of Lentinex® as a novel food ingredient has been established at the proposed conditions of use and the proposed levels of intake.

KEY WORDS

Lentinan, ß-glucan, Lentinula edodes, Shiitake, fermentation, novel food ingredient.

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[1] On request from the European Commission, Question No EFSA-Q-2009-00833, adopted on 9 July 2010.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on the safety of *'Lentinus edodes* extract".

The novel food ingredient (trade name Lentinex®) is an aqueous extract obtained from the Shiitake mushroom *Lentinula edodes* cultivated in a submerged fermentation. It consists of approximately 98 % water and has a dry matter of approximately 2 %. The dry matter is composed of the principle constituent of the novel food ingredient, the β-glucan lentinan (approximately 1g/L), free glucose and of N-containing constituents (e.g. proteins, amino acids).

The fruiting body of *Lentinula edodes* has an established history of consumption throughout the world. The applicant intends to use the novel food ingredient, produced from the mycelium of *Lentinula edodes*, in a wide range of products: dietary supplements, yoghurts, soft drinks, cooked and processed foods, and baked goods. Lentinex® would be marketed in the form of a capsule, a liquid, powder, gel or as part of a food product. The proposed daily intake of 2.5 mL Lentinex® containing 1 mg lentinan (β-glucan)/mL corresponds to 41.7 μg/kg body weight per day for a 60 kg person. The Panel noted that the intake of β-glucan resulting from the proposed use of the highly diluted novel food ingredient is low compared to the intake estimated from the consumption of the mushroom *Lentinula edodes* and of other β-glucan sources.

The animal and the human studies provided were primarily carried out to determine the efficacy of the novel food ingredient; they are supporting but only of limited value regarding a safety assessment.

Owing to the fermentative production of the novel food ingredient from the mycelium and the final application of a heat-induced sterilisation step, adverse effects reported after the consumption of the fruiting body of the Shiitake mushroom are not considered relevant.

The Panel considers that despite the low intake of lentinan at the proposed use levels it cannot be excluded that Lentinex® poses an allergenic risk to sensitive subjects. However, this risk is expected not to be higher than that resulting from the normal consumption of the fruiting body of the Shiitake mushroom.

The Panel notes the presence of soy peptides in the culture medium.

The Panel concludes that the novel food Lentinex® is safe as a food ingredient at the proposed conditions of use and the proposed levels of intake.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 19 December 2007, GlycaNova Norge AS submitted a request under Article 4 of the Novel Food Regulation (EC) No 258/97 to place on the market an 'extract of *Lentinus edodes'* as a novel food ingredient.

On 3 November 2008, the competent authorities of the United Kingdom forwarded to the Commission their initial assessment report, which came to the conclusion that the evidence provided was satisfactory subject to labelling requirements regarding the presence of soya derivatives.

On 7 January 2009, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted additional comments. The concerns of a scientific nature raised by the Member States can be summarised as follows:

- The description of the chemical composition of the novel food ingredient should be more exact.
- As the energy content is 14.4 kcal per 100 mL, the source of this energy cannot be explained solely by the information provided by the applicant. Information on dry matter content is lacking.
- Concerns were expressed on secondary metabolites.
- A detailed description of the production process is lacking.
- Knowledge in relation to the traditional uses of the mushrooms and the information available are insufficient to assess the safety of the novel food ingredient because the uses and preparation methods of the novel food ingredient differ considerably from the culinary tradition associated with the consumption of this mushroom. The transformation processes may, in particular, alter the toxicity of the products.
- Information provided as to the chemical composition of the novel food ingredient is insufficient to give an opinion on the nutritional properties of the novel food ingredient.
- Limited toxicological studies have been provided. No specific trial has been carried out on animal models to assess the toxicity of the novel food ingredient.
- The study carried out on healthy elderly volunteers did not reveal any adverse secondary effects but it was of short duration and was not carried out for toxicological purposes. Some findings of toxic effects of *Lentinus edodes* products are reported in literature, but do not seem to be reflected in the application or the assessment report (Ingestion of raw or insufficiently cooked raw, dried or freeze-dried raw fungi have given rise to various adverse effects in humans, including eosinophilia, abdominal symptoms, and Shiitake dermatitis, which is caused by a toxin and probably not immune-mediated. Other preparations of the fungus e.g. extract, boiled mushrooms and lentinan are reported to cause skin manifestations after oral intake. One paper ascribes the toxicity to a polysaccharide. One paper suggests a case of dermatitis that may be of allergic nature). These toxic effects should be further assessed.
- The natural level of formaldehyde measured in *Lentinus edodes* mushrooms is generally higher than in other species of cultivated mushroom. Given the toxicological characteristics and, in particular, the high reactivity of this compound, analytical data on the possible presence of formaldehyde in the novel food ingredient should be provided.
- As the novel food ingredient does not contain *Lentinus edodes* spores, the risk of an allergic reaction associated with consumption of the novel food ingredient may be ruled out. However, the

presence of soya peptone in the culture medium must be mentioned on the label in order to warn consumers who are allergic to soya, as required by Directive 2007/68/EC[4].

- The contents of aflatoxin and ochratoxin were assessed, but no explanation was given as to the choice of these particular mycotoxins.
- There is evidence to suggest that β-glucan may increase the immune response; potential contraindication for people with certain autoimmune disease should be considered.

In consequence, EFSA is asked to carry out the additional assessment and to consider the elements of scientific nature in the comments raised by the other Member States in accordance with Article 7, paragraph 1 of Regulation (EC) No 258/97.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for 'extract of *Lentinus edodes*' as food ingredient in the context of Regulation (EC) No 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of scientific nature in the comments raised by the other Member States.

[4] Commission Directive 2007/68/EC of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients.

ASSESSMENT

In accordance with the Commission Recommendation 97/618/EC, the 'extract of *Lentinus edodes'* is allocated to Class 2.1, „a complex (non-GM derived) novel food ingredient. The source of the novel food has a history of food use in the community". The assessment of the safety of this novel food ingredient is based on data supplied in the original application, the initial assessment by the competent authority of the United Kingdom, the concerns and objections of the other Member States and the responses of the applicant. The data are required to comply with the information required for the novel foods of Class 2.1, i.e. structured schemes I, II, III, IX, X, XI, XII and XIII of the Commission Recommendation 97/618/EC. This assessment concerns only risk that might be associated with consumption and is not an assessment of the efficacy of 'extract of *Lentinus edodes'* with regard to any claimed benefit.

1. Specification of the Novel Food (NF)

The novel food ingredient is an aqueous extract obtained from the Shiitake mushroom *Lentinula edodes* (formerly *Lentinus edodes*) cultivated in a submerged fermentation. The trade name is Lentinex®.

The principle constituent of the novel food ingredient is the ß-glucan lentinan (approximately 1 g/L). Lentinan is a β-(1-3) β-(1-6)-D-glucan which has a molecular weight of approximately 5 x 10^5 Daltons, a degree of branching of 2/5 and a triple helical tertiary structure.

Ethanol precipitation is used for the quantification of the polysaccharide from the *Lentinula edodes* extract. According to the applicant, the ratio of sugar moieties in the lentinan polymer is glucose:galactose:mannose (1:0.1:0.3); however, no further information on the composition of the polymer has been provided.

Typically, the novel food ingredient consists of approximately 98 % water and has a dry matter of approximately 2 %. The dry matter is composed of the polymer lentinan (approximately 4 %), of free glucose (approximately 66 %) and of N-containing constituents (e.g. proteins, amino acids; analytically determined by the Kjeldahl method, approximately 30 %).

The specification of the novel food ingredient as proposed by the applicant is outlined in Table 1.

Table 1 : Specification of the novel food ingredient Lentinex® proposed by the applicant

Parameter	Specification	Method
Appearance	Light brown, slightly turbid	Visual inspection
Microbiological data	Sterile	Total viability count on PCA and MDSA
Lentinan	1 g/L ± 0.2	Ethanol precipitation
Residual glucose	< 20 g/L	Enzymatic
Total protein	< 100 mg/L	Bradford
N-containing constituents	< 10 g/L	Kjeldahl
pH	3.0 - 4.0	pH-meter
Pesticides	Not detectable	Multimethod

Compositional data provided by the applicant for five arbitrarily chosen batches of the novel food ingredient are listed in Table 2.

Table 2 : Compositional data of five non-consecutive batches of the novel food ingredient

Batch	**Water** (%)	**pH**	**Lentinan** (g/L)	**Free glucose** (g/L)	**N-containing constituents** (Kjeldahl) (g/L)	**Protein** (Bradford) (mg/L)
10-071002	-	3.8	1.0	15.6	-	39
10-070921	-	3.8	1.1	12.3	< 3	56
10-070511	96.9	3.6	0.8	16.5	9	22
10-050530	-	3.8	1.1	21.0	-	50
10-061110	97.3	3.5	1.1	16.3	8	30
Mean	-	3.7	1.0	16.3	7.5	39
SD	-	0.1	0.2	2.8	1.5	15.0

The applicant also provided data on the content of fat (<40 mg/L), amino acids and related biogenic amines (totalling to 8.45 g/kg dry matter in one batch), ash (6 % in two batches), and ions (chloride: 101 mg/L, nitrate: 24 mg/L, nitrite: 0.18 mg/L, sulphate: 200 mg/L, ammonium: <0.2 mg/L, in five batches).

Data on the contents of water-soluble vitamins (B_1, thiamine: <0.01 mg/100 g; B_2, riboflavin: 0.05 mg/100 g; B_6, pyridoxine: 0.02 mg/100 g; B_3, niacin: 0.61 mg/100 g; B_5, pantothenic acid: 0.12 mg/100 g; B_8, biotin: 83 µg/100 g; B_9, folate: <5 µg/100 g; C, ascorbic acid: <1 mg/100 g), on β-carotene (<10 µg/100 g) and on ergosterol (0.02 mg/mL) were also provided for three batches.

Contaminants

For one batch of Lentinex® the following data on heavy metals were reported: cadmium <0.01 mg/kg, chromium 0.15 mg/kg, manganese 0.11 mg/kg, lead <0.02 mg/kg, nickel 0.87 mg/kg, mercury <0.02 mg/kg and copper 0.24 mg/kg.

For one batch the content of formaldehyde was shown to be below the limit of detection (<1 mg/kg).

Contents of 297 pesticides were below the limits of detection of the employed analytical method.

Analytical data on aflatoxins B1, B2, G1 and G2 (all <0.1 µg/kg) and ochratoxin (<0.2 µg/kg), as representatives of potential mycotoxins, were provided.

The analyses were carried out by an accredited laboratory using standard methods.

Secondary metabolites

According to the applicant, Lentinex is a growth-associated product, i.e. produced by growing *Lentinula edodes* cells. A kinetic analysis demonstrating the correlation between increase in biomass and production of β-glucan has been provided. The fermentation broth is harvested when the cells stop growing. According to the applicant, under these fermentation conditions the formation of secondary metabolites is unlikely.

The Panel supports the view expressed by the UK ACNFP in the initial assessment report that the culture conditions are unlikely to lead to the production of secondary metabolites as they are designed to optimise the production of β-glucan (primary metabolite). Moreover, in view of the history of consumption of the fruiting body, which is the most differentiated form of the mushroom and the one most likely to contain secondary metabolites, the information concerning the risk associated with secondary metabolites can be considered to be reassuring.

Stability

The applicant undertook stability studies with Lentinex®. Parameters such as the content of lentinan, free sugar, protein, pH and product appearance were included. The storage conditions were 25° C ± 2° C/60 % relative humidity (RH) ± 5 % and 40° C ± 2° C/75 % RH ± 5 % (accelerated conditions) for 5 months. The data provided suggest that the novel food ingredient is stable under these conditions.

2. Effect of the production process applied to the NF

The applicant is applying a production technology based on submerged cultivation of *Lentinula edodes* in sterilised liquid medium. *Lentinula edodes* mycelium is cultivated in a liquid aerobic fermentation process. The mycelium of the cultivated *Lentinula edodes* is submerged in defined medium, comprising glucose, malt extract, soy peptone and yeast extract; data on the purities of these materials have been provided. Controlled fermentation conditions (e.g. temperature, aeration rate, pH) are applied. The biomass is removed by filtration and the resulting fermentation liquid is the raw material for the lentinan-based products. Final concentrations of lentinan are adjusted by dilution with water. The liquid is sterilised by heat (20 minutes at 115° C). Sodium benzoate (E211) at 0.1 % is added as a preservative.

According to the applicant, the production of Lentinex® complies with GMP (Good Manufacturing Practice) Guidance used in the pharmaceutical industry, namely the ICH Harmonised Tripartite Guideline, "Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients, Q7; Current version". Written standard operating procedures (SOPs), Quality Control and Quality Assurance are applied in the manufacture.

All material employed for the safety studies was produced in a 750 L fermenter. According to the applicant, scale–up would not be expected to have an effect on the final product.

3. History of the organism used as a source

Lentinula edodes is indigenous to Japan, China and other Asian countries with temperate climates and usually grows on fallen deciduous trees. It is a common food in Asia (fresh and dried). The fresh mushrooms are now widely cultivated; the world production of *Lentinula edodes* amounted to 314,000 tons in 1986 and 1.6 Million tons in 1997, respectively (Chang, 1999).

The fruiting body of *Lentinula edodes* has an established history of consumption throughout the world. Consumption of 90 g of *Lentinula edodes* mushrooms (around 4-5 mushrooms) would result in an intake of approximately 1.8 g of lentinan.

4. Anticipated intake/extent of the use of the NF

The applicant intends to use the novel food ingredient in a wide range of products: dietary supplements, yoghurts, soft drinks, cooked and processed foods, and baked goods. Lentinex® would be marketed in the form of a capsule, a liquid, powder, gel or as part of a food product. The applicant did not provide an intake estimate based on consumption data of the proposed food categories to which the novel food ingredient is intended to be added.

The intention of the applicant for incorporating the novel food ingredient is to increase daily consumption of lentinan (β-glucan). The applicant wishes to market without restriction to the general population with a recommended daily intake of 1 - 2.5 mg of lentinan. The proposed intake of 2.5 mL Lentinex® containing 1 mg lentinan (β-glucan)/mL corresponds to 41.7 μg/kg body weight for a 60 kg person.

5. Information from previous exposure to the NF or its source

The applicant reported on many commercial products using β-glucan in concentrated form marketed in Asia, the USA and increasingly in Europe. Such products, which are available in a variety of dietary supplement forms, are obtained from mushrooms, including *Lentinula edodes*. According to the applicant, dietary supplements containing lentinan have been commercialised in the United States since the early 1990s. However, the applicant did not provide quantitative consumption or sales data on food supplements derived from *Lentinula edodes*.

ß-Glucans are also found in a number of foods other than mushrooms, with the most notable dietary sources being oats and other cereals, which contain (1-3)(1-4) β-D-glucans.

6. Nutritional information on the NF

According to the information provided by the applicant, Lentinex® contains less than 14.4 kcal per 100 mL.

Two feeding studies were conducted on broilers and weaned piglets. The results from these studies as regards weight gain and daily feed intake do not cause concerns.

Based on the information provided on the composition and the proposed use level, the Panel considers an intake of 2.5 mL Lentinex® per day as not nutritionally disadvantageous.

7. Microbiological information on the NF

The final step of production of the novel food ingredient involves heating for 20 minutes at 115° C. Sodium benzoate (E211) at 0.1 % is added as a preservative. The applicant provided certificates of analysis for five non-consecutive batches stating that the samples tested were sterile.

8. Toxicological information on the NF

8.1. Genotoxicity

The applicant did not provide studies on genotoxicity.

8.2. Animal studies

As result of a literature search, the applicant provided the following information (abstracts only) of toxicity studies performed with lentinan.

The acute toxicity of lentinan was studied using both sexes of mice (ICR) and rats (CD). LD_{50} values of 250-500 mg/kg body weight (bw) (intravenous administration) and greater than 2500 mg/kg bw (intraperitoneal, subcutaneous and oral administration, respectively) were determined (Moriyuki and Ichimura, 1980).

In a 6 months study, lentinan was given intravenously into the tail vein of rats (Shimazu et al., 1980). The effect of lentinan on the *in utero* foetal development and on postnatal development of the F1 offspring was investigated after intravenous administration to rats (Cozens et al., 1981 a, b). Owing to the route of administration, these studies were not considered relevant.

The applicant did not provide animal studies on the toxicity of the novel food ingredient in accordance with OECD Guidance or of comparable standards suitable to demonstrate the safety of a substance or an ingredient. However the applicant provided some animal studies that included a limited number of safety endpoints.

Four studies evaluated the effect of Lentinex® on the survival of Brown Norway rats in an acute Myelocytic Leukemia (BNML) model, when administered with or without cytostatic drugs. The Panel considers that these studies are not suitable to demonstrate the safety of the novel food ingredient.

A controlled study with 24 mice obtained from the Division of Laboratory Animal Medicine, Louisiana State University, examined the effect of daily 2 mg/kg and 10 mg/kg bw lentinan provided by GycaNova when administered intraperitoneally or via gavage, respectively, for 5 days (O'Reilly, 2005, unpublished). No effects were seen on the body weight. Other endpoints were the effect on the relative number of white blood cells (T-cells, B-cells, PBMN, macrophages) and the effect on the *in vitro* production of cytokines (IL-1β, IL-6, IL-12, TNFα) by peritoneal exsudate cells (PEC) and splenocytes after 12 hours of *ex vivo* culture with or without stimulation of *Escherichia coli* lipopolysaccharides (LPS). Increased production of TNFα by splenocytes and PEC with LPS stimulation, IL-1β by splenocytes and PEC with and without LPS stimulation, IL-6 by PEC without LPS, IL-6 by splenocytes with or without LPS stimulation, and IL-12 by PEC without LPS stimulation was reported. The applicant did not address the relevance of these findings with respect to the safety of the novel food ingredient. The Panel notes the short study duration, the study endpoints related only to the weight and immunological endpoints and the absence of any histological examinations. The Panel considers therefore that this study provides very limited evidence to support the safety of the novel food ingredient.

According to the treatment protocol of a study with ten male BN/Crl rats (120-140 g; provided by Charles River, France), three doses of Lentinex® (7.8 mg lentinan/kg, 9.2 mg/kg, 12 mg/kg bw) were administered until the age of 34 weeks (Gjertsen, no date, unpublished). A dose of 1.25 mg (approximately 7.8 mg/kg bw) was administered to 7 week old rats by gavage for three weeks, five day cycles with two days of rest in between. This dose (per kg bw) is approximately 190 times higher than the dose for human at the proposed intake level. At the age of ten weeks, the dose was increased to 2.48 mg (approximately 9.2 mg/kg bw) until the age of 21 week followed by another 15 weeks of 12 mg/kg bw dosage. A control was given only for the period of 17 – 26 weeks. Safety related endpoints studied were body weight, haematological parameters [number of platelets, red and white blood cells, and T-cells, T-helper cells and cytotoxic T-cells, and B-cells as % of peripheral blood mononuclear cells (PBMN), cytokines (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, INF-γ, TNFα, GM-CSF)]. In addition the rats were observed for lethargy and for toxic effects, ataxia and behavioural changes. The Panel notes the inappropriate application of the control, the limited number of safety endpoints, the absence of any histological examinations and the study design, which were not in compliance with OECD guidelines. The Panel considers therefore that this study provides very limited evidence to support the safety of the novel food ingredient.

8.3. Human studies

A randomised, placebo-controlled, double-blind trial with a cross-over design was conducted to study the efficacy and safety of Lentinex® in 42 healthy elderly humans over six weeks (Kristiansen et al., 2006, unpublished). Lentinex® was administered in a daily dose of two capsules of 540 mg (containing 1.25 mg lentinan). The primary objective was to evaluate the efficacy of Lentinex® to stimulate the cellular immune response by affecting the number of helper and cytotoxic T-cells in healthy elderly humans as compared to placebo over 2 x 6 weeks with a wash-out period of four weeks after the first six week period. Secondary endpoints were the number of NK-cells, T-cells, B-cells, numbers of different subpopulations of B- and T-cells, the effect on cytokine levels (IL-8, IL-10, IL-12, TNFα), CRP, and immunoglobulins (IgA, IgM, IgG), the frequency of infectious disease, and safety endpoints such as blood parameters (red and white blood cells including eosinophils, platelets, ALAT, ASAT, γGT, creatinine, bilirubin, triglycerides, total-, LDL- and HDL-cholesterol), adverse events and vital signs, defined as systolic and diastolic blood pressure and heart rate. Forty-one subjects were included in the intention to treat (ITT) analysis and 33 persons in the per protocol (PP) analysis. There was no difference between treatments regarding the incidence of adverse events (26 in

treatment group *versus* 27 in the placebo group) or adverse drug reactions. The most reported adverse event was nasopharyngitis (7 *versus* 9) that was not related to the treatment.
The Panel notes that the primary objective of this human trial was to study possible effects on the cellular immune response. The administered dose in this human study expressed in amounts of lentinan corresponded to the proposed intake level of 2.5 mg. Hence the study does not provide evidence on a possible safety margin. However, some safety endpoints have been studied and the results do not provide evidence for safety concerns.

A second randomised, placebo controlled, double blind clinical trial with 51 patients was conducted (Øfjord, 2008, unpublished). The primary objective was to investigate the safety profile of Lentinex® with increasing dose in a total period of eight weeks. Primary endpoints were vital signs (heart rate and sitting blood pressure), haematological (haemoglobin, leucocytes and platelets) and clinical chemistry parameters (haemoglobin, bilirubin, ALAT, ASAT). The nature, severity, frequency, date of onset and duration of adverse events and action taken were recorded in standardized forms. The secondary objective was to investigate if there are differences in immune-response related to the dose. Secondary endpoints were on the immune cell responses and cytokine production. Four different escalating dose levels, daily oral dose of 1 mg, 5 mg, 10 mg and 20 mg lentinan, provided by Lentinex® for eight weeks, were evaluated. Forty-one subjects received the supplement and ten subject received placebo. The summary on the outcome provided pooled the results for all four doses and compared the results to placebo. There were no statistically significant differences between the supplement and the placebo group for adverse effects.

The Panel considers that both human studies provide supporting but limited evidence for the safety of Lentinex®.

Adverse effects

A Member State expressed concerns as to a potential contraindication for people suffering from auto immune diseases, due to the potential ability of β-glucans to activate the immune system. The induction of autoimmune arthritis following high dose of intraperitoneally administered yeast wall derived β-glucans has been reported in the SKG mice, a strain with a point mutation which causes altered signal transduction in T-cells and leads to a spontaneous T-cell mediated chronic autoimmune arthritis (Yoshitomi et al., 2005; Hida et al., 2007; Sakaguchi et al., 2006). According to the applicant, no reports on autoimmune disease triggered by lentinan (Shiitake β-glucan) on have been published, even when lentinan is given parenterally, over extended periods.

Levy et al. (1998) studied the effects of intakes of 4 g freeze-dried Shiitake powder given in a capsule to ten healthy subjects for ten weeks. The exposure was repeated after three to six months. In each trial four participants responded with blood eosinophilia, increased eosinophil granule proteins in serum and stools, and gastrointestinal symptoms. In two subjects gastrointestinal symptoms coincided with eosinophilia. The Panel notes that this study used a product derived from Shiitake fruiting body and containing a considerably higher estimated amount of lentinan than Lentinex®.

The risk of adverse immunological effects due to the novel food ingredient, if any, is expected not to be higher than that resulting from the normal consumption of the fruiting body of the Shiitake mushroom.

8.4. Allergenicity

There are several case reports on photosensitivity, intolerance and allergic reactions related to the consumption of Shiitake mushrooms or products derived thereof. Kopp et al. (2009) reported a systematic allergic contact dermatitis due to the consumption of raw Shiitake mushrooms. Flagellate dermatitis was also reported by the German Bundesinstitut für Risikobewertung (BfR, 2006; Maier and Herzinger, 2007) and in addition photosensitivity related to the consumption of Shiitake was

described by Hanada and Hashimoto (1998). Although not directly proven, lentinan has been implicated as the causative substance for Shiitake dermatitis (BfR, 2004; Nakamura, 1992). According to the BfR (2004), despite the high consumption of Shiitake mushrooms, the incidence of severe allergic reactions appears to be low.

The Panel noted that the applicant did not provide any studies to evaluate the allergenicity of Lentinex®. The applicant pointed out that the intake of approximately 0.08 mg protein/day to be expected at the proposed use levels is much lower than the protein intake from Shiitake products causing allergic reactions reported in the literature.

The Panel considers that despite the low intake of lentinan at the proposed use levels it cannot be excluded that Lentinex® poses an allergenic risk to sensitive subjects. However this risk is expected not to be higher than that resulting from the normal consumption of the fruiting body of the Shiitake mushroom.

The Panel notes the presence of soy peptides in the culture medium.

DISCUSSION

The employed technology based on submerged cultivation of mycelium in sterilised liquid medium enables the reproducible and standardised production of a β-glucan-containing aqueous extract from *Lentinula edodes*. The Panel agreed with the view expressed by the UK ACNFP that the culture conditions are unlikely to lead to the production of secondary metabolites as they are designed to optimise the production of β-glucans (primary metabolite). Moreover, in view of the history of consumption of the fruiting body, which is the most differentiated form of the mushroom and the one most likely to contain secondary metabolites, the ANCFP considered the information concerning the risk associated with secondary metabolites to be reassuring. The Panel agrees with this view.

The Panel noted that the novel food ingredient is extracted from the mycelium and not from the fruiting body of *Lentinula edodes* that has an established history of consumption throughout the world.

The compositional data provided sufficiently characterise the β-glucan solution. The analytical data available do not allow to completely rule out the presence of secondary metabolites. However, owing to the growth-associated biosynthesis of the primary metabolite β-glucan and the resulting fermentation and production conditions, the Panel considers the formation of secondary metabolites unlikely.

The animal and the human studies provided were primarily carried out to determine the efficacy of the novel food ingredient; they are supporting but only of limited value regarding a safety assessment.

Owing to the fermentative production of the novel food ingredient from the mycelium and the final application of a heat-induced sterilisation step, adverse effects reported after the consumption of the fruiting body of the Shiitake mushroom are not considered relevant.

The Panel noted that the intake of β-glucan resulting from the proposed used of the highly diluted novel food ingredient is low compared to the intake estimated from the consumption of the mushroom *Lentinula edodes* and of other β-glucan sources.

CONCLUSIONS

The Panel concludes that the novel food Lentinex® is safe as a food ingredient at the proposed conditions of use and the proposed levels of intake.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on Lentinex® derived from the Mushroom *Lentinus edodes* received on 21 October 2009. Submitted by GlycaNova A/S on 19 December 2007. Additional data were provided on 31 May 2010 and on 14 June 2010.
2. Letter from the European Commission to the European Food Safety Authority with the request for an opinion on the safety of „an extract of *Lentinus edodes*". SANCO E4/AK/bs (2009) D/540619, dated 24 September 2009.
3. Initial assessment report carried out by UK: Advisory Committee on Novel Foods and Processes opinion on a β-glucan rich extract from *Lentinus edodes*.
4. Member States" comments and objections.
5. Response by the applicant to the initial assessment report and the Member States' comments and objections.

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GLOSSARY AND ABBREVIATIONS

ANCFP	Advisory Committee on Novel Foods and Processes
ALAT	Alanine Aminotransferase
ASAT	Aspartate Aminotransferase
BfR	Bundesinstitut für Risikobewertung
BNML	Brown Norway Myelocytic Leukemia rat
Bw	Body weight
CRP	C-reactive protein
GM	Genetically Modified
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
GMP	Good Manufacturing Practice
γGT	Gamma Glutamyl Transferase
HDL	High Density Lipoprotein
ICH	International Conference on Harmonization
IL	Interleukine
INF	Interferon
ITT	Intention To Treat
LDL	Low Density Lipoprotein
LPS	Lipopolysaccharides
MDSA	Medimush Double Strength Agar
PBMN	Peripheral Blood Mononuclear Cell
PCA	Plate Count Agar
PEC	Peritoneal Exsudate Cells
PP	Per Protocol
RH	Relative Humidity
SD	Standard Deviation
SOP	Standard Operating Procedure
TNF	Tumor Necrosis Factor