

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

BETA-GLUCAN RICH EXTRACT FROM *Lentinus edodes*

An application has been submitted to the UK Competent Authority for authorisation of a beta glucan-rich mycellial extract of *Lentinus edodes* under the Novel Foods Regulation (EC) 258/97. The Committee is asked to advise whether the available data provides an adequate basis for a safety assessment, and if it recommends authorisation of this novel ingredient.

Background

1. Glycanova (formally Medimush) has submitted an application for the authorisation of 'Lentinex' a beta glucan-rich mycelial extract of *Lentinus edodes* (Shiitake mushroom) as a novel food ingredient. The UK Competent Authority accepted the application in November 2007. In accordance with Article 6(3) of Regulation (EC) No 258/97, the UK has 3 months to prepare an initial assessment report on the above application. The European Commission will then circulate this initial assessment to the Competent Authorities in the other Member States for comment. The application dossier is attached at **Annex A**.
2. Members will recall that they are familiar with this novel ingredient (NI) which they have previously considered under the criteria for substantial equivalence. (ACNFP80P/2 ,ACNFP80/11, ACNFP81/5, ACNFP84P/1, ACNFP84/2). At the September meeting Members concluded that the data provided by the applicant was insufficient to confirm substantial equivalence. The applicant has now submitted a full novel food application for consideration.
3. The NI is a relatively rich source of Lentinan, a water-soluble β -glucan found in *Lentinus edodes* (otherwise known as Shiitake mushroom). This fungus is indigenous to Japan, China and other Asian countries with temperate climates and is usually found growing on fallen deciduous trees. Lentinan is a (1-3)(1-6) Beta-D-Glucan, with a molecular weight of 5×10^5 Daltons, a degree of branching of 2/5 and a triple helical tertiary structure. The NI has an energy value of 14.4kcal per 100ml.
4. The present application for authorisation of beta glucan-rich mycellial extract of *Lentinus edodes* was prepared pursuant to Commission Recommendation (97/618/EC) of 29 July 1997 concerning the scientific aspects and presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients. Beta glucan-rich mycellial extract of *Lentinus edodes* has been classified as a complex novel food from a non-GM source (class 2.2). The requirements for a submission for this class are as follows:

I	Specification of the NF	X
II	Effect of the production process applied to the NF	X
III	History of the organism used as the source of the NF	X
<i>IV</i>	<i>Effect of the genetic modification on the properties of the host organism</i>	-
<i>V</i>	<i>Genetic stability of the GMO</i>	-
<i>VI</i>	<i>Specificity of expression of novel genetic material</i>	-
<i>VII</i>	<i>Transfer of genetic material from GM microorganisms</i>	-

<i>VIII</i>	<i>Ability to survive in and colonise the human gut</i>	-
IX	Anticipated intake/extent of use of the NF	X
X	Information from previous human exposure to the NF or its source	-
XI	Nutritional information on the NF	X
XII	Microbiological information on the NF	X
XIII	Toxicological information on the NF	X

5. The application dossier has been placed on the FSA website to allow the public to input into the UK assessment and comments received after the ACNFP meeting will be forwarded to Members for consideration.

I. Specification of the novel food

Annex 1, pp 6-10

6. The applicant intends to market the NI as a nutritional food ingredient in a wide range of food categories¹. The NI, which is essentially sterilised fermentation liquor, is produced by the fermentation of a pure culture of *L. edodes* under controlled culture conditions and contains a relatively high concentration of lentinan, together with measurable quantities of glucose, protein and amino acids derived from the growth medium and/or the fungus. The mycellial biomass is removed as part of the production process.

7. The specification is given as follows.

Parameter	Specification
Appearance	Light brown, slightly turbid
Microbiological load	Sterile
Lentinan	1mg/ml ± 0.2
Residual glucose	<20mg/ml
Total proteins	<100µg/ml
pH	3.0 - 4.0
Protein incl free amino acids	<10mg/ml
Pesticides	Not detectable

¹ The earlier application for substantial equivalence was restricted to the existing use of the equivalent existing ingredient, i.e. limited to dietary supplement capsules.

8. The applicant provides data for five independent batches which show that each are produced within this specification (Annex 1 p 10) and states that a certificate of analysis will be provided for each batch of the NI (typical example is attached at Annex 1, Appendix E). The applicant has also provided data to show the absence significant levels of undesirable substances – e.g. heavy metals (Annex 1 Table 1.5) and pesticides. The applicant has also identified and quantified all the free amino acids present in the NI.
9. The applicant has provided data, attached at Annex 1 (Appendix A) which shows that the (sterile) product remains stable for a period of 5 months under accelerated storage conditions. The applicant therefore contends that there is no reason why the NI should not be sold with a 12m stability claim.

Effect of the production process applied to the novel food

Annex 1, pp11 – 15

10. A pure culture of *L. edodes* is grown in sterilised liquid medium comprising glucose (30g/l); malt extract (6g/l); soy peptone (10g/l); and yeast extract (6g/l), using a controlled fermentation process which regulates a number of key parameters such as temperature, pH etc. At a given time point the biomass is removed by filtration and the resulting fermentation liquor (the NI) is sterilised by heat. Details of the production process, and process controls are given in Annex 1 pp 13 – 14.
11. The NI is produced in accordance with Good Manufacturing Practice procedures employed in the pharmaceutical industry. The applicant works to a series of standard operating procedures, the most relevant of which are attached at Annex 1, Appendix H.
12. The Secretariat notes that some of the studies reported in the dossier use the NI grown in either lab or pilot scale fermenters. The applicant has commented that, as the fermentation is relatively tightly controlled, the scale has no effect on the final product, but has not included any data to demonstrate this.

III. History of the organism used as a source of the novel food

Annex 1, pp16-18

13. The source of the NI, *Lentinus edodes* (Shiitake mushroom) has an established history of consumption throughout the world including the EU, but consumption is restricted to the fruiting body (mushroom) form. The applicant contends that the mushroom is one of the leading sources of beta-glucans, and there are commercial products available that utilise beta-glucans in concentrated form. Such products, which are available in a variety of dietary supplement forms, are obtained from mushrooms, including *L. edodes*. The unique aspect of the NI is that the beta-glucan (lentinan) component is obtained from the mycellial form of the organism. The applicant states that this is a more reliable means to obtain the NI. Members have already considered detailed mycological evidence detailing the similarity of the mycelium to the fruiting body, which can be found at **Annex B**.
14. The applicant also notes that beta-glucans (including lentinan) are widely available in the diet (see paragraph 18 below).

IX. Anticipated intake/extent of use of the novel food

Annex 1, pp23-28

15. The applicant is proposing that the NI be considered for use as a nutritive ingredient in a wide range of products ranging from dietary supplements, to yoghurts, soft drinks, cooked and processed foods, and baked goods.
16. The purpose of incorporating the NI is to increase daily consumption of lentinan, which has perceived health benefits. Any health claims that are attributed to the consumption of lentinan are not considered as part of this application. Any health or nutrition claims that may be made are subject to a separate authorisation procedures under the terms of regulation (EC) 1924/2006.
17. The NI will be marketed without restriction to the whole population as a dietary supplement with a recommended daily intake of 1-2.5 mg of lentinan. The applicant does not provide any dietary survey data to demonstrate that these levels would not be exceeded, but they note that the levels are far lower than those seen if consumers were to regularly consume mushrooms (See X below).

X Information from previous human exposure to the novel food or its source

Annex 1, pp22-23

18. As noted in Section III the source of the novel ingredient is widely consumed, albeit as a mushroom. Consumption of 90g of *L. edodes* mushrooms (around 4-5 mushrooms) would contain approximately 1.8g (1800mg) of lentinan. The novel ingredient will be incorporated in products with a recommended daily consumption of 1-2.5mg (i.e. three orders of magnitude lower). Beta glucans are found in a number of food categories other than mushrooms. The most notable dietary sources are oats and other cereals, which contain (1-3)(1-4) beta-D-glucans, (lentinan is a (1-3)(1-6) beta-D-glucan).
19. A novel food application is a safety based application and any health or nutrition claims that may arise as a result of consumption of a novel food ingredient is now subject to other EU food legislation (See paragraph 16 above). Nevertheless the Secretariat notes that there is no explanation given by the applicant as to why the consumption of relatively small quantities of the NI is perceived to elicit the same health benefit as consumption of 90g of fresh *L. edodes* mushrooms.

XI. Nutritional information on the novel food

Annex 1, p 24

20. The applicant intends that the presence of the NI in the diet will be as a supplement (or supplemental ingredient) only and will not replace any existing foods or food ingredients. The applicant has carried out an analysis of the constituents of the NI in terms of recommended daily intakes, and whilst it could be argued that the figures used (which are US figures) are of limited relevance in the EU, it is clear that the amounts are so small that they are of little relevance in the total diet.

XII. Microbiological information on the novel food

Annex 1, pp35-36

21. The NI is sterilised and as such there is no cause for concern regarding microbial contamination. The applicant routinely analyses the NI for sterility by plating the NI

onto microbial growth medium and checking for the presence of colony forming units (CFU). As the applicant's presumption is that the product is sterile, the acceptance criterium is zero CFU, (denoted as OK in the stability report (Annex 1 p30)).

XIII Toxicological Information on the Novel Food

Annex 1, pp.37-70

22. The applicant has provided information from a series of toxicological studies. The same information was included in the earlier application (for substantial equivalence) when Members queried a number of points. The Secretariat has indicated Members' earlier concerns and the applicant's responses in the following paragraphs. Members previously indicated that, in terms of the requirements for substantial equivalence, the applicant's responses were adequate.
23. **Animal studies:** The applicant also provided results of studies in which the NI was administered to mice, rats and pigs. According to the applicant these studies provided evidence that the NI does not cause any harmful changes in metabolism in these species under the intended conditions of use. These results are in agreement with other studies on the safety of the NI.
24. Members previously noted that these are efficacy studies rather than toxicology studies and the end points were mainly haematological and immunological. Members therefore requested details of which other toxic/behavioural effects were monitored in the animals. Members also noted that the doses were significantly lower than the dose used in the human study and that this is inconsistent with the approach normally employed in order to provide reassurance of the safety of a food.
25. In response the applicant advised that all rats were monitored for a series of toxic effects including immunological variables and haematology, weight loss and lethargy. Hind limbs were also monitored for paralyses. In addition, the treated group was monitored for toxic effects, ataxia and behavioural changes. In all cases no abnormalities were observed. In terms of dosage, the applicant calculated that the tested doses provided a safety factor of between 15 and 560 compared with the human dose.
26. **Human studies:** The applicant has provided details of a cross-over placebo, controlled human study in which 40 elderly subjects ingested 2.5 ml of the NI daily for 6 weeks. The applicant suggested that this study demonstrated the safety of this product in healthy elderly consumers.
27. Members previously questioned the findings of this study, noting that an increase in serum C-reactive protein (CRP) was highlighted without any explanation, although this suggested an inflammatory response. Members also requested additional information on the adverse effects frequency which showed no difference in the incidence of adverse events, between placebo and treatment, but does not mention severity.
28. In response the applicant advised that increases in CRP levels were seen in both the NI and the placebo. When patients with high CRP values due to known infectious diseases were taken out of the analyses, no changes within or between

groups were observed for the NI and the placebo. The applicant also listed the adverse events, noting that the severity of the adverse events was mild, and there were no significant differences observed between NI and placebo.

Allergenicity & Labelling

Annex 1, p 71

29. Issues regarding the potential allergenicity of the NI were considered in the context of the unsuccessful substantial equivalence request. In their original application the applicant highlighted the almost complete absence of reports of allergenicity to shiitake mushroom (1 published report), despite its widespread consumption as a food. Whilst the applicant acknowledged that there are several reports of mushroom workers who experience lung reactions (mushroom worker's disease) from inhalation of shiitake spores, they maintain that, due to the production method, the NI does not contain spores or derivatives thereof. The applicant also noted that no allergic reaction was observed in the study of healthy elderly people described above (paragraph 26).
30. Based on this information, whilst there are measurable quantities of protein present in the NI, it is extremely unlikely that protein from *L.edodes* would give rise to any unusual allergic reaction compared with the fruiting body. The presence of soya peptone in the growth medium would mean that the NI would require labelling to highlight the presence of soya derivatives, in accordance with EU directives on allergen labelling.

Consumer access and choice

31. The Secretariat has considered the issues of access and choice in relation to beta glucan-rich mycellial extract of *Lentinus edodes*. If authorised, the NI would be available for use in products across the UK and subsequently in other EU Member States. In practical terms, access to foods fortified with beta glucan-rich mycellial extract of *Lentinus edodes* could be limited by a high price or by limited geographic distribution, which are both driven by commercial considerations that cannot be predicted at this stage.
32. It is envisaged that the introduction of foods fortified with beta glucan-rich mycellial extract of *Lentinus edodes* will increase existing consumer choice and, although it is not anticipated that other food ingredients will be displaced, the NI could displace other existing beta-glucan ingredients, derived from other sources, in dietary supplements. The consumer would be aware of the presence of beta glucan-rich mycellial extract of *Lentinus edodes* through the ingredient list and, most likely, through special marketing that highlights its contribution to the nutrient composition of the food.

COMMITTEE ACTION REQUIRED

33. The Committee is asked to consider whether the available data are adequate to determine whether the NI complies with the criteria for acceptance under the novel food regulation, namely:
- It does not present a danger to the consumer

- It does not mislead the consumer
- It is not nutritionally disadvantageous compared with foods which it might replace.

34. If so, the Committee is asked whether it is content to recommend approval for the NI to be used in the proposed food products.

35. If not, the Committee is invited to identify what further data should be provided.

**Secretariat
November 2007**

Annexes attached:

Annex 1 Application dossier – confidential version (Restricted)
Annex 2 Mycological Review

Annex 1 to ACNFP/85/3

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

Application Dossier – confidential version (restricted)

**Secretariat
November 2007**

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

Mycological review

**Secretariat
November 2007**

(Review provided in the context of the earlier application from Medimush for an opinion on substantial equivalence between the mycelial extract and dried fruiting bodies)

Basidiomycetes mycelium and fruiting bodies.

The life cycle of a Basidiomycetes fungus, such as *Lentinus edodes*, begins with and ends with the basidiospore – a tiny unicell containing a single haploid nucleus (+ or – sexually). Under suitable environmental conditions the basidiospore will germinate to form a limited thread-like hyphal growth and when compatible (+ and -) hyphae meet fusion or anastomosis will occur and from that will grow out a new tubular shaped hyphae, each cell of which contains two nuclei, one + and one -. At this stage the nuclei do not fuse and this is termed the dikaryotic phase, a unique feature in the living world. The dikaryotic mycelium will grow extensively by branching at the hyphal apex. This is now known as the vegetative mycelium and is the form that would be retained in stock cultures and this is the form of the Lentinex mycelium.

In nature, the dikaryotic mycelium can continue growing for weeks, months or years if suitable nutrients are available. In the commercial growing of *Lentinus edodes* the growing substrate is a mixture of sawdust and other suitable organics. After complete colonisation of the substrate, various environmental stimuli are applied to induce the vegetative mycelium to change in growth pattern, first producing a small irregular mass of hyphae which develop into an erect stock or stipe on which is developed the cap or pileus. This becomes the traditional carpophore or fruit-body of the mushroom and on the underside of the pileus special binucleate cells called basidio complete the life cycle. The haploid nuclei (+ 1 -) now fuse and undergo meiosis followed by mitosis to yield for haploid basidiospores (2+, 2-) which are then released into the atmosphere for dissemination.

Point to note: The vegetative mycelium and almost the entire fruit-body are composed of dikaryotic cells and there is a continuous cytoplasmic connection throughout the vegetative mycelium and the fruit-body.

Dikaryotic hyphae are divided into cells by regular cross-walls, and within each cell are the two haploid (+/-) nuclei. Each cross-wall has a pore through which cytoplasm can freely move but the pore is protected by a perforated structure (the dolipore) which prevents cross movement of the nuclei. Pore size allows free movement of cellular organelles such as mitochondria and various vesicles which will assist in cell wall synthesis and breakdown. Thus while in these hyphae there appears to be a form of compartmentalisation of the nuclei whereas the cytoplasm flows freely between the cells. In the vegetative dikaryotic hyphae all growth forward will occur at the apex or dome where active cell wall synthesis will occur. By this apical wall genesis a cylindrical, tubular growth form develops resulting in the typical hyphal growth form.

Hyphal walls of fungi have a chemically complex nature – they usually contain one or more polysaccharide as main constituents plus some protein and lipid. A dense network of microfibrils constitutes the skeletal support of the hyphal walls. The microfibrils appear to be embedded in an amorphous matrix. The structural polymers of the hyphal walls (chitin, chitosan, β -glucans and galactose - containing polymers)

are preferentially deposited at the apex. The building units for wall synthesis are carried in various types of vesicles developed further behind the expanding apex.

The main area of manufacture of wall components occurs within 1-2 μm of the apical pole and decreases sharply over a short distance corresponding approximately to the length of the apical dome. A residual but declining gradation of wall deposition persists in the tubular portion of the hyphae and will account for increases in girth and thickness of the hyphal tube. Wall synthesis probably parallels wall expansion. Thus while apical growth dominates wall growth in the hyphae intercalary wall synthesis can occur when required i.e. for repair or development of new outgrowths.

It is now firmly believed that cell wall polymer formation takes place both in the cellular cytoplasm of the hyphae and in the wall itself. Cytoplasmic vesicles have been shown to contain wall building materials. The microfibril network is assembled, if not entirely polymerised, *in situ*, while the matrix materials are probably prefabricated internally and need only be anchored to the wall. Accompanying wall synthesis there is also proof that wall lysis occurs. There exists strong evidence of the extreme of lytic enzymes in the apex region of the hyphae (strong evidence that such enzymes are used in anastomosis). In the control of wall growth a delicate balance exists between wall synthesis and wall lysis.

No attempt will be made here to describe the well known aspects of wall chemistry and structure apart from noting that the basics of wall synthesis apply throughout the fungal kingdom and help to explain the myriad of shapes and forms that fungi may achieve. While the final fungal form (such as of the Basidiomycete fruit-body) may appear complex, the biosynthetic method of achieving this is very similar to hyphal tip wall synthesis.

The actual shape and diameter of the hyphae is most probably determined by the spatial distribution of wall growth units and by the relative ratios of biosynthetic and lytic activity in these units. Excessive wall synthesis activity can lead to thick cell walls as exemplified in numerous examples in the fungal world. Particular examples are the upright aerial structures developed in the fungi for spore release and dissemination e.g. conidiophores in filamentous fungi and fruit-bodies in Basidiomycetes. In comparison, excessive lytic activity can result in cell wall bursting as can be seen often in filamentous cultures and the complete autolysis and breakdown of fruit-bodies after spore release. In nature, the components of fruit-body autolysis will return to the soil and be re-utilised by the vegetative mycelium.

Formation of the Basidiomycete carpophore or fruit-body

Two phases of growth can be distinguished in the formation of the Basidiomycete fruit-body. (a) an initial phase of release from the vegetative mycelium (initiation phase) followed by a morphogenetic phase. Various exogenous and endogenous factors are involved and are usually species dependent and have been extensively reviewed. It is not relevant to enter into a discussion on the nature of events that lead to a part of the vegetative mycelial network forming small cellular masses which quickly grow in an upright mode – the stipe or stalk. Rapid cell wall synthesis is obvious during fruit-body development. Rapid elongation of the stipe is due both to cell elongation and cell division and requires a continuous supply of water and nutrients from the vegetative mycelium during most of the growth period. This dependence decreases as the whole fruit-body matures and reaches a climax.

Although the results are somewhat fragmentary, there is now increasing evidence that passage from the vegetative phase of mycelial growth to the initiation phase of primordium formation involved certain metabolic changes. These changes appear to be related to variations in the amount of metabolite, particularly of carbohydrates and the growing substrate and in the mycelium.

The importance of rapid cell wall synthesis is obvious during fruit-body developments. Cell wall chitin content and chitin synthetase activity increase during stipe elongation and can be inhibited with Polyoxin D which specifically inhibits chitins synthetase activity. Autoradiographic studies have shown that cell growth of expanding stipes occurred by a uniform incorporation of cell wall material along the length of the cells. Electron microscopic autoradiography has located the site of chitin synthesis on the cell wall/plasma membrane region of the hyphal. This intercalary elongation contrasts in the predominantly polarized growth of the mycelial hyphae. However, as previously mentioned intercalary growth can also occur in vegetative hyphae when conditions require it. As the cap of pileus develops there is evidence of positive wall synthesis.

Once the initiation phase is accomplished, large fruit-bodies develops at the expense of carbohydrates remaining in the growth substrate or cellular constituents being stored in the mycelium and undeveloped primordia. Indeed the progression of the fruit-body morphogenesis depends on complex interactions between, for example, the growing medium and the vegetative mycelium, the vegetative mycelium and primordia, the developing fruit-bodies and aborting primordia and between the stipe and the cap of the fruit-bodies.

Cell wall hydrolytic enzymes – chitinase, β -1-3 glucanases etc. is produced in the pileus with much lower levels in the stipe area. Their synthesis occurs in the late stage of fruit-body development. Will maximum actively being obtained as the pileus starts to lyse. This enzyme production correlates with fruit-body morphogenesis and is independent of basidiospore formation, as sporeless mutants show the same pattern of enzyme production.

Conclusions:

The dominant feature of the Basidiomycete life cycle is the dikaryotic vegetative mycelium. By way of the epical dominance of the hyphae growth will be extensive through soil or sawdust mixture (in commercial productions). Nutrients will be derived by exoenzyme activity, absorbed into the hyphae and transported throughout the mycelial mass. Growth is indefinite and will continue while nutrients are available in the environment. Formation of the fruit-body will only occur under specific environmental conditions. Formation is rapid and controlled by genetic means. Some types of strains of vegetative mycelium do not have the genetic potential to form fruit-bodies. The mature fruit body will produce billions of basidiospores and will then deliquesce with the residual organic molecules being absorbed by the vegetative mycelium. The fruit body should be viewed as a collection of dikaryotic mycelium whose function is to form an aerial structure to release basidiospore to complete the organisms life cycle. While visually different, the structural components of the fruit-body are remarkably similar that of the parent vegetative mycelium.

Therefore the vegetative mycelium and the fruit body should be considered as substantial equivalent.