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Mr Andreas Klepsch
European Commission
By email

3 November 2008

Reference: NFU 695

INITIAL OPINION: A BETA-GLUCAN RICH EXTRACT FROM *Lentinus edodes*

Dear Mr Klepsch,

On 19 December 2007 the UK Competent Authority accepted an application from Glycanova for 'Lentinex,' a beta-glucan rich extract from *Lentinus edodes* as a novel food ingredient, in accordance with Article 4 of regulation (EC) 258/97. The Advisory Committee on Novel Foods and Processes (ACNFP) reviewed this application and their opinion is attached. I apologise for the delay in submitting this opinion as the ACNFP's evaluation was extended while we obtained additional information from the applicant.

In view of the ACNFP's opinion, the UK Competent Authority considers that the beta-glucan rich extract from *Lentinus edodes* meets the criteria for acceptance of a novel food defined in Article 3(1) of regulation 258/97.

I am copying this letter and the ACNFP's opinion to the applicant.

Yours sincerely,

(By e-mail only)

Dr Chris Jones

For the UK Competent Authority

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

OPINION ON A BETA-GLUCAN RICH EXTRACT FROM *Lentinus edodes*

Applicant	Glycanova
Responsible Person	Bjorn Kristiansen
EC Classification	2.2

Background

1. An application was submitted from Glycanova for the authorisation of 'Lentinex' a beta-glucan-rich mycelial extract of *Lentinus edodes* (Shiitake mushroom) as a novel food ingredient. This product was originally considered in 2006-7 under article 3(4) of (EC) 258/97 and at that time the Committee concluded that the data provided by the applicant were insufficient to enable an opinion on substantial equivalence to be issued.
2. The novel ingredient (NI) is a relatively rich source of lentinan, a water-soluble beta-glucan found in *L. edodes*. 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 in aqueous suspension has an energy value of 1.44 kcal per 10 ml.

I. Specification of the novel food

Application dossier, pp 6-10

3. The applicant intends to market the NI as a nutritional food ingredient in a wide range of food categories. 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 approximately 1 mg/ml lentinan, together with measurable quantities of glucose, protein and amino acids. The mycellial biomass is removed as part of the production process.
4. The specification is given as follows.

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

5. The applicant provided data for five independent batches all of which were within specification. The applicant provided data to show the absence of significant levels of undesirable substances (heavy metals, pesticides) and quantified all the free amino acids present in the NI. The applicant also provided data, which shows that the (sterile) product remained stable for a period of 5 months under accelerated storage conditions which they contend offers sufficient reassurance that the product could be marketed with a 12 month stability claim.
6. In response to a request from the Committee for a complete proximal analysis of the NI, the applicant provided some additional analyses for chloride, nitrate, and nitrite, sulphate, phosphate, together with copper, iron and aluminium, all of which are present at low levels. The applicant also provided data showing the absence of mycotoxins (aflatoxins and ochratoxin) in the NI. In response to a comment from the Committee regarding the relatively low levels of beta-glucans present, the applicant accepted that the levels present were low, compared to the starting material but viewed them to be sufficient for their proposed use.

Discussion. The Committee was content that the data provided by the applicant does not give cause for any concerns regarding the composition of the NI.

II Effect of the production process applied to the novel food

Application dossier, pp 11 – 15

7. A pure culture of *L. edodes* is grown in sterilised liquid medium comprising glucose, malt extract, soy peptone, and yeast extract 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.
8. 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 included in the application dossier.
9. The Committee queried whether the material used in the safety studies was produced in laboratory or pilot scale fermenters. The applicant confirmed that all the studies used material grown in a 750 litre fermenter (their current production fermenter) and was of the view that scale-up would have no effect on the final product, but did not include any data to demonstrate this.
10. The Committee also queried whether the applicant had considered whether the fermentation process may potentially give rise to the production of secondary metabolites. The applicant did not provide any data to demonstrate the absence of secondary metabolites but highlighted the absence of mycotoxins in the NI as indicative of the absence of toxic components. The applicant also viewed the widespread consumption of fruiting bodies (i.e. shiitake mushrooms) together with the lack of adverse reactions seen in human studies (see section XIII, below) as evidence that the production of secondary metabolites was an extremely unlikely scenario.

Discussion The Committee accepted that the NI was produced in accordance with GMP. With regard to the potential presence of secondary metabolites, the Committee noted that, whilst secondary metabolites can be produced by fungi in submerged

culture, this process requires a degree of physiological differentiation. Such differentiation would occur only under culture conditions which are the antithesis of those required for the production of the beta-glucans (primary metabolites) that are present in the NI. The Committee therefore concluded that secondary metabolites would be unlikely to be produced under the growth conditions described and the history of consumption of the fruiting body, the most differentiated form of the fungus and which would be most likely to contain any secondary metabolites that the species would be capable of synthesising, provides additional reassurance in this regard .

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

Application dossier, pp 16-18

11. The source of the NI *L. edodes* has an established history of consumption throughout the world including the EU, but consumption is restricted to the fruiting body. The applicant is of the view that mushrooms are one of the leading sources of beta-glucans (including lentinan) 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 applicant also noted that beta-glucans are widely available in the diet from other sources (see below).
12. The NI is novel solely by virtue of the fact that the beta-glucan (lentinan) component is obtained from the mycellial form of the fungus. The applicant states that this is a more reliable means to obtain the NI and that there is a mycological basis for accepting that beta-glucans from the fruiting bodies could be used as evidence of previous consumption.

***Discussion** The Committee considered detailed mycological evidence regarding the similarity of the mycelium to the fruiting body as the basis of the applicant's original application for an opinion on substantial equivalence. Whilst this did not provide adequate evidence of equivalence, the Committee accepted that there is widespread consumption of beta-glucans from shiitake mushrooms and shiitake mushroom per se.*

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

Application dossier, pp 23-28

13. The applicant is proposing that the NI be used as a nutritive ingredient in a wide range of products: dietary supplements, yoghurts, soft drinks, cooked and processed foods, and baked goods.
14. 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 by the applicant would be subject to a separate authorisation procedure under regulation (EC) 1924/2006.
15. The NI will be marketed without restriction to the whole population 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 consume mushrooms regularly (see Section X below).

***Discussion** The Committee viewed the proposed uses of the NI to be acceptable.*

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

Application dossier, pp22-23

16. As noted above the source of the novel ingredient is widely consumed in its mushroom form. Consumption of 90 g of *L. edodes* mushrooms (around 4-5 mushrooms) would contain approximately 1.8 g (1800 mg) of lentinan. The novel ingredient will be incorporated in products with a recommended daily consumption of 1-2.5 mg. Beta-glucans are also found in a number of food categories other than mushrooms with the most notable dietary sources being oats and other cereals, which contain (1-3)(1-4) beta-D-glucans, (lentinan is a (1-3)(1-6) beta-D-glucan).

Discussion The Committee accepted that there was evidence of consumption of both beta-glucans and the source material. The Committee highlighted the relatively low quantities of lentinan in the NI compared to the source material, but accepted that the applicant viewed the quantity to be sufficient for their proposed use.

XI. Nutritional information on the novel food

Application dossier, p 24

17. The applicant intends the NI will be used 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, using US data.

Discussion The Committee noted that whilst the US figures are arguably of limited relevance in the EU it was clear that, irrespective of the source of the dietary intake data, the amounts are so small that they are of little relevance in the diet as a whole.

XII. Microbiological information on the novel food

Application dossier, pp 35-36

18. The NI is marketed as a sterilised product. The applicant ensures sterility by carrying out a routine analysis which involves plating the NI onto two different microbial growth media, and checking for the presence of colony forming units (CFU). The applicant's presumption is that the product is sterile, and the acceptance criterion is zero CFU, (denoted as OK in the stability report (Application dossier p 30, Appendix H).

Discussion The Committee accepted that the product would be marketed in a sterile form and as such there was minimal risk of microbiological contamination.

XIII Toxicological Information on the Novel Food

Application dossier, pp 37-70

19. The applicant provided information from a series of toxicological studies.

20. **Animal studies:** The applicant detailed 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.

21. The Committee noted that these were efficacy studies rather than toxicology studies and the end points were mainly haematological and immunological. 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 paralysis. 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.

22. **Human studies:** The applicant 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 and viewed that the results of the study demonstrated the safety of this product in healthy elderly consumers. The Committee 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. The Committee also requested additional information on the adverse effects frequency, which showed no difference in the incidence of adverse events, between placebo and treatment but did not mention severity.
23. In response the applicant advised that increases in CRP levels were seen with 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.

Discussion The Committee was of the view that the toxicological studies were carried out primarily to determine the efficacy of the NI, and although they did not give any indication that consumption of the NI would give cause for concern, were of limited use in determining whether the product posed a toxicological risk. The Committee was of the view that in order for such reassurance to be obtained the applicant should carry out additional animal feeding studies carried out to OECD guidelines would be required.

Allergenicity & Labelling

Application dossier, p 71

24. 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 22).

Discussion The Committee accepted that whilst there are measurable quantities of protein present in the NI, as there is widespread consumption of the source material it is extremely unlikely that protein from L. edodes would give rise to any unusual allergenic reaction compared with the fruiting body. However, the Committee noted that the presence of soya peptone in the growth medium would mean that the NI should be labelled to highlight the presence of soya derivatives, whether or not this is a requirement of current EU legislation concerning allergen labelling.

Overall Discussion

The Committee noted that there was consumption of the fruiting bodies including extracted beta-glucan supplement forms in the EU but the production process employed for the NI meant that only limited reassurance could be gained from consumption of these products. The Committee's concerns regarding the paucity of toxicological safety studies carried out to international standards was in response to the applicant's view that studies carried out primarily to determine efficacy could also be used to allay concerns about safety. However the Committee accepted that the well characterised compositional profile of the NI, together with the relatively low quantities of beta-glucans compared to fruiting bodies, did not highlight any cause for concern. In addition the Committee was reassured that the fermentation conditions employed would be extremely unlikely to give rise to the production of secondary metabolites. In view of this the Committee accepted that the earlier concerns had been addressed and there was no requirement for the applicant to carry out additional safety studies.

Conclusion

The Advisory Committee on Novel Foods and Processes is satisfied by evidence provided by the applicant, Glycanova, that the range of uses for the novel ingredient beta-glucan rich extract from *Lentinus edodes* is acceptable subject to the labelling requirement described above.

October 2008