

## ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

BETA-GLUCAN RICH EXTRACT FROM *Lentinus edodes*

Members are asked to consider the response of the applicant to the concerns raised by the Committee at the last meeting

**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. This application has been considered by Members, on a number of occasions, initially in the form of a request for an opinion on equivalence. (ACNFP80P/2 ,ACNFP80/11, ACNFP81/5, ACNFP84P/1, ACNFP84/2, ACNFP 85/3, ACNFP87/3).
2. The NI is a relatively rich source of Lentinan, a water-soluble, (1-3)(1-6)  $\beta$ -D-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 and the fruiting bodies are widely consumed as foods.
3. At the April meeting the Committee was of the view that, due to concerns regarding the potential presence of secondary metabolites and a lack of safety studies, the applicant should provide additional information. The Committee was of the view that although the presence of secondary metabolites would be unlikely, the possibility could not be ruled out and additional animal feeding studies should be carried out, to OECD guidelines, to demonstrate that the product was safe.
4. The Secretariat advised the applicant of the Committee's view (letter attached at **Annex A**), however the applicant has requested that, prior to considering the feasibility of carrying out additional safety studies, they have the opportunity to respond to Members' concerns.
5. The response is in two parts. The applicant has provided a response to members concerns (attached at **Annex B**). This response complements the existing composition data (see below) with details of additional analyses for chloride, nitrate, and nitrite, sulphate, phosphate, together with copper, iron and aluminium, all of which are present at low level. With regard to secondary metabolites, the applicant suggests that, whilst their production cannot be ruled out, the data showing the lack of mycotoxins are sufficient to allay any theoretical concerns regarding their presence. The applicant also highlights a number of parameters that were monitored in their human (efficacy) study. The applicant contends these parameters provide sufficient supporting information to enable conclusions regarding the safety of the product to be drawn.

6. The Secretariat has collated all the compositional data previously provided by the applicant in responses to date (**Annex C**), which indicates that whilst there are batch variations, the product appears to comprise chiefly of small quantities of protein, free glucose, lentinan, also expressed as glucose, galactose and mannose. This table, together with a specification and additional data showing the absence of mycotoxins and the presence of trace amounts of vitamins in two production batches (referred to as Fermenteringsmedium) is attached in Annex B.

#### **Committee Action Required**

7. The Committee is asked whether the applicant's response provides sufficient information and adequately addresses the questions and concerns raised at the April meeting.
8. If not, the Secretariat will advise the applicant that the concerns as detailed in the letter attached at Annex A are still applicable and ask the applicant to confirm whether they intend to carry out the additional scientific studies.

**ACNFP Secretariat  
June 2008**

#### ***Annexes attached***

**Annex A:** Letter to the applicant, 14 April 2008

**Annex B:** Applicant's response

**Annex C:** Summary of compositional data provide by the applicant

**Letter from the Secretariat to the applicant, 14 April 2008**

**Beta Glucan Rich Extract From *Lentinus edodes***

Dear

As you are aware, the Advisory Committee on Novel Foods and Processes (ACNFP) considered your response to their questions regarding the above product at their meeting on 3 April. Members remain concerned about your product and I have highlighted the following points of concern below, each of which requires detailed clarification:

1. The additional compositional data you provided are based on the fermentation medium rather than the final product. This is unlikely to accurately reflect the composition at the end of the fermentation however.
2. The Committee commented on the growth conditions and noted that although unlikely, the possibility of secondary metabolite production could not be ruled out. Members noted that although the fruiting bodies of *Lentinus edodes* are widely consumed, they differ both in terms of growth conditions and physiology when compared to the mycelial mass that is fermented to produce your ingredient. The history of consumption of the fruiting bodies is therefore not of direct relevance in the consideration of the safety of your product. The Committee expressed concern that the fermentation conditions could, conceivably, lead to the production of secondary metabolites that would be expressed into the culture medium and may be present in the final product.
3. The Committee also noted that as your clinical studies are predominantly efficacy based, these do not appear to be suitable in determining the potential effect of secondary metabolites if they were to be present in your product. The Committee noted that a sub-chronic study carried out in laboratory animals in accordance with OECD safety assessment guidelines, together with appropriate genotoxicity studies would be necessary in order to make an informed judgement on the safety of the product.

The Committee's next meeting is on 4 June and we would ask you to reply, if possible by 3 May. Any response received after this date can be discussed at the subsequent meeting at the end of July.



**ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**

**Applicant's response**

# Food Standards Agency

Comments to letter of 14.04.2008.

(i) There seems to be a misunderstanding with respect to the additional compositional data that we provided prior to the meeting on 3 April. The data provided were from analysis of two product batches (not the fermentation medium). The production batch numbers are given on the certificates of analysis (10-080111 and 10-080130, resp.), whereas the term "fermenteringsmedium" is used only to describe the type of sample to the external laboratory that performed the analysis. Thus, the data previously provided does indeed reflect the composition of the product.

To further characterise our product, several new parameters have been analysed in five product batches, as shown in the enclosed tables:

Product batch	Chloride, mg/l	Nitrate, mg/l	Nitrite, mg/l	Copper, mg/l	Ammonium, mg/l
10-071025	102	23,4	0,19	0,63	<0,20
10-080111	95	22,4	0,204	0,83	<0,20
10-080130	95	24,4	0,172	0,75	<0,20
10-080211	105	27,4	0,202	0,99	<0,20
10-080220	107	21,4	0,152	0,68	<0,20
<b>Average</b>	<b>100,8</b>	<b>23,8</b>	<b>0,184</b>	<b>0,776</b>	<b>#DIV/0!</b>
<b>Stdav</b>	<b>5,59</b>	<b>2,30</b>	<b>0,02</b>	<b>0,14</b>	<b>#DIV/0!</b>

Product batch	Sulphate, mg/l	Iron, mg/l	Orthophosphate, mg/l	Total phosphate, mg/l
10-071025	200	1,49	<0,5	0,5
10-080111	190	1,15	<0,5	1,4
10-080130	190	1,11	<0,5	1,3
10-080211	210	1,38	<0,5	1,8
10-080220	210	0,95	<0,5	1,6
<b>Average</b>	<b>200</b>	<b>1,216</b>	<b>#DIV/0!</b>	<b>1,32</b>
<b>Stdav</b>	<b>10</b>	<b>0,22</b>	<b>#DIV/0!</b>	<b>0,50</b>

As documented by the standard deviations, batch-to-batch variations in these parameters are insignificant.

(ii) We notice that the Committee suggests that the possibility of secondary metabolite production cannot be ruled out. We have addressed this issue from two complementary perspectives; Firstly, we have analysed two product batches for the possible presence of five well-known fungal secondary metabolites (aflatoxin B1, B2, G1, G2 and ochratoxin A). As shown in the certificates of analysis provided in our previous letter, in all cases the content is below the detection limits of the analytical methods used (EN14123m and CEN 14132, resp.). Secondly, all available data (relevant scientific literature as well as our own previously submitted data on toxicology and safety) strongly argues against the presence of secondary metabolites of relevance to human consumption of our product.

(iii) The clinical study in healthy subject (Study number 10601, previously submitted) had a primary efficacy aim. However, a safety study would probably not have included a battery of immunology

tests, and not included more safety parameters. The study included tests for all vital system such as liver, kidney, blood lipids and blood cells. Heart rate and Blood Pressure were also followed. Additionally objective and subjective symptoms were recorded (ADEs and ADRs).

In our opinion, this study is fully comparable with a study which had safety as the primary aim. The tables give below contain all changes in the different immunological variables and organ function tests.

**Table 14 Helper and Cytotoxic T Cells - PP Population**

Treatment difference	Lentinex® Mean±SD	Placebo Mean±SD	p-value between treatments ANOVA / Wilcoxon
CD4+ (%)	-1.12±5.69 p=0.5274	-1.49±6.25 p=0.0357*	0.4655W
CD8+ (%)	0.41±2.97 p=0.4387	0.51±2.82 p=0.3051	0.8671A / 0.8442W
CD4+/CD8+ ratio	-0.155±0.693 p=0.3993	-0.409±1.959 p=0.0862	0.4176A / 0.4875W

**Table 15 Cellular Immune Response - PP Population**

Treatment difference	Lentinex® Mean±SD	Placebo Mean±SD	p-value between treatments ANOVA / Wilcoxon
CD3+ (%)	-1.41±5.96 p=0.4632	-0.94±4.13 p=0.0414*	0.9857W
CD19+ (%)	0.44±1.83 p=0.1785	-0.57±2.15 p=0.1372	0.0702A / 0.0368*W
CD56+ (%)	2.11±6.11 p=0.0465*	1.96±5.03 p=0.0323*	0.7078W

**Table 17 Humoral Immune Response - PP Population**

Treatment difference	Lentinex® Mean±SD	Placebo Mean±SD	p-value between treatments ANOVA / Wilcoxon
C3 (g/l)	-0.093±0.247 p=0.0418*	-0.077±0.176 p=0.0166*	0.7135A / 0.7367W
C4 (g/l)	-0.007±0.042 p=0.2187	0.001±0.035 p=0.6267	0.3295A / 0.4236W
IgG (g/l)	0.231±0.1.117 p=0.2444	0.273±0.853 p=0.0755	0.9845A / 0.9430W
IgA (g/l)	0.045±0.295 p=0.3927	-0.016±0.205 p=0.6551	0.2284A / 0.2959W
IgM (g/l)	-0.002±0.0.081 p=0.8977	-0.020±0.087 p=0.1958	0.3399A / 0.2724W

**Table 18 Inflammatory Markers - ITT Population**

Treatment difference	Lentinex® Mean±SD	Placebo Mean±SD	p-value between treatments ANOVA / Wilcoxon
IL-8 (pg/ml)	-0.8711±4.8836 p=0.1002	-0.4500±3.5880 p=0.2239	1.0000W
IL-10 (pg/ml)	-0.1581±1.0622 p=0.7908	2.4420±15.7327 p=0.5548	0.6543W
IL-10 without patient 129 (pg/ml)	-0.1622±1.0762 p=0.7908	-0.0429±0.7441 p=0.7897	0.8682W
IL-12 (pg/ml)	0.8469±17.7134 p=0.9020	3.1247±13.5194 p=0.1304	0.4123W
TNFα (pg/ml)	0.1084±0.4941 p=0.1788	-0.0042±0.3911 p=0.9465	0.2636A / 0.2987W
CRP (mg/l)	2.4±7.1 p=0.0206*	0.6±4.5 p=0.3450	0.4138W

**Table 27. Laboratory Variables Changes**

Treatment difference	Lentinex® Mean ± SD	Placebo Mean ± SD
Hb (g/dl)	0.10±0.61	0.06±0.46
Basophils (10 <sup>9</sup> /l)	0.01±0.05	0.01±0.05
Eosinophils (10 <sup>9</sup> /l)	0.01±0.06	0.05±0.08
Lymphocytes (10 <sup>9</sup> /l)	-0.05±0.36	-0.09±0.38
Monocytes (10 <sup>9</sup> /l)	-0.02±0.15	-0.01±0.18
Neutrophils (10 <sup>9</sup> /l)	0.30±1.71	0.11±0.97
Platelets (10 <sup>9</sup> /l)	-2.5±42.1	4.2±28.8
Leukocytes (10 <sup>9</sup> /l)	0.28±1.63	0.08±1.14
ALAT (U/l)	0.7±8.9	1.0±7.1
ASAT (U/l)	-0.3±5.2	0.3±5.7
γGT (U/l)	2.5±21.5	-1.5±14.4
Creatinine (μmol/l)	0.1±6.5	-1.1±5.2
Bilirubin (μmol/l)	-0.5±4.2	-0.0±4.5
TG (mmol/l)	0.040±0.371	0.040±0.501
Total cholesterol (mmol/l)	0.10±0.73	0.02±0.75
LDL cholesterol (mmol/l)	0.02±0.56	-0.10±0.63
HDL cholesterol (mmol/l)	0.00±0.20	0.06±0.23

**Table 29 Blood Pressure and Heart Rate - ITT Population**

Treatment difference	Period I		Period II	
	Lentinex® Mean±SD	Placebo Mean±SD	Lentinex® Mean±SD	Placebo Mean±SD
SBP (mmHg)	-1.36±11.04	0.50±9.58	-2.25±7.86	1.10±10.99
DBP (mmHg)	-0.45±7.06	-1.90±8.71	-0.40±7.26	-1.95±4.54
Heart rate (beats/min)	0.00±6.43	0.90±5.67	0.10±4.80	2.71±5.16

### ***Safety Conclusions***

There was no significant difference between treatments regarding the incidence of Adverse Events, Adverse Drug Reactions and Serious Adverse Events.

One of the safety laboratory variables eosinophils increased in the placebo group, but not in the Lentinex group. One patient showed changes in a safety laboratory value (cholesterol). This was reported as an AE.

There were no differences between treatments regarding the helper and cytotoxic T cells. A decrease in the number of helper T cells was observed within the placebo group, but not within Lentinex® group.

Among the different analyses performed a tendency for difference or significant difference between treatments in the number of B-cells was found depending on the statistical analysis chosen. This was confirmed in a subpopulation of subjects with starting CD19+ values lower than the median. Significant changes within treatment groups were observed for several parameters in both the placebo and Lentinex® groups. Lentinex® treatment gave an increase in CD56+, CRP and a decrease in C3, while subjects in placebo group showed an increase in CD56+ and a decrease in CD4+, CD3+ and C3 at the end of the study. No change in CRP and a tendency for increase in IgG within the Lentinex® group, and an increase in IgG within the placebo group were observed when the analysis was performed on a subpopulation where subjects with increased CRP due to known infectious disease were left out.

Period effects were observed for CD56+, C3, C4, IgG, IgA and IgM. These findings could contribute to masking of potential treatment effects.

The incidence and duration of infectious diseases did not differ between treatments.



**ACNFP/89/P1 Annex C**

**ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**

**Summary of Compositional data provided by the applicant**

**ACNFP Secretariat**

**June 2008**

**Table 1:**

Summary of major components present.

(Lentinan present at 1mg/ml +/- 0.2mg, in accordance with specification)

Batch no	Protein (B), (mg/l)	Protein (K), (mg/l)	Free Glucose (mg/ml)	Lentinan (mg/ml)*
10-070222	51.8			
10-070103	41.8			
10-SO1	38.7			
10-SO2	37.2			
10-061110	29.6	8	15.3	1.1
10-050530	41.8		21.0	1.1
10-070511	22.2	6	16.5	0.8
10-070921	61.8	<3	13.5	1.2
10-071002	39.2		15.6	1.0
10-070102				

(Data summarised by Secretariat)

Protein assay: (B) Bradford; (K) Kjeldahl

\* Lentinan analysed by HPLC after ethanol precipitation

**Table 2:**

Summary of major components present – product standardised to 0.5mg/ml Lentinan (i.e. approximately 1:1 dilution of the commercial product)

Batch no	Protein (B) (mg/l)	Free Glucose (mg/ml)	Glucose (mg/l)*	Galactose (mg/l)*	Mannose (mg/l)*
10-070222	32.4	6.0	283	71	152
10-061110	13.5	7.0	187	37	94
10-050530	20.9	10.4	253	21	102
10-070102	15.9	6.0	178	49	144

(Data summarised by Secretariat)

Protein Assay: (B) Bradford

\* HPLC after acid hydrolysis of the lentinan fraction

**Table 3:**  
Standardised Lentinan Product

Calories	<144cal/ml
Protein	<1%
Lentinan	1mg/ml
Fat (total)	Not detected
Cholesterol	Not detected
Sugars	<2.5%
Ash	<0.2%
Sodium (salt)	<0.035%

**Table 4:**  
Specification

Appearance	Light brown, slightly turbid
Microbiological data	Sterile
Lentinan	1 mg/ml +/-0.2
Residual Glucose	<20mg/ml
Total Protein	<100 µg/ml
pH	3-4
Protein	<10 mg/ml
Pesticides	Not detected