

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

BETA-GLUCAN RICH EXTRACT FROM *Lentinus edodes*

The Committee is invited to consider all the additional data provided by Medimush to finalise its opinion on this application.

Background

1. This request from Medimush for an opinion on the equivalence of their novel ingredient, a beta glucan-rich mycellial extract of *Lentinus edodes* (Shiitake mushroom), compared with an existing product, dried, pulverised fruiting bodies of *L. edodes* has been considered by members on a number of occasions (ACNFP80P/2 ,ACNFP80/11, ACNFP81/5, ACNFP84P/1).
2. During the latest postal review, some Members remained concerned that the data provided by the applicant may be insufficient to confirm substantial equivalence. The Secretariat therefore now drafted an opinion on equivalence for Members to consider (attached at **Appendix 1**).
3. The Secretariat notes that the discussions on this application have taken place over a significant period of time, including two postal considerations. This paper gives Members the opportunity to review a compilation of the additional information that the applicant has provided (**Appendix 2**). The application can be considered in the context of the ACNFP's guidelines on equivalence (**Appendix 3**).

Committee action required

4. The Committee is asked to consider whether the available information is sufficient to demonstrate that the two products are substantially equivalent, and to advise the Secretariat on the most appropriate text for the outstanding discussion points.

**Secretariat
September 2007**

Appendices attached

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| Appendix 1 | Draft Opinion (Restricted) |
| Appendix 2 | Compilation of information provided by the applicant |
| Appendix 3 | ACNFP Guidelines for Substantial Equivalence |

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

Draft opinion on substantial equivalence

**Secretariat
September 2007**

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

Compilation of data provided by the applicant:

- 1. Compositional comparison**
- 2. Mycology expert review**
- 3. Level of undesirable substances, animal and human studies**

**Secretariat
September 2007**

1 - COMPOSITION ANALYSIS

Our production process involves fermentation of *Lentinus edodes* in a defined medium. Since the yield of exopolysaccharides is typically above 1 mg/ml the fermentation liquid is diluted with distilled water to meet the specification of Lentinex, with a total polysaccharide content of 0.5 mg/ml. The following table presents data from four batches of Lentinex:

Batch no.	Composition, ug/ml			Protein, ug/ml	Free glucose, mg/ml
	Glc	Gal	Man		
Lentinex 10-050530	253	21	102	20,9	10,4
Lentinex 10-061110	187	37	94	13,5	7,0
Lentinex 10-070102	178	49	144	15,9	6,0
Lentinex 10-070222	283	71	152	32,4	6,0
	1	0.2	0.1(*)		
Average	225,2	44,7	122,9	20,7	7,3
StdAv.	51,1	21,2	29,4	8,4	2,1
%CV	22,7	47,3	23,9	40,6	28,6
Confidence interval (P=0,05)	184.3 - 266.1	27.8 - 68.2	99.4 - 146.4	14.0 - 27.4	5.6 - 9.0

Table 1. (*) value reduced for mannose in growth medium as previously reported (original value = 0.5).

To get the corresponding data on Bio-Life, 10.0 g of powder from capsules was extracted in 100 ml MQ water (standard procedure: 110°C, 1h), and analysed using the same methods as for Lentinex (see below):

Batch no.	Protein, ug/ml	Free glucose, mg/ml	Total polysacc. (HPLC), g/l	Composition		
				Glc	Gal	Man
Bio-Life 511432	129,1	2,7	4,7	4069	268	329
Bio-Life 710161	148,4	0,2	6,4	5308	507	540
				1	0.1	0.1
Average	138,8	1,4	5,5	4688,5	387,5	434,5
StdAv.	13,6	1,8	1,2	876,1	169,0	149,2
%CV	9,8	126,5	21,7	18,7	43,6	34,3
Konfidensintervall (P=0,05)	10,9	1,4	1,0	3987.5 - 5389.5	252.3 - 522.7	315.1 - 553.9

Table 2.

Since Lentinex is liquid and Bio-Life is a dry powder in capsules, the most relevant comparison is on a dry matter or daily dose basis. Using a dry matter content of 1.9% for Lentinex, recalculating the above data gives:

	Composition, % of dry matter	
	Bio-Life	Lentinex
Protein	0,14	0,11
Free glucose	1,4	38,4
Total polysaccharides (*)	5,5	2,1
Glc	4,7	1,2
Gal	0,4	0,2
Man	0,4	0,6

Table 3.

(*) Please note that the data on Bio-Life total polysaccharides probably overestimates biologically active ingredients due to the presence of filler materials added during the manufacturing process (details unknown).

Thus, the only significant difference between the two products is the content of glucose, which is approx. 25 times higher in Lentinex. However, taking the recommended daily dose into consideration the content of glucose is both of minimal nutritional importance and not significantly different in the two products.

Correspondingly, using 2ml daily dose of Lentinex and 2 capsules as daily dose of Bio-Life, data on daily dose comparison is presented in table 4.

	Content daily dose	
	Bio-Life	Lentinex
Protein, ug	1943,2	41,4
Free glucose, mg	19,6	14,6
Total polysacc., mg	77	0,7856
Glc, ug	65639	450,4
Gal, ug	5425	89,4
Man ug	6083	245,8

Table 4.

When evaluating the data in table 4 it is again important to keep in mind the presence of added filler materials (polysaccharides) to Bio-Life (quantity and quality unknown). Apart from this there is a significant difference with respect to protein content. However, the protein is not critical for the possible beneficial health effect of these nutraceuticals, and the data supports our claim that Lentinex does not contain proteinaceous material that is not present in the equivalent product.

The data in tables 1-4 are from our in-house QC and R&D laboratory, and the two products are compared using identical standard laboratory procedures. The methods used are the following:

Protein: standard Bradford assay (Bio-Rad)
Total polysacc. HPLC after acid hydrolysis, quantification of monosaccharides

Protein composition

Since the Committee has expressed concern about the possible existence of proteins in Lentinex that might not be present in the existing product (Bio-Life) we have compared total protein composition of the two products by SDS polyacrylamide gel electrophoresis. The gel was subjected to a standard silver stain procedure (Procedure LIT34 RevB from Bio-Rad Lab., based on Merril et al., Science, **211**, 1437 (1981)), with detection limit approx. 0.1 ng/mm² (see Fig. 1).

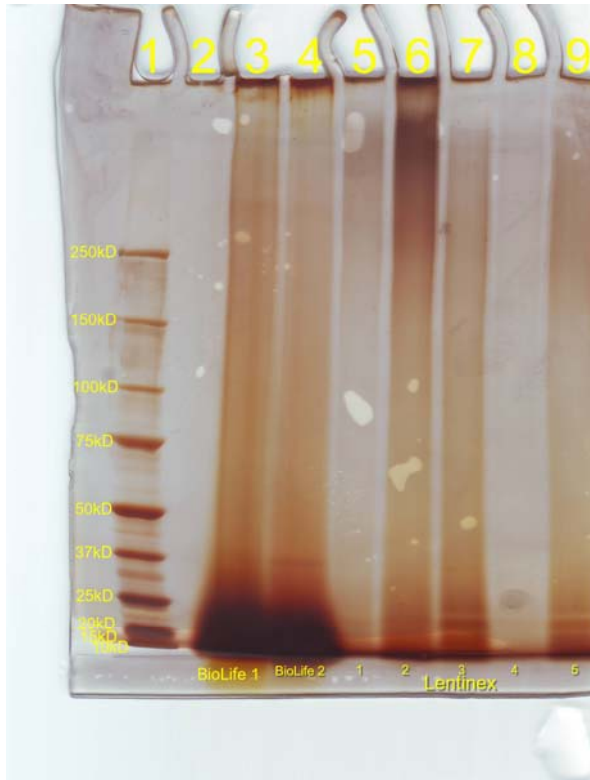


Fig. 1. Lane 1 is a molecular weight standard (Bio-Rad), lanes 3 and 4 are Bio-Life batches 710161 and 511432, resp., lanes 5-9 are Lentinex batches 10-S02, 10-S01, 10-070102, 10-061110 and 10-070222, resp.

Fig. 1 demonstrates that there are no protein bands that are unique to Lentinex. Also, only very small amounts of proteins are detected in Lentinex, primarily in the 10 – 25 kD mw-range. The relative amounts of proteins in Lentinex and Bio-Life in fig. 1 reflect the results from Bradford protein analysis (tables 1 and 2 above).

Response to Secretariat's request for clarification in June 2007

Secretariat Comment: Protein analysis

- You conclude that there are no proteins that are unique to your product, and that the only proteins present (I assume in both products) are small (range 10-25kD). It could be that the additional sensitivity offered by silver staining has masked the intrinsic resolution of the gel and we are not able to see these bands clearly. Do you have a better photo? perhaps the original Coomassie Blue stain shows this better. The presence of a 37kD protein that (as far as I can see) is only present in the Biolife product is a point that you may wish to comment on.

Applicant's Response

The photo gives a good representation of the gel. The part of the gel that was stained with Coomassie Brilliant Blue did not contain any bands that are not present in the silver stained part, as shown in the photo, reflecting the fact that the content of protein is very low. As far as the 37 kD band is concerned, this band is barely visible, and seems to be present in one of the Biolife samples only. Please remember that the samples applied to the gel is from a liquid extract of Biolife containing 10g powder in 100 ml of water (standard procedure for extraction from powders), i.e. 10% dry matter, while the Lentinex samples are as is, i.e. approx. 1.9% dry matter. Thus, even if the 37 kD band is really a standard ingredient in the Biolife product, it is not possible data to conclude that this ingredient is absent from Lentinex

Secretariat Comments: Compositional analysis

- Why are there large differences seen between each your four batches, which are produced under controlled fermentation conditions, using a defined medium?
- Table 3 gives the dry weight figures, to which you have applied a factor of 1.9% to give dry weight values for your product. As far as I can see the Biolife analysis contained 10g dry wt in 100ml, I am therefore not clear why this 1.9% figure is applicable, and why it is applicable to all samples. I am also struggling to understand how the you calculate the figures for dry weight, and question whether these are of any practical relevance (especially if next point is correct)
- If I understand it correctly (please confirm) Table 4 provides the most accurate comparison (albeit for mean values) as these refer to the composition of both products as they are to be consumed. If this is the case It would be useful to know why have you have you multiplied the Biolife product by 14 (presumably because this reflects the recommended dosage?). As I see it, in terms of composition your product contains approx 100x less polysaccharide than the existing product, the equivalent amount of glucose and 50x less protein. Is this correct?
- You refer to a filler material that is possibly present in the Biolife product. By law, the details must be on the label, if they are not I am not sure you can assume they are present.

Applicant's Response

The differences seen between our four batches are all acceptable, both according to product specifications as well as according to reasonable expectations for the output of the complex fermentation process. Our

production process is very different from conventional bacteria or yeast processes where smaller deviations are the norm

As a direct comparison between a liquid and a powder is not straightforward, tables 3 and 4 were included in our reply from May 16th to give a better basis for comparison. One could argue that the amount of water in our product is irrelevant to the comparison and the products should be compared on a dry weight basis. Thus, the data presented in table 3 is calculated using 1.9% dry matter for Lentinex and 10% (10g per 100 ml) for the extract of Biolife. Our comment about the probable overestimation of biologically active ingredient in Biolife due to the presence of filler material was, as you kindly point out in your reply, imprecise. The amount of filler material is declared on the Biolife box to be 100mg cellulose per capsule (i.e. 100mg cellulose per 700 mg product). When compared according to contents of dry matter (table 3) there appear to be a large difference between the two products with respect to glucose. However, this difference is insignificant when the products are compared on a daily dose basis. We apologise for not explaining explicitly how the data for Biolife in table 4 was calculated. As declared on the Biolife box, one capsule is 700 mg and recommended daily dose is 2 capsules, i.e. 1400 mg in total. As the Biolife data in table 3 are based on 100 mg/ml dry matter, the recommended daily dose is 14x higher. Thus, on a daily dose basis the Biolife product contains equivalent amounts of free glucose but significantly more polysaccharides and protein, whereas a comparison based on ingredients other than water reflects equivalent amounts of protein and polysaccharides, but higher free glucose in Lentinex

2 - EXPERT REVIEW

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 epical 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.

3 –Level of undesirable substances, animal and human studies

Regarding terpenes and alkaloids, it has been reported that these compounds may be found in fruiting bodies. Bio-Life does report on the level of terpenes or alkaloids in their product and we have not found significant levels in the MediMush product either (<1 mg/l).

All the MediMush data have been verified in five different batches

Level of undesirables

With respect to level of undesirables, we carry out product analysis in an independent approved laboratory and there is no trace of pesticides in our product. We also guarantee that our product is sterile and free of other micro-organisms. This information has been included in a corrected version of the main application document (enclosed).

Allergenicity

To our knowledge allergic reactions to orally taken Shiitake mushroom, is very seldom, taken the high consumption into consideration.

By a PubMed search, and search in a couple of governmental ADR-databases, only one case was found. A generalized exanthem developed in a patient after eating raw shiitake mushrooms. Reactions to prick and patch tests with shiitake mushrooms were negative. The skin eruption in this patient corresponded to the previously reported shiitake-induced toxicodermia reported in mushroom workers.

There are several reports of mushroom workers who by inhalation of shiitake spores experience lung reactions (mushroom worker's disease). There seem to be an agreement that the spores are responsible for the observed reactions.

Due to the production method, Lentinex does not contain spores, or derivatives thereof. The low content of protein (0,004 mg/ml) would probably have a low pro-allergic potential if any. In a study in 40 healthy elderly, ingesting 2,5 mg Lentinex® a day, for a period of 6 weeks, no allergic reaction was observed.

Additional Information

a) Animal studies

As mentioned in the application, all rats were monitored for toxic effects. In addition to the immunological variables and haematology, all rats were observed for weight loss, lethargy and hind limbs were monitored for paralyses. In addition, the Lentinex® group was monitored for toxic effects, ataxia and behavioural changes. No abnormalities were observed.

Comment on “The doses used were also significantly lower than the dose used in the human study and this is inconsistent with the approach normally employed in order to provide reassurance of the safety of a food.”

We apologise for the poor and unsatisfactory presentation of doses used in the different toxicological studies. To better understand the dose levels, doses should be presented as mg/kg/day of active substance. In the BN rat study 3 dose levels were

used, 7,8 mg/kg, 8,3 mg/kg and 12 mg/kg. In the BNML model studies, doses ranged from 0,5mg/kg to 20mg/kg. In comparison the daily dose given in the human study was 2,5mg corresponding to 0,04mg/kg. Thus the toxicological dose ranges between 15 to 560 times higher than the human dose, with no safety concern.

b) Human studies

Comment on *Increase in serum CRP*

1. CRP was a laboratory parameter with defined normal ranges. Three patients showed abnormal changes in CRP values during the course of the study and these changes were reported as AEs (table 1).

2. Table 1. Abnormal CRP values in 3 patients

Patient	Visit 1 value (Baseline)	Visit 3 value	Visit 4 value (After wash-out)	Visit 5 value	Normal range
119					<5 mg/l
	Lentinex®		Placebo		
119	1 mg/l	2 mg/l	1 mg/l	20 mg/l	
133	1 mg/l	19 mg/l	2 mg/l	18 mg/l	
	Placebo		Lentinex®		
138	1 mg/l	11 mg/l	<1 mg/l	37 mg/l	

As can be seen from the table, abnormal CRP values were observed both after Placebo and Lentinex®. All 3 subjects had levels inside the normal range at baseline and after the wash-out period.

3. When patients with high CRP values due to known infectious diseases were taken out of the analyses (i.e. pat. 101, 115, 120, 129, 133, 134, 138, 142) no changes within or between groups were observed ($p=0.4340$, $p=0.3223$ and $p=0.4674$ for Lentinex®, placebo and between groups respectively). Be aware that not all patients with infectious disease had abnormal CRP values.

4. No differences were observed between treatments with regard to infectious diseases, neither in duration, (table 2) nor in frequency or severity (table 3).

Table 2. Duration of Infectious Diseases

Variable		Lentinex®	Placebo	p-value between treatments (ANCOVA)
Duration (days)	Mean ± SE	14.,3±2.69	9.3±3.0	0.2278

Table 3. Infections and Infestations

	Mild		Moderate		Severe	
	Yes		Yes		Yes	
	n	%	n	%	n	%
N =42						
Placebo	3	7,1	7	16,7	0	0,0
Lentinex	6	14,3	6	14,3	0	0,0

Chi-square: $p=0,34$

Comment on "It would be useful to have more information on the adverse effects frequency":

The distribution of ADEs is given in table 4 (placebo), table 5 (Lentinex) and table 6 (total). Most symptoms were mild in intensity. Only one subject reported a severe symptom during the placebo period (nosebleed).

There are no differences between the placebo and Lentinex® groups with regard to reporting ADEs neither in frequency nor severity distribution or type of symptom.

Most frequent reported ADE was nasopharyngitis, 9 during placebo and 7 during Lentinex®.

Table 4. Distribution of ADEs and severity, placebo

System Organ Class / preferred Term	Number of patients	Cellulose (Placebo)											
		Mild				Moderate				Severe			
		No		Yes		No		Yes		No		Yes	
		n	%	N	%	n	%	n	%	n	%	n	%
gastrointestinal disorders	42	42	100	0	0,0	41	97,6	1	2,4	42	100	0	0,0
general disorders and administration site conditions	42	40	95,2	2	4,8	41	97,6	1	2,4	42	100	0	0,0
infections and infestations	42	39	92,9	3	7,1	35	83,3	7	16,7	42	100	0	0,0
injury, poisoning and procedural complications	42	41	97,6	1	2,4	42	100	0	0,0	42	100	0	0,0
investigations	42	40	95,2	2	4,8	42	100	0	0,0	42	100	0	0,0
musculoskeletal and connective tissue disorders	42	42	100	0	0,0	42	100	0	0,0	42	100	0	0,0
nervous system disorders	42	40	95,2	2	4,8	42	100	0	0,0	42	100	0	0,0
respiratory, thoracic and mediastinal disorders	42	40	95,2	2	4,8	42	100	0	0,0	41	97,6	1	2,4
skin and subcutaneous tissue disorders	42	42	100	0	0,0	42	100	0	0,0	42	100	0	0,0
vascular disorders	42	42	100	0	0,0	42	100	0	0,0	42	100	0	0,0
Total placebo (cellulose)				12	28,6			9	21,4			1	2,4

Table 5. Distribution of ADEs and severity, Lentinex

System Organ Class / preferred Term	Number of patients	Lentinex®									
		Mild				Moderate				Severe	
		No		Yes		No		Yes		No	
		n	%	n	%	n	%	n	%	n	%
gastrointestinal disorders	42	38	90,5	4	9,5	41	97,6	1	2,4	42	100
general disorders and administration site conditions	42	42	100	0	0,0	42	100	0	0,0	42	100
infections and infestations	42	36	85,7	6	14,3	36	85,7	6	14,3	42	100
injury, poisoning and procedural complications	42	42	100	0	0,0	42	100	0	0,0	42	100
investigations	42	41	97,6	1	2,4	41	97,6	1	2,4	42	100
musculoskeletal and connective tissue disorders	42	42	100	0	0,0	41	97,6	1	2,4	42	100
nervous system disorders	42	42	100	0	0,0	41	97,6	1	2,4	42	100
respiratory, thoracic and mediastinal disorders	42	41	97,6	1	2,4	42	100	0	0,0	42	100
skin and subcutaneous tissue disorders	42	41	97,6	1	2,4	42	100	0	0,0	42	100
vascular disorders	42	42	100	0	0,0	41	97,6	1	2,4	42	100
Total Lentinex				13	31,0			11	26,2		

Table 6. Total ADEs severity distribution.

Mild	Moderate	Severe
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	n	%	n	%	n	%	Chi-square
Placebo	12	(28,6)	9	(21,6)	1	(2,4)	p=0,98
Lentinex®	13	(31,0)	11	(26,2)	0	(0,0)	

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

ACNFP Guidelines for Substantial Equivalence

Secretariat
September 2007