

## ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

INSOLUBLE AND SOLUBLE YEAST *BETA*-GLUCANS**Issue**

The Irish Competent Authority has prepared an initial opinion on an application for the authorisation of Insoluble and soluble yeast *beta*-glucans as a novel food ingredient under the Novel Food Regulation (EC) No. 258/97. The Committee is asked whether it agrees with the initial opinion and whether it has any comments or objections. The Committee's advice will form the basis for the UK's formal response.

**Introduction**

1. On 19 January 2010, the European Commission forwarded the Irish Competent Authority's (CA) initial opinion on an application made by Biothera Inc., under Article 4(1) of Regulation (EC) 258/97, for the authorisation of insoluble and soluble yeast *beta*-glucans as novel food ingredients. Under the time scales set out in the regulation, the UK and other Member States have until 19 March 2010 to provide comments and/or reasoned objections to the initial opinion.
2. The Irish CA's initial assessment report is attached at **Annex 1** (Protect - Regulatory) and the original dossier is attached at **Annex 2** (Protect - Regulatory)<sup>1</sup>.

**Background**

3. This application from Biothera Inc., is for the placing on the market of 2 insoluble (BWGP and WGPD) and 1 soluble (WGPS) yeast beta-glucan ingredients, derived from baker's yeast, *S. cerevisiae* for use as food supplements and food ingredients. The applicant's beta-glucans are derived from the primary carbohydrate constituent (polysaccharide) of the cell wall of *S. cerevisiae* which has a long history of safe use in the production of bread, beer and wine.
4. In accordance with the European Commission Regulation 258/97, the Dutch CA considers that the novel ingredient (NI) can fall under the category described under Article 1(2)d. This corresponds to class 2.1 "Complex novel food from non-GM source which has a history of food use in the community" under Commission

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<sup>1</sup> The Protective Marking System is described in paper ACNFP/96/13.

Recommendation 97/518/EC, which sets out the guidelines for novel food applications. The requirements for a submission for class 2.1 are as follows:

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

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

The key issues for consideration are presented below under these headings.

## I. Specification of the novel food

Annex 2, p 6-16

5. The manufacturing process for the applicant's ingredient is divided into 2 stages. The final product obtained at the end of stage A is an insoluble product with a *beta*-glucan content of at least 70% (BWGP) or 75% (WGPD). The applicant provides product specifications for insoluble yeast *beta*-glucans in Table I.d-1. The final product obtained at the end of Stage B of the manufacturing process is a soluble yeast *beta*-glucan ingredient designated as WGPS. The applicant provides product specifications for this in Table I.d-2. Analytical data for 5 non-consecutive lots of insoluble and soluble yeast *beta*-glucans presented by the applicant show conformity to the product specifications.
6. The applicant is of the view that because insoluble yeast *beta*-glucans are used as the starting material in the production of soluble yeast *beta*-glucans, the analytical results for potentially toxic external contaminants obtained for insoluble yeast *beta*-glucans is expected to be similar and applicable to soluble yeast *beta*-glucans; therefore analysis of potentially toxic compounds is not included for the final soluble yeast *beta*-glucan ingredients (WGPS).
7. Volatile organic compounds (VOC) have been identified as a by-product of the production process, resulting from limited decomposition of lipid-derived fatty acids. In further information requested by the Irish CA, the applicant explains that VOC's are present in various yeast products and are produced as a result of the alkali washing of the yeast *beta*-glucans. The production of VOC's has been observed in fermentation of various foods and the applicant has provided the results of analysis for VOC in a number of yeast food ingredients for comparison

in Table I.e.2.1-3. The average methanol content in the NI was 243 µg/kg, ethanol 744 µg/kg, isopropanol 44 µg/kg, acetone 225 µg/kg and hexane 40 µg/kg. The Irish CA notes that the anticipated low intake levels of methanol and hexane are not considered to be of safety concern in the NI.

8. Three lots of insoluble yeast *beta*-glucan were analyzed for heavy metal content at the end of Stage A. The level of lead analysed was below the specified limit of 0.5 mg/kg. The mercury, arsenic and cadmium content were also assessed to ensure there would be no heavy metal contamination of the final product (Table I.e.3.1-1). The results of analysis for two lots of the insoluble yeast-*beta* glucan indicate the novel ingredient is free from contaminating pesticide residues (Table I.e.3.2-1)

## **II. Effect of the production process applied to the novel food**

Annex 2, p 17-22

9. The applicant provides a schematic representation of the two stages of its production process in Figure II.B.1-1 and Figure II.b.2-1. In Stage A of the production, the yeast *S. cerevisiae* is grown under controlled fermentation conditions and the manufacturing process is initiated by lysing the cells so that the beta-glucan component of the cell wall can be extracted and purified and dried to produce the final ingredient; beta-1,3-glucan (WGPD or BWGP, depending on the beta-glucan content). In Stage B of the process, the product from Stage A undergoes further processing in a series of solubilisation steps to produce soluble food grade WGPS ingredient.
10. The Irish CA notes that the applicant uses standard food grade chemicals in the isolation and purification steps. It also notes that the specifications of the insoluble and soluble beta-glucans confirm that the production process does not have any adverse effect on the quality or composition of the final product.

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

Annex 2, p 23-26

11. *Saccharomyces cerevisiae* has a long history of safe consumption for over a thousand years as a food ingredient and is commonly used in the production of bread and in the fermentation process of wine and beer, where it is more commonly referred to as brewers/baker's yeast. The applicant states that large quantities of *beta*-1, 3-glucans also occur naturally in a number of foods, such as various mushrooms (shiitake, Maitake etc).

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

Annex 2, p 28-36

12. The NI is intended to be used in food supplements, PARNUTS foods<sup>2</sup> and conventional foods. BWGP, WGPD and WGPS are proposed for use in food supplements at levels of between 250 and 500 mg per day, delivering approximately 175 to 137 mg yeast *beta*-glucans per day, and in PARNUTS foods where the level would not exceed 200 mg yeast *beta*-glucans per serving.
13. The NI is proposed for use in a range of conventional foods for the general population as presented in Table IX.a.3-1. The applicant explains that because BWGP, WGPD or WGPS contain approximately 70 or 75% yeast *beta*-glucans, the proposed maximum use-level of 200 mg yeast *beta*-glucans per serving is equivalent to 250 mg/serving of a trade ingredient.
14. The applicant uses data from the UK's National Diet and Nutrition Survey to estimate intake of the NI in the various food categories. However, the Irish CA notes that the estimates relate to general foodstuffs only and did not include intake from supplements or PARNUTS.
15. In summary, the highest mean and 97.5<sup>th</sup> percentile all-user intakes of the NI in all typical food uses in the EU was observed in male teenagers at 0.80 and 1.94 g/person/day respectively. Children had the highest mean and 97.5<sup>th</sup> percentile all-user intakes in relation to body weight of 49 and 105 mg/kg body weight/day, respectively. The Irish CA is of the view that the applicant makes a reasonable argument that the highest intake of 1.94g/person/day is the approximate equivalent of nine servings of foods containing added yeast *beta*-glucans, and is unlikely to be attained in practice by any of the user groups, particularly children.
16. The Irish CA notes that although foods containing the NI should be purchased only by those wishing to benefit from the functionality of the novel ingredient, the assumption that the NI would be consumed either through consumption of general foodstuffs or PARNUTS or dietary supplements, but not a combination of these is not well supported.
17. The Irish CA states that there are no firm grounds to believe that consumption of the NI through consumption of dietary supplements would preclude their consumption through general foodstuffs, particularly considering the broad range of general foods to which the applicant intends adding the novel ingredient into. The novel ingredient could be consumed through sports food (a category of PARNUTS) by active male teenagers who comprise the highest potential intake group and who are likely consumers of these products.

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<sup>2</sup> Foods for Particular Nutritional Uses, as defined in Council Directive 89/398/EEC.

18. The Irish CA notes, however, that even if these considerations resulted in worst case scenario intakes higher than those projected by the applicant, they would not be expected to present significant safety concerns.

19. The Irish CA notes that the estimated intakes calculated by the applicant are relatively close in value to the safe levels determined through animal and human studies. However this proximity in value is not an immediate cause for concern as the worst case scenario intake levels are not likely to be achieved by any of the user-groups, particularly children. In addition the Irish CA notes that the safe levels which resulted in no adverse effects were the highest levels tested and therefore may not represent the actual upper safe levels per se.

## **X. Information from previous human exposure**

Annex 2, p 37-40

20. *S. cerevisiae*, more commonly known as bakers/brewer's yeast, has been used for over a thousand years as a food ingredient, in the production of bread and in the fermentation process for wine and beer. In order to demonstrate the natural occurrence of yeast *beta*-glucans in foods, the applicant has analysed the yeast *beta* glucan content of various foods (Table X.a.1-1). The levels ranged from 2 to 1,004 mg/day. Marmite is derived from brewer's yeast and is expected to comprise approximately 1% yeast *beta*-glucan by weight. Typical serving sizes of marmite range from 1 to 18 g which would provide 10 to 180 mg yeast *beta*-glucans/serving. The applicant also states that *beta*-1, 3-glucans occur naturally in a range of foods, specifically a large quantity in mushrooms such as shiitake and maitake.

21. The Irish CA notes that the cell wall of *S. cerevisiae* which comprises approximately 30% of the total dry weight of the cell is made up of polysaccharides (85%) and protein (15%). Between 80-90% of the total cell wall polysaccharides are comprised of *beta*-glucans which means 15-25% of the cell is typically made up of *beta*-glucans. The Irish CA is therefore content with the applicant's conclusion that routine consumption of foods produced with *S. cerevisiae* such as breads, beer, wine and other foods naturally containing *beta*-glucans has led to a significant history of consumption of *beta*-glucans.

## **XI. Nutritional information on the novel food ingredient**

Annex 2, p 41-47

22. The applicant presents a number of immunologic responses observed with yeast *beta*-glucans in vitro in Table XII.a-1. The applicant reports that although the consumption of dietary fibre has been reported to reduce the bioavailability of various minerals, the estimated daily intake of yeast *beta*-glucans from the proposed use-levels of up to 200 mg per serving (i.e. 0.8 g/day) are well below the current recommendations for daily fibre intake (25-38 g per day) and therefore unlikely to have an adverse effect on mineral bioavailability within the human diet.

23. A study designed to investigate the effect of various yeast cell wall preparations on plasma cholesterol, faecal sterol excretion and short chain fatty acid production indicated no adverse effects on gastrointestinal function and the changes observed were thought to be of a beneficial nature. Other studies reported by the applicant indicated that brewer's yeast cell wall was an effective treatment for experimentally induced constipation in rats. No significant effects on the microbial populations of *Bifidobacterium* or Bacterioideceae were observed. It was concluded that brewer's yeast cell wall was fermented by intestinal bacteria and that the effect resulted in the alleviation of constipation and the improvement of the bowel environment.

24. The Irish CA points out that the novel ingredient is primarily a source of carbohydrate in the form of *beta*-glucans with relatively low proportions of protein and fat making up the remaining nutritional content. The Irish CA notes that the absorption of indigestible *beta*-glucans from the gastro-intestinal tract is extremely limited with the vast majority being excreted in the faeces. The Irish CA also notes that the evidence presented in the dossier supports the hypothesis that mineral bioavailability is improved by the consumption of fibres such as pectins and gums as well as indigestible carbohydrate polymers including *beta*-glucans.

## **XII. Microbiological information on the food ingredient**

Annex 2, p48-49

25. The Irish CA is content that the microbiological product analysis for several batches of insoluble and soluble yeast *beta*-glucans presented by the applicant in Tables XII.a-1 and XII.a-2 demonstrated that the product is free of microbial contamination and confirms to the product specifications.

## **XIII. Toxicological information on the novel food**

Annex 2, p 50-73

26. The applicant is of the view that consumption of insoluble and soluble yeast *beta*-glucans will not result in exposure to any substance that is not already present in the human diet, as brewer's yeast has been consumed for years in foods and significant levels of *beta*-1,3-glucans are present naturally in a number of foods. The applicant states that yeast *beta*-glucans are not absorbed to any physiological degree following consumption and systemic exposure is limited to trivial quantities that are taken up in the small intestine.

27. **Absorption, Distribution, Metabolism and Excretion (ADME):** The Irish CA notes that absorption of indigestible high molecular mass carbohydrates such as *beta*-glucans from the gastrointestinal tract is limited with the vast majority being excreted in the faeces. The applicant discusses a number of possible uptake pathways in the context of the animal studies cited, which demonstrate that the very small amounts of both soluble and insoluble *beta*-glucans are broken down and removed within hours or days in the system. The fate of the non-absorbed

beta-glucans is not explored in great detail. The Irish CA notes that some evidence exists demonstrating the reduction of beta-glucans through fermentation by gut microflora to short chain fatty acids, which are then removed by standard metabolic pathways.

28. **Toxicity:** The Irish CA notes that although the parenteral studies are of little significance to the safety assessment of yeast beta-glucans consumed as a food, they demonstrate a lack of toxicity when beta-glucans are administered at relatively high doses. No adverse results were observed from a single dose oral gavage study in rats using insoluble beta-glucans at 2000 mg/kg body weight. A 91 day repeat dose study of insoluble beta-glucans recorded a no observed adverse effect level (NOAEL) of approximately 75 mg beta-glucans/kg body weight/day, which was the highest level tested. Although a 52 week study of yeast beta-glucans at up to 200 mg/kg body weight/day in a mixed formulation produced some effects including diarrhoea, these effects were reversed after cessation of treatment and authors noted this was similar to effects seen as a result of exposure to certain sugar alcohol and some modified food starches.
29. The Irish CA noted that although the dossier did not provide any data on genotoxicity, carcinogenicity or reproductive effects, it was content that this was not a particular area of concern due to the history of safe consumption associated with yeast *beta*-glucans.
30. **Clinical studies:** The Irish CA notes the highest level of insoluble yeast beta-glucans used in the clinical studies (15 g/person/day) was consumed by obese hypercholesterolaemic males for an 8 week period. Adverse effects experienced included diarrhoea, abdominal discomfort and flatulence, which are not unexpected with the consumption of indigestible fibre. The Irish CA notes another human study demonstrated good tolerance with consumption of 500 mg of insoluble yeast beta-glucans (approximately 375 mg of beta-glucans) over a 10 day period. Only one human study involving parenteral administration of beta-glucans reported any significant toxicity. The study using intravenous administration of beta-glucans of up to 2 mg/kg body weight before surgery, recorded several minor adverse experiences which were possibly related to PGG-glucan<sup>3</sup> administration. The Irish CA however is of the view that these results have little significance for the safety assessment of yeast beta-glucans destined for the food chain.
31. **Allergenicity:** Reports of allergic responses resulting from the consumption of yeast by humans are rare, although allergic responses have been documented in individuals that have experienced *Candida* infections, or in people with atopic dermatitis. These responses are more generally associated with hypersensitivity to

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<sup>3</sup> poly-[1-6]-D-glucopyranosyl-[1-3]-D-glucopyranose glucan.

the enolase and/or mannan content of yeast rather than beta-glucans. The Irish CA points out that of some interest are the results of studies that demonstrate the ability of yeast *beta*-glucans to act as a strong adjuvant in antibody production and to potentiate delayed hypersensitivity in mice. The applicant claims that the low protein content in their novel ingredient would restrict the likelihood of its adjuvant potential, while the systematic dose of *beta*-glucans required to enhance delayed hypersensitivity suggests that there is limited risk from *beta*-glucans consumed as food.

32. The Irish CA notes that *S. cerevisiae* has a long and safe history of consumption therefore the relatively limited toxicological data presented in the dossier does not preclude an effective safety assessment. The immunostimulatory responses to *beta*-glucans are complex and could be considered a cause for concern by some people or a beneficial side effect by others. The Irish CA points out that considering the low absorption rate of *beta*-glucans, it is difficult to determine whether the addition of the novel ingredient to the intended food categories will have much of any immunostimulatory effect. The Irish CA proposes that some form of information label may be considered as part of risk management to address this issue.

33. The Irish CA concludes it does not have concerns about this safety of the novel ingredient provided the product specifications and intended use levels are maintained, the range of foodstuffs is limited to those presented in the dossier and any measures agreed as part of risk management are adhered to.

### **Committee Action Required**

34. The Committee is asked whether it agrees with conclusions of the Irish CA that yeast *beta*-glucans produced by Biothera Incorporated should be granted authorisation as a novel food ingredient for use in a number of food categories or whether it has any additional comments or objections.

**Secretariat**  
**February 2010**

Annexes attached:

**Annex 1 Irish Competent Authority's initial assessment report (Protect – Regulatory)**  
**Annex 2 Application Dossier (Protect - Regulatory)**

**ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**

**Irish Competent Authority's Initial Assessment Report (Protect – Regulatory)**

**Secretariat  
February 2010**



**ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**

**Application Dossier (Protect – Regulatory)**

**Secretariat  
February 2010**



