Initial Assessment

Insoluble and Soluble Yeast *beta*-Glucans

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Novel Food Classification: 2.1.

Introduction

A novel food application was submitted to the Food Safety Authority of Ireland (FSAI) in July, 2009, for the authorisation of insoluble and soluble yeast *beta*-glucans (collectively referred to as yeast *beta*-glucans from here). Following clarification of a number of technical issues by the applicant, the final dossier, submitted under *Article 4* of the novel food Regulation (EC) No. 258/97, was formally accepted by the FSAI on September 9th, 2009, by letter to Dr. Donald Cox of Biothera Inc. and copied to Mr. Andreas Klepsch of the European Commission.

The application for authorisation of yeast *beta*-glucans as a novel food ingredient was prepared pursuant to Commission Recommendation 97/618/EC concerning the scientific aspects and the presentation of information in support of an application to market novel foods and novel food ingredients in the EU. The applicant considers yeast *beta*-glucans to fall within the novel food category of "foods and food ingredients consisting of, or isolated from micro-organisms, fungi or algae", as set out in *Article 1.2.(d)* of the novel food Regulation. In order to assess wholesomeness, yeast *beta*-glucans are placed in Class 2.1. by the applicant; "Complex novel food from non-GM source which has a history of food use in the community", (Commission Recommendation 97/618/EC).

Biothera's yeast *beta*-glucans are derived from the primary carbohydrate constituent (polysaccharide) of the cell wall of *Saccharomyces cerevisiae*. *S. cerevisiae* has a long history of safe use in the production of bread, beer and wine which has resulted in it being more commonly referred to as baker's and brewer's yeast. Biothera is seeking authorisation to use yeast *beta*-glucans as a functional ingredient with potential immunomodulatory benefits in food supplements, certain PARNUTS categories and a range of general food products including beverages, cereal and milk products.

Using the schemes set out in Commission Recommendation 97/618/EC, the information addressing the safety of yeast *beta*-glucans is set out as follows. Additional questions and clarifications sought by FSAI experts, and answered by the applicant are provided in Annex A.

I. Specification of the novel food

Beta-glucans are complex, high molecular mass (100 – 200 kDa) sugars (polysaccharides), found in the cell wall of many yeasts and cereals. This novel food application is for the marketing of both insoluble [BetaRight WGP (BWGP) and

Wellmune WGP Dispersible (WGPD)] and soluble [Wellmune WGP soluble (WGPS)] yeast *beta*-glucans, isolated from *S. cerevisiae*. Yeast *beta*-glucans differ from their cereal counterparts in that they comprise a mixture of beta-1,3- and 1,6-glucans, compared to the cereal derivatives which are a mixture of beta-1,3- and 1,4-glucans. The insoluble products contain at least 70% (BWGP) or 75% (WGPD) carbohydrate in the form of *beta*-glucans while the soluble product (WGPS) contains at least 75% *beta*-glucans. Protein (insoluble: >10% and soluble: >3.5%) and fat (>20%: insoluble and >10%: soluble) make up the remaining nutritive elements of the *beta*-glucans novel ingredient. While specifications were set for lead content, no such specifications were set for cadmium and arsenic content, though the certificate of analysis show their levels to be low or non-detectable. The presence of a number of volatile organic compounds, purportedly the result of the limited decomposition of lipid-derived fatty acids is noteworthy. The average methanol content was 243 µg/kg, ethanol 744 µg/kg, isopropanol 44 µg/kg, acetone 225 µg/kg and hexane 40 µg/kg. Of most toxicological relevance is the presence of methanol and hexane, though the anticipated low intake levels would severely restrict consumer exposure, and therefore are considered not to be of safety concern.

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

The manufacturing process involves *Saccharomyces cerevisiae* as a starting material in the production of insoluble *beta*-glucans, which is then processed and purified further to yield soluble *beta*-glucans. The process is diagrammatically represented in Figure II.b-1 of the application dossier, and includes the use of standard food grade chemicals in the isolation and purification process. 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 the source of the novel food

Saccharomyces cerevisiae has a long history of safe consumption when used in the production of bread, beer and wine, to the extent that it is better known as bakers or brewers yeast.

IV. – VIII. Not applicable. No GMO involvement

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

The applicant intends to market the novel ingredient in a number of general food categories as well as in dietary supplements and foods for particular nutritional uses (PARNUTS-with the exception of foods for infants and small children). The intended use level as a food supplement is between 250 and 500 mg per day, delivering approximately 175 to 375 mg yeast *beta*-glucans. As a food ingredient or in PARNUTS the proposed intake per serving is 200 mg of *beta*-glucans, corresponding to approximately 250 mg of the products BWGP, WGPD and WGPS. In order to estimate potential consumer intake levels of *beta*-glucans, the applicant utilised consumption data from the UK National Diet and Nutrition Survey (NDNS).

However, this estimation related to general foodstuffs only and did not include intake from food supplements or PARNUTS.

Of the individual population groups considered, male teenagers were found to have the highest mean and 97.5th percentile all-user intake, at 0.80 and 1.94 g/person/day, respectively. Children had the highest mean and 97.5th percentile all-user intakes in relation to body weight of 49 and 105 mg/kg body weight/day, respectively. 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 by any of the user groups, particularly children.

The applicant also demonstrates that consumption of naturally occurring *beta*-glucans from other dietary sources should not have a significant impact on overall intake levels. Although the foods with added yeast *beta*-glucans should be purchased only by those wishing to benefit from the functionality of the novel ingredient, the assumption that yeast *beta*-glucans intake would be facilitated primarily through the consumption of either general foodstuffs, PARNUTS or dietary supplements, but not a combination of these, is not well supported. There are no firm grounds to believe that consumption of *beta*-glucans through dietary supplements would, under normal circumstances, preclude their consumption through general foodstuffs, particularly considering the broad range of general foods to which the applicant intends adding yeast *beta*-glucans. Additionally, *beta*-glucans could be consumed through sports foods (a category of PARNUTS) by active male teenagers who comprise the highest potential intake group and likely consumers of these products.

However, even if these considerations resulted in the worst case scenario intakes being somewhat higher than projected by the applicant, they would not be expected to present significant safety concerns.

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

The cell wall of *S. cerevisiae*, which comprises approximately 30% of the total dry weight of the cell, is predominantly made up of polysaccharides (85%) and protein (15%). Between 80% and 90% of the total cell wall polysaccharides are comprised of beta-glucans, which means that 15-25% of the cell is typically made up of *beta*-glucans. Therefore, it is safe to conclude that routine consumption of foods produced with *S. cerevisiae* such as breads, beer and wine has led to a significant history of consumption of yeast *beta*-glucans. Added to this is the dietary *beta*-glucans found in other naturally occurring foods including certain mushrooms, soybean, celery and many others.

XI. Nutritional information on the novel food

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 absorption of the characteristically high molecular mass indigestible *beta*-glucans from the gastrointestinal tract is extremely limited, with the vast majority being excreted in the faeces.

Though contrary to some reports, the evidence presented in the dossier supports the hypothesis that mineral bioavailability is improved by the consumption of indigestible fibres such as pectins and gums, as well as indigestible carbohydrate polymers including *beta*-glucans. The effects of yeast *beta*-glucans on the gastrointestinal system has not been studied to any great extent. However, the few studies cited in the application report possible beneficial effects on parameters such as plasma cholesterol, with no apparent adverse effects.

XII. Microbiological information on the novel food

Product specifications demonstrate a satisfactory microbiological profile for this novel ingredient.

XIII. Toxicological information on the novel food

The toxicological assessment of yeast *beta*-glucans is underpinned by the considerable history of safe consumption of yeast and yeast-derived ingredients in products such as bread, beer and wine, as well as the consumption of *beta*-glucans from other dietary sources.

Absorption, Distribution, Metabolism and Excretion (ADME)
Absorption of indigestible high molecular mass carbohydrates such as *beta*-glucans from the gastrointestinal tract is very limited, with the vast majority being excreted in the faeces. A number of possible uptake pathways are discussed, in the context of the animal studies cited, which demonstrate that the very small amounts of both soluble and insoluble *beta*-glucans taken up are sequestered and/or broken down and removed from the system within hours or days. The precise fate of the non-absorbed *beta*-glucans is not explored in any great detail. Some evidence exists demonstrating the reduction of *beta*-glucans to short chain fatty acids through fermentation by gut microflora, which are then absorbed and removed by standard metabolic pathways.

Toxicity
The data in the application dossier relating to safety testing in animals is limited to one oral and several intraperitoneal or intravenous acute toxicity studies, together with a 91 day repeat dose toxicity study in rats. 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 findings were identified from a single dose oral gavage study in rats using insoluble *beta*-glucans at 2,000mg/kg body weight. A 91 day repeat dose study of insoluble *beta*-glucans recorded a no observed adverse effect level (NOAEL) of approximately 75mg *beta*-glucans/kg body weight/day, which was the highest level tested. A 52 week study of yeast *beta*-glucans at up to 200mg/kg body weight/day in a mixed formulation including excipients did produce some effects including diarrhoea. However, the authors noted that these effects were reversed after cessation of the treatment and were characteristic of exposure to certain sugar alcohols, lactose, synthetic polydextrose and some chemically modified food starches.

Yeast *beta*-glucans would not be expected to have genotoxic, carcinogenic or reproductive effects, and thus the dossier does not provide any data on these safety aspects. However, this is not of particular concern due to the history of safe consumption associated with this novel ingredient.

Clinical studies

The outcomes of a number of clinical studies are discussed in the dossier. The highest level used in these studies (15g/person/day of insoluble yeast *beta*-glucans) was consumed by obese hypercholesterolaemic males for an 8 week period. The only adverse effects experienced included diarrhoea, abdominal discomfort and flatulence, which are not unexpected with the consumption of indigestible fibre. Another human study demonstrated a good tolerance, with no apparent adverse effects, to the consumption of 500mg of insoluble yeast *beta*-glucans (approx. 375mg of *beta*-glucans) over a 10 day period. Only one of a number of human studies involving parenteral administration of *beta*-glucans reported any significant toxicity. The study, using intravenous administration of up to 2 mg/kg body weight before surgery, recorded several minor adverse experiences which were possibly related to PGG-glucan administration. However, the results of these studies have little significance for the safety assessment of yeast *beta*-glucans destined for the food chain.

Allergenicity

Despite its extensive use in food production over many years, reports of allergenic responses resulting from the consumption of yeast by humans are rare. However, allergic responses by individuals that have experienced *Candida* infections, or people with atopic dermatitis have been documented, though they are generally associated with hypersensitivity to the enolase and/or mannan content of yeast rather than the *beta*-glucans.

Of some interest are the results of a number 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 relatively low protein content in their novel ingredient would restrict the likelihood of its adjuvant potential, while the considerable systemic dose of *beta*-glucans required to enhance delayed hypersensitivity would limit that risk from *beta*-glucans consumed as food.

Conclusions

S. cerevisiae has a long and safe history of use in the production of bread, wine and beer. A considerable history of safe use of *beta*-glucans also exists through the consumption of yeast, cereals and mushrooms among other foods, as well as food supplements. For these reasons, the relatively limited toxicological data presented in the application dossier does not form a barrier to conducting an effective safety assessment.

The estimated intakes calculated by the applicant attempt to present a worst case scenario, and are relatively close in value to the safe levels determined through animal and human studies. However, this proximity in values 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 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*.

The immunostimulatory response to *beta*-glucans is complex and could be considered a cause for concern by some people, or a beneficial side effect by others. Considering the low absorption rate of *beta*-glucans, it is difficult to determine whether the addition of this novel ingredient to the intended food categories will have much, if any immunostimulatory effect. Nevertheless, some form of information label may be considered as part of risk management to address this issue.

Recommendation

The FSAI is satisfied that the use of yeast *beta*-glucans in the food categories listed in the application dossier meets the criteria for novel food set out in *Article 3.1.* of the novel food Regulation EC (No) 258/97. The FSAI does not have concerns about the safety of this ingredient provided the product specifications and intended use levels are maintained, the range of foodstuffs is limited to those presented in the application dossier, and that any measures subsequently agreed as part of risk management are adhered to.

ANNEX A

Additional questions asked by FSAI experts and answered by the applicant

Question 1.

The exclusion of food supplements and, particularly, the PARNUTS categories from intake levels may need to be addressed. The concern would be of underestimation of intake of *beta*-glucans amongst young/teenage males who have already been identified as the highest potential intake group. This group would also be that most likely to be targeted and highest consumers of sports enhancing foods under the PARNUTS category and which have been identified for inclusion with *beta*-glucans.

Answer:

The response to the below is that indeed with 97.5th percentile for male teenagers approaching 2 g per day and target doses for beverages of maximum 200 mg per serving then that would be 10 servings of any food products all fortified with yeast *beta*-glucans. The mean is about 4 servings which seems pretty reasonable. If you look at the food codes in the intakes report, Appendix C page C-5 you will get the following codes under Sport beverages, which I think pretty much includes most of what you could consume whether normal foods or PARNUTS, so they were all covered in the intakes calculations.

So I would say we have probably drastically over-estimated consumption for teenage males rather than under. Biothera assure me that male teenagers are not the real target market for immune function type products.

Question 2.

What is the remaining carbohydrate (approximately 14%) in the soluble yeast *beta*-glucan? The *beta*-glucan content remains essentially similar to that in the insoluble material (approx 80%, there is a slight reduction) but the overall carbohydrate content increases to approx 92%, with reduction in protein content from ~ 2.8% to ~ 0.6%, and reduction in fat from 5-8% to 1.5 – 2%.

Answer:

We have now provided a footnote to the ingredient specifications and product analyses stating that the total carbohydrates represent the sum of *beta*-glucans (1,3/1,6), glycogen (*alpha*-1,4-linkages), and small amounts of *beta*-1,6-glucan and chitin. Also in Section I.c, we provided a description that "small amounts of beta-1,6-glucan and chitin are expected to be present in the final product." Please see the attached document with the added footnote. In addition, there are 2 reasons why the percentage of total carbohydrate is higher in the soluble ingredient in comparison to the insoluble. As mentioned in Section II.b.2, the soluble ingredient is manufactured by additional processing of the insoluble ingredient. During this processing, the total carbohydrate increases due to the removal of the fat and protein during the cook. In addition, some of the beta-glucans are hydrolysed to smaller chain carbohydrates, including the base glucose molecule, during the solubilisation of the insoluble ingredient. In general, it takes about 2 kg of WGPD to make 1 kg of WGPS.

Question 3.

Please provide a reference to VOC production in yeast food ingredients and beta-glucan supplements (p. 12)

Answer:
We did provide the analytical results of the VOC analyses in Appendix A of the dossier (also attached above) , so everything should be available to FSAI.

These analyses were conducted to support Biothera's hypothesis that the presence of VOCs were due to the decomposition of partially degraded fat triglycerides with increased reactivity present in Biothera's final ingredient. As a result, analyses from an external laboratory were conducted using 4 samples of Biothera's soluble ingredient, 3 samples of a competitor's ingredient, a sample of yeast extract from Japan, and 3 samples of fresh or dried yeast. The results of these analyses demonstrated that VOCs in varying amounts were present in all samples. Biothera, therefore, concluded with the support of the analytical results, that VOCs are present in various yeast products and are produced as a result of the alkali washing of the yeast *beta*-glucans. The production of volatile compounds also has been observed in the fermentation of various foods. Alcohols, acetates, and ethyl esters of C_4 to C_8 fatty acids are the major volatile compounds formed during wine production (Stashenko *et al*., 1992). The production of ethanol, propanol, and other volatile compounds were identified under varying fermentation conditions of sourdoughs started with *Lactobacillus brevis* lindneri CB1, *Lactobacillus plantarum* DC400, and *Saccharomyces cerevisiae* 141 (Gobbetti *et al*., 1995).

Question 4.

There is no analysis of "potential external toxic contaminants" in the soluble beta-glucan, assumed to be similar to insoluble?

Answer:
"Potentially external toxic contaminants" refers to the analyses conducted for heavy metals and pesticides. We did include a comment at the beginning of this section to state that results obtained from insoluble beta-glucans are relevant to soluble beta-glucans so this issue is addressed.

Question 5.

Please provide more details of the growth/production of the yeast from which the beta-glucan is derived.

Answer:
We did provide manufacturing details for the yeast under Section II.a.1 within the confidential dossier (21 Sept 09 final version)

Question 6.

A meaningless molecular weight of 240 is given on p. 24. 100kDa is given as average for soluble WGPS (p.15), so I assume this 240 is kDA, please clarify.

Answer:
Yes, kDa is correct. The references did not provide units either, but did explain the molecular weight as 240,000.