

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

ICE STRUCTURING PROTEIN PREPARATION

**Issue**

The Committee is invited to consider additional information provided by Unilever on their ice structuring protein preparation, and whether this allows the Committee to conclude its evaluation of this novel food ingredient.

**Background**

1. Members first considered Unilever's application for the authorisation of an ice structuring protein (ISP) preparation derived from a genetically modified baker's yeast as a novel food ingredient at their July 2006 meeting (ACNFP/78/2). Members requested additional information from Unilever on this application. This was considered by Members in September 2006 (ACNFP/79/5).
2. Following the discussion in September, a letter detailing the comments of the ACNFP was sent to the applicant on 16 October 2006 (see **Annex 1**) seeking clarification on the following points:
  - (i) The purpose of the inactive glycosylated form of the protein in the ISP preparation and whether there is a possibility of marketing a more purified ISP preparation of the active protein.
  - (ii) whether any information exists on the potential for the GM *Saccharomyces cerevisiae* proteins present in the preparation to induce allergic reactions in individuals sensitised to 'yeast' *Candida* or other fungi.
  - (iii) whether there is any data to demonstrate the absence of secondary integration sites in the genome,
  - (iv) whether the sequence analysis of the flanking regions of the insertion site(s) revealed the creation of any potential open reading frames in these regions.
3. Unilever replied to this letter on 30 October 2006 (see **Annex 2**). The Secretariat wishes to highlight the following points:
  - (i) Inactive glycosylated form of protein and possibility of marketing a more purified ISP preparation (Annex 2, p. 1)
    - The applicant has confirmed that the inactive glycosylated form of ISP protein has no function in the preparation. The application draws a parallel with the manufacture of food enzyme preparations, which generally

subjected to minimal processing in order to maintain high functional activity, resulting in varying degrees of purity. The applicant also points out that an extensive test regime has been carried out on the complete ISP preparation to ensure that it is safe for human consumption.

(ii) Potential for the GM *S. cerevisiae* proteins present in the preparation to induce allergic reactions in individuals sensitised to 'yeast' *Candida* or other fungi. (Annex 2, page 2-3)

- The applicant states that sensitisation to yeast proteins most via the respiratory tract and via the skin, and there is no evidence to indicate that it arises from the consumption of foods and drinks containing *S. cerevisiae*. This conclusion is supported by the fact that the three allergens in *S. cerevisiae* namely enolase, manganese super-oxide dismutase and cyclophin have only been associated with inhalant and/or skin allergies.
- It is recognised that people with atopic dermatitis which is associated with allergic reaction to yeasts such as *Candida albicans*, *Pytirisporum ovale* and *Malassezia furfur* are likely to cross-react to *S. cerevisiae* proteins when challenged in skin prick tests or RASTs. The applicant however refers to conclusions from Kortekangas-Savolainen et al (1994) that "the IGE-mediated allergy to baker's yeast should not lead to the denial of bakery, brewery and wine products".

(iii) Whether there is any data to demonstrate the absence of secondary integration sites in the genome (Annex 2, pp 6-7 and fig. 1 to 3 on pp 8-10)

- The applicant has not found any secondary integration sites in the genome of the *S. cerevisiae* used for producing the ISP preparation. Restriction maps of the DNA structure generated on integration of the cassette and the fragments detected are shown in figure 1 (Annex 2, p.8). Results obtained with five different restriction digests (Annex 2, see figs 2 and 3, p.9-10) have not shown the presence of a secondary integration site and the applicant is of the view that it is unlikely that this site would be masked. The applicant concludes that the rDNA is the sole location for integration of the expression cassette.

(iv) Whether the sequence analysis of the flanking regions of the insertion site(s) has been carried out and if so, whether this revealed the creation of any potential open reading frames in these regions (Annex 2, page 7).

- The applicant states that mechanism of integration regenerates the existing NTS1 sequence in rDNA, as confirmed by sequencing of boundary fragments. Integration therefore does not lead to generation of any open reading frames.

### **Additional information**

4. The applicant has recently informed the Secretariat of a new publication on the lack of immunogenicity of the ISP preparation administered by oral route to human volunteers (see **Annex 3**).

### **Committee Action Required**

5. The Committee is asked whether it is satisfied with the response provided by the applicant to the comments raised at the September meeting.
6. If so, the Secretariat will prepare a draft opinion reflecting the Committee's views on this application. This will be circulated to Members for comments before the March meeting.
7. If not, the Committee is asked to indicate what additional information is required.

**Secretariat  
January 2007**

### **Annexes attached:**

Annex 1 – Letter detailing ACNFP comments

Annex 2 – Applicant's response and extract from the application dossier (p.48-49)

Annex 3 – Publication on the lack of immunogenicity of ISP preparation administered by oral route to human volunteers



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Letter detailing ACNFP comments

**Secretariat  
January 2007**



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Applicant's response and extract from the application dossier (p.48-49)

**Secretariat  
January 2007**



**ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**

Pre-publication copy:

“Lack of immunogenicity of ice structuring protein type III HPLC12 preparation administered by the oral route to human volunteers”

Crevel, RWR et al

Food and Chemical Toxicology 45 (2007) 79-87

This paper will be available online at [www.sciencedirect.com](http://www.sciencedirect.com)

**Secretariat  
January 2007**