

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

FOOD USE OF GM MAIZE LINE 1507: FURTHER DATA

Issue

This paper seeks the Committee's views on additional information provided by the applicant in relation to concerns previously raised by this Committee in connection with an application under the Novel Food Regulation (EC) 258/97. This information forms part of the application recently considered by EFSA under the current Regulation (EC) 1829/2003. Members are asked if this information provides satisfactory answers to the concerns raised when the food use of this maize line was considered in February 2004.

Background

1. This insect-tolerant GM maize line was initially reviewed by Member States (MS) under Regulation 258/97, following an application from Pioneer Hi-Bred International/Mycogen Seeds that sought approval for the placing on the market of maize line 1507 for food use. In considering that application (ACNFP/64/05) the Committee raised two issues that were outlined in a letter to the Commission on 5 February 2004 (Annex 2), concerning the specificity of expression of novel genetic material and assessment of the allergenicity of novel proteins that might be present. As the dossier was not sufficiently advanced when Regulation 1829/2003 on GM food and feed came into force in April 2004, the dossier was re-presented for consideration under the new Regulation. Part of the transferred application consists of the Applicant's response to reasoned objections from various MS under Regulation 258/97, including the UK (Annex 3).
2. The GMO Panel of the European Food Safety Authority (EFSA) has evaluated the application in accordance with Article 6 of Regulation 1829/2003 and recently published its opinion. The Panel's opinion is attached for information as Annex 1. Under the new procedures for GM food and feed, the European Commission is required to draw up an authorisation decision, to be voted on by Member States, within 3 months, i.e. by 4 June 2005.
3. As notified at the November 2004 meeting (ACNFP/69/7) the Food Standards Agency does not intend to seek advice from the Committee on every opinion issued by EFSA. However, the Committee previously raised questions about this GM maize line and before the Agency decides its position on the authorisation of 1507 maize it needs to know whether the Committee considers that these questions have been adequately addressed by the additional data provided by the applicant.

Specificity of Expression of Novel Genetic Material – Applicant's Response

4. The applicant has provided the following explanations and data to clarify the expression patterns of the CRY1F and PAT proteins in maize tissues.

- a) *CRY1F doublet*: The applicant has put forward the argument that the doublet on the western blots from leaf, pollen, whole plant and grain tissue, can be explained by post-translational modification of the protein (p2-3, Annex 3). The difference in size of a full length CRY1F protein (Ca 68KDa) encoded by residues 1 – 605 and a trypsinolysed CRY1F protein (ca 65KDa) encoded by residues 28 – 605 (see p3 and p10 [Figure 4.MS] Annex 3) would account for the different mobilities of the proteins in SDS-PAGE. The Applicant has provided a detailed report on protein characterisation, which is attached as Annex 4.
- b) *PAT protein expression*: As requested the Applicant has carried out an additional Western blot analysis of pollen and leaf tissue using pre-absorbed polyclonal antisera to remove cross-reactivity with native proteins (p5 and p11 [Figure 5.MS] Annex 3). The applicant claims that the results confirm that expression of the PAT protein is not detectable at the level of sensitivity attained and that cross-reactivity with native proteins is negligible.

5. In addition to the above the Applicant has provided the following data:

- additional information regarding the sequence of the 1507 maize insertion event and flanking genomic DNA (Annex 5);
- Northern blot (Annex 6) and RT-PCR (p4-5 and figures 2 and 3 [p8-9], Annex 3);
- analyses to evaluate the potential transcription of the ORF3, ORF4, *cry1F* and *pat* sequences have also been carried out. The open reading frames ORF3 and ORF4 were identified as a result of the sequence analysis of the maize 1507 insert mentioned above. These results are summarised on p3-5 of Annex 3. The applicant notes that these additional analyses confirm the absence of unintended mRNA transcripts carrying the *cry1F* and *pat* sequences and presents them as further evidence for the absence of transcription of any potential fusion proteins from the 5' and 3' flanking regions of the 1507 maize insert.

6. The EFSA GMO Panel has concluded that the intended expression of the PAT and CRY1F proteins was demonstrated and there was no indication that the development of allergenic or toxic products would arise in the very unlikely event that the read-through mRNA is translated to the respective protein.

Potential Allergenicity of the Novel Food – Applicant's Response

7. As requested the Applicant has identified the 3 allergens that showed homology with CRY1F. These were the P7 allergen from *Dermatophagoides pteronyssinus* (dust-mite), a beta-1, 3-glucanase-like protein from the olive tree and a serum albumin precursor from *Canis familiaris* (p5, Annex 2). The Applicant argues that such matches of 6 contiguous amino acids can occur randomly and that current

evidence indicates that IgE binding epitopes of 8 - 12 amino acids are necessary to initiate an allergic response (p5-6, Annex 2).

8. Having considered all the available information the EFSA GMO Panel considered that any potential newly expressed proteins were not likely to be allergenic.

Committee Action Required

9. The Committee is asked whether the additional information provided is sufficient to address their earlier questions regarding the introduction of maize line 1507 for food use.

**Secretariat
March 2005**

Annexes attached:

Annex 1 – EFSA opinion on 1507 maize for food use

Annex 2 - Letter to the Commission with the ACNFP's comments on the novel food application

Annex 3 – Applicant's response to specific points raised by the ACNFP

Annex 4 – Study on the characterisation of proteins as expressed in *B.t.* CRY1F maize tissues

Annex 5 – Complete DNA sequence of the 1507 maize insert

Annex 6 – Northern analysis of transcription from potential ORFs in the insert and border regions of 1507 maize

Note: Annexes 3-6 are not for publication. However, the public can request access to this information by contacting EFSA.

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

EFSA opinion on 1507 maize for food use.

This document has been published on the EFSA website at:

http://www.efsa.eu.int/science/gmo/gmo_opinions/826_en.html

**Secretariat
March 2005**

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

Letter to the Commission with the ACNFP's comments on the novel food application.

**Secretariat
March 2005**

Mr Andreas Klepsch
DG SANCO Unit D/4
European Commission
Rue de la Loi 200
BRUSSELS
Belgium

B-1049

5th February 2004

NFU 308

Dear Mr Klepsch,

Application under Regulation (EC) 258/97 – 1507 maize line: UK response to Dutch CA's initial opinion

As the UK Competent Authority, the Food Standards Agency has sought comments from the Advisory Committee on Novel Foods and Processes (ACNFP) on the initial assessment report on this product, prepared by the Dutch CA under the novel foods regulation (EC) No 258/97.

The Committee was unable to agree with the positive initial opinion of the Dutch Competent Authority for the marketing of maize line 1507, and highlighted a number of concerns, as set out in the attached paper.

We cannot support the marketing of this product until these considerations have been satisfactorily addressed.

Yours sincerely,



Sonia Molnar
Novel Foods Division

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

1507 Maize line

Specificity of Expression of Novel Genetic Material

In section 5 of application dossier (p72) the applicants describe the safety assessment of this line based on a number of parameters, one of which is western blot analysis of the CRY1F and PAT proteins in transgenic plants. The applicants state that other than the expected bands there are no bands in the western blots to indicate either partial or fusion proteins in these lines. This is at odds with the western blot results presented in the application, and some of the conclusions cannot be made based on the cross-reactivity of the antisera:

- 1) There is not just one band for the CRY1F, there is a doublet. The applicants rely on a paper by Evans in 1998 to explain the origins of this doublet. There should be a more detailed analysis of this doublet in these exact plants so that a full safety assessment can be made.
- 2) The cross-reactivity in healthy pollen grains with PAT antisera is poorly explained and the existence of the cross-reactivity means that the firm conclusions made in Section 5 of the dossier cannot be upheld. A case could be made that the PAT protein is expressed in pollen of transgenic plants but it is modified to cause a shift in the molecular weight. This molecular weight could coincide with the same sized band that the PAT antisera cross reacts with in wild type and transgenic lines. Such a scenario may be regarded as unlikely, but the applicants have presented no evidence to either prove or disprove such an event (and such events can occur). Expression of these proteins is not impossible in pollen when using a CaMV35S promoter, which has variable reports in pollen expression studies. It is surprising that the applicants have relied on a source of antisera that gives such cross reaction with a host protein to base their conclusions on.

The applicants should repeat these experiments using pre-absorbed antisera to remove cross-reactivity, and/or using a better source of polyclonal antisera, and/or use a monoclonal antisera or specific phage display antisera. This could also be backed up by northern or RT-PCR experiments on RNA from wild type and transgenic pollen.

Toxicological information on the Novel Food

The initial opinion refers to a subsequent investigation of the CRY1F protein with a database of 2033 sequences of allergenic proteins (p76). Corresponding sequences of six contiguous amino acids were found in three proteins from the database used. The applicant should provide details of the three allergens which showed homology with CRY1F.