

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**GENETICALLY MODIFIED SPRING OILSEED RAPE EVENTS Ms8, Rf3 AND Ms8xRf3: PART C CONSENT UNDER DIRECTIVE 2001/18 (REF: C/BE/96/01)****Issue**

The Committee is asked to consider a favourable opinion (with limited scope) from the Belgian Competent Authority on a notification for **import, cultivation and processing** of oilseed rape genetically modified for the presence of transformation events Ms8 and Rf3, and Ms8xRf3 hybrids, under the Deliberate Release Directive (EC) No 2001/18. The Committee is asked whether or not it is content with the data provided in this notification and whether it agrees with the Belgian Competent Authority's Initial Opinion in relation to human health implications. The Committee's advice will form the basis of the Food Standards Agency's response to Defra.

Introduction

1. On 4th March 2004 the ACRE secretariat forwarded a notification with the Belgian Competent Authority's favourable opinion (with limited scope) on this Part C consent application under Directive (EC) No. 2001/18. In order for Defra to meet the 60-day deadline for response to the Commission a Food Standards Agency opinion is required by **26th March**.
2. The Belgian Competent Authority proposes to issue consent only for the purpose of import and processing. If consent is granted a number of conditions are proposed which cover labelling, reference material, validation and a post-market monitoring plan. Approval for cultivation is excluded for reasons relating to gene flow, co-existence and loss of biodiversity due to weed management practices.
3. This paper considers only the molecular characterisation and human health aspects of the application. Information on environmental risk assessment, post-market monitoring and detection method are dealt with by ACRE and can be provided on request.
4. The Belgian Competent Authority's assessment is included as Annex A. The non-confidential molecular characterisation parts of the application dossier are attached at Annex B. The sections of the application dossier dealing with human health issues are included as Annex C. Other parts listed at the end of this paper (including confidential business information on molecular characterisation) are available on request.

Background

5. This marketing application is from Bayer Bioscience for the import, cultivation and processing of Ms8, Rf3 and Ms8xRf3 oilseed rape and any progeny derived from these lines by conventional breeding methods with non-genetically modified oilseed rape. The application was originally submitted to the Belgian Competent Authority in 1996 under Directive (EC) No 90/220/EEC. In March 1997 ACRE advised that Ms8xRf3 did not pose a risk to human health or the environment and it had no objection to this product being placed on the market. In May 1998 the Scientific Committee on Plants of the European Commission also reached the same conclusion. However, in October 1999 ACRE issued further advice on the issue of the impact of changes in agricultural practice on farmland biodiversity. No vote was ever taken on this notification at a Regulatory Committee meeting and it was subsequently resubmitted by Bayer in October 2002 under Directive (EC) No 2001/18.
6. The notification specifically covers the growing and multiplication of seed for sale, field cropping for seed production for feed, food and industrial uses of non-living processed products and import of seed for processing for food animal feed and industrial uses. No consent is requested for use in animal feed or human feed. However oil derived from Ms8xRf3 hybrids already has clearance for placing on the market under Regulation 258/97, granted in 1999 on the basis of an opinion from the German Competent Authority that the oil was substantially equivalent to conventional rape seed oil. The ACNFP did not provide an opinion on this application.
7. The notification covers three genetically modified lines of oilseed rape:
 - a) Female (male sterile) oilseed rape line Ms8 and all progeny derived through traditional breeding crosses with non-GM oilseed rape
 - b) The male (fertility restoration) oilseed rape line Rf3 and all progeny derived through traditional breeding crosses with non-GM oilseed rape
 - c) Hybrid oilseed rape Ms8xRf3 obtained through traditional breeding from the parental lines containing events Ms8 and Rf3. This variety has been released in the UK under part B consents that allow limited experimental use since 1995 and was used in the recent Farm Scale Evaluations.

Molecular Characterisation

Annex B, pages 55-142

8. Ms8 (Annex B, pages 56-98) is produced using *Agrobacterium tumefaciens* transformation with plasmid pTHW107 and contains two gene cassettes (the *barnase* and *bar* gene cassettes). The *barnase* gene encodes for a ribonuclease resulting in a lack of viable pollen and conferring male sterility. The *bar* gene encodes the enzyme phosphinotricin acetyl transferase (PAT) which confers tolerance to glufosinate ammonium.

9. Rf3 (Annex B, pages 99-142) is also produced using *Agrobacterium tumefaciens* transformation with plasmid pTHW118. It too contains two gene cassettes (the *barstar* and *bar* gene cassettes). The *barstar* gene encodes fertility restoration by inhibiting the *barnase* gene function. As with Ms8, the *bar* gene confers tolerance to glufosinate ammonium.
10. Both the *barstar* and *barnase* genes are derived from *Bacillus amyloliquefaciens* and are controlled by the PTA29 promoter from *Nicotiana tabacum*, which limits expression of the inserted gene to the anthers. The *bar* gene is derived from *Streptomyces hygroscopicus*. This gene is under control of the pSSUAra promoter from *Arabidopsis thaliana*. No antibiotic resistance genes were inserted.
11. In both events the plasmid DNA is inserted at a single locus and expression is restricted to the intended genes. In Ms8 the inserted sequence is identical to the corresponding transforming plasmid DNA sequences. In Rf3 the insert is present in an inverted repeat with a second incomplete T-DNA copy. The sequence is identical to that in the plasmid DNA.
12. The analysis of flanking sequences for Ms8 and Rf3 is covered in the application dossier (Annex B) on pages 62-98 and 105-142 respectively. PCR analysis indicates that the sequences flanking both events are of oilseed rape origin. Blast analysis was not able to identify any significant sequence similarities in the 3' Ms8 and 5' Rf3 flanking sequences. However, the 5' Ms8 and 3' Rf3 flanking sequences did show some similarity with *Arabidopsis thaliana* (although this is not of concern as *A. thaliana* and oilseed rape are closely related). Further analysis also confirmed that the flanking sequences of Ms8 and Rf3 do not contain donor plasmid DNA.
13. Additional information to that provided in Annex B is available on the molecular characterisation events of Ms8 and Rf3 on request. This includes the full sequence of the inserted genetic material and is commercially confidential.

Human Health

Annex C, pages 36-39

14. Only the oil fraction of the plant is destined for direct human consumption. The application dossier states that the nutritional quality of food products derived from the transgenic oilseed rape is not different from those derived from non-transgenic oilseed rape and all proteins would be removed from the final product under quality control requirements. This was confirmed by numerous quality analyses, including oil content and composition, and bench top studies to simulate industrial processing practices. All results were within the typical range for oilseed rape. (The present application does not cover food use and as noted above, oil from the Ms8xRf3 hybrids is already authorised for use in the EU).

15. The applicant states that there are no indications that the transgenic oilseed rape would induce or change the intensity of an allergic reaction towards oilseed rape pollen in occupationally or daily exposed personnel. No special studies were carried out to test the allergenicity of the transgenic plant carrying the *bar*, *barnase* or *barstar* genes. This is because allergenicity to the respective proteins has not been reported in literature and the genes are not expressed at high levels in pollen (determined by Northern blot analysis and ELISA). An analysis of three databases for polypeptides homologous to those encoded by the inserted genes was carried out and homology found to be very low and scattered over the polypeptides. The applicant also states that many of their employees have been in contact with the transgenic plants during their development and no induction or change of intensity in allergic reaction noted.
16. The applicant rules out the possibility of transfer of inserted traits through sexual compatibility to wild relative plants sometimes consumed, non-commercially, as vegetables (indirect food use). This is provided that there are no commercial release plans for the region where wild relatives are prominent, the safety of the inserted genes and products has been documented and feeding studies do not reveal a negative effect. The applicant also notes that the presence of GM oilseed rape pollen in honey (up to 2%) can not be excluded. However, no adverse effects on human health are anticipated as the *bar*, *barnase* and *barstar* genes are linked to plant tissue specific promoters and not known to lead to gene expression in pollen. Even if new proteins were present these have already been assessed as having no toxicity to human beings, would be degraded during honey processing and are highly specific enzymes that do not bind to substrates other than their specific ones.

Committee Action Requested

17. The Committee is asked:
- If it is content with the data on molecular characterisation and human health implications provided in this application and
 - whether it agrees with the Initial Opinion from the Belgian CA that the product should be marketed under Directive EC No. 2001/18 (part C consent) with limited scope.

**Secretariat
March 2004**