ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT

Advice on a notification for marketing of insect resistant and herbicide tolerant GM maize

Notifier:	Syngenta seeds SAS
Notification reference:	C/F/96/05/10
Product:	Maize genetically modified for insect resistance and herbicide tolerance, transformation event Bt11.
Scope:	For the import and use of grain varieties derived from maize transformation event Bt11, and conventional hybrids, as for any other maize including cultivation, but without the utilisation of the herbicide tolerance trait.
Date:	4 February 2004

Advice of the Advisory Committee on Releases to the Environment (ACRE) under S.124 of the Environmental Protection Act 1990 (Part VI) to the Secretary of State for Environment, Food and Rural Affairs, Scottish Ministers, Ministers of the Welsh Assembly Government and the Department of Environment (Northern Ireland).

Secondary advice: ACRE has considered this notification for the import, cultivation and use of insect resistant and herbicide tolerant maize based on transformation event Bt11. ACRE considered the notifiers responses to several requests for further information (Appendix 1) and information provided in response to other Member States comments. ACRE have also taken account of the advice of the Advisory Committee on Animal Feedingstuffs Genetic Modification (ACAF GM) Sub-group.

The Committee concludes that the marketing of this product for cultivation in Europe will not pose a risk to human health and the environment. However since the scope of the notification has been limited to the use of Bt11 maize without the application of glufosinate ammonium herbicide, the Committee recommends that as a condition of consent the use of glufosinate ammonium herbicides on Bt11 maize is prohibited. ACRE appreciates that this product is unlikely to be grown in the UK for the foreseeable future. However ACRE does not regard the proposal for post market monitoring to be sufficient and so a more comprehensive case-specific monitoring plan is required before consent is issued.

Comment

ACRE considered the risk to human health and the environment posed by the marketing of Bt11 maize for import, cultivation and use. In its primary advice (Appendix 1) ACRE made several requests for additional information.

Molecular characterisation

- Further details of the data provided supporting the absence of the antibiotic resistance marker gene (*amp*) from line Bt11

- The sequences of the DNA flanking the single insertion site of the expression cassette should be provided together with bioinformatic analysis aimed at investigating the disruption of endogenous, or creation of novel, open reading frames.

- A detection protocol that will detect event Bt11 and no other transformation events

The notifier has provided the full sequence of the Bt11 insert and the flanking maize DNA. Analysis confirms that the *amp* gene is absent from the insert DNA. Taking this into account together with the Southern blot analysis provided previously, ACRE is now content that the antibiotic resistance marker is absent from Bt11. Bioinformatic analysis of the insert flanking regions identifies that the insert has occurred in a heterochromatic knob-associated region of the maize genome, which is not known to be expressed. Analysis did not identify any open reading frames in the flanking regions of the insert. ACRE concluded that they were content with the molecular characterisation of Bt11. In addition ACRE considered that the event-specific detection protocol described in Ronning *et al.* 2003[1] is truly event-specific for Bt11 maize.

Animal Feed Safety

- Data demonstrating that vegetative parts of maize containing Bt11 (as fresh green material and silage) is safe and nutritionally equivalent as other maize lines and Bt11 grain animal feed.

The Advisory Committee on Animal Feedingstuffs (ACAF) considered that additional information provided detailing compositional analysis of freshly cut whole Bt11 maize plants satisfactorily demonstrates that vegetative parts of Bt11 maize are substantially equivalent in composition to non-GM maize plants. In addition the animal feeding studies conducted with Bt11 maize forage and silage do not identify any differences giving cause for concern between animals fed Bt11 maize and non-GM maize.

Environmental risk assessment

- An environmental risk assessment of the impacts of altered management practices associated with the cultivation of Bt11 maize, including consideration of the impact of the insect resistance trait, altered herbicide regime and any potential cumulative effects for the two traits and associated changes in management.

ACRE considered the additional information provided by the Notifier with respect to the risk assessment. It was noted that the use of glufosinate ammonium herbicides on Bt11 will not be promoted, therefore the risk assessment does not take account of

[1] Ronning, S.B., Vaitilingom, M., Berdal, K.G., Host-Jenson, A.,. Event specific real-time PCR for genetically modified Bt maize (*Zea mays*) Eur. Food Res. Technol., 2003, 216: 347-354.

the altered management regimes associated with herbicide tolerant maize. In order to ensure that this is strictly adhered to ACRE require that as a condition of consent the use of glufosinate ammonium herbicides on Bt11 is prohibited.

With regard to the insect resistance trait ACRE is content with the risk assessment provided with respect to target and non-target organisms. The Committee is supportive of Syngenta's view that Bt11 maize will not be marketed for cultivation in the UK. ACRE agreed that there was no agronomic benefit to growing Bt11 maize in the UK as the insect pests to which this particular GM maize is resistant are not a problem in the UK. The Committee believes that notifiers and authorities have a responsibility to ensure that unnecessary selection pressure on target and non-target organisms in the agronomic environment is kept to a minimum.

Post market monitoring

- An improved post-market monitoring plan that takes into account any risks identified in the environmental risk assessment, and tests any assumptions made in the environmental risk assessment.

The post market monitoring plan is divided into two parts; case-specific and general surveillance. ACRE requested that the notifiers reconsider the case-specific part of the monitoring plan. Following further evaluation Syngenta conclude that no further case-specific monitoring is required in addition to that to monitor for insect resistance to Bt toxin as previously identified. The Company argues that the risk assessment does not identify any risks which require monitoring (except for development of insect resistance to Bt toxin) and further that monitoring to test assumptions made in the risk assessment are unnecessary as worst case scenario laboratory studies have already been carried out.

ACRE did not agree with the conclusion of the notifier and the Committee considers that details of further case-specific monitoring is a requirement before consent is granted. The study by Vendetti and Steffey[2] identifies that densities of natural enemies were substantially reduced in fields of Bt maize when compared with non-Bt maize fields in the US. It is therefore proposed that the use of non-Bt maize refuges within Bt maize fields is required not only for resistance management but also for conservation of natural enemies. Monitoring for the effectiveness of such refuges would appear an appropriate example of case-specific monitoring. ACRE also disagrees that the assumptions involved in the interpretation of case-specific field monitoring data would necessarily be similar to those used in the interpretation of laboratory studies. It also does not accept that the existence of these laboratory studies removes the need for case specific monitoring in cases like this one, ie when other field monitoring studies have clearly established that the assumptions of the risk assessment regarding impacts on natural enemies may be incorrect.

With respect to general surveillance a proposal was provided in the original notification provided in September 2003. Although ACRE was content with the general surveillance aspects of the post market monitoring plan, the Committee recommends that provision of appropriate detailed arrangements for general surveillance should be made a condition of any consent. These further details should include: (1) precisely who will be requested to provide information; (2) what type of

---

[2] Vendetti., M.E. & Steffey., K.L. (2002) Field effects of Bt corn on the impact of parasitoids and pathogens on European corn borer in Illinois.

information will be requested and the frequency of requests and (3) how the Company will ensure participation to ensure a robust assessment. Reports of the outcome of this monitoring should be provided on an annual basis after authorisation.

ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT

Advice on a notification for marketing of insect resistant and herbicide tolerant GM maize

Notifier:	Syngenta seeds SAS
Notification reference:	C/F/96/05/10
Product:	Maize genetically modified for insect resistance and herbicide tolerance, transformation event Bt11.
Scope:	For the import and use of grain varieties derived from maize transformation event Bt11, and conventional hybrids, as for any other maize including cultivation.
Date:	11 September 2003

Advice of the Advisory Committee on Releases to the Environment (ACRE) under S.124 of the Environmental Protection Act 1990 (Part VI) to the Secretary of State for Environment, Food and Rural Affairs, Scottish Ministers, Ministers of the Welsh Assembly Government and the Department of Environment (Northern Ireland).

Primary advice: ACRE has considered this notification for the import, cultivation and use of insect resistant and herbicide tolerant maize based on transformation event Bt11. The Committee does not consider that sufficient information has been provided by the notifier to allow a full assessment of potential risks to human health and the environment. In coming to this conclusion ACRE have taken account of the advice of the Advisory Committee on Animal Feedingstuffs Genetic Modification (ACAF GM) Sub-group who have recommended that further data be sought on the composition and nutritional value of vegetative parts of the maize in the assessment of this line. Before ACRE can complete its assessment the following information is required.

1. Further details of the data provided supporting the absence of the antibiotic resistance marker gene (*amp*) from line Bt11.

2. The sequences of the DNA flanking the single insertion site of the expression cassette should be provided together with bioinformatic analysis aimed at investigating the disruption of endogenous, or creation of novel, open reading frames.

3. Data demonstrating that vegetative parts of maize containing Bt11 (as fresh green material and silage) is safe and nutritionally equivalent as other maize lines and Bt11- grain animal feed.

4. An environmental risk assessment of the impacts of altered management practices associated with the cultivation of Bt11 maize, including consideration of the impact of the insect resistance trait, altered herbicide regime and any potential cumulative effects of the two traits and associated changes in management.

5. An improved post-market monitoring plan that takes into account any risks identified in the environmental risk assessment, and tests any assumptions made in the environmental risk assessment.

6. A detection protocol that will detect event Bt11 and no other transformation events.

Comment

Consent to import, store and process grain derived from maize containing transformation event Bt11 has previously been granted under Directive 90/220 (Ref: C/GB/96/M4/1). This consent was issued, in line with ACRE's advice, by the UK on 9 June 1998 in accordance with Commission Decision 98/292. This consent remains in effect, and the present application is concerned with extending consent to encompass cultivation.

Molecular characterisation

Molecular characterisation of Bt11 has been provided and ACRE remain content that the data provided support the conclusion that the inserted DNA sequences are present as a single copy and have not been subject to significant rearrangements. The Committee considered that two further points need to be addressed to complete the characterisation of the inserted DNA:

- The experiment designed to confirm that the antibiotic resistance marker (*amp*) is absent from Bt 11 (Appendix 3) uses DNA from line Bt10 as a positive control, demonstrating the ability to detect the *amp* sequence under the conditions of the experiment. However, the copy number of the amp gene in line Bt10 is not determined, so the value of this positive control is limited. The Committee requests further data supporting the use of DNA from line Bt10 as a positive control.
- The sequences of the DNA flanking the single insertion site of the expression cassette is not provided. These data are important because they allow examination of the sequence for the presence of disrupted endogenous open reading frames, or the presence of novel open reading frames generated by the insertion. The Committee requests that this sequence information is provided, together with a suitable bioinformatic analysis.

ACRE did not consider that the PCR detection protocol provided was truly event specific. The method is based on amplification of a sequence within the inserted DNA, so that any transformation event containing this insert would produce a positive result. The Committee consider that event specific detection methods should ideally involve amplification of sequences spanning the junction between the inserted DNA and endogenous plant DNA.

Animal feed safety

The safety and nutritional equivalence of grain derived from maize containing transformation event Bt11 was assessed as part of the application to import grain (C/GB/96/M4/1) and neither ACRE nor ACAF are aware of any new information which changes previous assessment. However, cultivation of Bt11 maize in the EU will allow the use of vegetative parts of maize (as fresh green material and/or silage) derived from this line in animal feed. While the safety of the expressed novel proteins in Bt11 maize have been demonstrated, compositional analysis and feeding studies have only been carried out with grain. Data demonstrating that silage and fresh maize containing Bt11 is safe and nutritionally equivalent as grain animal feed should be provided.

Environmental risk assessment

ACRE considered carefully the environmental risk assessment (ERA) for Bt11 maize provided by the notifier. The Committee did not consider that this assessment had been carried out in full. In particular, the ERA does not consider the possible indirect effects due to changes in management associated with the use of Bt11 maize. There are a number of areas that need to be addressed before the ERA is complete:

- Impact of altered weed management. The notifier states that the glufosinate tolerance trait is used only as a selectable marker. However, the presence of this trait in Bt11 maize would allow the use of an altered weed management approach based on this broad spectrum herbicide. This may have an impact on the biodiversity of organisms dependent on weeds for food, which, in turn, may impact on higher trophic levels. The results of the Farm Scale Evaluations of herbicide tolerant crops in the UK, which will be published shortly, will inform this part of the risk assessment.
- Impact of insect resistance trait on non-target insects. The notifier provides considerable evidence that the CRY1A toxin present in Bt11 maize has limited effect on organisms other than Lepidoptera, although the Committee notes that this is largely based on laboratory studies or data from North America rather than Europe. A number of European studies are cited but these are difficult to obtain – the Committee requests that the notifer provide copies of ABSTC (2002), Candolfi et al. (2002), Lozzia et al. (1999) and Lozzia and Rigamonti (1998) with translations into English as appropriate. A consideration of the wider biodiversity impacts of any effects on non-target insects should be included.
- Impact of insect resistance trait on target insects. The ERA does not consider fully the potential wider biodiversity impact of the insect resistance trait resulting from the effect on target insects. This consideration should not only include consideration of the primary targets *Ostrinia nubilalis* and *Sesamia nonagrioides* but also consider whether there are potential maize or grass feeding Lepidoptera in the UK that might be affected.
- Cumulative effect of changing management practices. As well as the assessment of the wider biodiversity impacts of the individual traits in Bt11 maize, and their associated management practices, the ERA should also consider the potential cumulative impact of the traits. For example, is the impact of the insect resistance trait on non-target Lepidoptera likely to exacerbated by altered floristic balance within fields caused by changes in herbicide use?

Post market monitoring

The aim of the case-specific part of the post market monitoring plan is to investigate risks identified in the ERA, and also to test any assumptions included in the ERA. The current plan for case-specific monitoring is focussed on monitoring to test the effectiveness of the Insect Resistance Management (IRM) strategy. While the Committee consider the IRM and monitoring of resistance to be adequate, the notifier will need to modify considerably the case-specific monitoring plan to take into account the additional requirements for the ERA outlined above. For example, depending on the outcome of the revised ERA, it may be appropriate to monitor changes in populations of target and non-target insects. In drawing up a revised case-specific monitoring plan the notifier should also consider the appropriate timeframe for reporting the outcome of monitoring to the regulatory authorities – this should be as frequent as is compatible with the type of monitoring study being carried out.