

# Opinion of the Scientific Committee on Plants Regarding the Genetically Modified, Insect Resistant Maize Lines Notified by the Monsanto Company

(NOTIFICATION C/F/95/12/02)

(Submitted by the Scientific Committee on Plants, 10 February 1998)

## 1. TITLE

Application for Consent to Place on the Market Insect-Resistant Transgenic Maize Expressing the Gene for **Btk** Toxin (Notification C/F/95/12/02)

## 2. TERMS OF REFERENCE

The Scientific Committee on Plants is asked to consider two issues relevant to this genetically modified organism (GMO):

1. Whether there is any reason to believe that the placing on the market of genetically modified **Btk** maize line MON810 and progeny thereof, with the purpose to be used as any other maize, is likely to cause any adverse effects on human health or the environment.
2. Whether the insect resistance management strategy as proposed in the application and supplemented with a programme in Italy aimed at validating the detailed provisions of the refuge strategy and coupled with the establishment, at European level, of an appropriate programme for monitoring resistance to **Btk**, satisfy the recommendation of the Scientific Committee for Pesticides in its evaluation of the Ciba-Geigy maize regarding the consideration of a resistance management strategy.

## 3. BACKGROUND

Directive 90/220/EEC requires an assessment to be carried out before a product containing or consisting of genetically modified organisms (GMOs) can be placed on the market. The aim of the assessment is to evaluate any risks to human health and the environment connected with the release of the GMOs. For genetically modified plants, the assessment must be based on information outlined in Annex IIB of Directive 90/220/EEC and take account of the proposed uses of this product.

Following the entry into force of the Regulation on Novel Foods and Novel Food Ingredients (EC No. 258/97) on 15 May 1997, in order for this maize seed and its derived products to be placed on the market for food purposes, the requirements of the Regulation will have to be satisfied. Such a regulation does not exist on Novel Feeds and Novel Feed Ingredients.

Member states have expressed a variety of concerns which have led the Commission to request the opinions of the Scientific Committee on Plants to examine the dossier as concerns safety matters within its remit.

## 4. PROPOSED USES

The products which are the subject of this application are seeds of an insect-protected maize line MON810 and seeds of any progeny (inbred or hybrids) derived from this line by conventional breeding methods. The application addresses the production of insect-protected maize in the European Community (EC), the import and processing of grain and maize products produced from insect-protected maize and their eventual use in food, feed and industrial products.

## 5. DESCRIPTION OF THE PRODUCT

Seeds of an insect-protected maize line MON810 and seeds of any progeny (inbreds or hybrids) derived from this line by conventional purposes. The insect-protected maize line was generated by particle acceleration technology using two plasmids; PV-ZMBK07 and PV-ZMGT10. The transgenic maize line produced expresses the **cry1A(b)** gene (origin - **Bacillus thuringiensis** subsp. **kurstaki**) which encodes a **cry1A(b)** insect control protein ( **Btk**).

## 6. OPINIONS OF THE COMMITTEE

### 6.1. Molecular/Genetic Aspects

6.1.1. **Transformation Technique:** Plasmid DNA was introduced into the maize line by the particle acceleration method. This is standard technology for maize transformation.

6.1.2. **Vector Constructs:** The maize line MON810 was produced with a DNA solution containing two plasmids: PV-ZMBK07 and PV-ZMGT10. PV-ZMBK07 contained the CaMV promoter with duplicated enhancer region (E35S); an intron from the maize **hsp70** (heat-shock protein) gene; the **cry1A(b)** gene encoding the nature identical **cry1A(b)** protein product; **NOS 3'** - a 3' non-translated region of the nopaline synthase gene (transcriptional termination; polyadenylation); **lacZ** (a partial **E. coli lacI** coding sequence, the promoter **Plac** and a partial coding sequence for b-D-galactosidase or **lacZ** protein from pUC119); **ori-pUC** (replication origin for pUC plasmids); the **nptII** gene (neomycin phosphotransferase type II confers resistance to aminoglycoside antibiotics).

Plasmid PV-ZMGT10 contained the E35S promoter; the **NOS 3'** terminator; the **hsp70** intron; the **lacZ** region; **ori-pUC**; the **nptII** gene. In addition, transit peptides CPT1 and CPT2 (from **Arabidopsis**); the CP4 EPSPS gene (from **Agrobacterium**) which allows for selection on glyphosate; the **gox** gene (encodes glyphosate metabolising enzyme).

6.1.3. **Transgenic Constructs in the GMO:** Evidence is provided that no sequences from the plasmid PV-ZMGT10 are integrated into the maize line. The line contains one integrated DNA which contains a single copy of the E35S promoter, the **hsp70** intron and the **cry1A(b)** gene. Evidence is provided that the **nptII** gene and the backbone sequences of plasmid PV-ZMBK07 are not integrated. Evidence for lack of integration of genes is provided using Southern blots. Western blots confirm that the **cry1A(b)** protein is accumulated in the transgenic maize line but that the CP4 EPSPS and **gox** gene products (proteins) are not.

On the basis of Southern and Western blot analyses the Committee accepts that the transgenic maize line MON810 contains the E35S promoter, the **hsp70** intron and the **cry1A(b)** gene. Also on this basis the Committee accepts that the likelihood of the integration of significant fragments of genes encoding **nptII**, **gox**, CP4 EPSPS and those found in the plasmid backbone is extremely remote.

However, the Committee encourages the company to provide further information on what remains of genes that may be undetectable by Southern blot analysis. Complementary data utilising specific PCR primers would be acceptable. Absence of these data does not prejudice the Committee's overall conclusions and opinions.

## 6.2. Safety Aspects

**6.2.1. Potential for Gene Transfer/Metabolism:** Since evidence is provided that **nptII**, CP4 EPSPS and **gox** genes are not integrated into the maize line MON810, the Committee accepts that there is no risk associated with gene transfer between organisms. This is particularly relevant for the antibiotic resistance marker gene **nptII**.

**6.2.2. Safety of Gene Products: Food and Feed:** The weight of evidence provided by the Company and available elsewhere leads the Committee to conclude that there is no significant risk to humans or livestock following ingestion of the gene product. No toxic effects have been observed in acute and short term toxicity studies. Widespread use of the natural **Btk** insecticides has not produced evidence of allergenic responses. Similarly no homologies have been found between **Btk** toxin and any known allergens. However, the Committee was of the opinion that the often applied **in vitro** methodology used to study the survival of **Btk** toxin can be improved. In particular, the use of the isolated protein in toxicity studies does not adequately model degradation of the same protein when fed as an integral component of the diet.

**6.2.3. Substantial Equivalence:** The Company has provided data on compositional analyses and agronomic performance from field trials in USA and Europe. These include fatty acid profiles, protein amino acid composition, crude fibre, ash, phytate and moisture contents determined for grain and silage of GM and non-GM plants. No significant nutritional differences could be detected between GM and non-GM materials. The Committee is of the opinion that the transgenic maize line is substantially equivalent to non-transgenic maize except for the transferred traits.

## 6.3. Environmental Aspects

**6.3.1. Potential for Gene Transfer/Gene Escape:** The risk of genetic escape from modified crop plants will be limited by poor dispersal and the absence of sexually-compatible plants either of the same or different species. **Zea mays** is not an invasive crop but is a weak competitor with limited powers of seed dispersal. Since pollen production and viability are unchanged by genetic modification in this wind-pollinated crop, dispersal and outcrossing frequency should be no different from other maize varieties. There are no plant species closely-related to maize in the wild in Europe and the risk of genetic transfer to other species appears remote.

**6.3.2. Treatment of Volunteers:** The risk of volunteer maize plants surviving is considered to be remote. In growing areas free from winter frost which will kill any residual plants, any volunteers may be controlled by agronomic practices including cultivation and the use of non-selective herbicides.

**6.3.3. Safety for Non-Target Organisms:** The target pest is the European corn borer **Ostrinia nubilalis**, a pyralid moth. The **cry1A(b)** crystal proteins are specifically toxic to **Lepidopteran** larvae on ingestion and appear non-toxic to other species of insects, either

directly or through secondary ingestion (predation). The endotoxin is (and has been for some 20 years) applied widely as an agricultural pesticide against **Lepidopteran** larvae, often on a broad scale e.g. on maize and in forestry, in many EU member states. Under the same growing conditions compositional data for grain and forage show that modified and unmodified plants are equivalent and no risk is identified to non-target herbivores including vertebrates. The **cry1A(b)** protein in modified plants is identical to the same protein in microbial formulations used safely as crop-protection sprays. Risks to soil organisms and soil function through degradation of modified plant material and contamination of ground water are considered to be extremely low.

**6.3.4. Resistance and Tolerance Issues:** The development of resistance in injurious target pests will be delayed by the rigorous adoption of a comprehensive resistance management strategy. To be effective this should require the active involvement of the notifying company to monitor for control failure, to provide technical support and to educate growers to implement the strategy.

The speed with which resistance to **Btk** toxin will develop in the target pest will depend on the rigour and efficiency of any insect resistance management strategy. Such a programme designed to delay resistance development requires adequate:

- 1. Knowledge of pest biology and ecology
- 2. Gene deployment strategy (full-season, constitutive, optimal dose **Btk** expression to control insects heterozygous for resistance alleles).
- 3. Refuges to support the development of **Btk** toxin-susceptible insects.
- 4. Monitoring and reporting of incidents of pesticide resistance development.
- 5. Employment of integrated pest management practices that encourage ecosystem diversity and provide multiple tactics for insect control.
- 6. Communication and education plan.
- 7. Development and deployment of products with alternative modes of action.

The plan proposed by the applicant addresses each of these points. Although it is not possible to determine optimal dose until resistant insects exist in the field, high protein levels appear present in all important plant tissues to provide season-long control. The success of the resistance management strategy will depend on the ability of any monitoring programme to detect resistance as soon as possible and the extent and quality of advice given to farmers. The proposed plan together with the validation programme in Italy should provide an adequate framework to delay the onset of resistance in the target pest.

It should also be noted that there is no substantiated evidence that reported incidents of losses of "Bollgard" cotton due to insect infestation is due to the increased resistance of the insects to the toxin **in planta**.

The Scientific Committee should be kept informed annually of the results of the proposed surveillance of resistance in the European corn borer in member states. Separately the Scientific Committee welcomes the initiative to monitor all lines of **Btk** maize to be placed on the market for the development of insect resistance and wishes to be kept informed of progress.

## **7. OVERALL ASSESSMENT**

The Commission requested the Scientific Committee on Plants to consider whether the production, import and processing of an insect-protected maize line MON810 (expressing the **Btk** endotoxin) and progeny derived thereof is likely to cause any adverse effects on human health or the environment. The Committee was also asked to assess the risk management strategies to be used to minimise the likelihood of resistance developing in the target pests. In the assessment of the dossier provided against the criteria set out in Directive 90/220/EC, the Committee has reached the following conclusions:

1. The Committee after examining and considering the existing information and data provided in the dossier, against the background of available knowledge in the areas concerned, considers that there is no evidence to indicate that the seeds of insect-resistant maize (expressing the **cry1A(b)** gene and protein) when grown, imported and processed in the manner indicated, are likely to cause adverse effects on human or animal health and the environment.
2. The Committee was also of the opinion that the proposed plan for risk assessment with regard to **Btk** endotoxin resistance development provides an adequate framework to delay the onset of such resistance in the target pest. The Scientific Committee should be kept informed of monitored progress in the field.