Opinion of the Scientific Committee on Plants regarding the submission for placing on the market of genetically modified, insect-resistant maize lines notified by the pioneer genetique S.A.R.L. Company (notification No C/F/95/12-01/B) (Submitted by the Scientific Committee on Plants, 19 May 1998)

1. TITLE

Application for Consent to Place on the Market Insect-Resistant Transgenic Maize Expressing the Gene for **Btk** Toxin (Notification No C/F/95/12-01/B).

2. TERMS OF REFERENCE

The Scientific Committee on Plants is asked to consider two issues relevant to this GMO:

1. Whether there is any reason to believe that the placing on the market of genetically modified **Btk** maize line MON809 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 the information outlined in Annex II B of Directive 90/220/EEC and take account of the proposed uses of the 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 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 MON809 and seeds of any progeny (inbred or hybrids) derived from this line by conventional breeding methods. The application addresses the growing of insect-protected maize in the European Union, the import and storage of grain from outside the EU, the processing of grain and maize products derived from insect-protected maize (including chopped green maize tissue forage/silage and the use of the GM maize in food, feed and industrial processes/products.

5. DESCRIPTION OF THE PRODUCT

Seeds of an insect-protected maize line MON809 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**). The maize also expresses the CP4-EPSPS gene and protein (5-enoylpyruvylshikimate-3-P synthase) as a selectable marker for growth of transgenics on glyphosate.

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. During particle acceleration portions of the same or different plasmids can become fragmented and rejoined.

6.1.2. Vector Constructs: The maize line MON809 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?-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: MON809 contains one I-DNA (ca 23Kb) which includes either complete or partial genes of **cry1A(b),** CP4 EPSPS and **gox.** Molecular analyses indicate that 2 **cry1(A)b** genes are inserted , one of the correct size and one truncated. It is concluded that it is the intact gene sequence which produces the **Btk** protein detectable in Western blots as no protein corresponding with the size of the truncated gene is detectable. Two copies of the CP4 EPSPS gene are inserted into MON809, both of the predicted size. The CP4 EPSPS protein product is detected in Western blots and is of the size

predicted. Southern blot analyses indicate that the **gox** gene is not inserted as a full length product and the **gox** gene product (protein) is not detectable by ELISA.

Based on Southern blot analyses the plasmid backbone of PV-ZMBK07 is absent from MON809. Both **npt11** and **ori-pUC** genes are detectable by Southern analysis. The **npt11** gene has a bacterial promoter which is non-functional in plants as confirmed by Western blots.

6.2. Safety Aspects

6.2.1. Potential for Gene Transfer/Metabolism: The kanamycin/neomycin resistance marker, **nptII**, is controlled by its own promoter. It is theoretically possible that DNA containing this gene could transform an intestinal bacterium resulting in the expression of the gene in a new host. However, the Committee is aware that natural resistance to kanamycin is common among bacteria. This together with the fact that kanamycin/neomycin are now relatively unimportant in clinical practice, makes the risk of interference with human or veterinary chemotherapy remote.

The **gox** and CP4 EPSPS genes encode for glyphosate resistance and are used as selection markers. No foreseeable risks are involved even in the unlikely event of their transformation into intestinal bacteria and their subsequent expression. The proteins encoded for by these genes do not have alternative substrates which can result in the production of toxic end-products. The **gox** gene product is not detectable in the GM plant, thus it is very unlikely that a functional gene is present.

6.2.2. Safety of Gene Products: Food and Feed: The **Btk** protein is present in leaves of the GM maize at between 0.88 and 2.37 µg/g FW and between 0.28 and 0.73 µg/g FW in the grain. CP4 EPSPS protein is present in leaves at between 4.5 and 43.7 µg/g FW and in grain at between 5.68 and 20.5 µg/g FW. Data is provided that this level of expression of CP4 EPSPS is too low to confer significant glyphosate resistance in the field. The **gox** protein is not detectable with the methods employed.

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 products. 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.

6.2.3. Substantial Equivalence: The Company has provided data on compositional analyses of GM and non-GM maize and on agronomic performance from field trials. These data include growth characteristics, yield and persistence, information on ash, carbohydrate, fat and fatty acid content, calorific value and amino acid composition of GM and non-GM plants. For those parameters quantified in grain and leaf materials the values obtained were within the boundaries of natural variation and no significant nutritional differences could be detected between GM and non-GM samples. All values fell within the ranges cited in published literature. The Committee was of the opinion that Bt-maize line MON809 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 outcropping 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 of 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 authorised as an agricultural pesticide. 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. Laboratory studies of honeybee larvae and adults, lacewing larvae, parasitic hymenoptera and adult ladybirds exposed to the **cry1A(b)** trypsin Â– resistant protein have not recorded adverse effects. Under the same growing conditions compositional data for grain show that modified and unmodified plants are equivalent and no risk is identified to non-target herbivores including vertebrates. From field examination of beneficial arthropods (the Anthocorid **Orius insidiosus** and spiders)in genetically modified **Btk** plants, it was concluded that any potential impact on non-target arthropods will be less than that from the use of conventional insecticides. Young quail fed with modified maize meal in their diet showed no adverse effects. Very little of the crop material remains after harvest for incorporation into the soil. Studies show that the endotoxin may become adsorbed to some soil fractions and that degradation is by microbial action. From the weight of evidence available the risks to organisms and soil function are considered to be very low. Contamination of ground water is unlikely. Thus the expectation is that the genetically modified maize will be at least as safe as, and perhaps safer than, traditional methods of insect control involving pesticides.

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:

- - knowledge of pest biology and ecology

- - gene deployment strategy (full-season, constitutive, optimal dose **Btk** expression to control insects heterozygous for resistance alleles).
- - refuges to support the development of **Btk** toxin-susceptible insects.
- - monitoring and reporting of incidents of resistance development.
- - employment of integrated pest management practices that encourage ecosystem diversity and provide multiple tactics for insect control.
- - communication and education plan.
- - development and deployment of products with alternative modes of action.

These points are addressed by the following plan proposed by the company:

- - deploying products with an effective dose of **Btk**
- - maintaining adequate refuges
- - monitoring control efficiency
- - educating seed distributors and farmers
- - continuing to conduct research

The success of the resistance management strategy will depend upon the ability of any monitoring programmes to detect resistance as soon as possible and the extent and quality of advice given to farmers. The proposed plan rigorously carried out with the active involvement of the company should provide an adequate framework to delay the onset of resistance in the target pest.

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 MON809 (expressing the **Btk** endotoxin) and progeny derived thereof is likely to cause any adverse effects on human health or the environment. The Committee were 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 health and the environment.

2. The Committee was also of the opinion that the proposed plan for risk management 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.