# Opinion of the Scientific Committee on Plants on the Genetically Modified Maize Lines Notified by the Novartis Company

(NOTIFICATION C/GB/96/M4/1)

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

## 1. TITLE

Application for consent to place on the market genetically-modified maize with **Btk** resistance to **Lepidoptera** and herbicide tolerance to glufosinate ammonium ( **Bt11**). Notification C/GB/96/M4/1.

## 2. TERMS OF REFERENCE

The Scientific Committee on Plants is asked to consider whether there is any reason to believe that the import and processing of hybrid seeds derived from the **Bt11** event is likely to cause any adverse effects on human health and the environment. This request does not cover the cultivation of hybrids or lines within the European Community (EC) containing the **Bt11** event in their genome. Cultivation of such material in the EC requires additional risk assessment by the Committee.

## 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.

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.

Applications for release of insect protected maize under Directive 90/220/EEC have been made by the applicant in Europe (reference n° B/F/94.01.06 ; B/F/95.01.03 ; B/It/95.16 ; B/F/95.12.04 ; B/It/96.13).

The Animal and Plant Health Inspection Service (APHIS) of the USDA published positive conclusions in the Federal Register Vol. 61 N° 19 on 29 January 1996 (pp 2789-2790) by declaring that **Bt11** and any progeny derived from hybrid crosses with other non-transformed corn varieties will be just as safe to grow as traditionally bred corn lines that are not regulated under 7CFR part 340.

## 4. PROPOSED USES

Import of hybrid seeds of insect protected maize and its use for processing and marketing for food and feed usages.

## 5. DESCRIPTION OF THE PRODUCT

The pollen of maize plants ( **Zea mays** L.) derived from transformation event **Bt11** was used to pollinate the female flowers of an inbred corn line. Descendants of the initial crossings have been successively back-crossed to evaluate different maize lines carrying the **Bt11** event. Hybrid lines were produced. The maize grains which is the subject of this application for consent are produced from these hybrid lines and are therefore descended from the initial **Bt 11** transformation event.

## 6. OPINIONS OF THE COMMITTEE

### 6.1. Molecular/Genetic Aspects

6.1.1. **Transformation Technique:** The genetic construct was introduced into protoplasts without a DNA carrier. Plants were then regenerated.

6.1.2. **Vector Construct:** The **Bt11** transformation event has been obtained using plasmid pZO1502 containing the following components

- a truncated synthetic **cry 1A(b)** gene encoding **Btk** endotoxin. It also contains a synthetic **pat** gene (to allow transformant selection on glufosinate ammonium). 35S CaMV is the promoter, **nos** 3Â'termination sequences are included and introns IVS 2 or IVS 6 are incorporated to enhance expression.

- the plasmid pZO1502 contains the **ampR** gene used as selectable marker when the plasmid was generated in **E. coli**.

- DNA from the well characterised plasmid pUC18 including portions of the **lac Z** and **lac** i genes and a segment of 1079 bp containing the bacterial origin of replication, **ori** .

- Small pieces of DNA containing useful restriction endonuclease sites, inserted and used to combine the various components above.

6.1.3. **Transgenic Construct in the Genetically Modified Organism:** The plasmid vector pZO1502 DNA was treated with the restriction endonuclease **Not**I in order to remove the **ampR** gene from the larger DNA fragment which contained the **Btk** gene fusion and **pat** gene fusion. This mixture of DNA fragments was then used to transform maize tissue.

The larger fragment contains the following :

1) the **pat** gene fusion (35S promoter - IVS 2 intron - PAT protein coding region - **nos** Â' termination sequence), which allows production in plants of the PAT enzyme for resistance to the herbicide, glufosinate.

2) the **Btk** gene fusion (35S promoter - IVS 6 intron - **Btk** HD-1 protein coding region - **nos** 3Â' termination sequence), which allows production in plants of the **Btk** protein to protect the plant from damage by larvae of European Corn Borer.

3) DNA, totalling about 1,400 bp, and including a bacterial origin ( **ori**) of replication, from the well characterised plasmid, pUC18.

4) small pieces of synthetic DNA, totalling about 120 bp and containing useful restriction endonuclease sites ; inserted and used to combine the various components above.

The smaller fragment from the **Not**1 digestion of pZO1502 contains the **ampR** gene. Southern blot and PCR analyses have shown that lines derived from the initial **Bt11** event do not carry the **ampR** gene. Thus, an antibiotic resistance gene is not present in the **Bt11** event, nor in the maize grain or grain products produced from them. The **Btk** gene fusion and **pat** gene fusion are stabily integrated as a single copy at a single locus in the long arm of chromosome 8.

**6.2. SAFETY ASPECTS**

6.2.1. **Potential for gene transfers: Antibiotic (ampicillin) resistance gene** Â— **ampR** gene was used in the construction of the vector. Before the final transformation event it was, however, removed from the plasmid by cutting with a restriction endonuclease. Consequently, the resulting GM-plant does not contain the **ampR** gene.

**pat gene Â— The gene is under the control of a plant promoter which is not functional in bacteria. Consequently, in the unlikely event of transformation, its expression would not occur. Even if, due to genetic recombination, the gene would be expressed in intestinal micro-organisms or in human or animal cells, the probability of which is remote, no negative effects are expected because the only known substrate of phosphinothricin acetyltransferase (PAT) is the herbicide glufosinate ammonium.**

6.2.2. **Safety of the gene product/metabolites (food and feed)**

Safety of gene products : Grain produced from the **Bt11** event contains **Btk** protein within a range of 5-25 mg/g fresh weight. PAT protein is present at around 80 ng/g fresh weight. Toxicity has been assessed on the residual core protein in rats. 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 gene products. PAT and **Btk** proteins are labile in **in vitro** assays with gastric juice of farm animals. Widespread use of natural **Btk** insecticides has not produced evidence of allergenic responses. Similarly no allergenic effect is predicted by comparing the new proteins ( **Btk** and PAT) with the structure of known allergenic proteins. However, the Committee is of the opinion that the often applied **in vitro** methodology to study the degradability of the **Btk** toxin (and phosphinotricin acetyl transferase) 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.

**Residue assessment: The principal residue identified in transgenic maize plants after post-emergence use of glufosinate ammonium was N-acetyl-glufosinate with lesser quantities of glufosinate and 3-methylphosphinico-propionic acid (MPP) which is also found in non-transgenic plants. In maize grain, which exhibits much lower residues than the other plant parts, the principal residue identified was MPP with lesser amounts of N-acetyl-glufosinate. In maize grain only 5% of about 300 samples analysed in US-trials exhibited residues $^{3}$ 0.05 mg/kg.**

The glufosinate-derived residues do not concentrate in any maize processed fraction which are relevant food or feed items. These include flour, starch, grits and oil. Residues are not detectable in crude and refined oil.

In ruminant and poultry feeding studies no detectable residues were found in meat, milk or eggs at the dose calculated to represent the highest residues in livestock feed under Good Agricultural Practice and taking into account the potential use of glufosinate herbicide in several tolerant crops.

It can be concluded, on the basis of the available data, that residues of glufosinate ammonium and its metabolites, N-acetyl-glufosinate and 3-methylphosphinico-propionic acid expressed as glufosinate free acid equivalents, will be below 0,2 mg/kg in imported field maize grain, the time-limited tolerance set by the US EPA (Federal Register vol. 62, n°24 p 5333, 1997). In food of animal origin from livestock animals fed with feedstuffs after application of glufosinate herbicide in tolerant maize no residues above the limit of determination are to be expected.

6.2.3. **Substantial equivalence:** Compositional analysis on seeds harvested from trials at a number of locations within the U.S. provided data on oil content, fatty acid composition, fibre and starch content and amino acids profiles. The composition of the genetically modified plants fall within the range observed for non-GM-plants and isogenic control varieties. On the basis of substantial equivalence it can be concluded that grain or products derived from imported grain harbouring **Bt11** event would be safe for food use.

### 6.3. ENVIRONMENTAL ASPECTS

6.3.1. **Potential for gene escape/gene escape:** Since the maize will not be grown in the EC, the only potential release is by spillage of grain during transport or processing. In the unlikely event of spilled grain becoming established, **Zea mays** is not invasive but is a weak competitor with limited powers of seed dispersal. There are no closely related wild plants in Europe. In areas free from winter frost which will kill residual plants, any subsequent volunteer plants may be controlled by cultivation and the use of non-selective herbicides. The risks of spread of the genetic traits are considered minimal.

6.3.2. **Treatment of volunteers:** Since this maize will not be cultivated in the EC, volunteers in following crops are not a potential problem.

6.3.3. **Safety of non-target organisms:** In view of the minimal risk of exposure (above) and the non-toxicity of any spilled grain to vertebrates, the risk to non-target and beneficial species in the environment from the proposed use of modified maize is considered remote.

6.3.4. **Resistance and tolerance issues:**Since this maize will not be cultivated within the E.U. and is extremely unlikely to escape, potential problems of **Btk**-resistance do not exist.

## 7. OVERALL ASSESSMENT

The import of genetically-modified maize seed (notification C/GB/96/M4/1) carrying the **Bt11** event can be considered as safe as utilising seed from non-genetically modified plants.