



## **ASSESSMENT REPORT**

### **NOTIFICATION NUMBER C/ES/01/01 TO MARKET GENETICALLY MODIFIED MAIZE (LINE 1507) RESISTANT TO LEPIDOTERAEE AND TOLERANT TO GLUFOSINATE-AMMONIUM HERBICIDE SUBMITTED BY THE COMPANY PIONEER, IN ACCORDANCE WITH DIRECTIVE 2001/18/EC.**

#### **Introduction**

On 11 July 2001, the companies Pioneer Hi-Bred Internacional, Inc. (represented by Pioneer Overseas Corporation) and Mycogen Seeds (c/o Dow AgroSciences LLC) submitted to the Competent Authority of Spain a notification in accordance with Directive 90/220/EEC to market genetically modified 1507 maize, resistant against certain lepidopteran insects and tolerant to glufosinate-ammonium herbicide. These characteristics have been conferred by inserting in its genome the genes *cry1F* and *pat*, respectively.

The notification has been evaluated during the last two years at several meetings of the Comisión Nacional de Bioseguridad (CNB), at which additional information was requested in order to complete the dossier.

On 17 December 2002, Pioneer submitted complementary information in accordance with the information required by Article 13 and Annexes II, IIIB, IV and VII of Directive 2001/18/EC consisting of the environmental risk assessment, proposal for a monitoring plan for the placing on the market, proposal for labelling of products consisting of or containing 1507 maize, and the new SNIF (Summary Notification Information Format).

At the meeting of the Comisión Nacional de Bioseguridad held on 9 May 2003, the CNB concluded the evaluation of the notification and considered that the information relative to the safety evaluation regarding the environment and human health of the product is adequately documented.

The notifiers have not declared as confidential business information any portion of the information contained in the Notification.

From the risk evaluation carried out it is concluded that there is no reason to believe that imports, production, processing and cultivation of 1507 maize line, resistant to lepidopterae and tolerant to glufosinate, will have any negative effects on human health or the environment.

Through the analysis of seed from this maize line, the safety of the expressed proteins, the composition, and the safety of the grain and derived products for animal feed and industrial uses have been evaluated.



## Structure of the Notification

The Notification is structured 10 Volumes and 39 Annexes as follows:

**Volume 1:** Consists of 7 Sections:

**Section 1:** Table of contents

**Section 2:** Information required in the notifications to market GMHP (Annex IIIB) of Directive 2001/18/EC.

**Section 3:** Complementary information in accordance with Annex IV.

**Section 4:** Environmental risk assessment in accordance with Annex II.

**Section 5:** Proposal for a monitoring plan for the placing on the market in accordance with Annex VII.

**Section 6:** Proposal for the labelling of products in accordance with Annex IV.

**Section 7:** Summary notification information format for GMHP.

**Volume 2 to Volume 10:** Annexes 1 to 39, corresponding to studies carried out on 1507 maize regarding molecular characterization, composition analysis, substantial equivalence, toxicity and allergenicity evaluation, non-target effects, insect resistance management plan (IRM), general surveillance plan, etc.

## Product description

The product is described as *B.t.* Cry1F maize (*Zea mays*) line 1507, also referred to as 1507 maize genetically modified to express CRY1F protein, conferring resistance to certain lepidopteran insect pests, and PAT protein, conferring tolerance to glufosinate-ammonium herbicide. The product also consists of progeny derived from conventional breeding between 1507 maize with any traditionally bred maize.

The genetic modification has been carried out by particle acceleration. The insert (PHI8999A) was obtained from plasmid PHP8999 following digestion of the plasmid DNA with the restriction enzyme *PmeI*. This insert contains the gene *cry1F* (from *Bacillus thuringiensis* subsp. *aizawai*), and the gene *pat* (from *Streptomyces viridochromogenes* strain Tü494) together with other sequences needed for expression.

## Scope of the commercialisation

Imports, processing and cultivation of maize resistant to lepidopterae and tolerant to glufosinate herbicide (Line 1507), for use as any other non genetically modified maize. The grain and products derived from this maize line will be distributed by maize traders and processors in the European Union.

The notifier informs that insertion of the *pat* gene conferring tolerance to glufosinate-ammonium herbicide has been used as a selectable genetic marker. In any case, the application of the above mentioned herbicide would require specific authorization for its use on this genetically modified



maize. Use of this genetically modified maize for human consumption is considered out of scope of this Notification.

The period for the first consent to market 1507 maize is requested for a maximum period of ten years after inclusion of the first variety from this maize in a National Catalogue of Plant Varieties in accordance with Council Directives 70/457/EEC and 70/458/EEC.

### **Identification and labelling**

Imports of commodity grain: The words “Contains genetically modified organisms” will be included on the label or accompanying documents.

The commercial name of the product will be transmitted to all intermediaries in the chain, together with the unique identifier and any other relevant information, including the procedure for public access to the GMO Register. **The unique identifier proposed by the notifiers for 1507 maize is DAS-Ø15Ø7-1.**

The PCR detection method specific for 1507 maize together with the corresponding reference material will be made available to the regulatory authority and the European Commission Joint Research Centre (JRC).

### **ENVIRONMENTAL RISK EVALUATION**

The Notification has been evaluated by the Comisión Nacional de Bioseguridad. From the assessment of the complete information submitted in the Notification and subsequent risk evaluation, it is considered that the product, given the current knowledge and for the proposed uses, does not represent any risk to human or animal health or for the environment.

The methodology followed in the risk evaluation arising from maize line 1507 has been the following:

1. Identification of the characteristics of the GMO and its use that could cause an adverse effect to human health and the environment.
2. Evaluation of the magnitude of the consequences of each adverse effect.
3. Evaluation of the likelihood of occurrence of each adverse effect identified.
4. Estimation of the risk posed by each adverse effect identified.
5. Application of management strategies.
6. Determination of the overall risk of the GMO.

The main aspects taken into account in order to evaluate the potential adverse effects on human and animal health and the environment were:

- Capacity of survival, establishment and dissemination.
- Potential for genetic transfer.
- Genetic and phenotypic stability.
- Expression products from the inserted sequences.



- Disease to humans and animals (including toxic and allergenic effects).
- Effects on other organisms.

### ***Capacity of survival, establishment and dissemination***

The introduced traits have not altered the plant reproductive morphology and therefore no difference in establishment, vigour and competitiveness is expected in 1507 maize with respect to other commercially available varieties. Except for the resistance against certain lepidopteran insects and tolerance to glufosinate-ammonium herbicide, the 1507 maize line does not show any differences in survival with respect to non-modified maize.

Maize (*Zea mays*) is not an invasive crop due to factors specific for this species, such as lack of dormancy, low survivability of the seed in the soil, and the sensitivity of the maize plantlets to frost. Maize production in the European Union requires the extensive application of agronomic practices. In any case, should volunteers occur in the cropping area, they are easily controlled by other non-selective herbicides and cultivation techniques.

### ***Potential for gene transfer***

The risks for dissemination and transfer are extremely low due to the poor dispersion and absence of sexually compatible plants. Gene flow through normal sexual transmission will depend on wind conditions, humidity and temperature. There are no known populations of wild relatives in Europe and transfer to other species is considered remote.

### ***Genetic and phenotypic stability***

The genetic modification in 1507 maize has been characterized in detail by Southern analysis and DNA sequencing. The molecular analysis, expression data and segregation of the progeny have confirmed the following:

- The simple insertion of the insert,
- There is one simple complete copy of the DNA fragment used in the transformation within the single insert,
- The genome of the 1507 maize line does not contain the *nptII* gene nor any other detectable sequence from the portion of plasmid PHP8999 not intended for transformation of this maize,
- The flanking maize genomic DNA sequences of the 5' and 3' borders of the 1507 maize insert have been sequenced in detail,
- The analysis of the Mendelian segregation of the introduced genes confirms that the *cry1F* and *pat* genes are inserted in the plant genome and that they are inherited as dominant genes.
- The expression levels of the PAT and CRY1F proteins are stable and within normal ranges of variation.



### ***Expression products from the inserted sequences***

The expression products from the 1507 maize insert consist of the CRY1F and PAT proteins. Expression of such proteins has been characterized by ELISAs (Enzyme Linked Immunosorbent Assay). The CRY1F protein is expressed in all tissues and throughout the development of the maize plant, while the PAT protein could only be measured in the V9 stage (9 leaves stage) of development. Different analytical techniques have been applied which confirm that the CRY1F and PAT proteins expressed in 1507 maize have the same molecular weight and immunoreactivity as the proteins produced in recombinant microorganisms. Additional confirmation regarding the equivalence between CRY1F protein derived from the recombinant microorganism *Pseudomonas fluorescens* and CRY1F protein expressed in 1507 maize has been obtained.

### ***Potential harm to humans, animals and plants***

The known function and ubiquity of the CRY1F and PAT proteins represent a history of safe use.

Studies of acute oral toxicity in mice with the CRY1F protein have demonstrated its safety to human and animal health. No mortality, toxicity or adverse clinical signs were observed with the highest dose tested of 5050 mg of sample per kg body weight (Kuhn, 1998), which when adjusted for purity (11.4%; Evans 1998) corresponds to a dose of 576 mg CRY1F per kg body weight.

No toxicologically significant differences were observed in a thirteen week (90 days) oral toxicity study in rats regarding body weight, food consumption, food efficiency, clinical signs of toxicity, ophthalmological observations, neurological behaviour, clinical pathology, organ weights and gross or microscopic pathology.

The safety of the PAT protein in terms of its absence of toxicity has already been determined in detail during the assessment of other glufosinate-ammonium tolerant maize. Toxicity studies carried out in rats and mice with the PAT protein have confirmed the absence of adverse reactions.

The allergenicity assessment has been carried out following the recommendations made by FAO/WHO (2000) and in accordance to the decision-tree by Metcalfe *et al.* (1996) developed for the assessment of the allergenicity of genetically modified crop plants. These studies have consisted of the comparison of the amino acid sequences of the CRY1F and PAT proteins with those of the known allergens, rapid degradation in simulated gastric fluid, relatively low expression level, lack of post-translational glycosylation and thermal susceptibility which confirm that the CRY1F and PAT proteins do not pose any significant potential allergenic risk.

Compositional analyses of protein, fibre, carbohydrates, fat, ash, minerals, vitamins, secondary metabolites, and anti-nutrients have been carried out in forage and/or grain from 1507 maize, which confirm the substantial equivalence of the products from this maize used in animal feed.

In addition, a feeding study has been carried out in poultry which showed that there were no significant differences between chickens fed with a 1507 maize diet and chickens fed with non-genetically modified maize, which confirms an equivalent nutritional value.



Finally and taking into account the results obtained from the experimental releases carried out in Spain, Italy, France and in other countries outside the EU, there is no evidence that the CRY1F or PAT proteins cause any disease to plants.

### ***Effects on non-target organisms***

The genetic modification does not cause any potential change in the interactions between 1507 maize and non-target organisms. This has been confirmed by the specificity of the activity of the CRY1F and PAT proteins, its ubiquity in the soil, limited soil persistence of the CRY1F protein and thorough studies of the absence of toxicity of the CRY1F protein to beneficial and non-target organisms: studies in non-target arthropods (*Chrysoperla carnea*, *Hippodamia convergens*, *Danaus plexippus*, *Nasonia vitripennis*, etc), bees (*Apis mellifera*), terrestrial organisms (*Eisenia foetida*, *Folsomia candida*), wildlife birds (*Colinus virginianus*) and aquatic organisms (*Daphnia magna*).

The possibility for any adverse effects on non-target organisms is considered negligible or insignificant.

## **MONITORING PLAN**

Taking into account that the proposed use for the placing on the market of this product is imports, processing and cultivation, mainly for use in animal feed, the monitoring plan is based on the identification, monitoring and traceability of grain from 1507 maize, by the provision of information through the chain of traders and processors of commodities from this maize, in order to be able to identify any unexpected effect in imports and processing of the product.

It also includes a process to inform the European animal feed sector and operators interested on the safety and general characteristics of the product. Such operators will be asked to inform the relevant authorities of any adverse effect to animal health that they gain knowledge of from national farmer and animal farming associations.

The Monitoring Plan proposes an insect resistance management plan (IRM) for early detection of the potential development of resistance to the Bt toxin in the borer populations. For this purpose, sampling and analysis will be carried out in a selection of representative areas with an elevated level of maize borer pests (*Ostrinia nubilalis* and *Sesamia nonagrioides*).

Finally, note that in order to authorize the registration of maize varieties of this transformation event in the Registro de Variedades Comerciales of Spain, a Monitoring plan for such varieties will need to be established so as to include studies on different aspects in relation with the potential effects on the environment posed by the cultivation of these genetically modified varieties.



## CONCLUSION

Taking into account that:

1º) No potential adverse effects to human and animal health or the environment have been identified from 1507 maize and that it is considered that there is only a limited potential development of resistance to the CRY1F protein in the target insect population for this crop,

2º) An adequate Resistance Management Plan will be applied,

3º) A Monitoring Plan will be carried out as part of the risk management strategy in order to confirm the safety of the commercial cultivation of 1507 maize or to detect unidentified potential adverse effects,

The Comisión Nacional de Bioseguridad, following the examination of the existing information and of the data provided by the companies Pioneer Hi-Bred International and Micogen Seeds, estimates that, for the considered uses, with the current level of knowledge, there is no scientific evidence to indicate that marketing of genetically modified 1507 maize line poses any risk to human or animal health or the environment.