Opinion of the Scientific Panel on Genetically Modified Organisms on an application (reference EFSA-GMO-NL-2004-02) for the placing on the market of insect-tolerant genetically modified maize 1507, for food use, under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International/Mycogen Seeds[1]

(Question No EFSA-Q-2004-087)

Opinion adopted on 19 January 2005

SUMMARY

This document provides an opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on 1507 maize, genetically modified to provide protection against specific lepidopteran pests. The maize also contains a gene providing tolerance to the herbicide glufosinate.

In delivering its opinion the Panel considered the application, additional information provided by the applicant and comments submitted by the Member States. Further information from other applications for placing 1507 maize on the market under current regulatory procedures were taken into account where appropriate, as were comments from the Member States. The information from other applications were notification C/ES/01/01 for cultivation, import, processing and use as any other maize (excluding food uses) and notification C/NL/00/10 for import and processing. For regulatory reasons the latter applications resulted in separate opinions.

1507 maize was assessed with reference to its intended use employing the appropriate principles as described in the 'Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed'. The scientific assessment included examination of the DNA inserted into 1507 maize and the nature and safety of the target proteins produced by the transgenic plants with respect to toxicology and allergenicity. Furthermore, a comparative analysis of agronomic traits and composition was undertaken and the safety of the whole food was evaluated. A nutritional and an environmental assessment, including monitoring plan, were both undertaken.

1507 maize has been developed for protection against specific lepidopteran pests such as the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. and for tolerance to the herbicide glufosinate. Insect resistance is achieved by production of a truncated Cry1F protein from *Bacillus thuringiensis* ssp. *aizawai* and tolerance to the herbicide is conferred by a phosphinothricin-N-acetyltransferase (PAT) from *Streptomyces viridochromogenes*. Maize embryos were transformed by particle bombardment to transfer a DNA fragment containing these two genes. As a result of the genetic modification, the 1507 event contains an insert bearing both *cry*1F and *pat* genes, under the control of the maize ubiquitin and the 35S promoters, respectively.

[1] For citation purposes: Opinion of the Scientific Panel on Genetically Modified Organisms on an application (reference EFSA-GMO-NL-2004-02) for the placing on the market of insect-tolerant genetically modified maize 1507, for food use, under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International/Mycogen Seeds, *The EFSA Journal* (2005) 182, 1-22.

Molecular analysis showed that 1507 maize contains one copy of the DNA fragment used for transformation and that this is present at a single locus in the nuclear genome of the GM plant. The complete DNA sequence of the insert was provided. In addition to the intact genes, the insert in 1507 maize includes DNA sequences originating from the fragment used for transformation as well as maize chloroplast and nuclear genome sequences at both ends of the inserted sequence. While these sequences may have resulted from the transformation process (insertional events), there were no indications that these additional fragments would result in the transcription of new RNA other than the mRNAs transcribed from the *cry*1F and *pat* genes. In the unlikely event that this does occur, bioinformatics analysis showed that any resulting peptides or proteins would have no homology to known toxins or allergens. Analysis of DNA sequences flanking both ends of the insert shows that they correspond to maize genomic DNA.

Analysis of kernel chemical composition from field trials in South America and Europe showed that 1507 maize was substantially equivalent to its non-GM comparator. Furthermore, appropriate animal feeding trials indicated that 1507 maize is nutritionally equivalent to its non-GM comparator.

Application EFSA-GMO-NL-2004-02 only concerns food uses for 1507 maize. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of the GM maize. The GMO Panel agrees that unintended environmental effects due to the establishment and spread of GM maize will not be different from those of maize bred traditionally. The monitoring plan provided by the applicant is in line with the intended uses for the GMO.

In conclusion, the GMO Panel considers that the information available for 1507 maize addresses the outstanding questions raised by the Member States and considers that 1507 maize will not have an adverse effect on human and animal health or the environment in the context of its proposed use.

This scientific opinion corresponds to the risk assessment report requested under Article 6(6) of Regulation (EC) No 1829/2003 and will be part of the overall opinion as required by Regulation (EC) No 1829/2003.

Key words: GMO, maize, *Zea mays*, 1507, insect protection, Cry1F, PAT, food safety, human health, cultivation, environment, import, Regulation (EC) 258/97, Regulation (EC) 1829/2003, Directive 90/220/EEC, Directive 2001/18/EC.

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BACKGROUND

On 10 June 2004 EFSA received from the Dutch Competent Authority an application submitted by Pioneer Overseas Corporation and Mycogen Seeds within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed (EC, 2003). The application was originally submitted to the Dutch Competent Authority under Article 4 of Regulation (EC) No 258/97 concerning novel foods and novel foods ingredients (EC, 1997) and covers foods consisting of or derived from genetically modified maize 1507. As indicated by the Dutch Competent Authority, the initial assessment was not finalised by 18 April 2004. In accordance with Article 46(1) of Regulation (EC) No 1829/2003, the application had, therefore, to be transformed into an application under Regulation (EC) No 1829/2003, following the procedures laid down in Regulation (EC) No 641/2004 (EC, 2004a). According to Article 5(5) of the latter Regulation, the transformed application shall be further processed as any other application under Article 5 of Regulation (EC) No 1829/2003. The application was named EFSA-GMO-NL-2004-02. Member States and the Commission were informed about the transformed application and the summary of the dossier was made publicly available on the EFSA website [2].

EFSA initiated a formal review of the application immediately, to check compliance of the dossier submitted with the requirements laid down in Article 5(3) of Regulation (EC) No 1829/2003. After receipt of additional information from the applicant on 20 August 2004 (requested by letter dated 9 August 2004), EFSA declared the application as valid and started the clock in accordance with Article 6(1) Regulation (EC) No 1829/2003 on 3 September 2004.

As initial steps in the administrative procedures and risk assessment, EFSA made the valid application available to the Member States and the Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC following the requirements of Article 6(4) Regulation (EC) No 1829/2003, to request their comments on the safety assessment of the genetically modified food. The Member State bodies had three months after the date of receipt of the request (until 3 December 2004) within which to make their opinion known. All comments were evaluated by the GMO Panel and taken into consideration for the further risk assessment. Comments on risk management issues, such as co-existence of different agronomic systems, were excluded from further considerations.

In delivering its opinion, the Panel considered the application, additional information provided by the applicant and comments submitted by the Member States. Further information from other applications for placing 1507 maize on the market under current regulatory procedures were taken into account where appropriate, as were comments from the Member States. The

[2] http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html

information from other applications referred to notification C/ES/01/01 for cultivation, import, processing and use as any other maize (excluding food uses) and notification C/NL/00/10 for import and processing. For regulatory reasons the latter applications resulted in separate opinions (EFSA, 2004b; EFSA, 2005).

In accordance with Article 6(1) of Regulation (EC) No 1829/2003 EFSA shall, in giving its opinion to the Commission, the Member States and the applicant, endeavour to respect a time limit of six months as from the receipt of a valid application. Apart from the requirements listed in Article 6(5) of Regulation (EC) No 1829/2003, the EFSA opinion shall include a report describing the assessment of the food and stating the reasons for its opinion and the information on which its opinion is based. This document is to be seen as the report requested under Article 6(6) of Regulation (EC) No 1829/2003 and thus will be part of the overall opinion as required by Regulation (EC) No 1829/2003.

TERMS OF REFERENCE

The GMO Panel was requested, in accordance with Article 6(6) of Regulation (EC) No 1829/2003, to carry out a scientific assessment of the genetically modified maize 1507 for food use.

Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Article 6(5)(e) of Regulation (EC) No 1829/2003.

The Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. The Panel did also not consider proposals for labelling and methods of detection which are matters related to risk management. The latter would include information on sampling and the identification of the specific transformation event in the food and/or foods produced from it.

ASSESSMENT

1. Introduction

GM 1507 maize was assessed with reference to its intended use and the appropriate principles described in the 'Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed' (EFSA, 2004c). In its evaluation the Panel also considered the issues that were raised by Member States during the initial assessment of the applications introduced under Directive 2001/18/EC and Regulation (EC) 258/97. The assessment presented here is based on the information provided in all available applications relating to GM 1507 maize submitted in the EU including additional information from the applicant in reply to Member States questions.

2. Molecular characterisation

2.1. Issues raised by Member States

(1) PCR analysis was requested to demonstrate the continuity of the DNA on both sides of the insert in comparison to the recipient plant; (2) a question over the presence of the detected sequences on both sides of the insert giving rise to instabilities of the insert was raised; (3) a question over the existence of a secondary insertion site detectable by Southern analysis was raised; (4) the possibility that very high levels of Cry1F toxin accumulated in specific tissues not subjected to analysis and which might be missed in the analyses was presented.

2.2. Evaluation of relevant scientific data

2.2.1. Transformation process and vector constructs

Embryogenic cells of Pioneer Hi-II maize were transformed using particle acceleration technology with tungsten particles coated with a purified linear fragment PHI8999A derived from plasmid PHP8999. For this purpose two restriction fragments of 6235 bp and 3269 bp were produced through *Pme* I-digestion of PHP8999. The larger fragment, named PHI8999A, was purified after agarose gel electrophoresis and the smaller fragment was discarded.

DNA fragment PHI8999A contains two adjacent plant gene expression cassettes. The first contains a truncated *cry*1F gene derived from the *Bacillus thuringiensis* ssp*. aizawai* sequence (Chambers *et al*., 1991). The coding sequence is regulated by a maize ubiquitin promoter and a maize ubiquitin intron sequence introduced upstream of the *cry*1F sequence. The 3' terminator sequence used is from the *Agrobacterium tumefaciens* mannopine synthase gene. The second expression cassette contains the *pat* gene from *Streptomyces viridochromogenes* (OECD, 1999) which is regulated by a CaMV 35S promoter and terminator. The coding sequence of both genes has been optimised to achieve a high level of expression in maize.

2.2.2. Transgenic constructs in the genetically modified plant

Southern transfer and hybridisation analysis showed the presence of a single insertion locus (comprising a complex structure of different fragments). The absence of vector backbone in the 1507 plants has been confirmed by Southern blotting using probes that cover the entire discarded 3269 bp fragment.

The nucleotide sequence of the insert in maize event 1507 has been determined in its entirety, as have sequences of the plant genome adjacent to the 3' and 5' sequences of the insert. Sequence analysis indicates that the insert comprises one almost complete copy[3] of fragment PHI8999A without internal rearrangements. Both *cry*1F and *pat* gene cassettes are intact within the transgenic event and the DNA sequences of the genes are identical to those in the original plasmid. The proteins produced in the GM plants are the ones intended, including a leucine residue (replacing a phenylalanine) at position 604 (of 605 amino acids in total) of Cry1F. This modification was introduced to create a specific restriction site for cloning purposes.

Southern analysis using a *cry*1F probe revealed the presence of two *cry*1F inserts. The first represents the intact gene from the expression cassette. The second insert is a truncated *cry*1F fragment of 335bp, which is located at the 5' end of the insertion locus. In addition, analysis of

[3] Base pairs 1-10 at the 5´ end and base pairs 6197-6235 at the 3´ end are missing. Both missing parts represent polylinker regions of fragment PHI8999A.

the sequences adjacent to the insert of fragment PHI8999A revealed DNA fragments that correspond to small segments from PHI8999A, including incomplete sequences from the *pat* gene, the maize ubiquitin promoter and the mannopine synthase terminator from *Agrobacterium*. Furthermore, different fragments of chloroplast DNA and a number of sequences with similarity to retrotransposons are also present in the border region of the insert.

PCR analyses indicated that the fragments in the flanking regions can also be found in the recipient line (Hi-II). No data documenting the intactness of the insertion site were shown. Therefore, a direct comparison of the insertion locus and the respective site in the recipient plant is not possible. Sequences found in the border regions showed a high degree of similarity to retrotransposon-like sequences that are considered to be very abundant throughout the maize genome. The design of PCR primers to provide unequivocal evidence that sequences detected in the flanking regions of the 1507 insert are also to be found as continuous sequences in the recipient plant is in general technically difficult. Thus, it cannot be assumed that DNA deletions have not occurred during the transformation process. There is, however, no indication that such a deletion produces any phenotypic effect in the transformed maize line (see Section 3.).

2.2.3. Information on the expression of the insert

Expression analysis of the Cry1F and the PAT proteins were carried out by Western analysis and ELISA. The tissues and plant samples examined were leaf, pollen, silk, stalk, whole plant and grain. The Cry1F protein was found in all tissues examined while the PAT protein could be detected only in leaf and whole plant.

Cry1F Western analyses with protein extracts from different plant tissues revealed a double band (65 to 68 kDa) in the range of the predicted size of 66 kDa which corresponds to the microbially produced Cry1F protein control. The smaller band detected in the 1507 protein extract is assumed to be the result of a limited N-terminal processing of the full size 1507 Cry1F protein during the extraction process by a plant protease with trypsin-like specificity. This assumption is supported by results from N-terminal amino acid sequencing of the protein which revealed a putative trypsin cleavage site starting at amino acid 28 (of 605) of the Cry1F protein. As no further bands were detected by Western analysis there is no evidence that unintended Cry1F-fusion proteins are expressed in 1507 maize.

As additional information, the applicant submitted tables including recalculated the data from Cry1F ELISA experiments. The data are presented on a ng Cry1F protein/mg tissue dry weight basis and show that the expression values fall within the same order of magnitude for cultivation in different years and at different locations. Maximum expression (on a tissue dry weight basis) was found in pollen (average 20.0 and maximum 29.3 ng Cry1F protein/mg tissue dry weight). The values for whole plant extracts ranged between 1.0 and 6.9 ng Cry1F protein/mg tissue dry weight and for kernels between 1.2 to 3.1 ng Cry1F protein/mg tissue dry weight. The expression of Cry1F was not influenced by the application of glufosinate.

Measurable expression levels of PAT protein were only found in leaves (<LOD[4] – 136.8 pg/µg TEP[5]) and whole plant extracts (<LOD – 38.0 pg/µg TEP) where the mean value for leaf was 42.0 pg/µg TEP and that for whole plant was below LOD. For kernels, all results were below LOD. Western analysis of PAT protein in leaves revealed only two bands of the expected size (ca. 22 kDa and 43 kDa [putative homodimer]). This indicates that no partial PAT proteins or fusion proteins were present at detectable levels.

[4] LOD = limit of detection

[5] TEP = total extractable protein

Bioinformatics analysis of the insert sequence indicates the presence, in addition to the two intended transcripts detected in the transgenic plant, of one further ORF of more than 300 bp length (ORF4: 630 bp) on fragment PHI8999A and a number of other ORFs (including ORF3, which is 753 bp long) spanning the junctions between maize DNA and DNA originating from the transformation fragment. This raises the possibility that new putative fusion proteins could be produced. A detailed analysis of the potential gene expression is provided for the two sequences longer than 300 bp (ORF3 and ORF4). No transcript corresponding to ORF3 was detected either by Northern or by RT-PCR analysis in experiments with mRNA from developing kernels. Northern analysis revealed no expression of ORF4 but a weak signal was detected using RT-PCR, which also indicated that the detected mRNA originates from a read-through product of the *cry*1F gene. In the very unlikely event that a protein were expressed from ORF4 on the read-through mRNA by using an alternative translation start codon or indeed if any of the other ORFs were transcribed and translated at a very low level, no adverse effects are expected as bioinformatics analysis revealed no significant homologies with known allergens. No known allergenic, toxic or gluten sensitive enteropathy-related proteins are encoded by these ORFs.

2.2.4. Inheritance and stability of inserted DNA

Event 1507 was produced in maize line Hi-II. The event was transferred to a Pioneer elite inbred line and the resulting plants backcrossed to the elite line for six generations. The Mendelian inheritance pattern of the traits was assessed together with the physical linkage of the target genes in resulting progeny. Southern blots and maintenance of the phenotype indicated genetic and phenotypic stability of the transgenic line and their progeny over several generations. No instability of the DNA sequences flanking the insert was observed.

2.3. Conclusion

GM maize line 1507 was generated through particle bombardment transformation of maize line Hi-II. Detailed molecular analysis of the insert and Mendelian inheritance of the trait indicated that one copy of fragment PHI8999A used for the transformation was inserted stably over several generations at a single locus in the maize nuclear genome. The inserted fragment is flanked by several fragments originating from the recipient maize plant chloroplast and nuclear genome and from fragment PHI8999A.

Evidence that the maize genomic DNA was contiguous with the flanking regions of the insert was not provided. The possibility of undetected deletions at the insertion site caused by the transformation process has been considered. The Panel is of the opinion that it is very unlikely that putative deletions or rearrangements at the insertion locus would result in undiscovered adverse effects. Firstly, a large proportion of the maize genome consists of non-coding sequences. Secondly, other elements of the overall risk assessment (see data provided in Section 3) show no indication of any unintended adverse effects. Thirdly deleted components will, in most cases, be complemented in commercial hybrids.

In conclusion, the Panel is of the opinion that the transgenic insert in 1507 maize was analysed and described sufficiently. None of the DNA stretches including the chloroplast DNA sequences detected in the insert region provide grounds for specific concern.

The intended expression of the PAT and Cry1F proteins was demonstrated and the expression levels were shown to be in the same range for different locations and growing seasons. The detection of a read-through mRNA comprising ORF4 sequences was shown. Bioinformatics assessment provided no indication that the development of allergenic or toxic products would arise in the very unlikely event that the read-through mRNA is translated to the respective protein.

Stability of inheritance of the newly inserted DNA and of the expression of the genes that code for Cry1F and PAT proteins in the transgenic plants was demonstrated.

3. Comparative analysis

3.1. Issues raised by Member States

(1) Additional data on lignin content were requested, based upon literature data indicating that these levels would be increased in transgenic maize lines expressing *B. thuringiensis* insecticidal proteins; (2) it was questioned whether levels of Cry1F in tissues of 1507 maize were significantly different over the locations and years; (3) data were requested on the levels of additional chemical substances including supplementary heavy metals, vitamins, and secondary metabolites.

3.2. Evaluation of relevant scientific data

3.2.1. Choice of comparator and production of material for the compositional assessment

1507 maize was compared with control hybrids that had not been genetically modified and that had background genetics representative of 1507 maize, except for the inserted genes.

Whole crops and maize tissues, including ears with kernels, were collected for compositional analysis from field trials. These field trials occurred during three seasons and at different locations (six locations in Chile (1998-1999), three locations in France and Italy (1999), and six locations in France, Italy and Bulgaria (2000). Maize plants in Chilean field trials were all treated with glufosinate, while those in the European field trials were split into treated and untreated groups.

3.2.2. Compositional analysis

For each season, the results of compositional analyses were provided for the individual and the combined locations.

The proximate and mineral analyses (fat, protein, acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, carbohydrate, phosphorus, and calcium) of forage from maize line 1507 (glufosinate-treated and untreated) were comparable to forage from the non-transformed version of the hybrid and within typical ranges reported in literature for commercial maize hybrids. Statistically significant differences were occasionally observed in some GM plants, for example increased overall levels of carbohydrates and decreased levels of fat in forage of maize line 1507 (both sprayed and non-sprayed) in the 2000 season. However, there were no differences that were consistently observed over years and at each location.

The compositional analysis of kernels of 1507 maize hybrid and its control included proximate analyses (as for forage above), fatty acid composition [palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3)], amino acids (twelve essential and six non-essential amino acids), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), vitamins (vitamin B1, vitamin B2, folic acid, and total tocopherols), secondary metabolites (inositol, raffinose, furfural, *p*-coumaric acid, and ferulic acid), and anti-nutrients (phytic acid and trypsin inhibitor). Kernels from the 2000 season were analysed additionally for crude fibre, arachidic acid, provitamin A, and vitamin E.

In summary, the analysis of nutrient composition of kernels from maize line 1507 (glufosinate-treated and non-treated) occasionally revealed statistically significant differences in some compounds. For example, kernels of 1507 maize contained higher overall levels of potassium, linoleic acid, linolenic acid, and tocopherols, as well as lower levels of fat, manganese, stearic acid, oleic acid, cysteine, methionine, and vitamin B1, than control kernels in the 1998-1999 season. The levels of protein, amino acids (Ala, Asp, Glu, Gly, His, Leu, Phe, Pro, Ser, Thr, Tyr, and Val), and potassium were increased, while the level of vitamin B2 was decreased, in kernels of 1507 maize (both sprayed and non-sprayed) compared with control kernels in 1999. In the 2000 season, ash, amino acids (Ala, Phe, Tyr), and potassium were increased, while manganese was decreased in kernels of maize line 1507 (both sprayed and non-sprayed) compared with controls. Across locations and between years, however, there were no consistent statistically significant differences. All analytical data were either very close to or within the ranges published in the literature.

It has been suggested that lignin levels might be increased in transgenic maize lines expressing *B. thuringiensis* insecticidal proteins (Saxena & Stotzky, 2001; Flores *et al*., 2005). However, a broader and more extensive study on lignin content in *Bt*-maize does not support this conclusion (Jung & Sheaffer, 2004). In addition, as mentioned above, the levels of ADF and NDF, which comprise lignin, in forage of 1507 maize were comparable with those in control maize and within the background range. Moreover, similar levels were observed for the lignin precursors *p*-coumaric acid and ferulic acid in kernels of 1507 maize and control maize, except for a small but statistically significant difference in *p*-coumaric acid between sprayed 1507 maize and control maize in the 2000 season.

Aside from minor modifications, the selection of compounds analysed followed the recommendations of OECD (OECD, 2002). During the Member State consultation under Article 6.4 of Regulation (EC) No. 1829/2003, it was suggested that additional compounds, including certain heavy metals, vitamins, and secondary metabolites, should be analysed. The Panel is of the opinion, however, that such additional information would not add value to the data that had already been provided, given, among other things, the high variability of the levels of some compounds like selenium and DIMBOA[6], due to environmental conditions or the stage of plant development.

3.2.3. Agronomic traits and GM phenotype

Studies of plant biology and canopy morphology complemented extensive agronomic data and confirmed the similarity of 1507 maize to its non-transgenic counterpart.

During field trials over several seasons and at different locations (USA in 1999, France, Italy, and Bulgaria in 2000, Spain in 2002) extensive agronomic data (germination as early stand counts, visual ratings of development, accumulated heat units to pollen shed and silking, stalk and root lodging, plant height, ear height, final population, date/time of leaf senescence, disease incidence, insect damage, grain moisture and density) were collected and confirmed the similarity of 1507 maize phenotype to its non-transgenic counterpart.

Slight differences in accumulated heat units to pollen shed and silking under infestation were reported and are regarded as indicative of small differences in the genetic background of the GM- and non-GM-hybrids. No differences in the general appearance of the plants or other phenotypical differences that would indicate unexpected pleiotropic effects of the genetic modification were found.

[6] DIMBOA = 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, a metabolite naturally formed by maize plants.

3.3. Conclusion

Based on the results of compositional analysis of samples from a representative range of environments and grown in three seasons, it is concluded that forage and kernels of 1507 maize are compositionally equivalent to those of conventional maize, except for the presence of Cry1F and PAT proteins in 1507 maize.

In addition, experimental field trials in the USA and Europe did not show indications for unexpected changes of agronomic characteristics and performance.

4. Food/feed safety assessment

4.1. Issues raised by Member States

(1) Bioinformatic analysis was requested to compare the conformations of MR872 (microbially produced, trypsinised *Bt* toxin) and the plant-expressed Cry1F protein; (2) it was argued that the Cry1F produced by plants might differ from the *Bt* toxin produced by bacteria, *e.g.* with regard to posttranslational modifications besides glycosylation; (3) further animal feeding studies, including tests on ruminants, laying hens, pigs, fish, and crustaceans, with whole products, including forage, derived from 1507 maize were requested; (4) additional toxicological studies comprising various trials, including chronic testing were requested; (5) clarification on the decreased average eosinophil counts in female rats fed diets containing 33% 1507 maize was requested.

4.2. Evaluation of relevant scientific data

4.2.1. Product description and intended use

Application EFSA-GMO-NL-2004-02 covers foods consisting of or derived from genetically modified maize 1507 . The feed uses of 1507 maize and the aspects of cultivation, import and processing are covered by other applications[7]. Maize plants and kernels are used mainly for animal feed but, on a smaller scale, sweet maize kernels are also used for direct human consumption. Products from maize kernels such as flour, starch (also transformed to sweeteners *e.g.* as syrups) and maize germ oil can be regarded as important base materials for food production.

As the modification in 1507 maize is only intended to improve the agronomic performance but not to influence nutritional aspects, production processes and overall use of maize as a crop are not expected to be influenced as a result of the introduction of the GM plants to the market.

4.2.2. Stability during processing

Experimental fish feed containing 38.7% maize meal was prepared in order to test the stability of Cry1F during processing. The Cry1F level in transgenic maize kernels was 2.2-3.5 ng/mg tissue dry weight prior to processing. The production of fish feed included an extrusion step, exposing feed ingredients to high pressure and temperature. Cry1F was not detectable in the final product, as established through an insect bioassay and immuno-assay (ELISA – LOD = 0.04 ng/mg tissue dry weight).

[7] Notifications C/ES/01/01 and C/NL/00/10 submitted under Directive 2001/18/EC

In addition, the thermostability of recombinant Cry1F protein produced by *Pseudomonas fluorescens* at elevated temperatures was assessed by heating solutions of 1.3 ppm Cry1F in phosphate buffer pH 7.5 at 60, 75, or 90ºC for 30 minutes. Samples were taken from these solutions and added to feed used in a bioassay for insecticidal activity on tobacco budworm (*Heliothis virescens*). It was thus observed that the Cry1F proteins heated at 75 and 90ºC had lost their insecticidal activity.

4.2.3. Toxicology

4.2.3.1. Cry1F and PAT proteins used for safety assessment

Given the low expression levels of Cry1F in 1507 maize, the applicant decided to use a trypsinised microbial analogue, MR872, of the truncated Cry1F protein expressed in maize line 1507 for safety testing. To this end, a fusion protein consisting of the non-truncated Cry1F (N-terminal) linked to Cry1Ab (C-terminal) was produced by recombinant *Pseudomonas fluorescens*. Trypsin cleavage sites in Cry1F are located between residues 28-29, 31-32, and 612-613. Enzymatic cleavage with trypsin of the fusion protein yielded a 'core' protein, MR872, identical to the truncated Cry1F protein expressed in 1507 maize, except for i) phenylalanine (Phe) instead of leucine (Leu) at position 604 and ii) a C-terminal extension of trypsinised MR872 with seven amino acid residues (606-612, Ala-Glu-Tyr-Asp-Leu-Glu-Arg). With regard to the conformation of Cry1F, it is considered unlikely that the substitution at position 604 would lead to conformational changes because both Phe and Leu are amino acids with hydrophobic side chains. The extension of the trypsinised MR872 protein with seven amino acids at the C-terminus of domain III is also present in native Cry1F from *B. thuringiensis*, as well as in other Cry proteins. Comparison of the crystal structure of Cry1Aa containing this extension (Grochulski *et al*., 1995) with that of Cry3A lacking this extension (Li *et al*., 1991) does not indicate differences in the overall structure of Domain III. It is therefore unlikely that this extension would affect the functional, toxicological, or allergenic properties of the protein.

Both bacterially produced Cry1F and plant-expressed Cry1F isolated from leaves and kernels of 1507 maize displayed a prominent 65 kDa band on Western blots, which corresponds to the N-terminally processed form of plant-expressed Cry1F as mentioned in Section 2.2.3. Glycosylation was analysed after SDS PAGE using a commercial staining kit. The results demonstrate that the plant-expressed Cry1F is not glycosylated. Moreover, MALDI-TOF mass spectrometry was performed on trypsin-digests of the recombinant Cry1F proteins produced by transgenic *P. fluorescens* and 1507 maize and separated by electrophoresis. Fragments were observed in the spectra of both types of Cry1F protein that concurred with the predicted masses of peptides derived from trypsin digestion, covering 34-39 percent of the total protein sequence (605 amino acids) encoded by the *cry*1F transgene in 1507 maize in various experiments. Data provided by the applicant on insect bioassays with recombinant Cry1F show no notable differences between preparations of this protein isolated from transgenic maize event 1360 (modified with Cry1F) and *P. fluorescens*.

Taking into account all the evidence provided, the Panel is of the opinion that the trypsinised MR872 analogue is an appropriate substitute of the Cry1F protein expressed in 1507 maize for safety testing.

Bacterially produced recombinant PAT showed the same electrophoretic mobility as PAT expressed in 1507 maize during Western blotting. As noted above, levels of PAT were not quantifiable in kernels of 1507 maize.

4.2.3.2. Toxicological assessment of expressed novel proteins in 1507 maize

(a) Acute oral toxicity

An acute oral study was performed in albino mice dosed with 576 mg truncated Cry1F/kg bodyweight (5050 mg/kg test material containing 11.4% Cry1F). No effects related to the administration of Cry1F were noted on bodyweight, gross necropsy, and mortality 14 days after the administration, except for one incidental finding out of 10 of lack of body weight gain between days 7 and 14.

For PAT, a study was performed, in which mice received 5000 mg PAT/kg bodyweight (equal to 6000 mg test material/kg). After two weeks, no effects on bodyweight and gross pathology were noted.

(b) Degradation in simulated digestive fluids

The trypsin-resistant core of the microbially produced Cry1F protein was rapidly degraded (<1 minute) in simulated gastric fluid at a Cry1F/pepsin molar ratio of 188:1 and 1:22. In the SDS PAGE gels of the incubation mixture, a 10-kDa band was visible that was relatively stable during the period of the experiments. This was probably a contamination of the microbial Cry1F preparation, as it was not detected in Western analysis with anti-Cry1F immune sera.

In simulated intestinal fluid (pancreatin), the trypsin-resistant Cry1F core protein proved stable over the entire exposure of 120 minutes.

For degradation of the PAT protein, reference is made to previous studies in which PAT was degraded within 5 seconds in simulated gastric fluid.

4.2.3.3. Toxicological assessment of new constituents other than proteins

Since no new constituents other than the above mentioned proteins were expressed in 1507 maize, nor were levels of endogenous compounds altered, a toxicological assessment is not applicable.

4.2.4. Toxicological assessment of the whole GM food/feed

4.2.4.1 Subchronic oral toxicity

A 90-day oral toxicity study has been performed on rats in five groups (12 animals/sex/group) fed diets containing 1507 maize (11 and 33%), a non transgenic control line with comparable genetic background (11 and 33%), and another non transgenic maize line as reference (33%). The diets were analysed for nutrients, antinutrients, mycotoxins, pesticides, heavy metals, transgenic DNA, and Cry1F (insect bioassay). Kernels used in this study were obtained from 1507 maize plants that had not been treated with glufosinate. The measurements on animals included feed consumption, body weight, clinical pathology (serum, blood, urine), and anatomical pathology (organ weights, histopathology).

A statistically significant increase in feed consumption was observed in male rats fed 33% 1507 maize compared with rats fed control maize, but not to those fed the reference maize (27.5 ± 2.6, 25.7 ± 1.7, and 27.3 ± 1.7 g per day, respectively). This effect is therefore not considered to pose concerns over the safety and nutritional value of 1507 maize. In addition, serum counts of eosinophil leukocytes were statistically significantly decreased in female rats fed 33% 1507 maize compared with those fed 33% near isogenic control and reference maize.

The observed differences were not considered to be biologically relevant, since (1) it was observed in one sex only, (2) this was an isolated finding in a series of haematological parameters, and (3) the inherent variability of the measured parameter. A number of histopathological changes were observed, in particular inflammation of the liver, nephropathy, and cardiomyopathy (kidney and heart damage) in animals of both sexes. To a lesser degree, inflammation of the prostate in males and the pancreas in females, fatty change in the liver of females, and atrophy of the pancreas in males were observed. These effects were not linked to the test-substance, since their incidences were not elevated substantially in the animals fed 1507 maize compared to control animals. This study, on the basis of presented results, is considered satisfactory and does not raise concerns over the safety of 1507 maize.

4.2.5. Allergenicity

The strategies in assessing the allergenic risk concentrate on characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food. A weight of evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (EFSA, 2004c; CAC, 2003).

4.2.5.1 Assessment of allergenicity of the newly expressed proteins

The PAT protein has been previously evaluated for its safety in the frame of other applications for the placing of PAT-expressing GM crops on the market. The potential allergenicity of the transgenic Cry1F protein and of the theoretical expression products of ORF4 (within the PHI8999A copy of the insert), and 24 ORFs (including ORF3) coding for putative fusion proteins in the regions adjacent to the PHI8999A copy of the insert were considered in this dossier.

The amino acid sequence of the Cry1F protein has been compared with the sequences of allergenic proteins compiled in an allergen database[8]. This comparison focused on two types of identity between Cry1F and allergens: (1) short linear stretches; with a relevant minimum size of eight contiguous amino acids and (2) overall identity of 80-amino-acid peptides of Cry1F (min. 35% identity relevant).

For both types of comparison, the FastA algorithm was applied, with appropriate settings. No outcomes were equal to or exceeded the minimum relevant size. The length of the longest identical short linear stretch, for example, was six amino acids.

In addition, comparison of the Cry1F sequence against a general protein database yielded predominantly homologies with other Cry-proteins (*e.g.* Cry1Ab with 52.4% identity over a 614 residue alignment overlap), except for three proteins from *Methanosarcina acetivorans*, *Saccharomyces cerevisiae,* and *Sinorhizobium meliloti*. These three proteins are not known to be toxic and therefore this result does not indicate any homology of the Cry1F with toxic proteins.

Three different linear six-amino acid stretches were found to be shared by Cry1F with allergenic proteins (Der p 7 from house dust mite, beta-1,3-glucanase-like protein from olive, and Can f 3 from dog dander). The EFSA panel is aware of studies that show that using a threshold of six amino acids for identical stretches between a given protein and allergens yields a high number of false positives, *i.e.* this threshold makes the comparison non-specific. Using a newly

[8] Applied update: March 2002 - comprises 2033 entries compiled from published lists supplemented through a search of public domain protein databases.

developed methodology (Soeria-Atmadja *et al*., 2004), the Swedish National Food Authority found that for Cry1F, many six-amino acid identities with non-allergenic proteins existed (data not published). Kleter & Peijnenburg (2002) further found that many transgenic proteins shared identical six- and seven-amino acid stretches with allergens. For the identical sequences that Cry1F shared with allergens (the same as found by the applicant) these authors found no indications that they were part of IgE-epitopes. Therefore it is unlikely that these identical stretches within Cry1F would induce allergic reactions.

In addition, the highest degree of similarity of 80-residue fragments of Cry1F was 33.8% identity (27 residues) with a pollen allergen (Syr v I) from *Syringa vulgaris* and with related olive pollen allergens.

Because the minimum relevant matches are eight-amino-acid linear sequences and 35% identity of 80-residue fragments, respectively, the search has yielded no outcomes that raise safety concerns for Cry1F.

The same methodology to search for short identical and larger similar stretches of homology to the proteins listed in the allergen database has been applied to assess the hypothetical peptides derived from ORF4 (within the copy of the PHI8999A sequence on the insert) and the 24 ORFs (including ORF3) coding for putative fusion proteins in the regions adjacent to the PHI8999A copy on the insert. In addition, the ORF3 and ORF4 sequences were compared with the sequences of a general protein database.

For ORF 4, the longest identical short linear stretch, for example, was six amino acids, shared with allergenic proteins from durum wheat (glutenin) and wheat (gamma-gliadin). An 80-residue fragment of ORF4 shared twenty-two identical residues (27.5%) with major hazel pollen allergen Cor a 1. In a comparison of ORF4 to general protein sequences, the protein from ORF VI of Cauliflower Mosaic Virus, followed by proteins from Carnation Etched Ring virus and *Plasmodium falciparum,* were most identical to the ORF4 sequence.

ORF3 shared two identical linear sequences of six amino acids with the allergen Gly m IA from soybean and with the allergens gamma-gliadin and alpha/beta-gliadin from wheat. In addition, an 80-residue fragment of ORF3 shared eighteen identical residues (22.5%) with the allergenic barley alpha amylase/trypsin inhibitor precursor and also with Sin a I allergen from white mustard. The highest scoring identities of the sequence of ORF3 with general protein sequences in a public database were those with chloroplast RNA polymerases of various plants and with phosphinothricin acetyltransferase enzymes. Some of the other 23 ORFs in the flanking regions shared six-amino acid identities with allergens. However, none of these ORFs shared relevant homologies with allergens consisting of identical linear sequences of a minimum of eight-amino acids or 35%-identities of 80-amino acid subsequences. In the comparison of these ORFs with a general protein database, none of the sequences sharing the most relevant identities with the ORFs were known to be toxic. The sequence homologies that have been found, therefore, do not raise concerns over the safety of 1507 maize that would justify additional studies regardless the fact that those ORFs are very unlikely to be transcribed and/or translated into peptides or proteins.

The degradation of gene products during processing at high temperature and in simulated digestive fluids, which is also relevant for the assessment of potential allergenicity, has been discussed in Sections 4.2.2 and 4.2.3.

Based on all information made available, the Panel considers that the newly expressed proteins are not likely to be allergenic.

4.2.5.2 Assessment of allergenicity of the whole GM plant or crop

Allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, for example through qualitative or quantitative modifications of the pattern of expression of endogenous proteins. This issue does not appear relevant to the Panel since maize is not considered a major allergenic food and possible over-expression of any endogenous protein that is not known to be allergenic would be unlikely to alter the overall allergenicity of the whole plant. The same considerations also apply for exposure by inhalation.

4.2.6. Nutritional assessment of GM food/feed

A 42-day feeding study was carried out with broilers to investigate nutritional equivalency. Diets contained on average 55% dry matter (DM) maize kernels from either the transgenic hybrid 1507 maize, the control hybrid maize Mycogen 7250, and four commercial maize hybrids. Each diet was fed to 35 animals (divided into 7 replicates of 5 animals). No statistically significant differences were observed for mortality, body weight, body weight gain, and feed conversion between the different maize lines.

Twenty lactating dairy cows were used in a single cross-over design in which there was 2 x 28-day feeding periods. The aim was to compare the effect of using maize silage and maize kernels derived from transgenic 1507 maize on feed intake and milk production when compared with maize silage and maize kernels derived from non-GM control hybrids.

Diets contained on average 43.0% DM maize silage and 22.1% concentrate of which 70.2% was in the form of ground maize. Other feed ingredients included alfalfa hay, soybean meal, and cotton seeds. The diet composition was analysed for proximates, minerals (Ca, P, Mg, K), mycotoxins and silage fermentation products and found to be similar for both treatment groups. Cry1F was detected in transgenic maize kernels and silage. PAT was not detectable in kernels, and ranged from not detectable to slightly above the detection threshold in forage, of 1507 maize.

The following measurements were made: (1) Physical (weekly): body weight, condition, temperature, pulse, feed intake; (2) Milk production (daily); (3) Milk composition (weekly): protein, fat, dry matter, lactose, urea N, somatic cell count, Cry1F; (4) Blood analysis (prior to and at the end of both trials): chemical and haematological.

One cow was positive for the presence of Cry1F in milk prior to and during both treatments, which can therefore be considered a false positive ELISA-reaction.

In conclusion, results showed no significant differences between dietary treatments and indicate nutritional equivalence between the transgenic 1507 maize and the non-GM control.

4.2.7. Post-market monitoring of GM food/feed

1507 maize is intended to have improved agronomic properties. From a nutritional point of view the maize is equivalent to conventionally bred hybrids. Therefore the GM plants will be used as any other maize and only replace a part of the overall maize products within the European market. The risk assessment concluded that no data have emerged to indicate that maize line 1507 is any less safe than its non-GM comparators. The opinion of the applicant that a post-market monitoring of the GM food/feed is not necessary is in line with the guidance document of the GMO Panel for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2004c) and is shared by the GMO Panel.

4.3. Conclusion

The transgenic Cry1F protein showed no adverse effects in an acute oral mouse study. In addition, Cry1F displayed instability towards conditions that prevailed during the production of fish feed including heating and was rapidly degraded in simulated gastric fluid.

The sequence of the transgenic Cry1F did not show any significant similarity with the sequences of known allergens. Neither the hypothetical peptide sequences corresponding to 24 ORFs that are present on the insert in 1507 maize nor ORF4 on fragment PHI8999A show significant similarity to allergens or toxins.

With regard to animal studies with the whole product, no oral toxicity of 1507 maize was observed in a 90-day rat study. In addition, nutritional data comprising target animal feeding studies with the whole maize kernel on broilers and dairy cows indicate that 1507 maize is nutritionally equivalent to other conventional maize cultivars. These animal studies therefore further support the findings of the compositional analysis of no effect beyond the intended introduction of the PAT and Cry1F proteins.

Based on the data provided, the Panel is of the opinion that there is no need for additional chronic toxicity testing, nor for testing in other target animal species.

5. Environmental risk assessment and monitoring plan

5.1. Issues raised by Member States

Concerns were expressed that (1) the environmental risk assessment did not adequately address environmental exposure and (2) that the monitoring plan provided by the applicant was not sufficient.

5.2. Evaluation of relevant scientific data

5.2.1. Environmental risk assessment

5.2.1.1. Potential unintended effects on plant fitness due to the genetic modification

Application EFSA-GMO-NL-2004-02 is for the placing on the market of foods consisting of or derived from 1507 maize only. Maize is highly domesticated and not generally able to survive in the environment without cultivation. Maize plants are not winter hardy in most parts of Europe, they have lost their ability to release seeds from the cob and they do not occur outside cultivated land in Europe, despite cultivation for many years. In addition, there are no cross-compatible wild relatives in Europe, and gene flow via pollen is largely restricted to neighbouring crops. Maize is a hybrid crop and thus imported grain will be a segregated F2 generation and not as fit as the F1 in case of accidental spillage. Field experiments carried out in France, Italy, Bulgaria and in South America demonstrated that 1507 maize has no altered survival, multiplication or dissemination characteristics. The Panel agrees with the assessment that the likelihood of unintended environmental effects due to the establishment and spread of 1507 maize will be no different to that of traditionally bred maize.

5.2.1.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, DNA in case of horizontal gene transfer and pollen in case of vertical gene flow through cross-pollination.

Exposure of microorganisms to transgenic DNA derived from GM maize plants takes place in the environment during natural decay of transgenic plant material, such as GM plant parts, in agricultural areas and/or pollen in nearby natural ecosystems as well as in cropped fields.

Transgenic DNA is a component of some or most of the food and feed products derived from the GM maize. Therefore microorganisms in the digestive tract of humans and animals (domesticated animals and other animals feeding on fresh and decaying GM plant material) may be exposed to transgenic DNA.

Transgenic pollen is shed and distributed from cultivated GM hybrids or from plants resulting from the adventitious presence of GM kernels in conventionally bred maize seeds. A further but less likely pathway of dispersal of transgenic maize pollen is the flowering of volunteer GM maize plants originating from accidental seed spillage during transport and/or processing. For *Zea mays* any vertical gene transfer is limited to other maize plants as populations of sexually compatible wild relatives of maize are not known in Europe.

(a) Plant to bacteria gene transfer

Based on present scientific knowledge and elaborated recently in more detail (EFSA, 2004d), gene transfer from GM plants to bacteria under natural conditions is extremely unlikely, and would occur primarily through homologous recombination in microbes.

The *cry*1F gene and the *pat* gene expressed in the 1507 maize are under the control of eukaryotic promoters with limited if any activity in prokaryotic organisms. Genes under control of prokaryotic regulatory elements conferring the same traits as expressed in the GM plants are widespread in microorganism in natural environments.

Taking into account the origin and nature of these genes and the lack of selective pressure in the intestinal tract and/or the environment, the likelihood that horizontal gene transfer would confer selective advantages or increased fitness on microorganisms is very limited. For this reason it is very unlikely that genes from 1507 maize would become established in the genome of microorganism in the environment or human and animal digestive tract. In the very unlikely event that such a horizontal gene transfer would take place, no adverse effects on human and animal health and the environment are expected as no principally new traits would be introduced into microbial communities.

(b) Plant to plant gene transfer

The extent of cross-pollination to conventionally bred hybrids will mainly depend on the scale of accidental release and/or adventitious presence in conventional seeds.

As shown in several field trials there are no indications for an altered ecological fitness of the GM maize in comparison to conventionally bred hybrids with similar genetic background.

The herbicide resistance trait can only be regarded as providing a selective advantage where and when glufosinate-ammonium containing herbicides are applied, *i.e.* mainly on arable land. Insect protection against lepidopteran pests is also not regarded as providing a selective advantage for maize in Europe, as the survivability is mainly limited by the absence of a dormancy phase, susceptibility to fungi and susceptibility to cold climate conditions. Therefore, as for any other maize cultivars, it is considered very unlikely that volunteers could survive until subsequent seasons or would establish undesirable populations under European environmental conditions.

5.2.1.3. Potential interactions of the GM plant with non-target organisms

There is an issue that gene products, particularly Cry proteins might enter the environment either from the gastrointestinal tracts of animals (manure and faeces), through horizontal gene flow to bacteria or as part of waste waters and/or dusts from food production. Data supplied by the applicant and other literature suggests that most protein would be denatured by enzymatic activity in the gastrointestinal tract so that little Cry toxin would survive to pass out in faeces. There would subsequently be further degradation of proteins in the manure due to microbial processes. Thus amounts of Cry proteins being distributed onto land in manure would be very low, minimising the possibility for exposure of potentially sensitive non-target organisms.

The GMO Panel considered possible differences between the plant expressed and the microbially-derived Cry1F protein regarding potential effects on non-target organisms. Equivalence tests showed that the activity and structure of Cry1F proteins derived from plant and microbe are comparable. Furthermore, the amino acid sequence of the biologically active core, immunoreactivity, glycosylation and biological activity were comparable between plant-expressed and microbially-produced protein.

5.2.1.4. Monitoring

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct and to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment. The scope of the monitoring plan provided by the applicant is in line with the intended uses for the GMO since the environmental risk assessment did not cover cultivation.

5.3. Conclusion

Application EFSA-GMO-NL-2004-02 only covers food uses of 1507 maize and thus there is no requirement for scientific information on environmental effects associated with cultivation. Maize is highly domesticated and not able to survive in the environment without cultivation. The Panel agrees that unintended environmental effects due to the adventitious establishment and spread of GM maize will be no different to that of traditionally bred maize. The scope of the monitoring plan provided by the applicant is in line with the intended uses for the GMO since the environmental risk assessment did not cover cultivation.

CONCLUSIONS AND RECOMMENDATIONS

Maize line 1507 has been developed for protection against lepidopteran pests by expressing the Cry1F Protein and for tolerance to glufosinate by the introduction of a *pat* gene. The GMO Panel has assessed information provided on molecular inserts within the transgenic event, on the safety of the proteins expressed and on the potential for risks associated with any changes to the nutritional, toxicological and allergenic properties of 1507 maize. Analysis of the chemical composition of the maize and field trial data were also used to assess the potential for changes to safety, nutritional as well as agronomic parameters. No data have emerged to indicate that maize line 1507 is any less safe than its non-GM comparators.

The GMO Panel considers that 1507 maize will have similar impacts as other comparable non-GM maize cultivars on the environment. The only adverse effect identified was the possibility that resistance to *Bt* toxin might evolve in corn borers exposed to 1507 maize following cultivation for some years. The Panel accepts the monitoring plan developed by the applicant to monitor specifically for resistance in corn borers and recommends that cultivation should be accompanied by appropriate risk management strategies to minimise exposure of both target and non-target insects to *Bt* toxins. In addition, the Panel accepts in principle the general surveillance plan submitted by the applicant.

The GMO Panel is therefore of the opinion that there is no evidence to indicate that placing of maize line 1507 and derived products on the market is likely to cause adverse effects on human or animal health or the environment in the context of its proposed use.

The authorisation of the complementary herbicide is not within the remits of this opinion and is covered by other legal frameworks of the EU and Member States.

The GMO Panel is of the opinion that, based on the outcome of the risk assessment, no specific conditions or restrictions should be imposed on the placing of 1507 maize on the market for food use. No specific conditions or restrictions for food use and handling, including post-market monitoring requirements regarding the use of 1507 maize for human consumption, are regarded as necessary. Furthermore, there is no need for specific conditions for the protection of particular ecosystems/environment and/or geographical areas.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Dutch Competent Authority (Ministerie van Volksgezondheid, Welzijn en Sport, Directie Voeding en Gezondheidsbescherming), dated 4 June 2004 concerning the submission to EFSA of application 1507 maize within the framework of Regulation (EC) 1829/2003 (Ref. VGB/VL 2487154).

2. Letter from Guy Van Den Eede (IHCP-JRC-Ispra/Italy), dated 27 July 2004, concerning the completeness check of application EFSA-GMO-NL-2004-02 in accordance with Article 5(3)(i) and (j) and Article 17(3)(i) and (j) of Regulation (EC) 1829/2003 (ref. JRC I06-BGMO/GVDE/D/2004 (56) 19688).

3. Letter from EFSA to applicant, dated 3 September 2004, concerning the "Statement of Validity" for application EFSA-GMO-NL-2004-02, 1507 maize submitted under Regulation (EC) 1829/2003 (Ref. SR/KL/jq (2004)631).

4. Submission of the application EFSA-GMO-NL-2004-02 by Pioneer/Mycogen Seeds to EFSA, containing:

   Part I – technical dossier
   Part II – summary
   Part III – Cartagena Protocol
   Part IV – labelling proposal
   Part V – samples and detection method
   Part VI – additional information for GMOs

5. The following application dossiers concerning 1507 maize including assessment reports, the respective Member States comments/objections and additional information submitted by Pioneer/Mycogen Seeds were considered where appropriate:

a. Notification (C/NL/00/10) to market products containing genetically modified organisms in accordance with Directive 2001/18/EC submitted by Pioneer/Mycogen Seeds to EFSA on 26 March 2004.
b. Application for placing on the market of novel foods and novel food ingredients containing genetically modified organisms in accordance with Regulation (EC) 258/97 submitted by Pioneer/Mycogen Seeds to EFSA on 26 March 2004.
c. Notification (C/ES/01/01) to market products containing genetically modified organisms in accordance with Directive 2001/18/EC submitted by Pioneer/Mycogen Seeds to EFSA on 19 May 2004.

REFERENCES

CAC, 2003. Codex principles and guidelines on foods derived from biotechnology. Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation, Rome. ftp://ftp.fao.org/codex/standard/en/CodexTextsBiotechFoods.pdf

Chambers, J.A., Jelen, A., Gilbert, M.P., Jany, C.S., Johnson, T.B.& Gawron-Burke, C., 1991. Isolation and characterization of a novel insecticidal crystal protein gene from *Bacillus thuringiensis* sbsp. *aizawai*. J. Bacteriol., 173(13), 3966-3976.

EC, 1991. Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ, L230, 1-32. http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31991L0414&model=guichett

EC, 1997. Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients OJ, L43, 1-6. http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31997R0258&model=guichett

EC, 2001 Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities, L106, 1-39. http://europa.eu.int/eur-lex/pri/en/oj/dat/2001/l_106/l_10620010417en00010038.pdf

EC, 2003. Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ, L268, 1-23. http://europa.eu.int/eur-lex/pri/en/oj/dat/2003/l_268/l_26820031018en00010023.pdf

EFSA, 2004a. Commission Regulation (EC) No 641/2004 of 6 April 2004 on detailed rules for the implementation of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the application for the authorisation of new genetically modified food and feed, the notification of existing products and adventitious or technically unavoidable presence of genetically modified material which has benefited from a favourable risk evaluation. OJ L102, 14-25. http://europa.eu.int/eur-lex/pri/en/oj/dat/2004/l_102/l_10220040407en00140025.pdf

EFSA, 2004b. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/NL/00/10) for the placing on the market of insect-tolerant genetically modified maize 1507, for import and processing, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds, The EFSA Journal, 124, 1-18. http://www.efsa.eu.int/science/gmo/gmo_opinions/663_en.html

EFSA, 2004c. Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. The EFSA Journal 99, 1-94. http://www.efsa.eu.int/science/gmo/gmo_guidance/660_en.html

EFSA, 2004d. Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants, The EFSA Journal 48, 1-18 http://www.efsa.eu.int/science/gmo/gmo_opinions/384_en.html

EFSA, 2005. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507, for import, feed and industrial processing and cultivation, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds, The EFSA Journal (2005) 181, 1-33. http://www.efsa.eu.int/science/gmo/gmo_opinions/catindex_en.html

Flores, S., Saxena, D. & Stotzky, G., 2005. Transgenic *Bt* plants decompose less in soil than non-*Bt* plants. Soil Biology & Biochemistry, in press.

Grochulski, P., Masson, L., Borisova, S., Pusztai-Carey, M., Schwartz, J.L., Brousseau, R., & Cygler, M., 1995. *Bacillus thuringiensis* CryIA(a) Insecticidal Toxin: Crystal Structure and Channel Formation. J. Mol. Biol. 254(3), 447-464.

Jung, H.G. & Sheaffer, C.C., 2004. Influence of *Bt* Transgenes on Cell Wall Lignification and Digestibility of Maize Stover for Silage. Crop Science, 44, 1781-1789.

Kleter, G.A. & Peijnenburg, A.A.C.M., 2002. Screening of transgenic proteins expressed in transgenic food crops for the presence of short amino acid sequences identical to potential, IgE-binding linear epitopes of allergens. BMC Structural Biology, 2, 8. http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=139984&action=stream&blobtype=pdf

Li, J.D., Carroll, J. & Ellar, D.J., 1991. Crystal structure of insecticidal delta-endotoxin from *Bacillus thuringiensis* at 2.5 A resolution. Nature, 353, 815-21.

OECD, 1999. Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide. Series on Harmonization of Regulatory Oversight in Biotechnology No. 11. Organisation for Economic Co-operation and Development (OECD), Paris. http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono(99)13

OECD, 2002. Consensus Document on Compositional Considerations for New Varieties of Maize (*Zea Mays*): Key Food and Feed Nutrients, Anti-nutrients and Secondary Plant Metabolites. Series on the Safety of Novel Foods and Feeds, No. 6, Organisation for Economic Co-operation and Development (OECD), Paris. http://www.olis.oecd.org/olis/2002doc.nsf/LinkTo/env-jm-mono(2002)25

Saxena, D. & Stotzky, G., 2001. *Bt* corn has a higher lignin content than non-*Bt* corn. Am. J. Bot., 88, 1704-1706.

Soeria-Atmadja, D., Zorzet, A., Gustafsson, M &Hammerling, U., 2004. Statistical evaluation of local alignment features for prediction of allergenicity using supervised classification algorithms. Int. Arch. Allergy Immunol., 133, 101-112.

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