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[1]

(Question No EFSA-Q-2004-072)

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.

The opinion is based on a question raised by the Commission relating to an application for the placing on the market of 1507 maize under Directive 2001/18/EC. The GMO Panel was asked to consider whether there is any scientific reason to believe that placing 1507 maize on the market, for cultivation, import, processing and use as any other maize (excluding food uses), is likely to cause any adverse effects on human health and the environment (Notification C/ES/01/01). The question followed a scientific assessment which was made initially by the Competent Authorities of Spain and evaluated subsequently by all other Member States. An assessment of the 1507 maize was requested by the Commission because of questions raised by several Member States following the evaluations at the national level. When this is the case, EU legislation requires that EFSA carries out a further assessment and provides an opinion.

In delivering its opinion the Panel considered the notification, additional information provided by the applicant and the specific questions and concerns raised by the Member States. Further information from other applications for the placing on the market of 1507 maize 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/NL/00/10 for import and processing and an application under the novel foods Regulation (EC) 258/97 which was transformed into application EFSA-GMO-NL-2004-02 for the authorisation of food products under Regulation (EC) No 1829/2003 on GM food and feed. 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' (EFSA, 2004b). The scientific assessment included examination of the DNA inserted

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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 feed 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.

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.

Notification C/ES/01/01 concerns cultivation, import, processing and use as any other maize, excluding food uses. 1507 maize is comparable with maize bred traditionally, except for the expression of tolerance to glufosinate herbicide and certain lepidopterans. Maize does not colonise and rarely survives outside the cultivated environment. It is winter-hardy only in parts of Southern Europe, and it has no cross-compatible wild relatives in Europe. Therefore, no unintended environmental effects due to the establishment and spread are anticipated. The likelihood of adverse effects on non-target organisms or on soil functions due to the expression of the *cry* gene or the *pat* gene is considered to be very low. The possible development of resistance of target organisms to *Bt* toxin has been identified as a potential risk due to large scale cultivation and/or long term exposure. Thus, an appropriate case-specific monitoring plan to record the development of resistance has been provided. In addition, the GMO Panel agrees in principle with the approach proposed by the applicant in the general surveillance plan.

In conclusion, the 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.

Key words: GMO, maize, *Zea mays*, 1507, insect protection, Cry1F, PAT, feed 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

The Commission received the summary notification (Reference C/ES/01/01) from Pioneer Hi-Bred International/Mycogen Seeds, on 13 February 2003. The full notification, together with a positive assessment report, was received by the Commission on 5 August 2003 from the lead Member State (Spain).

In accordance with Directive 2001/18/EC (EC, 2001), the notification was then transmitted to the Competent Authorities of the other Member States, a number of which have raised objections during the statutory 60-day period. The applicant provided the Member States with additional information in response to the objections raised during the 60-day period. The Member States had until 13 May 2004 to confirm or lift their objections. Where these objections are maintained, the Commission is required to consult the relevant Scientific Committees for opinion, now represented by EFSA. Some Member States maintained specific objections.

Article 18(1) of Directive 2001/18/EC states that the period of time during which the Commission is awaiting the opinion of the Scientific Committee shall not exceed 90 days. The evaluation by EFSA started on 27 May 2004, after receipt of the complete background information (request from the Commission, dossier of the applicant and final objections maintained by the Member States). During the 90-day period, EFSA requested further clarification from the applicant.

In delivering its opinion the Panel considered the notification, additional information provided by the applicant and the specific questions and concerns raised by the Member States. Further information from other applications for the placing on the market of 1507 maize 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/NL/00/10 for import and processing and an application under the novel foods Regulation (EC) 258/97 (EC, 1997) which was transformed into application EFSA-GMO-NL-2004-02 for the authorisation of food products under Regulation (EC) No 1829/2003 (EC, 2003) on GM food and feed. For regulatory reasons the latter applications resulted in separate opinions (EFSA, 2004a; EFSA 2005).

TERMS OF REFERENCE

EFSA was requested, under Article 29(1) and in accordance with Article 22(5)(c) of Regulation (EC) No 178/2002 (EC, 2002a), to provide a scientific opinion as to whether there is any scientific reason to believe that placing on the market of 1507 maize for import, feed and

industrial processing, and cultivation is likely to cause any adverse effects on human health and the environment within the scope of Directive 2001/18/EC.

In particular, EFSA was requested to take account of the scientific objections raised by the Competent Authorities of Member States in this context.

EFSA was not requested to give an opinion on the non-scientific objections raised by Competent Authorities in their replies, in the context of the entry into force of forthcoming legislation or requests for further legislative/implementing measures.

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, 2004b). 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[2] 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 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.

[2] 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.

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[3] – 136.8 pg/µg TEP[4]) 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.

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

[3] LOD = limit of detection

[4] TEP = total extractable protein

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, 2001a, 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[5], 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.

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.

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

4.2. Evaluation of relevant scientific data

4.2.1. Product description and intended use

Notification C/ES/01/01 covers cultivation, import and processing of 1507 maize and use as any other conventional maize. The food uses of 1507 maize are covered by another application[6]. Maize kernels are a rich source of carbohydrate, while starch production produces by-products, such as maize gluten and maize gluten feed, which are used as animal feed.

Maize kernel products are used in various animal feeds, including cattle, swine, poultry, and in fish feed.

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).

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

[6] Application EFSA-GMO-NL-2004-02 submitted under Regulation (EC) 1829/2003

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

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, 2004b; 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[7]. 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 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.

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

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 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

5.1 Issues raised by the Member States

(1) Direct and indirect effects of the Cry1F toxin on non-target organisms, specifically soil biota, arthropods, parasitoids of maize pests, butterflies, and other invertebrates, should be addressed; (2) more information on the general surveillance and monitoring of non-target effects was requested; in addition, a more detailed insect resistance management plan was demanded; (3) the lack of knowledge concerning the occurrence of lepidoptera and their sensitivity to the Cry1F toxin in and around maize fields was emphasised; (4) concerns about

potential harm to endangered Lepidopteran species were expressed and the possible need to protect endangered butterfly species was emphasised; (5) it was recommended that there should be consideration of potentially altered lignin contents and the biodegradability of plant litter as well as possible long-term persistence of the Cry1F protein; (6) dietary toxicity studies on non-target insects carried out with microbially-derived Cry1F protein were questioned due to a potentially higher toxicity of the toxin produced by GM plants[8]; (7) it was argued that the implications of the presence and use of *pat* gene, in addition to the *cry*1F gene, should be considered both in the environmental risk assessment (ERA) and in the post-market environmental monitoring plan (PMEM); (8) it was mentioned that the use of glufosinate in association with 1507 maize should be restricted to the regime used in the UK Farm Scale Evaluation trials; (9) the issue of outcrossing between GM and non-GM crops and related impacts on the co-existence of these crops was raised.

5.2. Evaluation of relevant scientific data

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

Maize is highly domesticated and not generally able to survive in the environment without cultivation. Maize plants are not winter hardy in many parts of Europe. They have lost their ability to release seeds from the cob and they do not occur outside cultivated or disturbed 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.

1507 maize has no altered survival, multiplication or dissemination characteristics except in the presence of glufosinate. The Panel is of the opinion 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.

5.2.2. 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.

[8] This issue is addressed in section 4.2.3.1.

(a) Plant to bacteria gene transfer

Based on present scientific knowledge and elaborated recently in more detail (EFSA, 2004c), 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.

5.2.3. Interactions between the GM plant and target organisms

According to the statement made by the Scientific Committee on Plants (SCP, 1999) and in line with Annex II of Directive 2001/18/EC the Panel considers that the evolution of resistance in target pests is an environmental and agronomic concern. Up to now, resistant *Ostrinia nubilalis* or *Sesamia nonagrioides* have not been found in fields in the US or in Europe (Evans 2002, Tabashnik *et al*., 2003, Bourguet *et al*., 2003, Farinós *et al*., 2004). Although laboratory tests showed that corn borer populations are capable of developing some degree of tolerance to the Cry1Ab protein (Huang *et al*. 2002), laboratory selection and F2 screening to generate highly resistant *O. nubilalis* strains have failed so far (Bourguet, 2004). However, another lepidopteran pest (*Plutella xylostella*) has developed resistance to *Bt* toxins (Tabashnik *et al*., 2003). The Panel concludes that large scale cultivation of 1507 maize over several years will increase the selection pressure on corn borers, which might result in the development of resistance. This could have several consequences including the use of alternative phytosanitary measures to control the pest including involving the use of insecticides other than *Bt* toxins. The Panel agrees that the likelihood of occurrence is low since, under field conditions and several years of

cultivation, no resistance has been reported. However, cultivation of *Bt* maize in Europe is currently on a small scale and limited to a few geographic regions. Thus it is difficult to predict future responses of corn borer populations in Europe. Therefore, the Panel advises that potential target pest resistance development should be monitored under case-specific monitoring using the methods submitted by the applicant as part of their general surveillance plan.

5.2.4. Interactions of the GM plant with non-target organisms

(a) Effects on predators and parasitoids of the target organisms

The abundance of non-target predators preying upon the target organisms *Ostrinia* or *Sesamia* will vary with the abundance of their prey. Thus, a reduction in prey either by cultivation of *Bt* maize or by insecticides may negatively effect the food source of predators like *Chrysoperla carnea* (Hilbeck *et al*. 1998a,b). However, current knowledge on toxicity and exposure give sufficient scientific evidence that *Bt* maize poses no risk to this predator (Dutton *et al*. 2003a, b; Romeis *et al.* 2004). Most field studies confirm that predator and parasitoid abundances and biocontrol functions are very similar in *Bt* and non-*Bt* fields (Candolfi *et al*. 2004, Pons & Stary, 2003, Musser & Shelton, 2003). Reductions of population densities of specialist *Ostrinia* predators and parasitoids are expected as this pest is the target to be controlled in *Bt* maize fields. Bourget *et al.* (2002) and Siegfried *et al.* (2001) have found that populations of specific natural enemies of *Ostrinia* are less abundant in *Bt* maize fields than in non-*Bt* maize fields. This is not thought to be due to the direct effects of the Cry toxin consumed while predating or parasitizing *Ostrinia* but is due to decreased availability of specific prey. Results of field studies comparing the effects of *Bt* maize with insecticide treatments against the target pest, show that broad-spectrum insecticides, like pyrethroids, reduce abundances of a range of predator and parasitoid species not specific to *Ostrinia*. Such effects have not been reported in *Bt* maize.

(b) Effects on other non-target organisms

It is well documented that a range of lepidopteran species may be affected by *Bt* toxins and some may be present in maize fields (Schmitz *et al*., 2003; for a review see Evans 2002). However, exposure of any populations of lepidoptera to the toxin is restricted to those consuming the *Bt* plant or its products. In the vicinity of the *Bt* maize field larvae may be most exposed to the toxin when *Bt* maize pollen is deposited on plants on which they are feeding. Maize, a recently introduced species into Europe, is not a significant food source for endemic lepidoptera and impacts due to pollen dispersal are likely to be transient and minor as demonstrated by studies on monarch butterflies in the USA (Dively *et al*., 2004). Published studies investigating potential effects of GMOs due to the expression of *Bt* toxins have been mainly performed with maize *Bt*11 and *Bt*176, both producing CrylAb. Generally similar effects on the environment due to the presence of different *cry* genes can be expected, however, the severity of potential effects will depend on the expression of the relevant gene and the toxicity of the resulting toxin. Hellmich *et al.* (2001) compared the toxicity of different *Bt* toxins and reported a >10.000 times lower toxicity of the Cry1F toxin (as produced in 1507 maize) on monarch butterfly first instars as compared with other Cry toxins (*e.g.* CrylAb). On the other hand, according to the data presented in the respective dossiers, Cry1F concentration in 1507 maize pollen is higher in comparison with CrylAb concentration in *Bt*11 pollen (1.3 ng Cry toxin mg^{-1} plant protein in *Bt*11 pollen compared with 160 ng Cry protein mg^{-1} plant protein in 1507 maize pollen). However, Hellmich *et al.* (2001) showed that monarch larvae were not affected by a diet consisting of 1507 maize pollen. Considering toxicity and exposure of Cry1F, the Panel agrees with the assessment of the applicant that risk of exposure of non-target lepidoptera to harmful toxin concentrations via 1507 maize pollen is negligible and that adverse impacts on populations are very unlikely.

Three year experimental studies of *Bt* maize (*Bt*176 expressing CrylAb) in Spain did not show effects on mortality, developmental and pre-reproductive times, fecundity, and intrinsic rate of population increase comparing the offspring of apterous aphids maintained on *Bt* or non-*Bt* maize for several generations (Lumbierres *et al.*, 2004), which is in line with the absence of *Bt* toxin in the phloem (Raps *et al.*, 2001).

Direct and indirect effects of GM plants in general on animals higher in the food chain including both invertebrates and vertebrates (birds, mammals) have been discussed in some publications (Kjellson & Strandberg, 2001; Firbank *et al.* 2003) No indications of intoxication have been reported or are indicated from first and second tier exposure studies or from feeding studies with diets containing *Bt* toxin. It should also be considered that under field conditions most animals higher in the food chain would be eating diets consisting of a range of food sources.

No evidence of accumulation of *Bt* toxins in the food chain has been reported and is not expected as the toxin is an easily degradable protein. In most situations the toxin appears to be degraded through passage of the gut, although detectable amounts of the *Bt* toxin can still be found in faeces and therefore pass into the environment. In cattle, the influence of CrylAb transgenic maize on rumen bacterial microflora was investigated compared with isogenic material through analysis of 497 individual bacterial 16S rDNA sequences. In principle, specific bacterial species could be identified in all bovine rumen extracts, but no significant influence of *Bt* maize feed (*Bt* 176) was found on the composition of the microbial population (Einspanier *et al.* 2004). It therefore appears that the environmental impact of *Bt* toxin through manure is negligible, as only very small amounts of the toxin are expected to be excreted to the environment through manure and significant long-lasting changes in the composition of microbial communities of the manure seem unlikely.

Reduction of prey/feed abundance can be a consequence of many types of crop management practices. The Panel has no reason to consider that 1507 maize will cause changes to non-target species that differ significantly from those caused by conventional farming.

5.2.5. Potential interaction with the abiotic environment and potential effects on biogeochemical processes

As a consequence of the cultivation of *Bt* maize the respective *Bt* toxins will be incorporated into the soil (root exudates, *Bt* toxin containing plant material like plant litter and pollen). Some scientific publications indicate that this might affect soil organisms. Assumptions were raised that the *Bt* toxin may persist and accumulate in soil during cultivation of *Bt* maize in subsequent years. Therefore, both direct and indirect impacts of the toxin or the *Bt* maize (*e.g.* potential increase of lignin content in combination with a possible delay in decomposition) on non-target organisms and soil function should be considered (Saxena *et al.* 2002, Zwahlen *et al.* 2003a). There was a concern that *Bt* maize might negatively affect species other than lepidoptera and consequently biodiversity. The suggested species range comprises soil and plant associated insects in food chains including those involved in plant decomposition.

Herman *et al.* (2002) showed that Cry1F produced In recombinant *Pseudomonas fluorescens* rapidly decomposed in soil studied under laboratory conditions which is in line with other publications on the degradation of Cry proteins in soil (Glare & O´Callaghan, 2000). Further data on potential effects of *Bt* plants are mainly available from maize expressing CrylAb such as *Bt*11. However, as effects of *Bt* plants expressing different Cry proteins are considered to be comparable, the GMO Panel takes published data on other *Bt* maize cultivars into account. Saxena & Stotzky (2001) reported Cy1Ab had no apparent effect on earthworms and nematodes in a 45-days study. Zwahlen *et al.* (2003b) reported a 200-day study investigating the impact of transgenic *Bt* maize event *Bt*11 (expressing Cry1Ab) on immature and adult *Lumbricus terrestris* in a single worst-case laboratory study and in a single small scale field test. At the end of the laboratory test the earthworms showed a significant weight loss of 18%

(compared with their initial weight) when fed (*Bt*+) maize litter whereas a weight gain of 4% occurred with non-GM control maize. No difference was found in the higher tier small scale field test. Due to the experimental design, the authors stated that they were unable to exclude the possibility that the weight loss of earthworms fed with Bt maize in the laboratory test was due to other factors.

The effects of 1507 and Bt11 maize on soil microbial community structure were assessed in growth chamber experiments using three soil types with different textures (Blackwood & Buyer, 2004). Very few significant effects on soil microbial communities due to the presence of the *Bt* toxins were found, whereas the soil type significantly influenced the composition of the soil microflora. Similarly, other studies on transgenic plants expressing Cry toxins did not reveal any negative, long-lasting impact on the soil or plant-associated microorganisms (Flores *et al.*, 2005; Devare *et al.*, 2004; Donegan *et al.*, 1995). Koskella & Stotzky (2002) reported that Bt proteins showed no toxicity to bacteria, fungi and algae. Turrini *et al.* (2004) reported that root exudates of *Bt*176 corn significantly reduced hyphal growth of arbuscular mycorrhizal fungi, a group of organisms that is fundamental for soil fertility and plant nutrition. In the same study, *Bt*11 did not affect the plant-mycorrhiza symbiosis (Turrini *et al.*, 2004). Blackwood & Buyer (2004) did not detect an effect due to the cultivation of 1507 maize on the abundance of arbuscular-mycorrhizal fungi.

For Bt11 maize, it has been suggested that biodegradation and mineralisation of plant litter was delayed by a higher lignin content (Zwahlen *et al*. 2003a, Saxena & Stotzky, 2001a). Zwahlen *et al*. (2003a) published the results of two field studies in the temperate maize-growing region of Switzerland investigating the degradation of Cry1Ab toxin in transgenic *Bt* maize leaves during autumn, winter and spring periods. Each of the two field trials (in 1999/2000 and 2000/2001) covered a period of 200 days. The results suggest that *Bt* toxin is not completely degraded within the period tested. The authors discuss their findings in the light of potential differences in lignification (Saxena & Stotzky, 2001a), although lignin content was not determined. A more comprehensive study suggests that the extent of lignification of Bt transgenic maize (several lines derived from MON 810 and *Bt*11) does not differ from the non-transgenic controls (Jung & Sheaffer, 2004). Compositional analysis provided by the applicant on 1507 maize of the lignin-containing acid and neutral detergent fibre content in forage, as well as the lignin precursors *p*-coumaric acid and ferulic acid in kernels, did not indicate altered lignification.

A four-year study on the decay of transgenic maize *Bt* toxin (event *Bt*176) was published (Hopkins & Gregorich, 2003). The authors followed the rate at which the toxin in *Bt*-maize leaves decomposed in soil from a field in which *Bt*-maize had been cultivated for four years. The results suggested that much of the *Bt* toxin in crop residues is highly labile and quickly decomposes in soil, but that a small fraction may be protected from decay in relatively recalcitrant residues. It is known from experience with conventional *Bt* sprays, that *Bt* toxins as crystals can persist in soils, *e.g.* for at least 28 months (Vettori *et al.*, 2003). Recently, the decomposition of different plant species expressing *Bt* toxins was analysed in laboratory experiments and results were discussed in relation to lignin contents and potential environmental consequences (Flores *et al.*, 2005). Generally, *Bt* plants showed less decomposition than non-*Bt* plants. However, this effect was not clearly related to lignification or reduced microbial activity in soil. The authors concluded that lower decomposition rates may be beneficial as organic matter derived from plants would persist for a longer period improving soil structure and reducing erosion. In addition, Flores *et al*. (2005) discussed potential effects on target and non-target insects due to the longer persistence of *Bt* toxins in soil. In relation to soil organic content, it has been shown that even distinct increases in decomposition resistant compounds such as lignin result in only modest increases in organic carbon in the topsoil. Changes in soil management have a much more pronounced effect (Sessitsch *et al*., 2004). Considering the available information on potential effects of *Bt* plants on the soil environment and in particular on soil non-target organisms, adverse effects due to slightly altered decomposition rates are unlikely.

The published results from laboratory and field trials showed that on short to medium time scales (up to 3 years) and under field conditions, the effects on soil functions and biodiversity (Blackwood & Buyer 2004; Motavelli *et al.*, 2004; Evans, 2002) does not exceed the range of the "natural" variability. No conclusive evidence has yet been presented that currently released transgenic *Bt* resistant crops are causing significant direct effects on the soil environment. The effects of transgenic *Bt* maize in these experiments were small, if they existed at all. In addition, the available data do not indicate a chain of events that might result in long-term effects. Therefore, it seems likely that in commercial cropping conditions, where crop rotations are used, the consequences of effects on soil functions and soil organisms are negligible. However, long-term effects may become detectable in cultivation systems without crop rotation where repeated cropping of 1507 maize might result in accumulation of effects.

5.2.6. Potential impacts of the specific cultivation, management and harvesting techniques

The environmental risk assessment made no comparisons of the environmental profile of the use of glufosinate on maize in comparison with other herbicides. Indeed, this would be difficult to do because of the range of other herbicides used and the range of agricultural systems and environments in which maize is grown and the wide diversity of weed species and associated flora and fauna that will be found in maize fields. Glufosinate is a contact, non-persistent and non-systemic broad-spectrum herbicide with activity against a wide range of plants though some tolerance occurs in some *Viola* species and some species of grasses. In the UK Farm Scale Evaluation study the glufosinate herbicide programmes studied on farms resulted in reduced biodiversity in spring oilseed rape but had less impact on biodiversity than the standard herbicide programmes used on maize (Champion *et al.* 2003). The most commonly used comparator in maize was atrazine for which authorisations had to be withdrawn in most EU countries by 10 September 2004 (EC, 2004a). However other herbicides were used and a recent report (Perry *et al.*, 2004) indicated that regimes applying glufosinate either had a better or similar biodiversity impact compared with these herbicides.

The Panel considers that the presence of the *pat* gene and the use of glufosinate is not likely to give an increased impact on biodiversity in most situations. The Panel therefore comes to the conclusion that case specific monitoring regarding any consequences due to the application of glufosinate in combination with the cultivation of 1507 maize is not required. The Panel, however, recommends that observation of general weed abundance and diversity should be included in the general surveillance plan.

5.3. Conclusions

The notification C/ES/01/01 for 1507 maize is for cultivation, and thus the environmental risk assessment and the monitoring plan have to consider the environmental impact of full scale commercialisation. The Panel is of the opinion that no significant risk has been identified in the environmental risk assessment with the exception of resistance development of the target insects, which affects the case-specific monitoring plan.

1507 maize has no altered survival, multiplication or dissemination characteristics except in the presence of glufosinate. 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 from that of traditionally bred maize.

Judging from the available data delivered either by the applicant or by literature survey, the likelihood of adverse effects on non-target organisms or on soil function is foreseen to be very low.

The Panel considers that the presence of the *pat* gene and the use of glufosinate is not likely to give an additional botanical diversity effect compared to other herbicides.

The safety of residues of glufosinate applied to 1507 maize and of any metabolites has to be evaluated under a different Directive (EC, 1991) before market approval, and is therefore not within the remits of this opinion.

The Panel is aware that glufosinate containing herbicides are currently being evaluated within the framework of the above mentioned Directive (EC, 1991).

6. Post-market environmental monitoring plan

6.1. Issues raised by the Member States

(1) It was stated that a detailed monitoring plan is required comprising general surveillance as well as case-specific monitoring. In addition, a more detailed insect resistance management plan was demanded; (2) it was argued that the implications of the presence and use of *pat* gene, in addition to the *cry1F* gene, should be considered in the PMEM plan.

6.2. Evaluation of relevant scientific data

Notification C/ES/01/01 for 1507 maize is for cultivation, and thus a monitoring plan is required that considers the environmental impact of full commercial scale, cultivation and production.

6.2.1. General aspects of monitoring

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC (EC, 2001) 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 that were not anticipated in the environmental risk assessment (EFSA, 2004b).

The Panel is of the opinion that the structure of the environmental monitoring plan provided by the applicant complies with the demands defined in the Directive 2001/18/EC, the Guidance Notes to Annex VII [EC, 2002b] and the Guidelines provided by EFSA (EFSA, 2004b). The monitoring plan (referring to both case-specific monitoring as well as general surveillance) describes objectives, responsibilities and tasks, flow of information and monitoring methods (including statistical approaches).

6.2.2. Interplay between environmental risk assessment and monitoring

From the ERA it can be concluded that the development of resistant corn borer populations could be induced by the cultivation of 1507 maize. Therefore, a case-specific monitoring of resistance development in corn borers is required, and an appropriate monitoring plan was provided by the applicant.

The GMO Panel considered whether the abundance of non-target lepidoptera in or close to maize fields should also be monitored. The ERA has not identified any risks specifically linked to *Bt* maize fields. The influence of 1507 maize on the variability of abundance of lepidoptera is expected to be minimal compared with other impact factors (general agricultural management; insecticide usage on neighbouring fields, weed abundance; climate). In addition it will be difficult to compare populations of lepidoptera in conventional maize fields (sometimes treated

with insecticides) with populations in *Bt* maize fields. Consequently, a significant and unequivocal correlation of detected differences with the cultivation of 1507 maize is highly unlikely. Furthermore, the recording of statistically sufficient data on the abundance of lepidoptera would demand a high input of personnel and costs (Lang, 2004), especially if larvae, as the most susceptible and immobile development stage, are to be monitored. In addition maize, a species recently introduced into Europe, is not a significant food source for endemic lepidoptera and impacts due to pollen dispersal are likely to be transient and minor as demonstrated by studies on monarch butterflies in the USA (Dively *et al*., 2004). The case-specific monitoring of the abundance of non-target lepidoptera in 1507 maize does not comply with the required cost-effectiveness according to Council Decision 811/2002/EG (EC, 2002b). However, management recommendations for the cultivation of 1507 maize, as given by the applicant to users of 1507 maize, considers measures to reduce exposure of non-target lepidoptera (as well as the target pest), such as the use of non-transgenic border rows as refugia for the target that would also reduce exposure of field margin weeds (and hence non-target lepidoptera) to pollen from *Bt* maize.

The Panel agrees with the risk assessment that no adverse effects on other non-target organisms are anticipated and thus this should not be included in the case-specific monitoring plan.

The Panel considers the spread of transgenes not relevant for an environmental monitoring regime since natural relatives of maize are not present in the EU. Furthermore horizontal gene transfer to microorganisms is extremely unlikely, and would occur primarily through homologous recombination in microbes.

The ERA provided by the applicant did not identify risks specific to the GMO associated with the *pat* gene or the management of herbicide tolerance. The GMO Panel agrees with this assessment. Thus, considering all the conclusions described above, the Panel considers that monitoring of target insect resistance is the only case specific monitoring requirement for 1507 maize.

6.2.3. Case-specific monitoring of 1507 maize

The case-specific monitoring plan clearly describes the responsibilities and activities of the applicant. These include organising the establishment and activities of this case-specific monitoring, co-ordinating third parties' contributions to the studies and establishing a reporting system to the EU and Competent Authorities of Member States. Since the development of resistance is more likely to occur with increased time and scale of cultivation, the monitoring period has been selected appropriately for the period of the market release. The applicant documented that previous commercial releases of *Bt* maize in Europe and North America have already been managed in order to reduce selection pressure. It is appropriate that resistance management strategies are fully integrated into PMEM plans so that information provided by the monitoring concerning resistance development is used to refine strategic options for managing resistance. In addition, the level of susceptibility and the geographical information of occurrence of resistance will be linked to a stepwise pest management strategy so that methodological improvements can be reviewed and adopted - if appropriate. The direct assessment of susceptibility in the corn borer populations allows the detection of resistance at an early stage of development, so that detection can be rapidly linked to pest management measures. Such a strategy of insect resistance management and monitoring should provide an efficient stewardship of 1507 maize and other similar maize cultivars, as well as an efficient pest control regime.

The Panel concludes that large scale cultivation of 1507 maize is likely to increase the selection pressure on corn borers to develop tolerance to its *Bt* toxin and possibly to others. This could have several consequences including the use of alternative phytosanitary measures to control

the pest including the use of insecticides other than *Bt* toxins. The Panel agrees that the likelihood of occurrence is low since under field conditions and several years of cultivation no resistance has been reported (Farinós *et al.*, 2004). However, cultivation of *Bt* maize in Europe is currently at small scale and limited to few geographic regions and thus it is difficult to predict future responses of corn borer populations in Europe.

6.2.4. General surveillance of the impact of 1507 maize

As part of an EFSA self-tasking activity EFSA has established a working group to study requirements for post-market environmental monitoring (PMEM WG) in order to produce guidance for both applicants and regulatory authorities. Based on its mandate, the PMEM WG initiated a series of consultation workshops with different stakeholders (applicants, environmental NGOs and scientific institutes, experts from Member States) to establish a rationale and general framework for general surveillance as a component of post-market environmental monitoring. The objective of general surveillance is to identify unforeseen adverse effects of the GM plant or its use on human health and the environment, which were not predicted in the risk assessment. The methods and approaches should be appropriate, proportional and cost-effective to allow for the detection of GMO effects. Potential data sources and related networks should be identified.

General surveillance is related to risk management, and thus a final adoption of the general surveillance plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific quality of the General Surveillance plan provided by the applicant.

The GMO Panel has suggested linking general surveillance to conservation goals such as sustainable agriculture and to environmental damage as defined in the new EU Directive on environmental liability (EC, 2004b; EFSA 2004d). During several stakeholder workshops, the PMEM working group has elaborated the possibility of using existing environmental surveillance networks supplementing general surveillance plans. Considering 1507 maize, the GMO Panel is not aware of any existing surveillance network that would substantially fulfil the scientific requirements for the detection of any unforeseen environmental effect in relation to 1507 maize cultivation. Thus the GMO Panel agrees with the proposal of the applicant not to use any existing surveillance network at this stage. However, the applicant should in principle consider access to any future surveys of conservation goals in farming regions cultivating *Bt* maize and investigate their suitability for providing data on potential changes in biota due to the cultivation of the *Bt* maize.

The GMO Panel welcomes the approach of the applicant to establish new general surveillance networks by using questionnaires as a reporting format. The questionnaires to farmers and others exposed to or utilising 1507 maize provided by the applicant are regarded as a good starting point for addressing several aspects of general surveillance. However, the applicant should broaden the farmer questionnaire to improve the scientific value of the records. Therefore the Panel makes the following recommendations:

1. the farmer should be requested to provide information before being asked to comment on any observed differences;

2. the questionnaire should request data on fertilizer usage, soil fertility, crop rotations, crop performance, crop yields, pests and diseases, pesticide use, and weed abundance;

3. the questionnaire should focus on sites (fields or group of fields) where 1507 maize is being grown and on years following cultivation;

4. the questionnaire should be structured to elicit detailed information. The questions should be presented in a way that the respondent can choose from a selection of

answers, and only has to mark boxes. Pre-formulated answers should consist of a classified scale of intensity (*e.g.* from 1 to 5 or none - low - mid - high) to allow analyses of any correlation between different factors;

5. an additional field for free answers or comments may follow the pre-formulated answers to allow comments on other factors not covered by the questionnaire;

6. the standard procedures of univariate or multivariate analysis of the questionnaire's key variables to be analysed by the applicant should be described precisely.

The EFSA GMO Panel considers the development of the format of questionnaires as an ongoing process. The Panel suggests at this early stage of market introduction a step-by-step approach: having a reporting and strategic summit every year where the applicants could share their experience with EFSA and Member States and improvements can be discussed.

Finally, the GMO Panel welcomes the intention of the applicant to carry out further field studies on non-target organisms. The GMO Panel is of the opinion that such a study is part of post-market biosafety research and thus is not regarded as part of a monitoring plan. However the GMO panel would be interested in receiving reports of results from this proposed study.

6.2.5. Reporting the results of monitoring

The Panel is content with the proposal made by the applicant on the reporting intervals and procedures.

6.3. Conclusion

An appropriate monitoring plan to record the development of *Bt* toxin resistance in target populations of lepidoptera has been provided by the applicant, which should be classed as a case-specific monitoring plan. The time period and design of this case-specific monitoring should consider the rate at which resistance is likely to evolve, resistance management strategies, the scale and the geographical dispersal of 1507 maize cultivation.

The GMO Panel has no objection in principle to the general methods and approach to the general surveillance plan. However, there are recommendations for improvement of the structure and format of the questionnaires in order to obtain data of greater scientific quality and value.

Management options for the cultivation of 1507 maize should include measures to reduce exposure of non-target lepidoptera and for delaying the development of resistance to the *Bt* toxin in target insects.

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 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 EFSA 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.

DOCUMENTATION PROVIDED TO EFSA

1. Note to Mr. Koëter, dated 10 May 2004 with ref. ENVB4/HM/KT/sf/D(04)341161, from Mr. J. Delbeke – advance copy of a request to EFSA concerning notification C/ES/01/01 (1507 maize).

2. Note to Mr. Koëter, dated 27 May 2004 with ref. ENVB4/HM/KT/bv/D(04)341254, from Mr. J. Delbeke – transmission of Member State objections concerning notification C/ES/01/01 (1507 maize).

3. Initial comments and final objections from Member States with regard to notification C/ES/01/01 (1507 maize).

4. E-mail from DG ENV, dated 1 June 2004 – comments from Estonia and Hungary which were received out of delay (45 day deadline) concerning notification C/ES/01/01 (1507 maize).

5. Submission from Pioneer/Mycogen Seeds (19 May 2004) to EFSA regarding the Notification to place on the market (including cultivation) products containing genetically modified organisms 1507 maize in accordance with Directive 2001/18/EC: Ref C/ES/01/01, containing:

    a) a letter from Pioneer/Mycogen Seeds to the Competent Authority of Spain concerning submission of the notification,
    b) the summary of the notification,
    c) the assessment report of the notification carried out by the Competent Authority of Spain,
    d) the notification submitted by Pioneer/Mycogen Seeds,
    e) additional information submitted by Pioneer/Mycogen Seeds in response to comments and objections raised by the Competent Authorities of Member States.

6. Letter from EFSA to applicant with request for clarification/additional information (ref. SR/ (2004) 436, 17 June 2004).
7. Additional information submitted by Pioneer/Mycogen Seeds on 25 June 2004 in response to EFSAs request for further information.
8. Letter from EFSA to applicant with request for clarification/additional information (ref. SR/ (2004) 949, 3 November 2004).

9. Additional information submitted by Pioneer/Mycogen Seeds on 2 December 2004 in response to EFSAs request for further information.

10. 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) Transformed application (EFSA-GMO-NL-2004-02) for authorisation of food products of 1507 maize in accordance with Regulation (EC) 1829/2003, submitted by the Dutch authorities to EFSA on 10 June 2004.

REFERENCES

Blackwood, C.B & Buyer, J.S., 2004 . Soil microbial communities associated with *Bt* and non-*Bt* Corn in three soils, Journal of Environmental Quality. 33, 832-836.

Bourguet D., Chaufaux J., Micoud, A., Delos, M., Naibo, B., Bombardes F., Marque, G., Eychenne N. & Pagliari, C., 2002. *Ostrinia nubilalis* parasitism and the field abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (*Zea mays*), Environ. Biosafety Res., 1, 49–60.

Bourguet, D., 2004. Resistance to *Bacillus thuringiensis* toxins in the European corn borer: what chance for *Bt* maize? Physiological Entomology 29: 251-256.

Bourguet, D., Chaufaux, J., Se´quin, M., Buisson, C., Hinton, J. L., Stodola, T. J., Porter, P., Cronholm, G., Buschman, L. L. & Andow, D. A., 2003. Frequency of alleles conferring resistance to *Bt* maize in French and US corn belt populations of the European corn borer, *Ostrinia nubilalis*. Theor. Appl. Genet. 106: 1225Ð1233.

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

Candolfi, M.P., Brown, K., Grimm, C., Reber, B. & Schmidli, H. 2004. A faunistic approach to assess potential side-effects of genetically modified *Bt*-corn on non-target arthropods under field conditions. Biocontrol Science and Technology, 14, 129-170.

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.

Champion, G.T., May, M.J., Bennett, S., Brooks, D.R., Clark, S.J., Daniels, R.E., Firbank, L.G., Haughton, A.J., Hawes, C., Heard, M.S., Perry, J.N., Randle, Z., Rossall, M.J., Rothery, P., Skellern, M.P., Scott, R.J., Squire, G.R. & Thomas, M.R., 2003. Crop management and agronomic context of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Phil. Trans. R. Soc. Lond., B 358, 1801.

Devare, M.H., Jones, C.M. & Thies, J.E. 2004. Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community: biomass, activity and diversity. J. Environ. Qual. 33:837-843.

Dively G.P., Rose, R., Sears, M.K., Hellmich, R.L. Stanley-Horn, D.E., Calvin, D.D., Russo, J.M. & Anderson, P.L. 2004: Effects on monarch butterfly larvae (Lepidoptera: *Danaidae*) after continuous exposure to Cry1Ab-expressing corn during anthesis. Environ.Entomol. 33(4): 1116-1125

Donegan, K.K., Palm, C.J., Fieland, V.J., Porteous, L.A., Ganio, L.M., Schaller, D.L., Bucao, L.Q. & Seidler, R.J. 1995. Changes in levels, species and DNA fingerprints of soil microorganisms associated with cotton expressiong the *Bacillus thuringiensis* var. *kurstaki* endotoxin. Appl. Soil Ecol. 2:111-124.

Dutton, A., Romeis, J. & Bigler, F., 2003a. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: *Bt*-Maize expressing Cr1Ab as a case study. BioControl 48, 611-636.

Dutton A., Klein H., Romeis J.& Bigler F., 2003b. Prey-mediated effects of *Bacillus thuringiensis* spray on the predator *Chrysoperla carnea* in maize. Biological Control 26, 209-215.

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, 2002a. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ, L31, 1-24. http://europa.eu.int/eur-lex/pri/en/oj/dat/2002/l_031/l_03120020201en00010024.pdf

EC, 2002b. 2002/811/EC: Council Decision of 3 October 2002 establishing guidance notes supplementing Annex VII to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ, L280, 27-36. http://europa.eu.int/eur-lex/pri/en/oj/dat/2002/l_280/l_28020021018en00270036.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

EC, 2004a. Commission Decision of 10 March 2004 concerning the non-inclusion of atrazine in Annex I to Council directive 91/414/EEC and the withdrawal of authorisation for plant protection products containing this active substance. OJ, L78, 53-55.

EC, 2004b. Directive 2004/35/CE of the European Parliament and of the Council of 21 April 2004 on environmental liability with regard to the prevention and remedying of environmental damage; Official Journal of the European Union L 143: 56-75. http://europa.eu.int/eur-lex/pri/en/oj/dat/2004/l_143/l_14320040430en00560075.pdf

EFSA, 2004a. 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, 2004b. 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, 2004c. 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, 2004d. Draft version of chapter 11.4 of the EFSA Guidance Document as agreed during the Meeting of the PMEM WG on 21 October 2004, see Annex I of the minutes of the consultation workshop Sept. 24, 2004 http://www.efsa.eu.int/science/gmo/gmo_consultations/732/minutes_1st_workshop_sept042.pdf

EFSA, 2005. Opinion of the Scientific Panel on Genetically Modified Organisms on an application 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. http://www.efsa.eu.int/science/gmo/gmo_opinions/catindex_en.html

Einspanier, R., Lutz, B. Rief, S., Berezina, O., Zverlov, V., Schwarz, W. & Mayer, J. 2004. Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgene maize European Food Research and Technology 218, 269-273.

Evans, H.F., 2002, Environmental Impact of Bt Exudates from Roots of Genetically Modified Plants. Defra-Report (EPG 1/5/156) http://www.defra.gov.uk/environment/gm/research/pdf/epg_1-5-156.pdf

Farinós G.P., de la Poza M., Hernández-Crespo P., Ortego F. & Castañera P., 2004. Resistance monitoring of field populations of the corn borers *Sesamia nonagrioides* and *Ostrinia nubilalis* after 5 years of *Bt* maize cultivation in Spain. Entomologia Experimentalis et Applicata, 110, 23-30.

Firbank, L.G., Heard, M.S., Woiwod, I.P., Hawes, C., Haughton, A.J., Champion, G.T., Scott, R.J., Hill, M.O., Dewar, A.M., Squire, G.R., May, M.J., Brooks, D.R., Bohan, D.A., Daniels, R.E., Osborne, J.L., Roy, D.B., Black, H.I.J., Rothery, P. & Perry, J.N., 2003. An introduction to the Farm-Scale Evaluations of genetically modified herbicide-tolerant crops. Journal of Applied Ecology 40(1), 2-16.

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* CrylA(a) Insecticidal Toxin: Crystal Structure and Channel Formation. J. Mol. Biol. 254(3), 447-464.

Hellmich, R.L., B.D. Siegfried, M.K. Sears, D.E. Stanley-Horn, M.J. Daniels, H.R. Mattila, T. Spencer, K.G. Bidne, & L.C. Lewis. 2001. Monarch larvae sensitivity to *Bacillus thuringiensis*-purified proteins and pollen. PNAS 98: 11925-11930.

Hilbeck, A., W.J. Moar, M. Pusztai-Carey, A. Filippini & F. Bigler, 1998a. Toxicity of *Bacillus thuringiensis* Cry1Ab toxin to the predator *Chrysoperla carnea* (Neuroptera: *Chrysopidae*). Environ. Entomol. 27: 1255–1263.

Hilbeck, A., M. Baumgartner, P.M. Fried & F. Bigler, 1998b. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: *Chrysopidae*). Environ. Entomol. 27: 480–487.

Hopkins, D.W. & Gregorich E.G. 2003. Detection and decay of the *Bt* endotoxin in soil from a field trial with genetically modified maize. European Journal of Soil Science, 54, 793-800

Huang, F., L. L. Buschman, R. A. Higgins, & H. Li. 2002. Survival of Kansas Dipel-resistant European corn borer (Lepidoptera: *Crambidae*) on *Bt* and non-*Bt* corn hybrids. J. Econ. Entomol. 95: 614Ð621.

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.

Kjellson, G. & Strandberg, M., 2001. Monitoring and surveillance of genetically modified higher plants – Guidelines for procedures and analyses of environmental effects. Birkhäuser Publishing – Basel, Boston, Berlin. 119 pages.

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

Koskella, J. & Stotzky, G. 2002. Larvicidal toxins from *Bacillus thuringiensis* subsp. *kurstaki*, *morrisoni* (strain *tenebrionis*), and *israelensis* have no microbicidal or microbiostatic activity against selected bacteria, fungi, and algae *in vitro*. Can. J. Microbiol. 48, 262-267.

Lang, A. 2004. Monitoring the impact of *Bt* maize on butterflies in the field: estimation of required sample sizes Environ. Biosafety Res. 3: 55-66

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.

Lumbierres, B., Albajes, R. & Pons, X. (2004) Transgenic *Bt* maize and *Rhopalosiphum padi* (Hom., *Aphididae*) performance. Ecological Entomology vol. 29 (3) p.309-317

Motavalli, P.P., Kremer, R.J., Fang, M. & Means, N.E. 2004; Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations. Journal of Environmental Quality 33, 816-824

Musser, F.R. & Sehlton, A.M., 2003. *Bt* sweet corn and selective insecticides: Impacts on pests and predators. J. Econ. Entomol. 96, 71-80.

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, 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

Perry J.N., Firbank L.G., Champion L.G., Clark S.J., Heard M.S., May M.J., Hawes C., Squire G.R., Rothery P., Woiwod, I.P. & Pidgeon, J.D., 2004. Ban on triazine herbicides likely to reduce but not negate relative benefits of GMHT maize cropping. Nature, 428, 313-316.

Pons, X. & Stary, P. 2003. Spring aphid-parasitoid (Hom.*Aphididae*, Hym. *Braconidae*) association and intractions in a Mediterranean arable crop ecosystem, including *Bt* maize. J. Pest Sci. 76, 133-138.

Raps, A. , Kehr, J., Gugerli, P., Moar, W. J., Bigler, F. & Hilbeck, A. 2001. Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. Molecular Ecology 10: 525-533

Romeis J., Dutton A. & Bigler F. 2004. *Bacillus thuringiensis* toxin (Cry 1 Ab) has no direct effect on larvae of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: *Chrysopidae*). Journal of Insect Physiology 50, 175-183.

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

Saxena, D. & Stotzky, G. 2001b. *Bacillus thuringiensis* (*Bt*) toxin released from root exudates and biomass of *Bt* corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. Soil Biology and Biochemistry 33:1225 –1230

Saxena, D., Flores, S. & Stotzky, G. 2002. *Bt* toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. Soil Biology and Biochemistry, 34, 133–137.

Schmitz, G., Pretscher, P. & Bartsch, D. 2003. Selection of relevant non-target herbivores for monitoring the environmental effects of *Bt* maize pollen. Environmental Biosafety Research 2, 117-132.

SCP (1999). Opinion of the SCP on *Bt*-resistance monitoring. 4 March 1999. http://europa.eu.int/comm/food/fs/sc/scp/out35_en.html

Sessitsch, A., Smalla, K., Kandeler, E. & Gerzabek, M.H. 2004. Effects of transgenic plants on soil microorganisms and nutrient dynamics. In Plant Microbiology (Eds. M. Gillings & A. Holmes), BIOS Scientific Publishers, London and New York.

Siegfried, B.D., Zoerb, A.C. & Spencer, T. 2001. Development of European corn borer larvae on Event 176 *Bt* corn: influence on survival and fitness. Entomol. Exp. et Appl.100, 15-20.

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.

Tabashnik, B.E., Carrie`re, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M. & Zhao, J.Z., 2003. Insect resistance to transgenic *Bacillus thuringiensis* crops: lessons from the laboratory and field. Journal of Economic Entomology 96, 1031–1038.

Turrini, A., Sbrana, C., Nuti, M.P., Pietrangeli, B. & Giovannetti, M. 2004. Development of a model system to assess the impact of genetically modified corn and aubergine plants on arbuscular mycorrhizal fungi. Plant & Soil, in press.

Vettori C., Paffetti D., Saxena D., Stotzky G., Giannini R., 2003. Persistence of toxins and cells of *Bacillus thuringiensis* ssp. *kurstaki* introduced in sprays to Sardinia soils. Soil Biology and Biochemistry, 35: 1635-1642.

Zwahlen, C., Hilbeck, A., Gugerli, P. & Nentwig, W. (2003a). Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. Molecular Ecology 12, 765-775.

Zwahlen, C., Hilbeck, A., Howald, R. & Nentwig, W. (2003b). Effects of transgenic *Bt* corn litter on the earthworm *Lumbricus terrestris*. Molecular Ecology 12, 1077–1086.

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