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Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-NL-2005-12) for the placing on the market of insect-resistant genetically modified maize 59122, for food and feed uses, import and processing under Regulation (EC) No 1829/2003, from Pioneer Hi-Bred International, Inc. and Mycogen Seeds, c/o Dow Agrosciences LLC.

(Question No EFSA-Q-2005-045)

Opinion adopted on 23 March 2007

SUMMARY

This document provides the opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on genetically modified insect-resistant maize 59122 (Unique Identifier DAS-59122-7) developed to express the CRY34Ab1, CRY35Ab1 and PAT proteins.

In delivering its opinion the GMO Panel considered the application EFSA-GMO-NL-2005-12, additional information provided by the applicant (Pioneer Hi-Bred International, Inc. and Mycogen Seeds, c/o Dow Agrosciences LLC) and the scientific comments submitted by the Member States. The scope of the application is for food and feed uses, import and processing of maize 59122 and does not include cultivation. The GMO Panel assessed maize 59122 with reference to the intended uses 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. The scientific assessment included molecular characterization of the inserted DNA and expression of the target proteins. A comparative analysis of agronomic traits and composition was undertaken and the safety of the new proteins and the whole food/feed was evaluated with respect to nutritional quality, potential toxicity and allergenicity. An assessment of environmental impacts and the post market environmental monitoring plan were undertaken.

Maize 59122 was transformed by *Agrobacterium*-mediated gene transfer technology and expresses CRY34Ab1, CRY35Ab1 and PAT proteins. The molecular characterisation data established that maize 59122 contains a single insert of the T-DNA. The structure of the insert in maize 59122 was determined by Southern analysis and DNA sequencing. No vector backbone sequences were detected. BLAST sequence analysis revealed that border regions of the maize event 59122 show significant homology to maize genomic DNA and EST sequences. None of the EST sequences showed homology to known toxin or allergen encoding sequences. Analysis of ORFs spanning the two junction regions was performed by bioinformatic analysis and no novel ORFs with sequence similarity to known toxins or allergens were identified.

The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of maize 59122 does not raise safety concerns, and that sufficient evidence for the stability of the insert structure was provided.

Based on the results of compositional analysis of samples from a representative range of environments and seasons, the GMO Panel concludes that forage and kernels of maize 59122 are compositionally equivalent

to those of conventional maize, except for the presence of CRY34Ab1, CRY35Ab1 and PAT proteins. In addition, field trials did not show indications for unexpected changes of agronomic performance and phenotypic characteristics.

The CRY34Ab1, CRY35Ab1 and PAT proteins induced no adverse effects in acute and repeated dose oral toxicity studies in rodents. In addition, these proteins are rapidly degraded in simulated gastric fluid and inactivated during heat treatments.

A 90-day feeding study of rats fed a diet including kernels from maize 59122 at a level of 35% indicated no adverse effects. A feeding study of broilers did not indicate differences in the nutritional value of maize 59122 versus the non-GM comparator. These animal studies support the findings of the compositional analysis of no effect beyond the intended introduction of the CRY34Ab1, CRY35Ab1 and PAT proteins.

The application EFSA-GMO-NL-2005-12 concerns food and feed uses, import and processing of maize 59122. There is therefore no requirement for scientific information on possible environmental effects associated with the cultivation of maize 59122. There are no indications of increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of 59122 seeds during transportation and processing. Also, the low levels of environmental exposure through other routes indicate that the risk to target and non-target organisms is likely to be extremely low. The scope of the monitoring plan provided by the applicant is in line with the intended uses of maize 59122.

In conclusion, the GMO Panel considers that the information available for maize 59122 addresses the scientific comments raised by the Member States and that maize 59122 is as safe as its non genetically modified counterparts with respect to potential effects on human and animal health or the environment. Therefore the GMO Panel concludes that maize 59122 is unlikely to have any adverse effect on human and animal health or on the environment in the context of its intended uses.

Key words: GMO, maize, insect resistance, glufosinate-containing herbicide, tolerance, CRY34Ab1, CRY35Ab1, PAT protein, *pat* gene, DAS-59122-7, human and animal health, environment, import, food, feed, Regulation (EC) No 1829/2003.

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BACKGROUND

On 26 January 2005 EFSA received from the Dutch Competent Authority an application (Reference EFSA-GMO-NL-2005-12), for authorisation of the insect-resistant genetically modified maize 59122 (Unique Identifier DAS-59122-7), submitted by Pioneer Hi-Bred/Dow Agrosciences within the framework of Regulation (EC) No 1829/2003 on genetically modified (GM) food and feed (EC, 2003) for food and feed uses, import and processing.

After receiving the application EFSA-GMO-NL-2005-12 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission and made the summary of the dossier available to the public on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 16 September 2005 EFSA declared the application as formally valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC (EC, 2001) following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member State bodies had three months after the date of receipt of the valid application (until 16 December 2005) within which to make their scientific comments known.

The GMO Panel carried out a scientific assessment of maize 59122 taking account of 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, 2006a).

On 10 February, 24 May, 12 July 2006 and 26 January 2007 the GMO Panel asked the applicant for additional data or clarifications on maize 59122. The applicant provided the requested information on 3 August, 12 December 2006 and 5 February 2007, respectively. After receipt and assessment of the full data package, the GMO Panel finalized its risk assessment of maize 59122.

The GMO Panel carried out the scientific assessment of the genetically modified maize 59122 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into consideration the scientific comments of the Member States and the additional information provided by the applicant.

In giving its opinion on maize 59122 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003 EFSA has endeavoured to respect a time limit of six months from the receipt of the valid application. As additional information was requested by the GMO Panel, the time limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, the EFSA opinion shall include a report describing the assessment of the food and feed and stating the reasons for its opinion and the information on which its opinion is based. This document is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the overall opinion in accordance with Articles 6(5) and 18(5).

TERMS OF REFERENCE

The GMO Panel was requested to carry out a scientific assessment of the genetically modified maize 59122 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)e of Regulation (EC) No 1829/2003.

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

ASSESSMENT

1. Introduction

The genetically modified (GM) maize 59122 (Unique Identifier DAS-59122-7) was assessed with reference to its intended uses taking account of the appropriate principles described in the Guidance document (EFSA, 2006a). In its evaluation the GMO Panel also considered the comments that were raised by Member States. The assessment presented here is based on the information provided in the application relating to maize 59122 submitted in the EU including additional information from the applicant. The scope of the application is for food and feed uses, import and processing of maize 59122. Cultivation is not included in the scope.

1.1. Description of the traits and mechanism of action

Maize 59122 expresses CRY34Ab1 and CRY35Ab1 proteins rendering maize 59122 resistant to certain coleopteran pests and the PAT (phosphinothricin-N-acetyltransferase) protein which was used as a selectable marker and confers tolerance to glufosinate-containing herbicides. PAT acetylates the active compound L-phosphinothricin present in the glufosinate-containing herbicide and gives rise to the formation of the inactive product N-acetyl-L-phosphinothricin.

The CRY34Ab1 and CRY35Ab1 proteins are derived from *Bacillus thuringiensis.* CRY34Ab1 is a 14 kDa protein comprising 123 amino acids and CRY35Ab1 is a 44 kDa protein comprising 383 amino acids. Both are expressed in maize 59122 and act as a binary toxin to confer resistance to certain coleopteran species, such as corn rootworm larvae (*Diabrotica* spp.). A study showed that CRY35Ab1 protein alone is not active against corn rootworm larvae and that CRY34Ab1 alone causes mortality and growth inhibition to corn rootworm larvae, but for maximal insecticidal activity both the CRY34Ab1 and CRY35Ab1 proteins are required. The binary protein formulation enhances the insect toxicity (Herman et al. 2002). The hypothetical mode of action for this kind of association (i.e. binary toxins) is that CRY34Ab1 is responsible for specific binding to receptors on the insect midgut epithelium while CRY35Ab1 is active on membrane pore formation (de Maagd, 2003).

2. Molecular characterisation

2.1. Issues raised by the Member States

Comments were given regarding the statistical analysis of the expression data and the observed variability of expression levels in terms of gene-environment interactions as well as the size of potential ORFs (Open Reading Frames) to be considered.

2.2. Evaluation of relevant scientific data

2.2.1. Transformation process and vector constructs

The maize event 59122 was produced by means of *Agrobacterium*-mediated transformation of the maize line Hi-II using the binary vector PHP17662 in the *Agrobacterium* strain LBA4404. PHP17662 contains between the left and right T-DNA borders the *cry*34Ab1 coding sequence under the control of *ubi*1ZM promoter (from *Zea mays*) and the *pin*II terminator (from *Solanum tuberosum*), the *cry*35Ab1 coding sequence under the control of the wheat peroxidase promoter (from *Triticum aestivum*) and the *pin*II terminator and the *pat* coding sequence (from *Streptomyces viridochromogenes*) under the control of the 35S promoter and 35S terminator (from cauliflower mosaic virus). The *cry*34Ab1 and *cry*35Ab1 were cloned from *Bacillus thuringiensis* strain PS149B1 and the coding sequence of both genes has been adapted to the codon usage in maize as to achieve expression in the maize plant. The vector backbone portion contains among others a spectinomycin resistance gene, the ColE1 *ori*, *tetA* and *tetR* genes (tetracycline resistance) and several *vir* genes.

2.2.2. Transgenic constructs in the genetically modified plant

The insert of maize event 59122 in the nuclear genome has been entirely sequenced, including the 3' and 5' flanking maize genome sequences. The sequence analysis indicates that the insert comprises one complete copy of the T-DNA of PHP17662 without internal rearrangements. All three gene cassettes, *cry*34Ab1, *cry*35Ab1 and *pat*, are intact within the transgenic event. The DNA sequences of the genes in 59122 are identical to those in the original plasmid except for two nucleotide differences in the wheat peroxidase promoter. At the 5' T-DNA end a deletion of 22 bp is observed and at the 3' T-DNA end a deletion of 25 bp is observed. The plant DNA flanking the insert was sequenced: 2593 bp of the 5' flanking region and 1986 bp of the 3' flanking region. The absence of the tetracycline and spectinomycin resistance genes, the *vir*G gene and regions immediately outside the left and right T-DNA borders was shown by Southern analysis which demonstrates the absence of the vector backbone in maize 59122. The GMO Panel concludes that in the insert of maize 59122 all intended genes (*cry*34Ab1, *cry*35Ab1 and *pat*) are intact.

2.2.3. Information on the expression of the insert

2.2.3.1. Expression of the introduced genes

Expression analysis of the proteins CRY34Ab1, CRY35Ab1 and PAT was carried out by ELISA. Plant material was collected at eleven locations in three countries and at four developmental stages (see section 3.2.1).

The whole plant (above-ground parts) as well as individual samples of leaf, pollen, root, stalk and grain were examined. The CRY34Ab1 and CRY35Ab1 proteins were found in all parts examined whereas the PAT protein was mainly found in the leaf and whole plant samples and not in pollen.

The concentrations of CRY34Ab1 and CRY35Ab1 and their ratio differ depending on the plant tissues/organs where the proteins are expressed. CRY35Ab1 is expressed in low or not detectable levels in pollen whereas CRY34Ab1 is present at concentrations ranging from ca. 50 to 74 µg/g dw (dry weight). Variability is also observed between the different field trials, likely due to environmental conditions. The coefficients of variations in levels of gene expression observed are not uncommon in plants.

With regards to kernels the concentration of CRY34Ab1 and CRY35Ab1 proteins were 61.8 ± 16.5 and 2.34 ± 0.475 µg/g dw, respectively (ratio ~ 26) in Europe; 49.7 ± 16.2 and 0.99 ± 0.33 µg/g dw (ratio ~ 50) in Chile and 36.4 ± 8.9 and 2.0 ± 0.7 µg/g dw (ratio ~ 20) in USA and Canada. The differences in the concentration of the newly expressed proteins in various tissues of maize 59122 untreated and treated with glufosinate-containing herbicide were small with respect to the variation observed in the field trials.

The concentrations of PAT protein were always very low in every part of the plant and detected in the European field trials at a concentration of 0.0807 ± 0.0800 µg/g dw.

SDS-PAGE and Western analysis were used to determine if the CRY34Ab1, CRY35Ab1 and PAT proteins expressed in maize 59122 were of the expected molecular weight and immunoreactivity. For CRY34Ab1, no other bands indicative of partial CRY34Ab1 protein or a fusion protein of greater molecular weight were observed in maize 59122. For CRY35Ab1 two bands were present, the expected 44 kDa band and a truncated 40 kDa band. The identical N-terminal sequence of the intact and the truncated protein suggests that the truncation occurs at the C-terminus.

The PAT protein, expressed in maize 59122, was detected by Western analysis as a band of approximately 23 kDa. No other bands indicative of a partial PAT protein or a fusion protein of greater molecular weight were observed in maize 59122.

2.2.3.2. Putative cryptic open reading frames (ORF) in maize 59122

Bioinformatic analysis for the assessment of novel, putative ORFs created within the maize event 59122 insert was carried out. All potential ORFs have been considered and one novel ORF of 45 amino acids spanning the right T-DNA border was identified. The deduced amino acid sequence of this ORF shows no significant similarity to known toxins or allergens.

BLAST sequence analysis revealed that border region sequences flanking the insert in maize event 59122 have significant homology to maize genomic DNA (the 3' untranslated end of genomic *Zea mays* alcohol dehydrogenase (*adh*1) genes) and several EST (expressed sequence tags) sequences. None of these showed homology to any sequence with a known function, including known allergens or toxins.

2.2.4. Inheritance and stability of inserted DNA

The event 59122 was produced in the maize line Hi-II. The original germplasm was then crossed to a Pioneer elite inbred line PH09B and the resulting plants crossed and back-crossed twice to line 581 and then self-crossed. The Mendelian inheritance pattern of the traits (herbicide tolerance and CRY34Ab1 expression) was demonstrated in the 5th generation resulting progenies. Southern blot patterns obtained for

these plants and maintenance of the phenotype indicated genetic and phenotypic stability of the event 59122 over five respectively four generations.

2.3. Conclusion

The molecular characterisation data established that maize 59122 contains a single insert of the T-DNA. The structure of the insert in maize 59122 was determined by Southern analysis and DNA sequencing. No vector backbone sequences were detected. BLAST sequence analysis revealed that border regions of the maize event 59122 show significant homology to maize genomic DNA and EST sequences. None of the EST sequences showed homology to known toxin or allergen encoding sequences. Analysis of ORFs spanning the two junction regions was performed by bioinformatic analysis and no novel ORFs with sequence similarity to known toxins or allergens were identified.

The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of maize 59122 does not raise safety concerns, and that sufficient evidence for the stability of the insert structure was provided.

3. Comparative analysis

3.1. Issues raised by Member States

Comments were given regarding the choice of comparator, the number of locations/seasons and the statistical outcome of the analyses performed during field trials. Comments on the concentration and range of variations of some nutrients and of CRY34Ab1 and CRY 35Ab1 were also raised, as well as on the composition of the maize used to prepare the diets for the 90-day rat feeding study.

3.2. Evaluation of relevant scientific data

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

Maize 59122 was compared to control non-GM lines with a similar genetic background. Upon request of the GMO Panel, the applicant provided additional information on the different breeding schemes used to produce the control non-GM maize lines for field trials. The pedigree of the control non-GM maize lines showed that the controls were representative of the different crosses and backcrosses that were used to produce maize 59122 in each trial. The GMO Panel concluded that the choice of comparators was appropriate for each field study.

Field trials were carried out at six locations in Chile during the 2002-2003 growing season, at three locations in the USA during 2003, and at two locations in Canada during 2003. Additional data was requested by the GMO Panel and was provided by the applicant for three locations in Bulgaria during 2003 and 2004, and three locations in Spain during 2004. Maize 59122 plants treated with glufosinate-containing herbicide, untreated and the non-GM control maize were included in these field trials. The data from field trials performed in Europe were used by the GMO Panel as the primary source for the comparative assessment of

the composition of maize 59122. Whole crops (forage) and maize tissues, including kernels, were collected from field trials for compositional analysis.

3.2.2. Compositional analysis

Compositional data were obtained by analysis of forage and kernels harvested from field trials performed in maize growing regions of Europe in 2003 and 2004. Statistical analysis of supplied data was performed on both individual and combined locations. The GMO Panel is of the opinion, that this set of compositional data is in compliance with the principles described in the Guidance document (EFSA, 2006a), and the selection of compounds follows the recommendations of OECD (2003).

The proximate and mineral analyses (fat, protein, total carbohydrate, acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, phosphorus, and calcium) of forage from maize 59122 (treated and untreated) were compared to forage from the non-GM control and to typical ranges reported in literature for commercial maize (ILSI, 2006; OECD, 2003). The compositional analysis of kernels of maize 59122 and its control harvested in European field trials included proximate analyses (fat, protein, ash, moisture, carbohydrates, starch), fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acid), amino acids (eighteen amino acids including aromatic amino acids), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, selenium and zinc), vitamins (vitamin B1, vitamin B2, folic acid, β-carotene, vitamin E), anti-nutrients (phytic acid, raffinose and trypsin inhibitor) and other secondary metabolites (inositol, furfural, p-coumaric acid, and ferulic acid).

Statistically significant differences between maize 59122 and the control were observed for some parameters in the forage analysis, such as ash, carbohydrate, crude protein and phosphorous contents. However, no differences were consistently observed over years at any location. In addition, the levels of the compounds found to be statistically different from the corresponding control were within the literature ranges reported for commercial maize varieties.

The analysis of composition of kernels from maize 59122 (treated and untreated) and its control occasionally revealed statistically significant differences in some compounds such as the folic acid and phosphorous contents. However, none of these differences was consistently observed over years and at each location. In addition, the levels of those compounds which were different to the level in the corresponding control were within the literature ranges reported for commercial maize varieties.

The GMO Panel noted that the range provided for the phenylalanine contents of maize 59122 (untreated) grown at a single location in Europe was rather broad (0.445 – 2.96 % dry weight) and outside the range reported in literature for commercial maize (0.24 – 0.94 % dry weight). The GMO Panel accepted the explanation that the 2.96 % dry weight phenylalanine value for a single untreated maize 59122 grain sample can be considered as an outlier due to an experimental error that occurred during sample processing, rather than as a true biological effect. In addition, no consistent statistically significant differences between untreated maize 59122 and the non-GM comparator were observed for phenylalanine and for other amino acids that share the same biosynthesis pathway, such as tryptophan and tyrosine.

Maize 59122 kernels and forage (from treated and untreated plants) and the corresponding non-GM control which had been grown in Chile, USA and Canada were analysed for the same spectrum of compounds as described for the material grown in the European field studies. Some differences in composition were occasionally detected at single field sites, but no consistency over locations was observed. All levels fell within ranges reported in recent literature (ILSI, 2006; OECD, 2003).

Nutrient composition was also determined for the diets to be fed in the subchronic rat feeding study (see section 4.2.4). The GMO Panel noted that the content of vitamin B6 in the rat diet containing 35 % maize 59122 was lower than in diet containing the non-GM control. Therefore the applicant was requested to comment on the observed differences and to provide relevant data on the concentration of vitamin B6 in the GM and non-GM lines. The information provided showed no consistent statistically significant differences between the vitamin B6 contents of maize 59122 and the corresponding non-GM controls were observed for the material grown in Chile, USA and in Canada.

3.2.3. Agronomic traits and GM phenotype

During field trials over several seasons and at different locations (six locations in Chile during the 2002-2003 growing season, three locations in the USA during 2003, two locations in Canada during 2003, three locations in Bulgaria in 2003, three locations in Spain and three locations in Bulgaria in 2004) extensive agronomic data (e.g. grain yield, number of emerged plants, ear height, plant height, early population, final population), were collected for maize 59122 (treated and untreated) and for the corresponding non-GM control. Some statistically significant differences were detected, *e.g.* for mean early population, final population, plant height, and ear height in the European field trials during 2004 growing season. None of these differences were consistently observed over locations and years. The GMO Panel concludes that the agronomic performance and phenotypic characteristics of maize 59122 are comparable to its non transgenic counterpart except for the introduced traits.

3.3. Conclusion

Based on the results of compositional analysis of samples from a representative range of environments and seasons, it is concluded that forage and kernels of maize 59122 are compositionally equivalent to those of conventional maize, except for the presence of CRY34Ab1, CRY35Ab1 and PAT proteins in maize 59122. In addition, experimental field trials in Europe, as well as in Chile, USA, and Canada, did not show indications for unexpected changes of agronomic performance and phenotypic characteristics.

4. Food/feed safety assessment

4.1. Issues raised by Member States

Comments were given regarding how the poultry feeding trial was conducted. One Member State requested further explanation on the significance of an increase of liver weights in female chicken fed the GM-maize diet in the 42-day poultry feeding trial. Some questioned the 90 day subchronic toxicity study on rodents and noted that only one dose of the test material (i.e. maize 59122) was delivered and that the statistical analysis should be complemented with an analysis comparing maize 59122 directly to its non GM comparator. They also noted that statistically significant changes in haematological parameters were observed. A question regarding the identity of material used for the acute toxicity studies was also raised.

4.2. Evaluation of relevant scientific data

4.2.1. Product description and intended use

The genetic modification in maize 59122 is intended to improve the agronomic performance and not to influence nutritional characteristics or production processes. The overall uses of maize as a crop are not expected to be influenced as a result of the introduction of the GM plants into the market.

A dietary exposure assessment assuming that 100% of consumed maize is derived from maize 59122 was carried out. Based on an average maize consumption for the European population of 8.8 g/person/day (Food Balance Sheet compiled by FAO), the applicant calculated a theoretical daily intake for adult European consumers of 60 kg bodyweight. This would correspond to 0.0062 mg/kg bw/day and 0.00024 mg/kg bw/day for the CRY34Ab1 and CRY35Ab1 proteins respectively. It refers to the mean CRY34Ab1 and CRY35Ab1 expression levels in maize 59122 grains presented above. With regards to PAT protein which is expressed at a very low level, the daily intake would be negligible.

4.2.2. Stability during processing

According to the applicant, maize 59122 will be used for production and manufacturing of food and feed products as any other commercial maize. The production methods should not be affected by the genetic modification.

The CRY34Ab1, CRY35Ab1 and PAT proteins expressed in maize 59122 are readily inactivated by heating. Indeed a mixture of CRY34Ab1 and CRY35Ab1 proteins lost biological activity (i.e. capacity to induce mortality or decreased growth in southern corn rootworm *(Diabrotica undecimpunctata howardi*) after exposure to heat treatment (30 minutes at 60°C, 75°C and 90°C) while the group treated with the positive control (non heat-treated binary toxin) showed a growth reduction of 70%. Thus CRY34Ab1 and CRY35Ab1 are likely to be denatured and degraded during the production and processing of foods and feeds made of or derived from maize 59122. Rapid denaturation during heat treatments of the PAT protein is already known (OECD, 1999).

4.2.3. Toxicology

4.2.3.1. Cry and PAT proteins used for safety assessment

Because it was not possible to extract and purify sufficient amounts of proteins from maize 59122 to perform the toxicity studies, CRY34Ab1 (14 kDa) and CRY35Ab1 (44 kDa) proteins were produced in recombinant *Pseudomonas fluorescens* strains MR1253 and MR1256 respectively. Whereas *P. fluorescens* strain MR1253 expresses the native Bt CRY34Ab1 protein, also expressed in maize 59122, *P. fluorescens* strain MR1256 expresses a CRY35Ab1 protein slightly different from that in maize 59122, missing the 4 amino acids at the C-terminal end of the protein. The truncated 40-kDa protein retains the biological activity of the full-length CRY35Ab1 protein.

The microbial-produced CRY34Ab1 and CRY35Ab1 proteins were compared for their structure and biochemical properties with those expressed in the maize 59122. For the comparison CRY34Ab1 and CRY35Ab1 proteins were extracted and purified from maize 59122 leaves and *P. fluorescens*. The purified

protein fractions were analysed by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and showed 2 characteristic bands at molecular weights of ca. 14 and 44 kDa, respectively. In addition a band was observed at ca. 40 kDa for the *P. fluorescens*-derived CRY35Ab1 protein preparation, most likely produced by cleavage of the CRY35Ab1 peptide by proteases removing the C-terminal 4 amino acid peptide. Detection of carbohydrates possibly covalently linked to leaf-derived CRY34Ab1 and CRY35Ab1 proteins were assessed by a Glycoprotein Staining Kit but no glycoproteins were found, indicating a lack of post-translational modification (glycosylation). A proteomic analysis of CRY34Ab1 and CRY35Ab1 proteins derived from maize 59122 leaves was performed using i) electrophoretic separation, ii) in-gel digestion by trypsin, then iii) analysis of the resulting peptide mixture by MALDI-TOF MS (matrix assisted laser desorption/ionization-time of flight mass spectrometry). The peptide mass fingerprints for both the CRY34Ab1 and CRY35Ab1 correctly matched with those theoretically expected from the sequence of CRY34Ab1 and CRY35Ab1 proteins. Unidentified peptides were observed and some expected peptides were not detected, due to random cleavage or formation of fragments during hydrolysis. However this analysis provides good indications on a high degree of homology between the proteins of both sources. The first 10 N-terminal amino acid residues of the maize-derived CRY34Ab1 and CRY35Ab1 proteins were sequenced and compared to the sequence of the microbe-derived proteins. For both the *P. fluorescens* and the maize-derived CRY34Ab1 protein, the first amino acid (methionine) was missing, but the subsequent ten amino acids matched the expected protein sequence. This result is accounted for by the fact that the N-terminal end can be cleaved by aminopeptidases in both prokaryotes and eukaryotes. In conclusion there is a strong weight of evidences that the *P. fluorescens-produced* CRY34Ab1 and CRY35Ab1 proteins used in the experimental studies are equivalent to those extracted from leaf material of maize event 59122.

For toxicity studies the PAT protein was produced in a recombinant *E. coli* strain and was compared to that produced by maize 59122 using SDS PAGE, Western blotting, and ELISA. Analysis of the PAT microbial protein by SDS-PAGE showed the characteristic band at ca. 23 kDa MW. Western blots also showed the same immunoreactive band of expected MW in preparations of the microbe-derived PAT protein and in extracts from 5 samples of maize 59122 leaves. The ELISA determination was confirmatory. This indicates that both the maize 59122 plant extracts and the microbe-derived recombinant PAT contained the intact full length PAT protein.

4.2.3.2. Toxicological assessment of expressed novel proteins in maize 59122

(a) Search for amino acid sequence homology to known toxins

The amino acid sequences of the CRY34Ab1, CRY35Ab1 and PAT proteins expressed in maize 59122 were compared to protein sequences available in public databases using the BLASTP algorithm for the comparison. These searches identified a total of 10, 22 and 148 protein sequences similar to the amino acid sequence of the transgenic proteins, mostly CRY proteins. None of the identified sequences showed any biologically significant sequence homology to known toxins.

A similar comparison was performed with the putative 45 amino acid protein corresponding to the identified ORF spanning the right T-DNA border of the maize 59122 insert (See also section 2.2.3.2). The BLASTP search for this ORF showed no significant sequence homology to known toxins or allergens.

(b) Resistance to proteolysis

The proteolytic degradation of the CRY34Ab1 and CRY35Ab1 proteins by simulated gastric fluid (SGF) was studied by estimating the concentrations of undigested protein and incompletely digested

fragments after various times of exposure, using SDS-PAGE electrophoresis and Western blotting for analysis. Assuming that the pepsinolysis follow a first order kinetic reaction model under the protein:pepsin ratio used, the time at which 90% of the protein had been degraded (DT90) was 6.5 minutes for the CRY34Ab1 protein and less than 5 minutes for the CRY35Ab1 protein. Whereas no degradation fragments were observed during CRY34Ab1 digestion, fragments of 40 and 15 kDa were observed after a 1 minute digestion of CRY35Ab1 protein. No proteolysis resistant degradation fragments were detected for the CRY34Ab1 or CRY35Ab1 protein after 20 min SGF digestion. These DT values are consistent with those observed with other Bt proteins, such as CRY1Ab. The PAT protein was shown to be rapidly degraded to non-detectable levels in simulated gastric fluid containing pepsin.

(c) Acute oral toxicity

Acute oral toxicity studies were carried out in mice using the microbial-produced CRY34Ab1 and CRY35Ab1 proteins separately as well as in combination. There were no indications of adverse effects when the CRY34Ab1 protein was administered by oral gavage to 5 male mice at a dose of 2700 mg per kg body weight. There were also no indications of adverse effects when the CRY35Ab1 protein was administered at a dose of 1850 mg per kg body weight. In another study, a mixture of both proteins at an equimolar ratio, corresponding to a dose of 482 mg CRY34Ab1 and 1520 mg CRY35Ab1 per kg body weight, respectively, was administered to 5 male and 5 female mice by oral gavage. The test material did not induce toxicologically relevant effects.

The applicant provided an acute oral toxicity study performed in 5 male and 5 female mice at a dose of ca. 5000 mg PAT per kg body weight. According to the study report, no treatment-related clinical signs were observed. There were no gross pathologic lesions for any animal. All mice except one female gained body weight over the duration of the study. The other animals developed normally, and the GMO Panel did not consider this effect as being induced by administration of the PAT protein. In addition the applicant refers to toxicity studies with PAT already reported (OECD, 1999).

(d) Repeated dose oral toxicity study

The GMO Panel requested the applicant to provide a repeated dose 28-day oral toxicity study in rodents with the CRY34Ab1 and CRY35Ab1 proteins. This study included five groups of ten mice each (five females and five males). Three test groups received a mixture of the microbial-produced CRY34Ab1 and CRY35Ab1 proteins in the diet at the following doses: 1.97/0.078 (low-dose), 19.7/0.78 (intermediate-dose) or 197/7.8 (high-dose) mg CRY34Ab1/CRY35Ab1 proteins per kg body weight per day, respectively. A control group was fed a standard rodent diet that did not contain the CRY34Ab1 and CRY35Ab1 proteins. In addition, a bovine serum albumin (BSA) protein control group received a diet containing 204.8 mg BSA per kilogram body weight per day to represent an equivalent increase in protein content as in the high-dose CRY34Ab1/CRY35Ab1 group.

The high-dose level selected for this study was based on a 1000-fold margin of exposure over a worst case scenario for human consumption of the CRY34Ab1 and CRY35Ab1 proteins expressed in maize 59122 grains. The average expression of the CRY34Ab1 and CRY35Ab1 proteins in maize 59122 kernels was ca. 41.9 µg/g tissue dry weight for the CRY34Ab1 protein and 1.66 µg/g tissue dry weight for the CRY35Ab1 protein. Assuming that 100% of consumed maize (i.e. highest global level of consumption estimated at 4.7 g maize/kg bw/day by the applicant who refers to WHO GEMS Cluster Diet Data) is derived from maize 59122 and that there is no degradation of the CRY34Ab1 and CRY35Ab1 proteins by food and feed processing, worst case daily human exposure to the CRY34Ab1 and CRY35Ab1 proteins would be 0.197 and 0.0078 mg/kg bw/day, respectively. The doses administered in the 28 day study correspond to 10, 100 and

1 000 times this anticipated maximum exposure of humans in the low dose, intermediate dose and high dose group, respectively.

Body weights and feed consumption were recorded regularly. Detailed clinical observations were conducted on all animals before the beginning of the treatment and once per week throughout the study. At the end of the feeding period, mice were sacrificed and haematology, clinical chemistry and urine examinations, organ weight determinations as well as macroscopic and histopathological examinations of selected organs and tissues were carried out.

All animals consuming diets containing the CRY34Ab1/CRY35Ab1 proteins survived the 28-day test period. Some transient fluctuations in the weight gain of females were observed in the low dose and intermediate dose groups. They were considered unrelated to treatment because they were not observed in the high-dose group and they occurred only at two time points at the beginning of the study. There were no statistically significant differences in body weights of males or females between the CRY34Ab1/CRY35Ab1 groups and the BSA protein control in the overall observation period. Mean feed consumption for males in all CRY34Ab1/CRY35Ab1 groups at day 2-3 was statistically different from the BSA protein control group. However, this observation only occurred once at the beginning of the study suggesting that the observed effects were unrelated to treatment. There were no treatment-related effects in clinical signs, ophthalmic, hematology, organ weights, clinical chemistry parameters and gross or histopathologic observations in male and female mice following 28 days of dietary exposure to the CRY34Ab1/CRY35Ab1 proteins at any of the dose levels tested.

The design of this study is appropriate with regard to the safety evaluation of kernels from maize 59122 containing CRY34Ab1 and CRY35Ab1 proteins in a specific ratio allowing a synergistic action.

A repeated dose toxicity study feeding rats the PAT protein during 14 days was provided. The rats received diets containing 0 (control), 5 and 50 g of PAT protein/kg of diet which corresponded to average maximum intakes of ca. 7.6 and 7.9 g/kg bw/day for males and females, respectively. The animals were examined daily for signs of toxicity, and food consumption and body weights were recorded regularly. Food consumption and body weight were not influenced by the PAT treatment with no occurrence of mortality. At the end of the treatment period, haematology, clinical chemistry and urine examinations, organ weight determinations as well as macroscopic and histopathological examinations of selected organs and tissues were carried out. The GMO Panel concluded that feeding the PAT protein to rats for 14 days revealed no indications for adverse effects up to the highest dose tested.

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 maize 59122, and levels of endogenous compounds were not altered, a toxicological assessment for new constituents is not applicable.

4.2.4. Toxicological assessment of the whole GM food/feed

Subchronic feeding study

A thirteen-week (90-day) feeding study in rats has been carried out with kernels from maize 59122 and recently published (Malley *et al.*, 2007). Groups of 12 male and 12 female rats were fed for 90 days diets

containing ca. 35% kernels from maize 59122, the non-GM control maize or a commercial non-GM maize. Two additional groups received a standard commercial rodent diet.

The animals were regularly examined for clinical signs, and body weights, food consumption and food efficiency were recorded. Neurobehavioural and ophthalmological examinations were performed at the beginning of the study and at the end of the treatment period. Blood and urine samples were taken at approximately day 45 and day 90 and haematology, clinical chemistry and urine analyses (clinical pathology) were carried out. At the end of the treatment period, the rats were sacrificed, the weights of selected organs and tissues were determined and gross and histopathological examinations were conducted on all animals.

According to the original study report, no adverse diet-related differences were observed with respect to clinical signs of toxicity, opthalmological observations and neurobehavioural assessments, clinical pathology, organ weights and gross or microscopic findings in rats receiving the maize 59122 diet compared with the four combined control groups. In addition, there were no adverse, diet-related differences in mean body weight, body weight gain, food consumption or food efficiency.

However the GMO Panel did not consider the statistical analysis as adequate, because the comparisons were made between groups fed maize 59122 and the four combined control groups. Therefore a new statistical analysis was requested. In addition, information regarding the origin of the non-GM control maize with comparable genetic background was requested.

The new statistical analysis revealed no significant differences in final body weight, body weight gain, food consumption and food efficiency between rats fed the maize 59122 diet compared with the non-GM control maize. In the clinical pathology examinations, male rats receiving the maize 59122 diet showed statistically significant decreases in absolute reticulocyte count and red cell distribution width as well as increases in mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Females showed an increase in platelet count. These differences were small, and the values were generally comparable with those of other control groups in this study and/or fell within the ranges for the historical control means for rats of the same strain in other subchronic feeding studies. In addition, there were no statistically significant differences in other parameters which are expected to be affected in case of relevant effects. The GMO Panel therefore does not consider the observed differences as toxicologically relevant.

There were no remarkable findings in the histopathological examinations of selected organs and tissues. Organ weight determinations revealed a statistically significant increase in uterus weight in females receiving the maize 59122 diet. This effect was explained by the observation that a larger proportion of females in this group were in the estrus or proestrus whereas a larger proportion of the controls were in the metestrus and diestrus (determined by estrus staging). In addition, the values fell within the ranges for the historical control means, and the histopathological examinations did not reveal adverse effects in this organ. In males a higher weight of the adrenal glands was found. This effect was small and not statistically significant when the values were compared with those of the three other control groups. No relevant differences between groups were found in the histopathological examinations of this organ. Therefore the GMO Panel does not consider these organ weight changes as toxicologically relevant.
The GMO Panel noted that only one dose level was administered in this study. According to the respective OECD Guideline (408), which was designed for the testing of chemicals, this is acceptable under certain conditions. In this study, the percentage of maize kernels in the diet was ca. 35%, which approximates to the highest level normally used in 90-day feeding studies with maize in rats. Since no toxicologically relevant effects were observed, the GMO Panel considered the study as acceptable.

4.2.5 Allergenicity

For the assessment of the allergy risk, two issues are taken into consideration by the GMO Panel: 1) exposure to newly expressed protein(s) that can be present in edible parts of the plants or in the pollen and 2) alterations to the allergenicity of the whole plant and derived products *e.g.* due to over-expression of natural endogenous allergens as an unintended effect of the genetic modification.

4.2.5.1. Assessment of allergenicity of the newly expressed proteins

Allergenicity of CRY34Ab1, CRY35Ab1 and PAT proteins was assessed, using a weight of evidences approach, through i) information regarding the allergenicity of the source of the transgenes, ii) the search of any sequence homology between the newly expressed proteins and common allergens and iii) studies on resistance to *in vitro* simulated digestibility. In addition, the applicant provided information on the glycosylation and on the heat stability of the newly expressed proteins.

B. thuringiensis (the source of the *cry*34Ab1 and *cry*35Ab1 genes) and *S. viridochromogenes* (the source of the *pat* gene) are not commonly known to cause allergy including occupational allergy in workers producing or using *B. thuringiensis* or derived products.

The comparison of sequences focused on two types of homology between CRY34Ab1 or CRY35Ab1 and allergens: short linear stretches (i.e. contiguous 8 amino acid fragments) corresponding to IgE binding epitopes and overall identity (e.g. 35% or higher) within 80 amino-acid peptides. None of the searches identified homology with known allergens.

A similar comparison of the amino acid sequence of the PAT protein to known protein allergens was also carried out. The results confirmed that the PAT protein shares no significant amino acid homology with known protein allergens, as already noted in previous opinions.

A hypothetical peptide translated from an ORF sequence was also compared to sequences in allergen database in order to identify amino acid sequences that may represent linear epitopes. No stretches of six or more contiguous amino acids of the putative ORF were found to be identical to sequences present in the known protein allergens.

In addition the applicant recalled that i) the CRY34Ab1, CRY35Ab1 and PAT proteins are not glycosylated when expressed in the maize 59122 grain where they only represent very small amounts, ii) they are completely degraded during in vitro digestion by simulated mammalian gastric fluids and loose biological activity when exposed for 30 minutes at temperatures of 60°C and higher (already discussed in sections 4.2.3.2. and 4.2.2., respectively). Based on all information made available, the GMO Panel considers that the newly expressed CRY34Ab1, CRY35Ab1 and PAT proteins are not likely to be allergenic.

4.2.5.2. Assessment of allergenicity of the whole GM crop

Allergenicity of the whole crop could be increased as an unintended effect of the genetic modification, for example through qualitative or quantitative modification of the pattern of expression of endogenous proteins. However there is no such indication from the compositional analysis (see section 3.2.2.). Moreover this issue does not appear relevant to the GMO Panel since maize is not considered a common allergenic

food. Food allergies to maize are of low frequency and mainly occur in populations of specific geographic areas. Rare cases of occupational allergy to corn dust have been reported. Therefore a possible over expression of any endogenous protein, which is not a common allergen, would be unlikely to alter the overall allergenicity of the whole plant. In addition, there is no reason to expect that the use of GM maize 59122 will significantly increase the intake and exposure to maize and consequently increase the allergy risk.

4.2.6. Nutritional assessment of the whole GM food/feed

Poultry feeding study (42 days)

A poultry feeding study over a period of 42 days was carried out on 600 broiler chickens (50% males and 50% females) with grain from maize 59122, grain from a non-GM near-isogenic control maize (091 maize) and grain from 3 commercial non-GM maize. There were 10 broilers per pen (5 males and 5 females) with 12 pens per treatment. Broilers were fed their respective dietary treatments from time of hatching (Trial Day 0) to 42 days of age; day 0-21 a starter diet (53% maize), day 22 to 35 a grower diet (58% maize), and day 36 to 42 and finisher diet (70% maize). Homogeneity and stability of the CRY34Ab1, CRY35Ab1 and PAT proteins in the diet were evaluated using specific ELISA. Actual concentrations were : 13 ± 1.27, 12.4 ± 0.63 and 18.3 ± 0.75 ng CRY34Ab1 /g diet ; 0.68 ± 0.08, 0.75 ± 0.04 and 1 ± 0.04 ng CRY35Ab1 /g diet in the starter, grower and finisher diet respectively. In all diets, the concentration of PAT protein was below the limit of detection (i.e. 0.034 ng/g diet).

Regarding growth performance (body weight and gain, mortality, and feed efficiency) there was no observable effect of the different dietary exposure groups particularly between the non-GM control maize and maize 59122 groups.

No statistically significant difference in carcass yields and organ weights were observed between the non-GM control maize group and the maize 59122 group except for liver weight in females which was higher for broilers fed with maize 59122 diet than those fed the control diet. However, after consideration of the multiplicity of the tests performed (McNaughton, 2007) and the variability calculated from data relating to the non-GM commercial maize varieties, the GMO Panel considers that the difference is unlikely to be of any biological significance.

In conclusion, results showed no consistent differences between dietary treatments with the maize 59122 and the non-GM control maize which is in line with the absence of significant differences observed in the compositional analysis.

4.2.7. Post-market monitoring of GM food/feed

Maize 59122 is intended to have improved agronomic properties. From a nutritional point of view the maize is equivalent to conventionally bred varieties. 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 59122 is any less safe than its non-GM comparators. The opinion of the applicant that a post-market monitoring of GM food and GM feed products containing, consisting of or derived from maize 59122 is not necessary is in line with the Guidance document (EFSA, 2006a) and is shared by the GMO Panel.

4.3. Conclusion

The transgenic CRY34Ab1, CRY35Ab1 proteins induced no adverse effects in acute and repeated dose oral toxicity studies in mice at doses that are up to 1 000 times higher than the exposure for humans in a worst case scenario. In addition, they are rapidly degraded in simulated gastric fluid and inactivated during heat treatments. The PAT protein is expressed at very low levels in maize 59122. It has also been proved to be safe in toxicity studies and it is rapidly degraded by proteases.

The sequence of the transgenic CRY34Ab1, CRY35Ab1 and PAT proteins did not show any significant similarity with the sequences of known toxins or allergens. With regard to animal studies with the whole product, there were no indications of adverse effects in a 90-day feeding study in rats fed a diet including kernels from maize 59122 at a level of 35%. In addition, nutritional data comprising a target animal feeding study with maize 59122 grains on broilers indicate that maize 59122 is nutritionally equivalent to the non-GM comparator. These animal studies therefore further support the findings of the compositional analysis of no effect beyond the intended introduction of the CRY34Ab1, CRY35Ab1 and PAT proteins.

The GMO Panel is of the opinion that maize 59122 is as safe as its non GM counterparts and that the overall allergenicity of the whole plant is not changed and concludes that maize 59122 is unlikely to have any adverse effect on human and animal health in the context of its intended uses.

5. Environmental risk assessment and monitoring plan

5.1. Issues raised by the Member States

Comments were given regarding the potential development of resistance in target organisms, the mode of action and the potential toxicity of the CRY34Ab1 and CRY35Ab1 proteins on non-target organisms, the possible use of glufosinate-containing herbicides and the need for data on potential increased fitness. Further comments were raised with respect to the need for case specific monitoring and a more detailed general surveillance plan including specific management measures in case of accidental release of maize 59122 into the environment.

5.2. Evaluation of relevant scientific data

5.2.1. Environmental risk assessment

Maize 59122 has been developed for protection against specific coleopteran pests, such as the western corn rootworm larvae (*Diabrotica virgifera virgifera* LeConte), and tolerance to glufosinate-containing herbicides. The insect resistance is achieved by expression of CRY34Ab1 and CRY35Ab1 proteins from *Bacillus thuringiensis* and tolerance to the glufosinate-containing herbicides is conferred by phosphinothricin-N-acetyltransferase (PAT) from *Streptomyces viridochromogenes*.

The scope of the application is for food and feed uses, import and processing of maize 59122 and excludes cultivation. Considering the proposed uses of maize 59122, the environmental risk assessment is concerned mainly with indirect exposure through manure and faeces from the gastrointestinal tracts of

animals fed on the GM maize and with accidental release into the environment of GM seeds during transportation and processing for food and feed uses.

The scope of the application excludes cultivation; therefore concerns regarding glufosinate treatments to maize 59122 apply only to imported and processed maize products that may have been treated with glufosinate-containing herbicides in the countries of origin. Glufosinate-containing herbicides are also used in Europe and the regulation and risk assessment of these herbicides is within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market (EC, 1991).

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

Maize is highly domesticated and generally unable to survive in the environment without cultivation. Maize plants are not winter hardy in most regions of Europe, they have lost their ability to release seeds from the cob and they do not occur outside cultivated land or disturbed habitats 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.

The herbicide tolerance trait can only be regarded as providing a selective advantage for the GM maize plant where and when glufosinate-containing herbicides are applied. Insect resistance against certain coleopteran pests, such as corn rootworm larvae (*Diabrotica* spp.), provides an advantage in cultivation. However survival of maize outside cultivation in Europe is mainly limited by a combination of poor competitive ability, absence of a dormancy phase, susceptibility to diseases and to cold climate conditions. Since these general characteristics of this GM maize are unchanged, insect resistance is not likely to provide a selective advantage outside cultivation in Europe. Therefore it is considered very unlikely that plants of this GM maize or its progeny will differ from conventional maize varieties in their ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

Field trials with maize 59122 were carried out at 6 locations in Chile (2002-2003), at 5 locations in USA and Canada (2003), at 3 locations in Bulgaria respectively in 2003 and in 2004 and at 3 locations in Spain in 2004. The field data provided in the application do not show increased invasiveness or enhanced weediness and fitness, except in the presence of glufosinate-containing herbicides. In addition to the data presented by the applicant, the GMO Panel is not aware of any scientific report of increased spread and establishment of the maize 59122 and any change in survival capacity, including over-wintering.

Since maize 59122 has no altered survival, multiplication or dissemination characteristics except in the presence of the specific glufosinate-containing herbicides or target organisms, the GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this maize will not differ from that of conventional maize varieties.

5.2.1.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via seed dispersal and cross-pollination.

(a) Plant to bacteria gene transfer

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

In the case of accidental release and establishment of maize 59122 in the environment, exposure of microorganisms to transgenic DNA derived from GM maize plants would take place during natural decay of GM plant material and/or pollen in the soil of areas where feral plants established.

Food and feed products derived from the GM maize could contain transgenic DNA. Therefore microorganisms in the digestive tract of humans and animals may be exposed to transgenic DNA.

The *cry34Ab1* and *cry35Ab1* genes are under the control of eukaryotic promoters (see Section 2.2.1) therefore no significant gene expression is expected in prokaryotes in the unlikely event of horizontal gene transfer.

The *pat* gene is known to be widespread in soil microbial populations (Herouet *et al.*, 2005) and the *cry34Ab1/cry35Ab1* genes, which occur naturally in bacterial populations (Schnepf *et al.*, 2005), were cloned from a naturally occurring *B. thuringiensis* (strain PS149B1). Taking into account the origin and nature of the *cry34Ab1/cry35Ab1* and *pat* genes and the lack of selective pressure in the intestinal tract and/or the environment, the likelihood that horizontal gene transfer of the *cry34Ab1/cry35Ab1* and *pat* genes would confer selective advantage or increased fitness to microorganisms is very limited. For this reason it is very unlikely that genes from maize 59122 would become transferred and established in the genome of microorganisms in the environment or human and animal digestive tract. In the very unlikely event that such horizontal gene transfer would take place, no adverse effects on human and animal health or the environment are expected, as no principally new traits would be introduced or expressed in microbial communities.

(b) Plant to plant gene transfer

The extent of cross-pollination to conventional maize varieties will mainly depend on the scale of accidental release during transportation and processing. For maize, any vertical gene transfer is limited to other *Zea mays* plants as populations of sexually compatible wild relatives of maize are not known in Europe (OECD, 2003).

Maize 59122 has no altered survival, multiplication or dissemination characteristics except in the presence of the specific glufosinate-containing herbicides or target organisms. Tolerance to glufosinate-containing herbicides and insect resistance against certain coleopteran pests, such as corn rootworm larvae (*Diabrotica* spp.), provide agronomic advantages. However survival of maize outside cultivation in Europe is mainly limited by a combination of poor competitive ability, absence of a dormancy phase, susceptibility to diseases and to cold climate conditions. Since these general characteristics of this GM maize are unchanged, herbicide tolerance and insect resistance are not likely to provide selective advantages outside cultivation in Europe. Therefore, as for any other maize varieties, GM plants would only survive in subsequent seasons in the warmer regions of Europe and are not likely to establish feral populations under European environmental conditions (see Section 5.2.1.1).

The flowering of the sporadic feral GM maize plants originating from accidental release occurring during transportation and processing is unlikely to disperse significant amounts of GM maize pollen to other maize plants. Since enhanced survival, multiplication or dissemination are only likely when maize 59122 is

cultivated in the presence of the specific herbicides or target organisms, the GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this maize in Europe will not differ from that of conventional maize varieties.

5.2.1.3. Potential interactions of the GM plant with target organisms

The maize 59122 was transformed to co-express CRY34Ab1 and CRY35Ab1 proteins from *Bacillus thuringiensis*. This binary insecticidal toxin is made of two components, the CRY34Ab1 and the CRY35Ab1 proteins, acting together in the control of certain coleopteran pests, such as the western corn rootworm (*Diabrotica virgifera virgifera* LeConte), the northern corn rootworm (*D. barberi* Smith & Lawrence) and the southern corn rootworm (*D. undecimpunctata howardi* Barber) (Masson *et al*., 2004).

However, considering that the proposed use of maize 59122 specifically excludes cultivation, the environmental exposure is mainly limited to the rare occurrence of sporadic feral plants due to accidental release of GM seeds during transportation and processing for food and feed uses. Thus the level of exposure of target organisms to CRY34Ab1 and CRY35Ab1 proteins is likely to be extremely low and of no ecological relevance.

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

Considering the proposed uses of maize 59122, the environmental risk assessment is concerned mainly with indirect exposure through manure and faeces from the gastrointestinal tracts of animals fed on the GM maize and with accidental release into the environment of GM seeds during transportation and processing.

The GMO Panel assessed whether CRY34Ab1 and CRY35Ab1 proteins might potentially affect non-target organisms by entering the environment in manure and faeces from the gastrointestinal tracts of animals fed on the maize 59122. Data supplied by the applicant (Herman *et al.*, 2003) and literature on other CRY proteins (Ahmad *et al*., 2005 and references therein; Lutz *et al*., 2005) indicate that most CRY proteins are degraded by the enzymatic activity in the gastrointestinal tract so that very low amounts of CRY protein would remain intact to pass out in faeces. There would subsequently be further degradation of these proteins in the manure and faeces due to microbial processes. Exposure of soil and water environments to these CRY toxins from disposal of animal wastes is likely to be very low and localized. Thus exposure of potentially sensitive non-target organisms (e.g. coprophagous Coleoptera species) to CRY34Ab1 and CRY35Ab1 proteins is likely to be very low and of no ecological relevance.

In conclusion the GMO Panel considers that the level of exposure of any potential non-target organisms to the binary CRY34Ab1 and CRY35Ab1 proteins in combination with the PAT protein would be of no ecological relevance.

5.2.1.5. Potential interaction with the abiotic environment and biogeochemical cycles

This point was not considered an issue by the Member States or by the GMO Panel. The level of exposure would be so low that potential effects on the abiotic environment and biogeochemical cycles are unlikely.

5.2.2. Monitoring

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct and to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment.

Monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific quality of the monitoring plan provided by the applicant (EFSA, 2006b). The potential exposure to the environment of maize 59122 would be through manure and faeces from the gastrointestinal tracts of animals fed on the GM maize or through accidental release into the environment of GM seeds during transportation and processing.

No specific environmental impact of this GM maize was indicated by the risk assessment and thus no case specific monitoring is required.

In the general surveillance plan provided in the application, the applicant explains that i) a monitoring system will be used by including all the operators involved in the handling and use of viable maize 59122, ii) substantial accidental releases of viable maize 59122 will be monitored for any potential adverse effects and iii) the operators will be required to report to the applicant any unanticipated adverse effects due to environmental exposure of the GM maize. The applicant will submit a general surveillance report on an annual basis. In case of adverse effects altering the conclusions of the environmental risk assessment, the applicant will immediately inform the European Commission.

The GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize 59122 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan. The GMO Panel advises that appropriate management systems should be in place to restrict seeds of maize 59122 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

5.3. Conclusion

The scope of the application is for food and feed uses, import and processing of maize 59122 and excludes cultivation. Considering the proposed uses of maize 59122, the environmental risk assessment is concerned mainly with indirect exposure through manure and faeces from the gastrointestinal tracts of animals fed on the maize 59122 and with accidental release into the environment of 59122 seeds during transportation and processing.

There are no indications of increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of 59122 seeds during transportation and processing for food and feed uses. Only extremely low levels of gene transfer to other maize plants are predicted with no adverse effects. Taking into account the scope of the application, only rare occurrence of sporadic feral plants and the low levels of exposure through other routes indicate that the risk to target and non-target organisms is considered negligible.

The scope of the monitoring plan provided by the applicant is in line with the intended uses of maize 59122 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. Furthermore the GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel was requested to carry out a scientific risk assessment of the maize 59122 for food and feed uses, import and processing.

The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of maize 59122 does not raise safety concerns, and that sufficient evidence for the stability of the insert structure was provided.

Comparative analysis has shown that maize 59122 is compositionally and agronomically equivalent to conventional maize lines, except for the introduced transgenic traits. The risk assessment included an analysis of data from analytical studies, bioinformatics, and *in vitro* and *in vivo* studies. The GMO Panel concluded that the maize 59122 is as safe as its non GM counterparts and that the overall allergenicity of the whole plant is not changed.

The application EFSA-GMO-NL-2005-12 concerns food and feed uses, import and processing of maize 59122. There is therefore no requirement for scientific information on possible environmental effects associated with the cultivation of maize 59122. There are no indications of increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of 59122 seeds during transportation and processing. Also, the low levels of environmental exposure through other routes indicate that the risk to target and non-target organisms is likely to be extremely low. The scope of the monitoring plan provided by the applicant is in line with the intended uses of maize 59122.

In conclusion, the GMO Panel considers that information available for maize 59122 addresses the comments raised by the Member States and considers it unlikely that maize 59122 will have any adverse effect on human and animal health or on the environment in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Dutch Competent Authority (VROM), dated 26 January 2005, concerning a request for placing on the market of maize 59122 in accordance with Regulation (EC) 1829/2003.

2. Letter from JRC to EFSA, dated 20 June 2005, with complete application (ref. JRC 106-BGMO/GVDE/SC/D(2005)(154)15011).

3. Letter from EFSA to applicant, dated 2 August 2005, with request for clarifications/additional information (ref. SR/AC/sp (2005) 1001).

4. Letter from applicant to EFSA, dated 9 September 2005, providing additional information upon EFSA request.

5. Letter from EFSA to applicant, dated 16 September 2005, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2005-12, maize 59122 submitted by Pioneer under Regulation (EC) 1829/2003 (ref. SR/AC/jq (2005) 1149).

6. Letter from applicant, dated 16 September 2005, providing EFSA with an updated version of the application EFSA-GMO-NL-2005-12 submitted by Pioneer under Regulation (EC) 1829/2003:

   Part I – Technical dossier

   Part II – Summary

   Part III – Cartagena Protocol

   Part IV – Labelling and Unique Identifier

   Part V – Samples and Detection

   Part VI – Additional information for GMOs

7. Letter from EFSA to applicant, dated 10 February 2006, with request for additional information (ref. SR/AC/jq (2006) 1366685).

8. Letter from applicant to EFSA, dated 21 March 2006, providing additional information upon EFSA request.

9. Letter from EFSA to applicant, dated 24 May 2006, with request for additional information (ref. SR/KL/jq (2006) 1540176).

10. Letter from applicant to EFSA, dated 20 June 2006, providing additional information upon EFSA request.

11. Letter from EFSA to applicant, dated 12 July 2006, with request for additional information (ref. SR/CP/jq (2006) 1636132).

12. Letter from applicant to EFSA, dated 3rd August 2006, providing additional information upon EFSA request.

13. Letter from applicant to EFSA, dated 11 December 2006, providing additional information upon EFSA request.

14. Letter from EFSA to applicant, dated 26 January 2007, with request for additional information (ref. SR/CP/shv (2007) 1937382).

15. Letter from applicant to EFSA, dated 5 February 2007, providing additional information upon EFSA request.

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SCIENTIFIC PANEL MEMBERS

Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Lieve Herman, Marc De Loose, Niels Hendriksen, Sirpa Kärenlampi, Jozsef Kiss, Ilona Kryspin-Sørensen, Harry Kuiper, Ingolf Nes, Nickolas Panopoulos, Joe Perry, Annette Pöting, Joachim Schiemann, Willem Seinen, Jeremy Sweet, and Jean-Michel Wal.

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