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SCIENTIFIC OPINION

Application (Reference EFSA-GMO-UK-2005-11) for the placing on the market of insect-resistant genetically modified maize MIR604 event, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds S.A.S on behalf of Syngenta Crop Protection AG[1]

Scientific Opinion of the Panel on Genetically Modified Organisms

(Question No EFSA-Q-2005-046)

Adopted on 2 July 2009

PANEL MEMBERS[*]

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SUMMARY

Following a request from Syngenta Seeds S.A.S on behalf of Syngenta Crop Protection AG within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the authorisation of the insect-resistant genetically modified maize MIR604 (Unique Identifier SYN-IR6Ø4-5) for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the new application EFSA-GMO-UK-2005-11, additional information provided by the applicant and the scientific comments submitted by the Member States. The scope of application EFSA-GMO-UK-2005-11 is for food and feed uses, import and processing of genetically modified maize MIR604 and all derived products, but excluding cultivation in the EU.

The EFSA GMO Panel assessed maize MIR604 with reference to the intended uses and 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

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[*] (minority opinion) This opinion is not shared by 0 members of the Panel. / (conflict of interest) 0 members of the Panel did not participate in (part of) the discussion on the subject referred to above because of possible conflicts of interest.

feed. The scientific assessment included molecular characterisation of the inserted DNA and expression of the newly expressed proteins. A comparative analysis of agronomic traits and composition was undertaken, and the safety of the new proteins and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plan was also undertaken.

Maize MIR604 was engineered with a modified *cry3A* coding sequence *(mcry3A)* derived from *Bacillus thuringiensis* subsp. *tenebrionis* that encodes an insecticidally active mCry3A protein confering resistance to the Western Corn rootworm (WCR) *(Diabrotica virgifera virgifera)* and other related coleopteran pests of maize like the Northern Corn rootworm (NCR) (*Diabrotica barberi*). In addition maize MIR604 was engineered with the *pmi* (*manA*) gene from *Escherichia coli*, which encodes the enzyme PMI (PhosphoMannose Isomerase) as a selectable marker. PMI allows transformed maize cells to utilize mannose as a sole carbon source, while maize cells lacking the *pmi* gene fail to grow with mannose as single carbon source.

The molecular characterisation data established that a single insert with one copy of the expression cassette containing the *mCry3A* gene and the *pmi* gene is integrated in the maize genomic DNA. Appropriate analyses of the integration site including sequence determination of the inserted DNA and flanking regions. Bioinformatic analysis of junction regions demonstrated the absence of any potential new ORFs coding for known toxins or allergens. The expression of the gene introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The EFSA GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of maize MIR604 does not raise any safety concern, and that sufficient evidence for the stability of the genetic modification was provided.

Based on the results of the comparative analysis of samples from a representative range of environments and growing seasons, it is concluded that maize MIR604 is compositionally, phenotypically and agronomically equivalent to conventional maize varieties, except for the presence of the PMI and mCry3A proteins.

The functional characteristics and the potential toxicity and allergenicity of the newly expressed PMI protein have been explored through various studies, for which *in-vitro*, *in-vivo*, and bioinformatic-supported methods have been employed. It was concluded that the PMI protein did not show characteristics that would indicate potential toxicity or allergenicity.

A subchronic (90-day) feeding study revealed no indications of adverse effects in rats fed diets containing grains from maize MIR604. In addition, a feeding study in broiler chickens provided evidence of nutritional equivalence of maize MIR604 to conventional maize. These studies, therefore, support the conclusion of the compositional and agronomical comparison that the genetic modification resulted in no unintended effects.

The application EFSA-GMO-UK-2005-11 concerns food and feed uses, import and processing, but excludes cultivation in the EU. There are no indications of an increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of maize MIR604 viable grains during transportation and processing for food and feed uses. Taking into account the scope of the application, both the rare occurrence of feral plants and the low levels of exposure through other routes indicate that the risk to non-target organisms is negligible. The scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MIR604 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. Furthermore the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

In conclusion, the EFSA GMO Panel considers that the information available for maize MIR604 addresses the scientific comments raised by Member States and that maize MIR604 is as safe as its conventional counterpart with respect to potential effects on human and animal health or the environment. Therefore the EFSA GMO Panel concludes that maize MIR604 is unlikely to have any adverse effect on human or animal health or on the environment in the context of its intended uses.

Key words: GMO, maize (*Zea mays*), MIR604, insect-resistant, mCry3A, PMI, import, processing, food safety, feed safety, human and animal health, environment, Regulation (EC) No 1829/2003

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BACKGROUND

On 12 January 2005, EFSA received from the Competent Authority of the United Kingdom an application (Reference EFSA-GMO-UK-2005-11) for authorisation of the genetically modified insect-resistant maize MIR604 (Unique Identifier SYN-IR6Ø4-5) submitted by Syngenta Seeds S.A.S on behalf of Syngenta Crop Protection AG within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed (EC, 2003) for food and feed uses, import and processing.

After receiving the application EFSA-GMO-UK-2005-11 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States as well as the European Commission and made the summary of the dossier publicly available 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 19 August 2005 EFSA received additional information requested under completeness check and on 16 September 2005 EFSA declared the application as 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 17 December 2005) within which to make their opinion known.

The EFSA GMO Panel carried out a scientific assessment of genetically modified (GM) maize MIR604 taking into account 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 3 April 2006, 26 October 2006, 14 March 2007, 15 June 2007, 24 September 2007, 26 November 2007 and 14 March 2008, the EFSA GMO Panel asked for additional data on maize MIR604. The applicant provided the requested information respectively on 29 June 2006, 30 January 2007, 26 March 2007, 4 July 2007, 23 August 2007, 14 November 2007, 1 April 2008, 13 May 2008, 3 November 2008 and 7 April 2009. On April 29, the EFSA GMO Panel gave the possibility for technical experts of the applicant to clarify specific issues. After assessment of the full data package, the EFSA GMO Panel finalised its risk assessment of maize MIR604.

The EFSA GMO Panel carried out a scientific assessment of the GM maize MIR604 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 GM maize MIR604 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 EFSA 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 scientific opinion shall include a report describing the assessment of the food and feed and stating the reasons for its scientific opinion and the information on which its scientific 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 EFSA GMO Panel was requested to carry out a scientific assessment of the genetically modified maize MIR604 for food and feed uses and 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/environments 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 EFSA GMO Panel was not requested to give an opinion on information required under Annex II of the Cartagena Protocol, nor on the 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 GMO risk management.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Groups on Molecular Characterisation, Food/Feed and Environment, as well as the *ad hoc* experts: Hans-Jörg Buhk and Angela Sessitsch, and the following members of its staff: Anna Christodoulidou, Ana Gomes and Sylvie Mestdagh for the preparation of this opinion.

ASSESSMENT

1. Introduction

Maize MIR604 (Unique Identifier SYN-IR6Ø4-5) is assessed with reference to its intended uses and 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). The risk assessment presented here is based on the information provided in the application relating to maize MIR604 submitted in the EU, including additional information from the applicant, and scientific comments that were raised by Member States.

2. Issues raised by Member States

Issues raised by Member States are addressed in Annex G of the overall opinion.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs

Event MIR604 was produced by genetic transformation of a hybrid between two maize inbred lines (NP2500 and NP2499). Immature maize embryos were transformed using *Agrobacterium tumefaciens* strain LBA4404 containing the plasmid pZM26. Two expression units were present between the left and the right borders of the T-DNA in pZM26.

One expression unit comprises a modified version of a *Cry3A* gene (*mCry3A* gene) derived from *Bacillus thuringiensis* subsp. *tenebrionis* under the control of the MTL-promoter from a metallothionein-like gene from *Zea mays* and the NOS terminator of *A. tumefaciens*. The *mCry3A* gene was modified in order to enhance toxicity to target insects. This was achieved by incorporating a cathepsin-G serine protease recognition site and by N-terminal deletion of the *Cry3A* protein. Also, codon usage was optimised for expression in maize.

The other expression unit comprises the *man*A gene as a selectable marker encoding phosphomannose isomerase (PMI) from *E. coli* under regulation of the promoter and first intron region of the *Zea mays* polyubiquitin gene and the NOS terminator of *A. tumefaciens*. A functional *manA* gene allows selection of transformed cells on media containing mannose as the sole carbon source.

3.1.2. Transgenic constructs in the genetically modified plant

Southern analysis combined with sequencing and inheritance studies establishes integration of a single T-DNA copy. The absence of the vector backbone in the MIR604 plants has been confirmed by Southern analysis using probes that cover the entire backbone sequence of 5309 base pairs (bp).

The nucleotide sequence of the insert in maize event MIR604 has been determined in its entirety. The intact T-DNA copy has been inserted with the exception of 43 bp and 44 bp deletions at the left and right borders respectively. In addition, three base pair changes have occurred in the insert, two of which result in amino acid substitutions in the PMI protein.

Sequences flanking the 5' and 3' regions of the MIR604 event have been determined, extending at least 1 Kb into the host genome. A recent (2008) BLASTN analysis of the 5' and 3' flanking sequences showed no significant homology with any known *Zea mays* sequences. ORF analysis of all six potential reading frames at both the 5' and 3' flanking regions revealed the presence of one putative novel ORF. This is 258 bp in length, begins in the NOS terminator and extends through the T-DNA into the 3' flanking sequence. The ~240 bp upstream of this putative ORF is terminator sequence, and no promoter elements have been found. Therefore, transcription of this putative ORF is unlikely. In the unlikely event that the ORF were to be transcribed, bioinformatic analysis indicates no sequence homologies to known toxins or allergens.

3.1.3. Information on the expression of the insert

Expression analysis of the mCry3A and the PMI proteins in maize plants derived from event MIR604 was carried out by ELISA in material at four growth stages from field-grown plants in the USA (see also section 4.1.1). The samples examined were leaf, root, whole plant (above ground parts), kernels, silk and pollen. Expression studies have been conducted in different genetic backgrounds (hybrids and inbred line).

The mCry3A protein was found in all plant parts analysed except for pollen, while the PMI protein could be detected in all analysed plant parts.

The data provided for mCry3A are presented on a µg mCry3A protein/g tissue dry weight and a µg mCry3A protein/g tissue fresh weight basis. Maximal expression level was found in leaves of a MIR604-derived inbred line at anthesis (average 93.5 and maximum 107.6 µg mCry3A protein/g dry weight). The average values for whole plant extracts of MIR604-derived hybrids ranged between 7.3 and 23.8 µg mCry3A protein/g tissue dry weight and for kernels between 0.8 to 2 µg mCry3A protein/g tissue dry weight. The level of mCry3A protein in pollen was below the detection level (0.15 µg/g dry weight).

The concentration of PMI protein based on ELISA analysis was generally lower than the mCry3A. The data are presented on a µg PMI protein/g tissue dry weight and a µg PMI protein/g tissue fresh weight basis. Maximum expression (on a tissue dry weight basis) was found in leaves of MIR604-derived hybrids and inbred line at whorl stage (average 2.14 and maximum of 2.56 µg PMI protein/g tissue dry weight). The average values for whole plant extracts ranged from below the limits of detection to 2.01 µg PMI protein/g tissue dry weight and for kernels from below the limits of detection to 0.5 µg PMI protein/g tissue dry weight.

3.1.4. Inheritance and stability of inserted DNA

Genetic stability of event MIR604 was investigated by Southern, PCR and ELISA analysis of backcross (with inbred line NPH8431) generations BC4 to BC6. The presence of a single copy of the gene encoding mCry3A in the analysed material indicates stable inheritance over several generations. The expected inheritance ratio of 3:1 was observed for PMI and mCry3A, indicating the presence of a stable single Mendelian locus. The expression of mCry3A and PMI was demonstrated to be stable over four backcross generations.

3.2. Conclusion

Appropriate analysis of the integration site including flanking sequences and bioinformatic analysis have been performed to analyse the construct integrated in the genetically modified plant. The expression of the genes introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The molecular characterisation provided for the transformation event MIR604 is sufficient for the

safety assessment of this transformation event. The EFSA GMO Panel considers this to be an adequate analysis and the molecular characterisation assessment does not indicate any safety concerns.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

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

Maize MIR604 was compared with control non-GM lines with comparable genetic background maize during field trials in multiple locations in the USA that had been carried out for two seasons (i.e., 2002 and 2003). Kernels as well as forage consisting of the above-ground parts were obtained from these field trials for use in the compositional analysis As requested by the EFSA GMO Panel, the applicant has provided further details on the breeding history of the non-GM maize line NP894 as the most appropriate comparator in the two growing seasons. Supplementary data were provided where maize MIR604 was compared with two additional non-GM comparators (lines NPH8431 and NP904), each of which was only used in a single growing season.

For the analysis of mono- and disaccharides, including phosphorylated forms of these saccharides, the applicant provided, at the EFSA GMO Panel's request, additional data from field trials with maize MIR604 and a non-GM, near-isogenic control, which had been performed at six locations in the USA in 2006. Kernel samples from the plots within each location were processed into flour for subsequent analysis.

4.1.2. Compositional analysis

The composition of forage samples from both years (2002 and 2003) was analysed for macronutrients, and fibre, while the kernel samples were analysed for macronutrients, fibre, vitamins (provitamin A, vitamins E, B1, B2, B6, niacin, folic acid), major elements (Ca, Mg, P, K, Na) and trace elements (Cu, Fe, Zn, Cr, Mn, Se), amino acids, and fatty acids. In 2003, a number of supplementary analytes were measured, including total carbohydrates and minerals in forage and some additional vitamins (pantothenic acid, C), cryptoxanthin, antinutrients (trypsin inhibitor), phytosterols (cholesterol, campesterol, stigmasterol, beta-sitosterol), and other secondary metabolites (phytic acid, inositol, raffinose, ferulic acid, p-coumaric acid, furfural) in kernels. This choice of analysed compositional parameters is in line with those recommended by the OECD Consensus Document on key nutrients, anti-nutrients, and secondary plant metabolites (OECD, 2002). At the EFSA GMO Panel's request, the applicant provided further details of the statistical analysis with respect to data from each location, adding to the existing analysis of combined data from all field trial sites. In addition, the applicant provided supplementary data on the standard error and ranges for each parameter analysed, and on the background range of phytosterol values in maize kernels.

A number of statistically significant differences ($p<0.05$) were observed in the comparison between the GM lines and their non-GM comparators. In the analysis of data obtained from the maize lines grown for two years (2002 and 2003), the sole difference that was observed in both years was an increased level of the fatty acid oleic acid (C18:1) in kernels of the transgenic maize. Additional differences noted in this comparison occurred only in the second year, including moisture and several minerals in forage, as well as various proximates, minerals, vitamins, amino-acids and fatty acids, in kernels.

Higher levels of oleic acid (C18:1) were reported for kernels derived from a MIR604 maize line that had been field-tested only in 2002. The kernels of the same GM maize line also showed differences in another fatty acid and a single amino acid whilst forage of this line contained less ash than forage from the control. Kernels of another pair of GM and non-GM lines tested in 2003 showed differences in various proximates, minerals, vitamins, amino acids, phenolic acids, and phytosterols.

These observed differences between maize MIR604 and its non-GM comparators all fell within the range of natural variability reported in literature, except for: i) campesterol in kernels of one GM line, which was slightly above the upper boundary of background values in one location; and ii) for values of oleic acid in kernels of two control lines and one GM line, which fell below the range of natural variability. With the exception of higher oleic acid levels, none of these differences were consistently observed over the seasons tested.

For analytes that had been measured in only one season, statistically significant differences occurred in the levels of two phenolic compounds, i.e. ferulic acid and p-coumaric acid, and two sterols, i.e. campesterol and stigmasterol (see above) when analysed across field trial sites. None of the statistically significant differences in the combined locations were statistically significant in each separate location of the particular year. The differences in phenolics appeared relatively large, but the levels fell within the wide range of natural variability. The levels of these phenolic acids in kernels can vary widely across varieties and are influenced both by genotype and by environment (e.g., Chetrit et al., 1998). In addition, following a request to the applicant from the EFSA GMO Panel, supplementary information on the content of these phenolic acids in kernels, data for two "stacked events" containing the MIR604 event (MIR604 x GA21 and Bt11 x MIR604) tested in the USA in one growing season were provided. Whilst the average levels of phenolic acids in Bt11 x MIR604 were statistically significantly lower than in the non-GM comparator, their levels were higher in MIR604 x GA21 although these higher levels were not statistically significant.

The EFSA GMO Panel also considered the possibility that the expression of the PMI enzyme interfered with the formation of downstream metabolites of mannose-6-phosphate and fructose-6-phosphate, including glycans attached to glycoproteins. The applicant provided further details corroborating the findings of Privalle (2002) that a PMI-producing maize did not show any changes in the electrophoretic profiles of glycoproteins. In compounds that could theoretically be linked to PMI (e.g., starch and other carbohydrates), no consistent compositional differences were observed in the comparison between maize MIR604 and its non-GM comparators.

In the additional field trials in 2006, an analysis of monosaccharides and disaccharides, and their phosphorylated forms, was carried out on flour derived from kernels of maize MIR604 and its non-GM comparators. No statistically significant difference were observed for the levels of fructose-6-phosphate; mannose-1-phosphate/mannose-6-phosphate; sucrose-6-phosphate; glucose-6-phosphate; fructose-1,6-diphosphate; fructose; glucose; sucrose; myo-inositol; and various unidentified saccharides.

Taking into consideration the relatively minor magnitude of most observed differences and the inherent variability of the composition of maize in general, and fatty acids in particular (e.g., Reynolds et al., 2005; Dunlap et al., 1995), the EFSA GMO Panel concludes that the observed differences do not raise any safety concern.

4.1.3. Agronomic traits and GM phenotype

The agronomic performance of transgenic maize MIR604 and controls was analysed in multiple field and greenhouse trials that were carried out during two years, *i.e.* 2002 and 2003, in various locations in the USA. The parameters tested included corn rootworm damage, pathogen

infestation, yield and other physiological characteristics. Corn rootworm damage was lower in MIR604 compared with the non-GM comparators, and yields of maize MIR604 were higher in locations where corn rootworm and drought were prevalent. No other consistent differences in agronomic performance and pathogen infestation were observed. The EFSA GMO Panel therefore concludes that, with the exception of expected differences in agronomic performance linked with the introduced insect-resistance trait of maize MIR604, the phenotypic and agronomic performance of this maize is equivalent to that of the non-GM comparators.

4.2. Conclusion

Based on the results of the comparative analysis of samples from a representative range of environments and growing seasons, and literature data, it is concluded that maize MIR604 is compositionally, phenotypically and agronomically equivalent to conventional maize, except for the presence of the PMI and mCry3A proteins in maize MIR604.

5. Food/Feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Product description and intended use

The scope of application EFSA-GMO-UK-2005-11 includes the import and processing of maize MIR604 and its derived products for use as food and feed. Thus, the possible uses of maize MIR604 include the production of animal feed and food products such as, starch, syrups and oils.

The genetic modification of maize MIR604 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, processing characteristics and overall use of maize MIR604 as a crop.

5.1.2. Effect of processing

Processed maize fractions were analysed by ELISA for the presence of mCry3A. The mCry3A protein was detectable in all fractions of dry-milled maize whilst it was not detected in refined maize oil and corn tortilla chips. In some fractions of wet-milled maize (e.g., medium and fine fiber and gluten meal) mCry3A was also detectable, but not in germ, starch and steep water.

In silage made from whole chopped maize plants of maize MIR604, the level of mCry3A was measured by means of an ELISA assay in samples taken before ensiling (day 0) and after 15, 29, and 75 days of ensiling. The protein was still detected after 75 days of ensiling, albeit at lower levels than in the pre-silage sample. Whilst PMI was also measured in the same experiment, the ELISA method was not sufficiently sensitive to measure the levels of PMI in the silage samples.

Moreover, at the EFSA GMO Panel's request, the applicant provided a study on the presence of the newly expressed PMI in processed fractions of a stacked maize event containing MIR604, i.e. Bt11 x MIR604, with similar expression levels of PMI to the single event MIR604. Both dry-milled and wet-milled fractions were analysed for the presence of PMI by ELISA and enzyme activity assays. The protein was present in the kernels used as starting material and also in the germs and flour fractions obtained after dry milling, whilst neither PMI protein nor its activity could be detected in wet-milled fractions consisting of gluten, starch, and dried germ. Moreover, PMI was not detectable by ELISA in the germ fractions after the moistened germs had been heated to 100° for 30 minutes, which simulates processing conditions during oil extraction.

Since maize MIR604 is compositionally equivalent to the control maize, except for the newly expressed proteins (see section 4.1.2), the effect of processing on maize MIR604 is not expected to be different compared to that on conventional maize.

5.1.3. Toxicology

5.1.3.1. Protein used for safety assessment

Given the low levels of mCry3A and PMI proteins expressed in maize MIR604 plant tissues, and the difficult task of isolating a sufficient quantity of purified proteins from this maize for safety testing, proteins produced in a recombinant *E. coli* strain were used.

The mCry3A protein produced in *E. coli* bacteria was a mixture of two forms of mCry3A, one was the expected mCry3A protein, and the other had an additional N-terminal extension of 16 amino acids derived from the cloning vector used in the bacteria. The plant-expressed and the microbial proteins displayed similar activities in an insect bioassay. In addition, a comparison of the bacterially and plant-produced forms of mCry3A by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE), immunoblotting, and mass spectrometry confirmed their similarity. In addition it was shown that none of these forms was glycosylated.

The influence of temperature on the mCry3A protein was studied in a bio-assay by determining its insecticidal activity on insect larvae after incubation of the enzyme at 4, 25, 37, 65 and 95 ºC for 30 minutes. After incubation at 95 ºC for 30 minutes at pH 7.5, no activity was detected.

The applicant employed a PMI protein produced in *E.coli*, named PMI-0105 that contained the same two amino acid substitutions as the plant produced protein for acute toxicity testing and *in vitro* digestibility and temperature stability assays. The identity of the PMI-0105 protein has been confirmed by Western blotting, molecular weight determination by mass spectrometry, N-terminal sequencing, and enzymatic activity assay. At the EFSA GMO Panel's request, further data have been provided by the applicant on the equivalence of PMI-0105 to the newly expressed PMI present in maize MIR604. This showed that both PMI-0105 and the PMI from MIR604 have similar specific enzymatic activities and that both have the same molecular size based on identical electrophoretic mobilities as detected by immunoblotting. Immunoblots of PMI-0105 also show a faint band that likely corresponds to a dimeric form of PMI-0105. The enzymatic activity of the microbially derived PMI-0105 (see section 5.1.3.2) was almost completely lost (97% reduction) after incubation at pH 7.0 and 65 ºC for 30 minutes, whilst the immunoreactivity of PMI-0105, as measured by ELISA, was completely lost under these conditions.

In addition, another PMI protein has been produced in *E. coli*. This protein, named PMI-0198, had an additional N-terminal T7-tag extension compared to the plant PMI protein, due to the technique used to express it in *E.coli.* This protein lacked the two amino acid substitutions that had occurred in the plant-expressed PMI protein, i.e. valine to alanine at position 61 and glutamine to histidine at position 210, and, apart from the N-terminal extension, it can be considered identical to the native protein encoded by the bacterial *manA* gene. PMI-0198 has been used as substance for acute toxicity testing and *in vitro* digestibility and temperature stability assays. The data on the testing with this protein were considered supplementary by the EFSA GMO Panel.

The EFSA GMO Panel accepts the use of the bacterially produced mCry3A and PMI proteins for safety testing.

5.1.3.2. Toxicological assessment of expressed novel protein in maize MIR604

The mCry3A protein has not been assessed previously by the EFSA GMO Panel, although there is significant experience in dealing with the safety assessment of other Cry proteins, e.g., Cry3Bb1, Cry1Ab and Cry1Ac.

The PMI enzyme has not been assessed previously for its safety by the EFSA GMO Panel. PMI catalyses the conversion of mannose-6-phosphate to fructose-6-phosphate and vice versa, and these two compounds are the only known substrates of PMI enzymes (Freeze, 2002). This has been further confirmed by a study performed by the applicant, in which various saccharides were incubated with PMI-0105, the bacterially produced analogue of the newly expressed PMI in maize MIR604. Whilst PMI catalysed the interconversion between fructose-6-phosphate and mannose-6-phosphate at pH 7.5, no reaction occurred when fructose-1,6-diphosphate, mannose-1-phosphate, glucose-6-phosphate, fructose, or mannose were added as substrates. PMI enzymes occur in a wide range of organisms including prokaryotes and eukaryotes such as bacteria, yeasts, animals, and humans, as well as plants, in which PMI is involved, for example, in glycoprotein synthesis. In MIR604 maize plants expressing PMI, no change in glycoprotein profiles has been observed (see section 4.1.2), indicating no effects of the introduction of PMI on the host plants' protein glycosylation (Reed et al., 2001). At the EFSA GMO Panel's request, the applicant also provided data on the pH-activity profile of PMI-0105, the bacterially produced analogue of the newly expressed PMI enzyme in maize MIR604. PMI-0105 showed enzymatic activity across the pH range tested (pH 5.0 to 10.0) with a pH optimum of 7.5.

(a) Acute toxicity testing

The proteins mCry3A and PMI-0105 did not induce adverse effects in acute oral toxicity studies using mice after administration of a single dose of 2377 mg mCry3A/kg body weight and 2072 mg PMI/ kg body weight, respectively.

(b) Degradation in simulated digestive fluids

The *in vitro* digestibility of the mCry3A and PMI proteins was studied in model systems employing solutions of the protease pepsin in diluted hydrochloric acid, also referred to as simulated gastric fluid (SGF).

Both microbially produced and plant-derived mCry3A were incubated in SGF (pH 1.2) at a pepsin : mCry3A ratio of approximately 2.7:1 (w/w). Both proteins were degraded within two minutes, as measured by SDS-PAGE and immunoblotting.

In addition, the sensitivity of PMI-0105 to pepsin degradation was measured by incubation in SGF (pH 1.2) at a pepsin: PMI ratio of 2.9:1 (w/w). PMI-0105 was completely degraded by pepsin within one minute, as shown in immunoblots following electrophoretic separation of the incubation samples. Incubation of PMI-0105 in simulated intestinal fluid (SIF; pH 7.5) at a pancreatin:PMI ratio of 38:1 (w/w) showed that PMI was immediately completely degraded, as shown by immunoblots. Additional incubations with SIF that had been diluted 10 and 100 fold showed that, in 10-fold diluted SIF, PMI-0105 was completely degraded within 30 minutes, whilst a faint band of intact PMI was still present in immunoblots of samples from incubations of PMI-0105 in 100-fold diluted SIF.

(c) Bioinformatic studies

Comparison of the amino acid sequences of the mCry3A and PMI proteins expressed in maize MIR604 with the sequences stored in a general protein sequence database identified no similarities with known toxic proteins.

5.1.3.3. Toxicological assessment of new constituents other than proteins

No new constituents other than the mCry3A and PMI proteins are expressed in maize MIR604 and no relevant changes in the composition of maize MIR604 were detected in the comparative compositional analysis (see section 4.1.2).

5.1.3.4. Toxicological assessment of the whole GM food/feed

Subchronic oral toxicity

A 90-day rat feeding study with kernels from maize MIR604 was carried out with rats of a Wistar-derived strain (Alpk:APfSD). There were four groups of rats, consisting of 12 animals of either gender, two of which received diets containing kernels from maize MIR604 at 10% or 41.5% (w/w) inclusion rates, while the other two groups received diets containing kernels from a non-GM control maize at the same inclusion levels.

During the experimental period, animals were checked daily for clinical signs, food consumption and body weight were recorded weekly, and functional capability and motor activity tests were carried out at the end of the treatment period. Clinical pathology measurements at study termination included haematology, serum chemistry, organ weight determinations, macroscopic and microscopic examinations.

There were a number of statistically significant differences compared with the controls, many of which occurred in the group fed diets with 10% maize MIR604 (compared with the 10% control group) and not in the groups fed 41.5% maize. In particular, average bodyweights of males fed 10% transgenic maize lagged consistently behind those of the controls during most of the experiment. The EFSA GMO Panel considers that those differences, which occurred only between the 10% groups, were not dose-related, and therefore not related to the administration of maize MIR604.

Differences that occurred in the group fed 41.5% GM maize compared with the controls included lower average bodyweights of female animals during the second, fifth, sixth and tenth weeks of the experiment. At the same inclusion level of transgenic maize, feed consumption was decreased in females receiving the GM maize during one week. Since, the differences in body weights were small and final body weights did not differ, the EFSA GMO Panel does not consider them as toxicologically relevant.

With regard to haematology, lower platelet counts were observed in males receiving 41.5% maize MIR604 compared with the corresponding controls. The average platelet count values of particularly the control- but also the MIR604-fed groups were above the average values of historical controls, which were provided on request of the EFSA GMO Panel. All of the individual values for MIR604-fed rats nonetheless fell within the historical control ranges, whilst the range for the control-fed animals partially exceeded the historical range. In addition, there were no differences in related parameters (prothrombin time, activated partial thromboplastin time). Therefore, this difference is regarded as incidental and probably due to a relatively high control value. In the results of serum chemistry analysis, males receiving 41.5% transgenic maize showed higher mean plasma cholesterol levels. There was a relatively wide variation in the range of

individual values in this group and the mean value was higher than the range of the historical control means. Since there were neither any differences in related blood parameters nor any related findings in the microscopic liver examinations, and males of the control group also had relatively high cholesterol levels, this difference is not regarded as toxicologically relevant and probably not related to administration of maize MIR604. A lower mean plasma creatinine kinase activity in females fed 41.5% maize MIR604 was apparently due to a relatively high mean value in the corresponding control group including two animals showing very high values. After removal of these outliers, the difference was no longer statistically significant.

The organ weight determinations of groups that had received diets containing 41.5% GM maize showed higher heart weights in females (adjusted for bodyweight) and testes weights in males compared with the corresponding controls. Mean and individual values for heart weights in females fell within ranges of the historical controls. Mean absolute testes weight was slightly higher than the range of historical control means, but lower than in the group fed 10% non-GM control maize. In addition, there were no findings in the histopathological examinations of hearts and testes. Therefore, these differences are not regarded as toxicologically relevant.

5.1.4. Allergenicity

Strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the newly expressed protein, the potential of the newly expressed protein to induce sensitization or to elicit allergic reactions in already sensitized 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 (CAC, 2003; EFSA, 2006a).

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

The mCry3A and PMI proteins originate from sources that have no documented history of allergenicity. In addition, these proteins are rapidly degraded in simulated gastric fluid (see section 5.1.3.2.(b)).

When the criterion of an identical 8-aa contiguous amino acids stretch was applied in bioinformatic-supported studies using databases of known allergens, the mCry3A sequence yielded no positive outcomes, whereas one identical stretch of 8 amino acids was observed that occurred in both PMI and a frog leg allergen. However, no reaction occurred between the PMI protein and IgE serum from a human subject that was allergic towards frog legs as demonstrated by immunoblotting. This serum was taken from the same subject that had been reported in scientific literature to react with the pertinent frog allergen (Hilger et al., 2002). A positive control with the pertinent frog leg allergen reacted positively.

Another bioinformatic analysis was carried out on possible similarities of the sequences of the plant-expressed mCry3A and PMI proteins to known allergens. Codex alimentarius (CAC, 2003) as referenced by the EFSA Guidance Document recommends considering potential IgE cross-reactivity if there is more than 35% identity in a segment of 80 or more amino acids (EFSA, 2006a). No peptides having 35% identity in an 80-amino-acid window to an allergen sequence of similar size were identified for mCry3A while one window was found to be similar to the latex allergen Hev b 13 in PMI. This allergen has been described to loose its IgE-serum-binding characteristics after deglycosylation (Arif et al., 2004). Unlike Hev b 13, the plant-expressed PMI is not glycosylated (see sections 3.1.2 and 5.1.3.1). Whilst this does not definitely rule out a possible cross reaction, the EFSA GMO Panel also considered the fact that the 35% level of identical amino acids was reached within a single window of 80 amino acids. The alignment of this

window to Hev b 13 required the introduction of many sequence gaps that lower the percentage of identity to 28% when included in the denominator for the calculation. The EFSA GMO Panel thus concluded that the outcomes of the alignment of PMI with Hev b 13 do not indicate potential cross reactivity of the newly expressed PMI protein.

The EFSA GMO Panel also noted that PMI derived from the *manA* gene in *E. coli* is a member of the superfamily of "cupins," which are proteins with a specific 3-D structure. Some members of this superfamily are known to be allergens (Breiteneder and Radauer, 2004; Dunwell et al., 2001; Mills et al., 2004). The EFSA GMO Panel noted that bioinformatic analysis did not reveal any relevant sequence homology between the PMI expressed in maize MIR604 and known allergens of the cupin superfamily (see above). At the EFSA GMO Panel's request, the applicant has provided a risk assessment of the potential allergenicity, including the capacity for sensitisation, of the newly expressed PMI protein being a member of the cupin superfamily. This included the construction of a three-dimensional, spatial structure of the newly expressed PMI protein using a computer algorithm, and a comparison of this spatial structure with that of the cupin allergen Ara h 1, which naturally occurs in peanut. A comparison of these spatial structures showed that, whilst the proteins share a core with the typical barrel structure inherent to cupins, the remainder of the structures was different. For example, various parts of the Ara h 1 protein that are known to act as IgE-binding epitopes do not have corresponding counterparts in the spatial structure of the newly expressed PMI.

The applicant also provided supplementary data in the form of a report on the outcome of a study in which rats, sensitised with ovalbumin, were fed diets containing either maize MIR604 or a non-transgenic control maize. The EFSA GMO Panel did not consider this study as relevant with respect to the assessment of the sensitizing potential of maize MIR604 and its newly expressed proteins.

The EFSA GMO Panel considers that the available data do not provide indications of the newly expressed PMI having possible cross-reactivity with known allergens or a *de novo* sensitising potential.

The EFSA GMO Panel also considered possible immunogenicity and adjuvanticity of Cry proteins. After intraperitoneal (i.p.), intranasal (i.n.) or intragastric administration of Cry1Ac and i.p. and i.n. administration of Cry3A to mice at relatively high dosage, IgG, IgM and mucosal IgA response were induced, but no IgE response was reported (Guerrero et al., 2004; Vazquez-Padron et al., 1999; 2000). This demonstrates that Cry1Ac and Cry3A have no allergenic potential under the conditions used.

Furthermore, Cry1Ac has been shown to act as an adjuvant e.g., it enhances the mucosal and/or the systemic antibody response to an antigen, i.e. hepatitis B surface antigen or the capsular polysaccharide of *Streptococcus pneumoniae*, where co-administered with the Cry protein through the i.g., i.p., and i.n. routes (Vazquez et. al., 1999; Moreno-Fierros et al., 2003). The EFSA GMO Panel is of the opinion that, as maize is not a common allergenic food, the adjuvant effect of Cry proteins, observed after high dosage intragastric or intranasal administration, is unlikely to raise any concerns regarding allergenicity.

5.1.4.2. Assessment of allergenicity of the whole GM plant

The issue of a potential increased allergenicity of maize MIR604 does not appear relevant to the EFSA 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 maize dust have been reported. There is no reason to expect that the use of GM maize will significantly increase the intake and exposure to maize. Therefore a

possible over-expression of any endogenous protein, which is not known to be allergenic, would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

5.1.5. Nutritional assessment of GM food/feed

A feeding study with rapidly-growing broilers has been performed. Three groups of each 75 males and 75 females received diets contained transgenic maize MIR604, a non-GM control maize with comparable genetic background, or conventional maize. Starter, grower, and finisher diets were administered to the animals with maize inclusion rates varying between 55-66% during 49 days. During the experiment, lighting had been reduced in order to slow down growth and thus reduce affections related to rapid growth. Body weights were measured at 0, 16, 31, and 49 days, in addition to feed intake. The carcass characteristics that were measured in six male and six female animals per group included body weight and weights of fat pads, drums, thighs, wings, pectoralis major and pectoralis minor. A statistically significant difference was observed in thigh weights of female animals, which were slightly higher in animals that had received transgenic MIR604 than in those that had received the non-GM control maize, but not different from those that had received the reference diet. The EFSA GMO Panel concludes that this difference is minor and not biologically relevant and that maize MIR604 is as nutritionally wholesome as conventional maize.

5.1.6. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that maize MIR604 is any less safe than its non-GM comparator. In addition, no biologically relevant agronomic and compositional changes were identified in maize MIR604. Therefore, in line with the guidance document (EFSA, 2006a), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

5.2. Conclusion

No toxicity of the mCry3A protein was observed in an acute oral toxicity study in mice. The mCry3A protein expressed in maize MIR604 showed no homology to known toxic proteins and allergens. Furthermore, it was rapidly degraded in simulated gastric fluid.

The functional characteristics and the potential toxicity and allergenicity of the newly expressed PMI have been explored through various studies, including substrate specificity testing; an assay of the pH-activity relationship; a thermal stability test; bioinformatic-supported comparisons of the protein with toxins and allergens, *in vitro* resistance to proteases and an acute oral toxicity study using mice. In addition, the spatial structure of the newly expressed PMI has been compared with that of an allergenic protein from peanut, Ara h 1, both being members of the cupin superfamily of proteins. PMI did not show characteristics that would indicate potential toxicity or allergenicity of PMI.

A subchronic (90-day) feeding study revealed no indications of adverse effects in rats fed diets containing grains from maize MIR604. In addition, a feeding study in broiler chickens provided evidence of nutritional equivalence of maize MIR604 to conventional maize. These studies, therefore, support the conclusion of the compositional and agronomical comparison that the genetic modification resulted in no unintended effects.

The EFSA GMO Panel is of the opinion that maize MIR604 is as safe as conventional maize varieties and considers it unlikely that the overall allergenicity of the whole plant is changed. Maize MIR604 and derived products are unlikely to have any adverse effect on human and animal health in the context of its intended uses.

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

6.1.1. Environmental risk assessment

The scope of application EFSA-GMO-UK-2005-11 is for food (e.g., syrup, starch, oil)/feed (e.g., meal, oil) uses, import and processing of maize MIR604 and does not include cultivation. Considering the intended uses of maize MIR604, the environmental risk assessment is concerned with exposure through manure and faeces from the gastrointestinal tracts of animals fed maize MIR604 and with accidental release of maize MIR604 viable grains into the environment during transportation and processing.

Maize MIR604 has been developed for protection against specific coleopteran pests, such as the Western corn rootworm larvae (*Diabrotica virgifera virgifera*). The insect resistance is achieved by expression of the modified Cry3A protein from *Bacillus thuringiensis* subsp. *tenebrionis*.

6.1.1.1. 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 agricultural landscapes of Europe, despite cultivation for many years.

Insect resistance against certain coleopteran pests, such as corn rootworm larvae (*Diabrotica* spp.), provides a potential agronomic advantage in cultivation under *Diabrotica* spp. infestation conditions. 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 or volunteers of maize MIR604, 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 were carried out by the applicant at 22 locations in US over two growing seasons (2002-2003). The field data provided in the application showed enhanced biomass production in conditions of *Diabrotica* spp. infestation but do not show changes in plant characteristics that indicate altered fitness and invasiveness of maize MIR604 plants. In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased spread and establishment of maize MIR604 and any change in survival capacity, including over-wintering. Besides the ability to utilize mannose can only be regarded as selective advantage where and when mannose is available as carbon source, which is not the case in soils.

Since maize MIR604 has no altered survival, multiplication or dissemination characteristics, except under infestation conditions of target pests, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this GM maize will not differ from that of conventional maize varieties.

6.1.1.2. 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

Current scientific knowledge (see EFSA, 2009 for further details) suggests that gene transfer from GM plants to microorganisms under natural conditions is extremely unlikely, and that its establishment would occur primarily through homologous recombination in microorganisms. *Cry*3A and *man*A genes, as expressed in maize MIR604, are of bacterial origin. As the functional genes are already present in microorganisms in the natural environment, homologous recombination and acquisition of these genes by microorganisms will not alter the gene pool of the natural microbial community.

In addition, the modified version of the *cry3A* gene and the *man*A gene in maize MIR604 are under the control of eukaryotic promoters with limited, if any, activity in prokaryotic organisms (see section 3.1.1).

Transgenic DNA is a component of many food and feed products derived from 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 although DNA becomes degraded in the human or animal digestive tract.

In the case of accidental release and establishment of maize MIR604 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 GM plants establish.

The ability to utilise mannose is a common metabolic trait in several soil/aquatic microorganisms but mannose is not a common carbon source for soil/aquatic microorganisms. The presence of the *manA* gene, encoding phosphomannose isomerase (see section 3.1.1), therefore cannot be considered a fitness enhancer for microorganisms.

Taking into account the microbial origin and/or nature of the modified *cry*3A gene and the lack of selective pressure in the intestinal tract and/or the environment, the likelihood that horizontal gene transfer would result in increased fitness on microorganisms or other selective advantages is very small. For this reason it is very unlikely that genes from maize MIR604 would become established in the genome of microorganisms 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 or the environment are expected, as no principally new traits would be introduced into or expressed by natural microbial communities.

(b) Plant to plant gene transfer

The extent of cross-pollination of other 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 (Eastham and Sweet, 2002; OECD, 2003).

The flowering of occasional 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. Field observations performed on GM maize volunteers in Spain revealed that maize volunteers had a low vigour, rarely had cobs and produced pollen that cross-pollinated neighbour plants only at low levels (Palaudelmàs et al., 2009).

Insect resistance against certain coleopteran pests, such as corn rootworm larvae (*Diabrotica* spp.), provides an agronomic advantage in cultivation under infestation conditions of the specific target organisms. However survival of maize outside cultivation in Europe is mainly limited by a

combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens and frost. Since these general characteristics are unchanged in maize MIR604, insect resistance is not likely to provide a selective advantage outside cultivation in Europe. Therefore, as for any other maize varieties, GM plants would only survive in subsequent seasons in warmer regions of Europe and are not likely to establish feral populations under European environmental conditions.

In conclusion, since maize MIR604 has no altered survival, multiplication or dissemination characteristics, except under infestation conditions of target pests, the EFSA 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.

6.1.1.3. Potential interactions of the GM plant with target organisms

Maize MIR604 was transformed to express a modified version of the Cry3A protein from *Bacillus thuringiensis* subsp. *tenebrionis*. This insecticidal protein is active in the control of certain coleopteran pests, such as the Western corn rootworm (*Diabrotica virgifera virgifera*) and the Northern corn rootworm (*Diabrotica barberi*).

The intended uses of maize MIR604 specifically exclude cultivation and environmental exposure to maize MIR604 plants is limited to the accidental release of viable grains into the environment during transportation and processing. The EFSA GMO Panel considers that it would need successful establishment and spread of high numbers of maize MIR604 to enable any significant interaction with target organisms, which is very unlikely (see section 6.1.1.1).

Environmental exposure to Cry3A protein is otherwise limited to manure and faeces from the gastrointestinal tracts of animals fed maize MIR604. Data supplied by the applicant suggest that only small amounts of the modified Cry3A protein enter the environment due to low expression in kernels. Moreover, most Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only low amounts of Cry proteins would remain intact to pass out in faeces (e.g., Einspanier et al., 2004; Lutz et al., 2005, 2006; Wiedemann et al., 2006; Guertler et al., 2008). It can thus be concluded that the level of exposure of target organisms to the Cry3A protein is likely to be extremely low and of no biological relevance.

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

The EFSA GMO Panel assessed whether the Cry3A protein might potentially affect non-target organisms by entering the environment through manure and faeces from the gastrointestinal tracts of animals fed maize MIR604. Due to the selectivity of Cry proteins, non-target organisms most likely to be affected by the Cry3A protein are those belonging to a similar taxonomic group as that of the target organisms.

Data supplied by the applicant suggest that very low amounts of the modified Cry3A protein enter the environment due to low expression in kernels. Moreover, most Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only low amounts of Cry proteins would remain intact to pass out in faeces (e.g., Einspanier et al., 2004; Lutz et al., 2005, 2006; Wiedemann et al., 2006; Guertler et al., 2008). There would subsequently be further degradation of the Cry proteins in the manure and faeces due to microbial processes.

Exposure of soil and water environments to this Cry protein from disposal of animal wastes or accidental spillage of maize kernels is likely to be very low and localized. While Cry proteins can bind to a certain extent to clay minerals and humic substances in soil, thereby potentially reducing

their availability to microorganisms for degradation, a number of studies revealed that there is no persistence and accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky, 2008).

Considering the scope of the application that excludes cultivation and the intended uses of maize MIR604, it can be concluded that the exposure of potentially sensitive non-target organisms (e.g., coprophagous *Coleoptera* species) to the Cry3A protein is likely to be very low and of no biological relevance.

6.1.1.5. Potential interaction with the abiotic environment and biogeochemical cycles

Considering the scope of the application and the intended uses of maize MIR604 and due to the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

6.1.2. Monitoring

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are (1) 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 (2) 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 MIR604 would be through manure and faeces from the gastrointestinal tracts of animals fed the GM maize or through accidental release of maize MIR604 viable grains into the environment during transportation and processing

No specific environmental impact of maize MIR604 was indicated by the environmental risk assessment and thus no case specific monitoring is required.

The general surveillance plan proposed by the applicant includes (1) the description of an approach involving operators (federations involved in maize import and processing), reporting to applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of the information recorded by the various operators (Lecoq et al., 2007; Windels et al., 2008); and (3) the use of networks of existing surveillance systems. The applicant proposes a general surveillance report on an annual basis and a final report at the end of the consent.

The EFSA 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 MIR604 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

6.2. Conclusion

The scope of the application is for food/feed uses, import and processing of maize MIR604 and excludes cultivation. Considering the intended uses of maize MIR604, the environmental risk assessment is concerned with exposure through manure and faeces from the gastrointestinal tracts

of animals fed maize MIR604 and with accidental release of maize MIR604 viable grains into the environment 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 maize MIR604 viable grains 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, both the rare occurrence of maize plants and low levels of GM plants and Cry3A protein exposure through other routes indicate that the risk to 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 MIR604 since the environmental risk assessment excluded cultivation and identified no potential adverse environmental effects. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel assessed maize MIR604 for food and feed uses, import and processing.

The EFSA GMO Panel is of the opinion that the molecular characterisation provided for maize MIR604 is sufficient for the safety assessment. The bioinformatic analysis of the inserted DNA and flanking regions does not raise any safety concern. The expression of the genes introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The EFSA GMO Panel considers that the molecular characterisation does not indicate any safety concern.

Based on results of the comparative analysis, the EFSA GMO Panel concluded that maize MIR604 is compositionally, phenotypically and agronomically equivalent to conventional maize varieties, except for the presence of mCry3A and PMI proteins. In addition, there are no indications of potential toxicity and allergenicity of the mCry3A and PMI proteins expressed in maize MIR604. A subchronic (90-day) feeding study revealed no indications of adverse effects in rats fed diets containing grains from maize MIR604. In addition, a feeding study in broiler chickens provided evidence of nutritional equivalence of maize MIR604 to conventional maize. The EFSA GMO Panel considers that maize MIR604 is as safe and as nutritious as its non-GM counterpart and conventional maize varieties and that it is unlikely that the overall allergenicity of the whole plant is changed by the genetic modification.

Considering the intended uses of maize MIR604, which exclude cultivation, there is no requirement for scientific assessment of possible environmental effects associated with the cultivation of this GM maize. In case of accidental release into the environment of maize MIR604 viable grains during transportation and processing, there are no indications of increased likelihood of establishment or survival of feral maize plants. Also, the low levels of environmental exposure through other routes indicate that the risk to non-target organisms is likely to be extremely low. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize MIR604.

The EFSA GMO Panel considers that maize MIR604 is as safe as its conventional counterpart with respect to effects on human and animal health and the environment, and thus concludes that this maize is unlikely to have any adverse effect on human and animal health and the environment in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of United Kingdom dated 12 January 2005, concerning a request for placing on the market of genetically modified maize MIR604 in accordance with Regulation (EC) 1829/2003, submitted by Syngenta Seeds S.A.S on behalf of Syngenta Crop Protection AG.
2. Acknowledgement letter dated 16 September 2005, from EFSA to the Competent Authority of United Kingdom.
3. Letter from EFSA to applicant dated 13 July 2005 with request for clarifications under completeness check.
4. Letter from Applicant to EFSA dated 17 August 2005 providing EFSA with an updated version of the Application EFSA-GMO-UK-2005-15 submitted by Syngenta Seeds S.A.S on behalf of Syngenta Crop Protection AG under Regulation (EC) 1829/2003.
5. Letter from EFSA to Applicant dated 16 September 2005 delivering the "Statement of validity" for Application EFSA-GMO-UK-2005-11 for authorisation of the genetically modified maize MIR604 submitted by Syngenta Seeds S.A.S on behalf of Syngenta Crop Protection AG under Regulation (EC) No 1829/2003.
6. Letter from EFSA to Applicant dated 3 April 2006, with request for additional information.
7. Letter from Applicant to EFSA dated 29 June 2006 providing additional information.
8. Letter from EFSA to Applicant dated 26 October 2006, with request for additional information.
9. Letter from Applicant to EFSA dated 30 January 2007 providing additional information.
10. Letter from EFSA to Applicant dated 14 March 2007, with request for additional information.
11. Letter from Applicant to EFSA dated 26 March 2007 providing additional information.
12. Letter from EFSA to Applicant dated 15 June 2007, with request for additional information.
13. Letter from Applicant to EFSA dated 4 July 2007 providing additional information.
14. Letter from Applicant to EFSA dated 23 August 2008 providing additional information.
15. Letter from EFSA to Applicant dated 24 September 2007, with request for additional information.
16. Letter from Applicant to EFSA dated 14 November 2007 providing additional information.
17. Letter from EFSA to Applicant dated 26 November 2007, with request for additional information.
18. Letter from EFSA to Applicant dated 14 March 2008, with request for additional information .
19. Letter from Applicant to EFSA dated 1 April 2008 providing additional information.
20. Letter from Applicant to EFSA dated 13 May 2008 providing additional information.
21. Letter from Applicant to EFSA dated 3 November 2008 providing additional information.
22. Letter from EFSA to Applicant, dated 2 April 2009, inviting Syngenta to a technical hearing with the GMO Working Group on 29 April.

23. Letter from Applicant to EFSA dated 7 April 2009 providing additional information.
24. Letter from EFSA to Applicant dated 22 June 2009, restarting the clock.

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