SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-UK-2007-48) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MIR604 x GA21 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds[1]

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ABSTRACT

This scientific opinion reports on an evaluation of a risk assessment for placing on the market the genetically modified insect resistant and herbicide tolerant maize MIR604 x GA21 for food and feed uses, import and processing. Conventional crossing methods were used in the production of maize MIR604 x GA21 from lines of the respective single maize events. The structure of the inserts in the single maize events, as well as the phenotypes were both retained in the stacked maize events. The expression levels of the mCry3A, PMI and mEPSPS proteins in maize MIR604 x GA21 were demonstrated to be comparable with those of the respective single maize events. The comparative analysis of compositional, phenotypic and agronomic characteristics indicated equivalence of maize MIR604 x GA21 with its conventional counterpart, except for the newly expressed proteins which provided resistance to certain coleopteran target pests and tolerance to glyphosate-based herbicides. The safety assessment identified no concerns regarding potential toxicity and allergenicity of maize MIR604 x GA21. Considering the intended uses of maize MIR604 x GA21, which excludes cultivation within the European Union, no scientific assessment of potential environmental effects associated with cultivation of maize MIR604 x GA21 was required. In case of accidental release of viable maize MIR604 x GA21 grains into the environment during transportation and processing, there are no indications of increased likelihood of establishment or survival of feral maize plants, except in the presence of glyphosate-based herbicides. It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts. In conclusion, the EFSA GMO Panel considers that information available for maize MIR604 x GA21 addresses the scientific comments raised by Member States, and that the maize MIR604 x GA21, as assessed in this application, is as safe as its conventional counterpart and other appropriate comparators with respect to potential effects on human and animal health and the environment in the context of its intended uses. The EFSA GMO Panel concludes that maize MIR604 x GA21 is unlikely to have an adverse effect on human and animal health and environment in the context of its intended uses.

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1 On request from the Competent Authority of the United Kingdom for application (EFSA-GMO-UK-2007-48) submitted by Syngenta Seeds, Question No EFSA-Q-2007-196, adopted on 29 April 2010.

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3 Acknowledgement: The GMO Panel wishes to thank the members of the Working Groups on Molecular Characterisation, Food/Feed and Environment for the preparation of this opinion and EFSA's staff members Yann Devos, Zoltán Divéki and Ellen Van Haver for the support provided to this EFSA scientific output.

Suggested citation: EFSA Panel on Genetically Modified Organisms; Scientific Opinion on application (EFSA-GMO-UK-2007-48) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MIR604 x GA21 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds. EFSA Journal 2010; 8(5):1611. [30 pp.]. doi:10.2903/j.efsa.2010.1611. Available online: www.efsa.europa.eu

KEY WORDS

GMO, maize (*Zea mays*), MIR604 x GA21, insect resistance, herbicide tolerance, stacked events, risk assessment, food and feed safety, environmental safety, food and feed uses, import, processing, Regulation (EC) No 1829/2003

SUMMARY

Following the submission of an application (Reference EFSA-GMO-UK-2007-48) under Regulation (EC) No 1829/2003 from Syngenta Seeds, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of insect resistant and herbicide tolerant genetically modified (GM) maize MIR604 x GA21 (Unique Identifier SYN-IR6Ø4-5 x MON-ØØØ21-9) for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-UK-2007-48, additional information provided by the applicant and scientific comments submitted by Member States. Further information from applications for placing the single maize events Bt11, MIR604 and GA21, as well as the stacked maize events Bt11 x MIR604 x GA21 on the market under EU regulatory procedures was taken into account. The scope of application EFSA-GMO-UK-2007-48 is for food and feed uses, import and processing of maize MIR604 x GA21 and all derived products, but excludes cultivation in the EU. The EFSA GMO Panel evaluated maize MIR604 x GA21 with reference to the intended uses and appropriate principles described in the EFSA GMO Panel guidance documents for the risk assessment of GM plants and derived food and feed, and for the risk assessment of GM plants containing stacked transformation events. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of target proteins. A comparative analysis of agronomic traits and composition was undertaken, and the safety of the new proteins, as individual proteins and in combination, and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An evaluation of environmental impacts and the post-market environmental monitoring plan was undertaken.

Maize MIR604 x GA21 has been produced by conventional crossing methods between lines containing the single maize events MIR604 and GA21 to combine both the resistance trait against certain coleopteran target pests and the ability to use mannose as sole carbon source in maize MIR604 with tolerance to glyphosate-based herbicides in maize GA21. These single maize events have been the subject of separate risk assessment evaluations by the EFSA GMO Panel. No new genetic modifications were introduced in maize MIR604 x GA21.

Molecular analysis of DNA present in maize MIR604 x GA21 confirmed that maize MIR604 and GA21 inserts are present and that their structures are retained. The expression levels of the mCry3A, PMI and mEPSPS proteins in maize MIR604 x GA21 were comparable to those of the respective single maize events.

The comparative agronomic analysis showed that maize MIR604 x GA21 was agronomically equivalent to its conventional counterpart, except for the new traits. The comparative assessment of maize MIR604 x GA21 was based on the compositional analysis of forage and grain derived from maize Bt11 x MIR604 x GA21 which is in accordance with the EFSA GMO Panel's guidance document on GM plants containing stacked transformation events. Based on all data available, and in this specific case, the EFSA GMO Panel accepts the use of maize Bt11 x MIR604 x GA21 for the comparative compositional analysis and concludes that forage and grain from maize MIR604 x GA21 are likely to be compositionally equivalent to those of its conventional counterpart, except for the presence of the newly expressed proteins.

The mCry3A and PMI proteins expressed in maize MIR604 and the mEPSPS protein expressed in maize GA21 have been assessed previously as described in the scientific opinions of the EFSA GMO Panel on the single maize events, and no safety concerns have been identified. Regarding the safety and nutritional properties of whole food and feed products derived from maize MIR604 x GA21, the EFSA GMO Panel considers it unlikely that interactions among the single maize events will occur that may impact on the food and feed safety and the nutritional properties of maize MIR604 x GA21. The EFSA GMO Panel bases this consideration on the known functional characteristics of the newly expressed proteins and on the outcomes of the comparative analysis of compositional, phenotypic and

agronomic characteristics. In addition, the EFSA GMO Panel considers it unlikely that the overall allergenicity of maize MIR604 x GA21 has been altered.

The EFSA GMO Panel concludes that the maize MIR604 x GA21 assessed in this application is as safe and nutritious as its conventional counterpart in the context of its intended uses.

The application EFSA-GMO-UK-2007-48 concerns food and feed uses, import and processing, but excludes cultivation in the EU. Therefore, there is no requirement for scientific assessment of possible environmental effects associated with the cultivation of maize MIR604 x GA21. There are no indications of an increased likelihood of establishment and spread of feral maize plants in case of accidental release into the environment of viable maize MIR604 x GA21 grains during transportation and processing, except in the presence of glyphosate-based herbicides. Taking into account the scope of the application, the rare occurrence of feral maize plants and the low levels of exposure through other routes, the risk to non-target organisms is considered 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 x GA21. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

The EFSA GMO Panel considers that the information available for maize MIR604 x GA21 addresses the scientific comments raised by Member States and concludes that the maize MIR604 x GA21, assessed in this application, is as safe as its conventional counterpart and other appropriate comparators. In addition, the EFSA GMO Panel is of the opinion that crossing of maize MIR604 and GA21 results in no interaction between the single maize events, which would affect the safety of maize MIR604 x GA21 with respect to potential effects on human and animal health, and on the environment in the context of its intended uses. The EFSA GMO Panel concludes that maize MIR604 x GA21 is unlikely to have an adverse effect on human and animal health and on the environment, in the context of its intended uses.

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BACKGROUND

On 14 November 2007, the European Food Safety Authority (EFSA) received from the Competent Authority of the United Kingdom an application (Reference EFSA-GMO-UK-2007-48) for authorisation of genetically modified (GM) maize MIR604 x GA21 (Unique Identifier SYN-IR6Ø4-5 x MON-ØØØ21-9), submitted by Syngenta Seeds within the framework of Regulation (EC) No 1829/2003 on GM food and feed. After receiving the application EFSA-GMO-UK-2007-48 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed Member States and the European Commission, and made the summary of the application 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 3 March 2008, EFSA received additional information (requested on 20 December 2007) and declared the application as formally valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003 on 12 March 2008.

EFSA made the valid application available to Member States and the European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member State bodies had three months after the date of receipt of the valid application (until 12 June 2008) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out an evaluation of the scientific risk assessment of maize MIR604 x GA21 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. When carrying out the safety evaluation, the EFSA GMO Panel took into account the appropriate principles described in the EFSA GMO Panel guidance documents for the risk assessment of GM plants and derived food and feed (EFSA, 2006a), and for the risk assessment of GM plants containing stacked transformation events (EFSA, 2007a), the scientific comments of Member States and the additional information provided by the applicant. Further information from applications for placing the single maize events Bt11, MIR604 and GA21, as well as the stacked maize events Bt11 x MIR604 x GA21 on the market under EU regulatory procedures were also taken into account (EFSA, 2005; 2007b; 2009a,b; 2010).

The EFSA GMO Panel requested from the applicant additional information on 12 March 2008, 5 October 2009 and 11 January 2010, and the applicant provided the additional information on 4 November 2008, 18 November 2009, 7 April 2009 and 8 February 2010, respectively.

The risk assessments of the single maize events MIR604, GA21 and Bt11 have been the subject of separate evaluations by the EFSA GMO Panel. The EFSA GMO Panel has concluded that they are unlikely to have any adverse effect on human and animal health and the environment, in the context of their intended uses (EFSA, 2005, 2007b, 2009a,c).

- Application EFSA-GMO-UK-2005-11, submitted under Regulation (EC) No 1829/2003, for food and feed uses, import and processing of maize MIR604 has been evaluated by the EFSA GMO Panel (EFSA, 2009c) and recently approved by the Commission Decision 2009/866/EC (EC, 2009).

- Applications EFSA-GMO-UK-2005-19 and EFSA-GMO-RX-GA21, both submitted under Regulation (EC) No 1829/2003, concerning, respectively, food and feed uses, import and processing, and the renewal of the authorisation for continued marketing of existing products produced from maize GA21 have been evaluated by the EFSA GMO Panel (EFSA, 2007b). The use of maize GA21 for food and feed uses, import and processing has been approved by the Commission Decision 2008/280/EC (EC, 2008). Previously, the use of food and food ingredients produced from maize GA21 has been evaluated by the Scientific Committee on

Food (SCF, 2002a) and approved under Regulation (EC) No 258/97 by the Commission Decision 2006/69/EC (EC, 2006). Other commercial uses have been evaluated under Directive 2001/18/EC by the Scientific Committee on Plants (SCP, 2000a).

- Notification C/F/96/05.10 submitted under Directive 2001/18/EC covering cultivation, feed uses, import and processing of maize Bt11 has been evaluated by the EFSA GMO Panel (EFSA, 2005). Previously, maize Bt11 has been evaluated by the Scientific Committee on Plants (SCP, 1998) and approved for feed uses, import and processing by the Commission Decision 98/292/EC (EC, 1998). The cultivation of maize Bt11 has been evaluated under Directive 90/220/EEC (SCP, 2000b). Food uses of sweet maize Bt11 have been approved according to Regulation (EC) No 258/97 by the Commission Decision 2004/657/EC (EC, 2004) after an evaluation by the Scientific Committee on Food (SCF, 2002b). An application for renewal of the authorisation for continued marketing of existing products produced from maize Bt11 made under Articles 11 and 23 of Regulation (EC) No 1829/2003 has been evaluated by the EFSA GMO Panel (EFSA, 2009a).

In giving its scientific opinion on maize MIR604 x GA21 to the European Commission, 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 acknowledgement of the valid application. As additional information was requested by the EFSA GMO Panel, the time limit of six 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, this scientific opinion 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 EFSA overall opinion in accordance with Articles 6(5) and 18(5).

Maize MIR604 contains a modified *cry3A* coding sequence (m*cry3A*) derived from *Bacillus thuringiensis* subsp. *tenebrionis* that encodes an insecticidal mCry3A protein conferring resistance to the Western corn rootworm (*Diabrotica virgifera virgifera*) and other related coleopteran target pests such as the Northern corn rootworm (*Diabrotica barberi*). Maize MIR604 also contains the *pmi* (*man*A) gene from *Escherichia coli* which encodes the phosphomannose isomerase (PMI) as a selectable marker protein. 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.

Maize GA21 expresses a modified *epsps* gene derived from maize, encoding a modified 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS) which confers tolerance to glyphosate-based herbicides.

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific risk assessment of maize MIR604 x GA21 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 EFSA GMO Panel was not requested to give a scientific opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA 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 maize MIR604 x GA21 (Unique Identifier SYN-IR6Ø4-5 x MON-ØØØ21-9) was evaluated with reference to its intended uses, taking into account the principles described in the EFSA GMO Panel guidance documents for the risk assessment of GM plants and derived food and feed (EFSA, 2006a), and for the risk assessment of GM plants containing stacked transformation events (EFSA, 2007a). The evaluation of the risk assessment presented here is based on the information provided in the application relating to maize MIR604 x GA21 submitted in the EU, including additional information from the applicant and scientific comments submitted by Member States. Further information for placing the single maize events Bt11, MIR604 and GA21, as well as the stacked maize events Bt11 x MIR604 x GA21 on the market under EU regulatory procedures was taken into account (EFSA, 2005; 2007b; 2009a,c; 2010).

In this application, the comparative compositional assessment was based on the analysis of forage and grain derived from maize Bt11 x MIR604 x GA21 and its conventional counterpart. This is in line with the EFSA GMO Panel's guidance document on GM plants containing stacked transformation events, which states that, as long as each event in the highest number of stacked events has been risk assessed, the risk assessment of the stacked events might also be applicable to GM stacks containing fewer of these events, provided that potential interactions between the stacked events are taken into account (EFSA, 2007a). The EFSA GMO Panel is of the opinion that the use of maize Bt11 x MIR604 x GA21 is acceptable in this case because:

- All single maize events have been evaluated and were considered as safe as their conventional counterparts. No biologically relevant compositional and agronomical differences have been identified with respect to the conventional counterparts (EFSA, 2005; 2007b, 2009a,c);
- There are no indications that crossing maize Bt11, MIR604 and GA21 to produce maize Bt11 x MIR604 x GA21 results in specific interactions causing compositional or agronomic changes (EFSA, 2010);
- In addition, in the present application, there are no indications of differences in agronomic and phenotypic characteristics of maize MIR604 x GA21 compared to its conventional counterpart (section 4.1.4), or in the levels of the newly expressed proteins (section 3.1.4).

2. Issues raised by Member States

The scientific comments raised by Member States are addressed in Annex G of the EFSA overall opinion and have been considered in this EFSA GMO Panel scientific opinion[4].

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Method of production of maize MIR604 x GA21

Conventional crossing methods were used to develop maize MIR604 x GA21 and no new genetic modification was involved. The two inserts that are present in maize MIR604 x GA21 were derived

[4] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-196

from lines containing the single maize events MIR604 and GA21. Each of these single maize events was the subject of a previous safety evaluation and separate opinions for each of them have been published (EFSA, 2007b, 2009c).

3.1.2. Summary of the evaluation of the single maize events

Maize MIR604

Maize MIR604 was developed by using *Agrobacterium*-mediated transformation and as a result expresses a modified version of a *cry3A* gene (m*cry3A*) derived from *B. thuringiensis* subsp. *tenebrionis* conferring resistance to certain coleopteran target pests and a *man*A gene encoding phosphomannose isomerase (PMI) from *Escherichia coli* as a selectable marker.

Molecular characterisation data established that maize MIR604 contains a single copy of the T-DNA, and that vector backbone sequences are absent.

Sequences flanking the 5' and 3' regions of maize MIR604 have been determined and recent (2008) BLASTN analysis of the 5' and 3' flanking sequences has revealed no significant homology with any known maize sequences. Analysis of putative open reading frames (ORFs) at the 5' and 3' flanking regions indicated no sequence homologies to known toxins or allergens.

Southern, PCR and ELISA analyses of maize MIR604 indicated genetic and phenotypic stability of the event over multiple generations.

Maize GA21

Maize GA21 was developed through particle bombardment using a purified plasmid fragment and as a result expresses a modified maize *epsps* gene (m*epsps*) conferring tolerance to glyphosate-based herbicides.

Molecular characterisation data established that maize GA21 contains a single insertion locus consisting of six contiguous complete or truncated versions of the purified plasmid fragment used for the transformation. Molecular analysis indicated that vector backbone sequences are absent.

The sequences of the plant genome adjacent to the 3' and 5' ends were determined. Bioinformatic analysis of the 3' sequence did not indicate that the insertion event occurred in a functional maize gene. The 5' flanking sequence was shown to be of chloroplast origin. Bioinformatic analysis also revealed no biologically relevant homology to allergens or toxins for any of the putative polypeptides that might be produced from ORFs spanning the junction regions.

Southern analysis of maize GA21 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations.

3.1.3. Transgene constructs in maize MIR604 x GA21

Maize MIR604 x GA21 has been produced by conventional crosses between lines containing the single maize events MIR604 and GA21. The integrity of the individual inserts present in maize MIR604 x GA21 was investigated using Southern analyses. This involved the use of restriction enzymes and DNA probes specific for maize MIR604 and GA21 (including the junction regions). The predicted DNA hybridisation patterns from each single maize event were retained in the stacked maize events MIR604 x GA21, demonstrating that integrity of the inserts was maintained.

3.1.4. Information on expression of the insert

Maize MIR604, GA21 and MIR604 x GA21 were grown, together with a conventional counterpart, in 2005 in Illinois (US) using standard agronomic practices. Leaf, root, pollen and grain tissues were analysed by ELISA to compare the concentrations of mCry3A, PMI and mEPSPS proteins produced in the plants. Since the scope of the application covers food and feed uses, import and processing, only protein data related to the seed/grain is considered relevant. The levels of the proteins mCry3A, PMI and mEPSPS detected in mature grain of the single maize events MIR604 and GA21 and the stacked maize events MIR604 x GA21 are shown in Table 1.

Mean mCry3A and PMI concentrations were comparable in maize MIR604 and MIR604 x GA21. Similarly, mEPSPS concentrations were comparable in maize GA21 and MIR604 x GA21. Although some statistically significant differences were seen, these are small and do not raise any safety concerns.

Table 1: Summary of protein expression levels in maize MIR604 x GA21, MIR604 and GA21 grains (expressed in µg/g dry weight).

Protein		MIR604 x GA21	MIR604	GA21
mCry3A	Mean	0.5	0.5	--
	Range	0.4 – 0.5	0.3 – 0.8	--
PMI	Mean	1.5	1.6	--
	Range	1.2 – 1.7	1.2 – 2.0	--
mEPSPS	Mean	2.7	--	3.3
	Range	2.4 – 3.0	--	2.8 – 3.6

3.1.5. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in maize MIR604 and GA21 was demonstrated previously (EFSA, 2007b, 2009c). The Southern data indicate that the structure of the inserts in the single maize events is retained in the stacked maize events MIR604 x GA21. Furthermore, each of the traits has been conserved in maize MIR604 x GA21.

3.2. Conclusion

As conventional crossing methods were used in the production of maize MIR604 x GA21, no additional genetic modification was involved. Southern analyses demonstrated that the structure of the inserts in maize MIR604 and GA21 is retained in maize MIR604 x GA21. The genetic stability of the integrated DNA has been demonstrated in the single maize events. The levels of mCry3A, PMI and mEPSPS proteins expressed in mature grain of maize MIR604 x GA21 were comparable with those expressed in maize MIR604 and GA21.

The EFSA GMO Panel concludes that the molecular characterisation does not indicate safety concerns.

4. Comparative analysis

4.1. Evaluation of the relevant scientific data

4.1.1. Summary of the previous evaluation of the single maize events

Maize MIR604

Maize MIR604 was compared with control non-GM lines with a genetic background comparable to maize MIR604 during field trials in multiple locations in the US for two seasons (i.e., 2002 and 2003). In addition, analysis of mono- and disaccharides, including phosphorylated forms of these saccharides, in maize MIR604 and a non-GM near-isogenic control, has been performed by the applicant at six locations in the US in 2006, following a request from the EFSA GMO Panel. The composition of forage and grain samples from 2002 and 2003 was analysed and the selected constituents were in line with those recommended by the OECD consensus document on key nutrients, anti-nutrients, and secondary plant metabolites of maize (OECD, 2002). 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. In compounds that could theoretically be linked to PMI activity (e.g., starch and other carbohydrates), no consistent compositional differences were observed in the comparison between maize MIR604 and its non-GM comparators. Based on the results of the compositional analysis, the EFSA GMO Panel concluded that forage and grain of maize MIR604 were compositionally equivalent to conventional maize, except for the presence of the PMI and mCry3A proteins (EFSA, 2009c).

Moreover, agronomic performance and phenotypic characteristics were analysed in multiple field trials in the US during two years (2002 and 2003). The EFSA GMO Panel concluded that the phenotypic and agronomic performance of maize MIR604 is equivalent to that of the non-GM comparators, except for the introduced traits (EFSA, 2009c).

Maize GA21

Maize GA21 was compared with near-isogenic non-GM controls. Forage and grain were collected for compositional analysis from field trials conducted over several seasons and at different locations: five locations in the US (1996); seven locations in the US (1997); four locations in Italy and Spain (1997); and six locations during two seasons in the US (2004 and 2005). Maize GA21 plants treated with glyphosate herbicides, as well as plants untreated with the target herbicide were included in these field trials. Based on the results of compositional analysis of these samples, it was concluded that forage and grain of maize GA21 are compositionally equivalent to those of conventional maize, except for the presence of the mEPSPS protein in maize GA21 (EFSA, 2007b).

In addition, field trials over several seasons and at different locations (US in 1999 and 2004; Brazil in 2003) did not show changes in phenotypic characteristics and agronomic performance, except for the introduced trait (EFSA, 2007b).

Maize Bt11

Maize Bt11 was compared with isogenic non-transgenic comparators. Forage and grain were collected for compositional analysis from field trials. These field trials were conducted in the US (studies involving 3-6 sites in 1995) and France (two locations in 1998). Based on the results of the compositional analysis, the EFSA GMO Panel concluded that forage and grain of maize Bt11 were compositionally equivalent to those of conventional maize, except for the presence of the proteins Cry1Ab and PAT in maize Bt11.

In addition, field trials over several seasons and at different locations in the EU (Spain, France, Italy and Portugal between 1994 and 2003) did not show indications for unexpected changes of agronomic characteristics and performance (EFSA, 2005). In 2009, the EFSA GMO Panel concluded that no new information has appeared since 2005 which would indicate differences in the composition of products derived from maize Bt11, as compared to its non-GM maize counterpart (EFSA, 2009a).

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

The comparative assessment of the maize MIR604 x GA21 was based on the compositional analysis of forage and grain derived from maize Bt11 x MIR604 x GA21 (see section 1). Forage and grain of maize Bt11 x MIR604 x GA21 and its conventional counterpart were collected from field trials at six locations in the US in 2006. The field trial design in each location included three replicates of blocks containing test maize Bt11 x MIR604 x GA21 and its conventional counterpart. All fields underwent similar agronomic treatments, except for additional treatment of maize Bt11 x MIR604 x GA21 with glufosinate-ammonium and glyphosate-based herbicides. Given the fact that the previous assessment of the herbicide tolerant single maize events GA21 and Bt11 considered both plants treated with the target herbicide and plants treated with conventional herbicides, the EFSA GMO Panel does not consider it necessary to ask for additional data on the composition of maize Bt11 x MIR604 x GA21 treated with only conventional herbicides. Samples were taken from each replicate from maize Bt11 x MIR604 x GA21 and its conventional counterpart, and were analysed for composition.

For the comparison of agronomic and phenotypic characteristics of maize MIR604 x GA21, two varieties of maize, each containing the stacked maize events MIR604 x GA21, i.e., an early-maturing and a mid-maturing maize variety, plus the corresponding conventional counterparts were grown in different locations in the US in 2005. In four of these locations, the early-maturing maize variety was grown, while the mid-maturing maize variety was grown in the remaining six locations. Each location contained four replicates of each material. Both test and control maize fields underwent the standard agronomic treatment with regard to management of insect pests, weeds and diseases.

4.1.3. Compositional analysis

The compositional parameters analysed for forage and grain of maize Bt11 x MIR604 x GA21 and its conventional counterpart are in line with those recommended by the OECD consensus document on key compositional parameters of maize (OECD, 2002). Forage has been analysed for proximates (moisture, crude protein, total fat, ash and carbohydrates by calculation); fibres [acid detergent fibre (ADF) and neutral detergent fibre (NDF)]; calcium; and phosphorus. Analysis of grains has been carried out for proximates (moisture, crude protein, total fat, ash, carbohydrates by calculation); fibres [ADF, NDF and total detergent fibre (TDF)]; starch; minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P, Se, Zn); amino and fatty acids; (pro-)vitamins [β-carotene, B1(thiamine), B2 (riboflavine), niacin, B6 (pyridoxine), folic acid, E (α-tocopherol)]; and secondary metabolites, including anti-nutrients (ferulic acid, p-coumaric acid, furfural, inositol, raffinose, trypsin inhibitor, phytic acid). At the EFSA GMO Panel's request, the applicant provided a statistical analysis of the comparison between the test maize and the conventional counterpart on a per-location basis, supplementing the across-location statistical analysis that had already been provided with this application.

In the across-location statistical analysis of the composition of forage, no statistically significant differences were observed between maize Bt11 x MIR604 x GA21 and its conventional counterpart. In the per-location analysis, only one parameter showed a statistically significant difference at a single location. In the across-location statistical analysis of the composition of grains, statistically significant differences were observed in the levels of protein (10.4% by dry weight in maize Bt11 x MIR604 x GA21 versus 10.9% by dry weight in the conventional counterpart), and similar differences were observed for most amino acids. Significant differences were also observed for zinc and vitamin B1. All the different average values across locations were within the compositional ranges of conventional maize varieties collected in the ILSI crop composition database (ILSI, 2006) and close to the means of

those ranges. A number of parameters showed statistically significant differences in separate locations in the per-location analysis, but none of them in each location. Levels below the limit of quantitation precluded statistical analysis of vitamin E, sodium, raffinose, and furfural across- or in separate-locations.

The EFSA GMO Panel concludes that forage and grain from the maize Bt11 x MIR604 x GA21 are compositionally equivalent to those of its conventional counterpart, except for the presence of the newly expressed proteins.

4.1.4. Agronomic traits and GM phenotype

The analysis of agronomic and phenotypic characteristics of maize MIR604 x GA21 and its conventional counterpart included a range of parameters related to plant morphology, physiology, appearance and performance, including disease susceptibility and plant health. In the across-location analysis, a statistically significant difference was observed in the moisture content of the early-maturing maize variety containing the stacked maize events MIR604 x GA21, as compared to the conventional counterpart. Since this difference was relatively small and not observed in the mid-maturing maize variety, the EFSA GMO Panel does not consider it biologically relevant. A number of parameters showed statistically significant differences in the per-location statistical analysis of the comparison between maize MIR604 x GA21 and its conventional counterpart, but none of these differences was consistently observed in each location.

4.2. Conclusion

The comparative assessment of maize MIR604 x GA21 was based on the compositional analysis of forage and grain derived from maize Bt11 x MIR604 x GA21 which is in accordance with the EFSA GMO Panel's guidance document on GM plants containing stacked transformation events (EFSA, 2007a). Forage and grain from maize Bt11 x MIR604 x GA21 were shown to be compositionally equivalent to those of its conventional counterpart, except for the presence of the newly expressed proteins (EFSA, 2010). In addition, the outcome of the phenotypic and agronomic analysis of maize MIR604 x GA21 did not show biologically relevant differences compared with its conventional counterpart, and the maize MIR604 x GA21, assessed in this application, was considered phenotypically and agronomically equivalent, except for the new traits. Based on the assessment of the data available, the EFSA GMO Panel is of the opinion that crossing MIR604 and GA21 to produce maize MIR604 x GA21 does not result in interactions between the single maize events which cause compositional or agronomic changes. Therefore, in this case, the EFSA GMO Panel accepted the use of maize Bt11 x MIR604 x GA21 for the comparative compositional analysis and concludes that forage and grain from the maize MIR604 x GA21, assessed in this application, are likely to be compositionally equivalent to those of its conventional counterpart, except for the presence of the newly expressed proteins.

5. Food/Feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Summary of the previous evaluation of the single events

Maize MIR604

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 for the safety testing after their equivalence to the plant-expressed proteins had been demonstrated experimentally.

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

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 known toxins and allergens; *in vitro* digestion using simulated gastrointestinal fluids containing proteases; and an acute oral toxicity study using mice. Because the newly expressed protein PMI is a member of the cupin superfamily of proteins, which also includes some allergens, additional information was provided by the applicant upon request of the EFSA GMO Panel. Among others, the 3D structure of PMI was compared with that of an allergenic cupin protein from peanut, Ara h 1. In this comparison with Ara h 1, PMI 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 up to 41.5% grains from maize MIR604. In addition, a 49-day feeding study in broiler chickens provided evidence of nutritional equivalence of maize MIR604 to conventional maize. These studies supported the conclusion of the compositional and agronomical comparison that the genetic modification resulted in no unintended effects.

The EFSA GMO Panel was of the opinion that maize MIR604 is as safe and as nutritious as its non-GM counterpart and conventional maize varieties, and considered it unlikely that the overall allergenicity of the whole plant is changed. Maize MIR604 is therefore unlikely to have any adverse effect on human and animal health in the context of its intended uses (EFSA, 2009c).

Maize GA21

The mEPSPS protein expressed in maize GA21 differs from the native maize EPSPS protein in two of a total of 445 amino acids. Bioinformatics-supported studies demonstrated that the amino acid sequence of the mEPSPS protein shows no homology to known toxic proteins and allergens. For the safety testing a mEPSPS protein produced in a recombinant *E. coli* strain was used after it had been demonstrated experimentally that the protein was equivalent to that produced in maize GA21. The protein was rapidly degraded in simulated gastric fluid and did not induce adverse effects in a study on acute oral toxicity in mice.

With regard to animal studies with the whole product, there were no adverse effects in a subchronic (90-day) rat feeding study using diets containing grains from maize GA21. In addition, a 49-day feeding study with broiler chickens provided evidence of nutritional equivalence of maize GA21 to conventional maize. The EFSA GMO Panel concluded that maize GA21 is as safe as conventional maize, and that the overall allergenicity of the whole plant is not changed. Maize GA21 was considered unlikely to have any adverse effect on human and animal health in the context of the intended uses (EFSA, 2007b).

5.1.2. Product description and intended use

The scope of application EFSA-GMO-UK-2007-48 includes the import and processing of maize MIR604 x GA21 and its derived products for use as food and feed. Thus, the possible uses of maize MIR604 x GA21 include the production of animal feed, as well as valuable food products, such as starch, syrups and oils.

The genetic modification of maize MIR604 x GA21 is intended to improve agronomic performance only, and is not intended to influence the nutritional properties, processing characteristics and overall use of maize as a crop.

5.1.3. Effects of processing

Since maize MIR604 x GA21 is likely to be compositionally equivalent to its conventional counterpart, except for the newly expressed proteins (see section 4.2), the effect of processing on maize MIR604 x GA21 is not expected to be different compared to that on conventional maize.

5.1.4. Toxicology

5.1.4.1. Toxicological assessment of expressed novel proteins in maize MIR604 x GA21

The mCry3A and PMI proteins expressed in maize MIR604, and the mEPSPS protein expressed in maize GA21 have been assessed for their safety previously (EFSA, 2007b, 2009c), and no safety concerns were identified. The EFSA GMO Panel is not aware of any new information that would change this conclusion.

No new genes in addition to those occurring in maize MIR604 and GA21 have been introduced in maize MIR604 x GA21.

Following a request from the EFSA GMO Panel, the applicant submitted an updated bioinformatic analysis comparing the amino acid sequences of the newly expressed proteins mCry3A, PMI and mEPSPS expressed in maize MIR604 x GA21 with the sequences of known toxic and general proteins using an updated database. These analyses confirmed the results of the previous studies, which showed no similarities between the newly expressed proteins mCry3A, PMI and mEPSPS and known proteins toxic to mammals.

Determination of the levels of the newly expressed proteins in grains of maize MIR604 x GA21, MIR604 and GA21 showed comparable expression levels in the stacked maize events and the respective single maize events (see section 3.1.4). Based on the known function and mode of action of the newly expressed proteins mCry3A, PMI and mEPSPS, the EFSA GMO Panel considers the occurrence of interactions between these proteins unlikely.

5.1.4.2. Toxicological assessment of new constituents other than proteins

No new constituents other than the mCry3A, PMI and mEPSPS proteins have been identified in maize MIR604 x GA21, and relevant changes in the composition of maize MIR604 x GA21 are unlikely.

5.1.4.3. Toxicological assessment of the whole GM food/feed

Maize MIR604 and GA21 have previously been found as safe as their conventional counterparts for human and animal consumption (EFSA, 2007b, 2009c). In the present assessment, it was found that the structural integrity of the inserts in maize MIR604 x GA21 was not changed in comparison with maize MIR604 and GA21, respectively, and expression analysis of the proteins revealed that the overall levels of the proteins mCry3A, PMI and mEPSPS in maize MIR604 x GA21 were generally similar to the levels in maize MIR604 and GA21 (see section 3.2). Moreover, based on the assessment of the data available, the EFSA GMO Panel is of the opinion that crossing MIR604 and GA21 to produce maize MIR604 x GA21 does not result in interactions between the single maize events which cause compositional or agronomic changes (see section 4.2). The EFSA GMO Panel considered all the data available for maize MIR604 x GA21, and the compositional data of maize Bt11 x MIR604 x

GA21 and the newly expressed proteins mCry3A, PMI and mEPSPS, and is of the opinion that interactions between the single maize events that might impact on the food and feed safety of maize MIR604 x GA21 are unlikely.

Therefore, the EFSA GMO Panel does not consider additional animal safety studies with the whole GM food/feed necessary.

5.1.5. Allergenicity

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation, or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (CAC, 2003; EFSA, 2006a).

5.1.5.1. Assessment of allergenicity of the newly expressed proteins

The newly expressed proteins (mCry3A, PMI and mEPSPS) present in maize MIR604 x GA21 have been evaluated previously and it was found unlikely that they are allergenic (EFSA, 2007b, 2009c). At the request of the EFSA GMO Panel, the applicant submitted an updated bioinformatic analysis comparing the amino acid sequences of the newly expressed proteins mCry3A, PMI and mEPSPS with the sequences of known allergens using an updated version of the FARRP allergen database. These analyses confirmed the results of previous studies.

Based on the information provided, the EFSA GMO Panel considers it unlikely that potential interactions occur that might change the allergenicity of the newly expressed proteins.

5.1.5.2. Assessment of allergenicity of the whole GM plant or crop

The issue of a potential increased allergenicity of maize MIR604 x GA21, as compared to the single maize events MIR604 and GA21 and to conventional maize varieties, does not appear relevant to the EFSA GMO Panel, since maize is not considered a common allergenic food. However, rare cases of occupational allergy to maize dust have been reported in the scientific literature. The EFSA GMO Panel is also aware that few cases of food allergy to maize have been specifically observed in some geographically restricted areas where maize is a common food and that, in the few cases reported, the major maize allergens have then been identified. In the context of the present application, the EFSA GMO Panel considers it unlikely that any interactions between the newly expressed proteins and metabolic pathways of maize would alter the pattern of expression of endogenous proteins/potential allergens and thereby significantly change the overall allergenicity of the whole plant. In addition, given all the available information, the EFSA GMO Panel sees no reason to expect that the use of maize MIR604 x GA21 would significantly increase the intake and exposure to maize.

5.1.6. Nutritional assessment of GM food/feed

Based on the outcome of the compositional, phenotypic and agronomic analysis (see section 4.2), the EFSA GMO Panel does not consider a nutritional feeding study with the whole GM food/feed necessary.

The applicant provided a 43-day feeding study with broiler chickens to analyse the nutritional value of grains from maize MIR604 x GA21, in relation to grains from its conventional counterpart and one commercial non-GM maize variety. However, this study was not considered by the EFSA GMO Panel because of relevant deviations from Good Agricultural Practice (e.g., ILSI, 2007), in particular very

high temperatures and high animal losses in the finishing period as well as large differences in crude protein content of grower diets.

5.1.7. Post-market monitoring of GM food/feed

An evaluation of the risk assessment concluded that there are no data to indicate that maize MIR604 x GA21 is any less safe than its conventional counterpart. In addition, maize MIR604 x GA21 is, from a nutritional point of view, equivalent to its conventional counterpart. Therefore, and in line with its guidance document for the risk assessment of GM plants and derived food and feed (EFSA, 2006a), the EFSA GMO Panel is of the opinion that post-market monitoring of the food/feed derived from maize MIR604 x GA21 is not necessary.

5.2. Conclusion

The mCry3A and PMI proteins expressed in maize MIR604 and the mEPSPS protein expressed in maize GA21 have been assessed previously, as described in the scientific opinions of the EFSA GMO Panel on the single maize events, and no safety concerns have been identified. Regarding the safety and nutritional properties of whole food and feed products derived from maize MIR604 x GA21, the EFSA GMO Panel considers it unlikely that interactions between the single maize events will occur that may impact on the food and feed safety and the nutritional properties of maize MIR604 x GA21. The EFSA GMO Panel bases this consideration on the known functional characteristics of the newly expressed proteins and on the outcomes of the comparative analysis of compositional, phenotypic and agronomic characteristics (see section 4.2). In addition, the EFSA GMO Panel considers it unlikely that the overall allergenicity of maize MIR604 x GA21 has been altered.

The EFSA GMO Panel concludes that the maize MIR604 x GA21 assessed in this application is as safe and nutritious as its conventional counterpart. The EFSA GMO panel concludes that maize MIR604 x GA21 is unlikely to have an 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

The scope of the application is for food and feed uses, import and processing of maize MIR604 x GA21 and does not include cultivation. Considering the proposed uses of maize MIR604 x GA21, the environmental risk assessment is concerned with the exposure through manure and faeces from gastrointestinal tracts of animals fed maize MIR604 x GA21 and with the accidental release into the environment of maize MIR604 x GA21 grains during transportation and processing.

As the scope of the present application excludes cultivation, environmental concerns related to the use of glyphosate-based herbicides on maize MIR604 x GA21 apply only to imported and processed maize products that may have been treated with those herbicides in countries of origin. The EFSA GMO Panel is aware that the risk assessment of active substances falls within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market.

6.1.1. Evaluation of single maize events MIR604 and GA21

In its previous scientific opinions, the EFSA GMO Panel was of the opinion that the single maize events MIR604 and GA21 are as safe as their conventional counterparts, and that the placing on the market of maize MIR604 and GA21 for food and feed uses, import and processing is unlikely to have an adverse effect on human or animal health, or the environment (EFSA, 2007b, 2009c). Furthermore,

post-market environmental monitoring plans for maize MIR604 and GA21, including general surveillance, were proposed by the applicant and considered in line with the EFSA GMO Panel scientific opinion on post-market environmental monitoring (EFSA, 2006b) by the EFSA GMO Panel.

6.1.2. Environmental risk assessment

6.1.2.1. Unintended effects on plant fitness due to the genetic modification

Maize is highly domesticated and generally unable to survive in the environment without management intervention. Maize plants are not winter hardy in many 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. In cultivation, maize volunteers may arise under some environmental conditions (mild winters). Observations made on cobs, cob fragments or isolated grains shed in the field during harvesting indicated that grains may survive and overwinter in some regions, resulting in volunteers in subsequent crops. The occurrence of maize volunteers was reported in Spain and other European regions (e.g., Gruber *et al*., 2008). However; maize volunteers have been show to grow weakly and flower asynchronously with the maize crop (Palaudelmàs *et al*., 2009).

Applicant's field trials have shown that there are no indications of an altered fitness of the single maize events MIR604 and GA21 as compared to their conventional counterparts. In addition to the field trials carried out with the single maize events MIR604 and GA21 (EFSA, 2007b, 2009c), a series of field trials with maize MIR604 x GA21 were conducted across ten US corn belt locations in 2005. Information on phenotypic and agronomic characteristics was provided to assess the agronomic performance of maize MIR604 x GA21 in comparison with its conventional counterpart. These field trial data showed enhanced biomass production when glyphosate-based herbicides were applied and/or under infestation of target pests, but did not show changes in plant characteristics that indicate altered fitness and invasiveness of maize MIR604 x GA21 plants. The EFSA GMO Panel is not aware of any scientific report of increased establishment, spread or any change in survival capacity including overwintering of maize MIR604 x GA21, or maize with comparable properties such as single maize events.

The insect resistance against certain coleopteran target pests provides a potential agronomic advantage in cultivation under infestation of target pests. Likewise, the herbicide tolerance traits can only be regarded as providing a potential agronomic and selective advantage for this GM maize plant where and when glyphosate-based herbicides are applied. However, survival of maize plants outside cultivation or other areas where glyphosate-based herbicides could be applied in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens, herbivores and cold climatic conditions. Since these general characteristics are unchanged in maize MIR604 x GA21, herbicide tolerance and insect resistance are not likely to provide a selective advantage outside cultivation in Europe. Therefore, it is considered very unlikely that maize MIR604 x GA21 will differ from conventional maize varieties in their ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

Since maize MIR604 x GA21 has no altered survival, multiplication or dissemination characteristics except when glyphosate-based herbicides are applied and/or under infestation of target pests, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the accidental release into the environment of viable maize MIR604 x GA21 grains will not differ from that of maize MIR604 and GA21 or that of conventional maize varieties.

6.1.2.2. 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

Genomic DNA is a component of many food and feed products derived from maize. It is well documented that DNA present in food and feed becomes substantially degraded in the process of digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to microorganism in the digestive tracts of humans, domesticated animals, and other animals feeding on maize MIR604 x GA21 is expected.

Current scientific knowledge indicates that horizontal gene transfer of non-mobile DNA fragments between unrelated organisms (such as plants to microorganisms) is extremely unlikely to occur under natural conditions (see EFSA, 2009b for further details). In addition to the low concentration of DNA in the gastrointestinal tracts and the lack of competence of most bacteria to take up foreign DNA, a major barrier to such inter-domain transfer is the lack of sufficient DNA sequence similarity for homologous recombination to occur in bacteria.

With the exception of the m*epsps* gene from *Zea mays* expressed in maize GA21, all other inserted genes (m*cry3A* and *pmi* (*man*A)), as expressed in maize MIR604 x GA21 are of bacterial origin. Thus, in theory, the m*cry3A* and *pmi* genes of the recombinant DNA insert could provide sufficient DNA similarity for homologous recombination with genes from environmental bacteria. However, such hypothesised horizontal gene transfer event is not likely to be maintained in bacterial populations due to a predicted lack of efficient expression and no identified selective advantage for gene transfer recipients in the unlikely case of their expression.

In case of illegitimate recombination into environmental bacterial genomes, it is unlikely that recombinant genes (m*cry3A* and *pmi*) regulated by eukaryotic plant promoters in maize MIR604 x GA21 would be expressed. Moreover, no selective advantage of a hypothesised bacterial uptake of the above mentioned genes is anticipated, because *cry* and *pmi* genes are already occurring in various bacterial species in the environment. Thus, the hypothesised low level exposure of environmental bacterial communities to the maize MIR604 x GA21 m*cry3A* and *pmi* genes must be seen in the context of the natural occurrence and level of exposure to alternative sources of genetically diverse *cry* and *pmi* genes to which bacterial communities are naturally exposed.

The m*epsps* gene is of plant origin, but with minor nucleotide modifications in the coding region and altered combinations of plant regulator sequences. A plausible selective advantage of bacteria receiving the m*epsps* gene extending beyond those that can be hypothesised for any native maize gene has not been identified.

The wide environmental presence of genetically diverse natural variants of the recombinant DNA coding sequences, the use of regulatory sequences optimised for expression in eukaryotes, and the absence of an identified plausible selective advantage, suggest it is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts (EFSA, 2009b).

(b) Plant to plant gene transfer

The extent of cross-pollination to other maize varieties will mainly depend on the scale of accidental release during transportation and processing, and on the successful establishment and subsequent flowering of GM maize plants. 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 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 maize volunteers after GM maize cultivation 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 and herbicide tolerance provide agronomic and selective advantages under infestation of target pests and/or in areas where glyphosate-based herbicides are applied. Even though the occurrence of some GM maize plants outside cropped area have been reported in Korea due to grain spillage during import, transportation, storage, handling and processing (Kim et al., 2006; Lee et al., 2009; Park et al., 2010), survival of maize plants outside cultivation in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens, herbivores and frost. Since these general characteristics are unchanged in maize MIR604 x GA21, insect resistance and herbicide tolerance are not likely to provide selective advantages outside cultivation or other areas where glyphosate-based herbicides could be applied and/or under infestation of target pests in Europe. Therefore, as for any other maize varieties, these GM maize plants would only survive in subsequent seasons in warmer regions of Europe and are not likely to establish feral populations under European environmental conditions.

The EFSA GMO Panel takes into account that this application does not include cultivation of maize MIR604 x GA21 within the EU so that the likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low. However, in countries cultivating maize MIR604 x GA21 and producing seed for export, there is a potential for admixture in seed production and thus the introduction of GM seeds through this route. Hence, it is important that appropriate management systems are in place to restrict seeds of maize MIR604 x GA21 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, maize MIR604 x GA21 has no altered survival, multiplication or dissemination characteristics, except under infestation of target pests and/or when glyphosate-based herbicides are applied. 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 in Europe will not differ from that of maize MIR604 and GA21, or of other maize varieties.

6.1.2.3. Interactions of the GM plant with target organisms

The intended uses of maize MIR604 x GA21 specifically exclude cultivation and the environmental exposure to maize MIR604 x GA21 is limited to the accidental release of grains into environment during transportation and processing. The EFSA GMO Panel considers that it would need successful establishment and spread of high numbers of maize MIR604 x GA21 plants to enable any significant interaction with target organisms, which is very unlikely.

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

The intended uses of maize MIR604 x GA21 specifically exclude cultivation and the environmental exposure to maize MIR604 x GA21 is limited to the accidental release of grains into environment during transportation and processing. The EFSA GMO Panel considers that it would need successful establishment and spread of high numbers of maize MIR604 x GA21 plants to enable any significant interaction with non-target organisms, which is very unlikely.

In addition, the EFSA GMO Panel evaluated whether the mCry3A protein might potentially affect non-target organisms by entering the environment through manure and faeces from the gastrointestinal tracts of animals fed maize MIR604 x GA21. Due to the specific insecticidal selectivity of Cry proteins, non-target organisms most likely to be affected by the mCry3A protein belong to the same or closely related taxonomic groups as those of the target organisms.

Data supplied by the applicant suggest that only low amounts of the mCry3A protein enter the environment due to low expression in grains. Moreover, this Cry protein is degraded by enzymatic activity in the gastrointestinal tract of animals fed on GM maize or derived feed products (see section 5.1.1), meaning that only low amounts of this protein would remain intact to pass out in faeces. This has been demonstrated for Cry1Ab (Einspanier *et al.*, 2004; Lutz *et al.*, 2005, 2006; Wiedemann *et al.*, 2006; Guertler *et al.*, 2008; Paul *et al.*, 2010). It is expected that there would subsequently be further degradation of the Cry protein in the manure and faeces due to intrinsic microbial proteolytic activity. Therefore, exposure of soil and aquatic environments to the mCry3A protein from disposal of animal wastes or accidental spillage of maize grains is likely to be very low and localised. While Cry proteins may bind to clay minerals or humic substances in soil, thereby reducing their availability to microorganisms for degradation, there are no indications of 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 x GA21, it can be concluded that the exposure of potentially sensitive non-target organisms to the mCry3A protein is likely to be very low and of no ecological relevance.

6.1.2.5. Interactions with the abiotic environment and biochemical cycles

Considering the scope of the application (that excludes cultivation) and the intended uses of maize MIR604 x GA21 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.

6.1.3. Post-market environmental 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 also related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA 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 x GA21 would be mainly through manure and faeces from gastrointestinal tracts of animals fed maize MIR604 x GA21 and/or through accidental release into the environment of GM maize grains during transportation and processing.

No specific environmental impact of maize MIR604 x GA21 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 the 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; and (3) the use of networks of existing surveillance systems (Lecoq

et al., 2007; Windels *et al.*, 2008). 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 x GA21 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. 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 includes food and feed uses, import and processing of maize MIR604 x GA21 and excludes cultivation. Considering the intended uses of maize MIR604 x GA21, the environmental risk assessment is concerned with indirect exposure mainly through manure and faeces from gastrointestinal tracts of animals fed maize MIR604 x GA21 and with the accidental release into the environment of maize MIR604 x GA21 grains during transportation and processing.

There are no indications of an increased likelihood of establishment and spread of feral maize plants in case of accidental release into the environment of viable maize MIR604 x GA21 grains during transportation and processing, except in the presence of glyphosate-based herbicides. Taking into account the scope of the application, both the rare occurrence of feral maize plants and low levels of mCry3A protein exposure in maize MIR604 x GA21 grains or through other routes indicate that the risk to non-target organisms is extremely low. It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts.

The scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MIR604 x GA21, 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.

CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out an evaluation of a scientific risk assessment of maize MIR604 x GA21 for food and feed uses, import and processing. The EFSA GMO Panel evaluated maize MIR604 x GA21, which has been produced by conventional crossing methods between lines containing the single maize events MIR604 and GA21, for food and feed uses, import and processing. Both single maize events MIR604 and GA21 have been evaluated previously by the EFSA GMO Panel (EFSA, 2007b, 2009c). In evaluating maize MIR604 x GA21, the EFSA GMO Panel considered the application EFSA-GMO-UK-2007-48, additional information provided by the applicant and scientific comments submitted by Member States. Further information from applications for placing the single maize events Bt11, MIR604 and GA21, as well as maize Bt11 x MIR604 x GA21 on the market under EU regulatory procedures was taken into account (EFSA, 2005; 2009a,c; 2010).

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for maize MIR604 x GA21 produced by conventional crossing are sufficient to conclude on this part of the evaluation. The bioinformatic analysis of the inserted DNA and the flanking regions of the single maize events MIR604 and GA21 do not raise safety concerns. The expression of mCry3A, PMI and mEPSPS proteins in maize MIR604 x GA21 has been analysed and the stability of the genetic modification has been demonstrated. The EFSA GMO Panel considers that the molecular characterisation does not indicate any safety concern.

The comparative agronomic analysis showed that maize MIR604 x GA21 is agronomically equivalent to its conventional counterpart, except for the new traits. The comparative assessment of maize MIR604 x GA21 was based on the compositional analysis of forage and grain derived from maize Bt11 x MIR604 x GA21 which is in accordance with the EFSA GMO Panel's guidance document on GM plants containing stacked transformation events (EFSA, 2007a). Based on all of the data available, the EFSA GMO Panel accepted in this case the use of maize Bt11 x MIR604 x GA21 for the comparative compositional analysis and concludes that forage and grain from maize MIR604 x GA21, assessed in this application, are likely to be compositionally equivalent to those of its conventional counterpart, except for the presence of the newly expressed proteins.

The mCry3A and PMI proteins expressed in maize MIR604 and the mEPSPS protein expressed in maize GA21 have been assessed previously, as described in the scientific opinions of the EFSA GMO Panel on the single maize events, and no safety concerns have been identified. Regarding the safety and nutritional properties of whole food and feed products derived from maize MIR604 x GA21, the EFSA GMO Panel considers it unlikely that interactions between the single maize events will occur that may impact on the food and feed safety and the nutritional properties of maize MIR604 x GA21. The EFSA GMO Panel bases this consideration on the known functional characteristics of the newly expressed proteins and on the outcomes of the comparative analysis of compositional, phenotypic and agronomic characteristics. In addition, the EFSA GMO Panel considers it unlikely that the overall allergenicity of maize MIR604 x GA21 has been altered.

The EFSA GMO Panel concludes that the maize MIR604 x GA21, assessed in this application, is as safe and nutritious as its conventional counterpart in the context of its intended uses. Considering the intended uses of maize MIR604 x GA21, 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 viable maize MIR604 x GA21 grains during transportation and processing, there are no indications of an increased likelihood of establishment and spread of feral maize plants, except in the presence of glyphosate-based herbicides. Also, the low levels of environmental exposure to these GM maize plants and the mCry3A protein through other routes indicate that the risk to non-target organisms is extremely low. It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize MIR604 x GA21.

The EFSA GMO Panel recommends that appropriate management systems should be in place to restrict seeds of maize MIR604 x GA21 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

The EFSA GMO Panel considers that the information available for maize MIR604 x GA21 addresses the scientific comments raised by Member States and concludes that the maize MIR604 x GA21, assessed in this application, is as safe as its conventional counterpart and other appropriate comparators. In addition, the EFSA GMO Panel is of the opinion that crossing of maize MIR604 and GA21 results in no interaction between the single maize events, which would affect the safety of maize MIR604 x GA21 with respect to potential effects on human and animal health, and on the environment in the context of its intended uses.The EFSA GMO Panel concludes that maize MIR604 x GA21 is unlikely to have an adverse effect on human and animal health and on the environment, in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the United Kingdom (FSA), dated 14 November 2007, concerning a request for placing on the market of maize MIR604 x GA21 in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 30 November 2007, from EFSA to the Competent Authority of the United Kingdom.

3. Letter from EFSA to applicant, dated 20 December 2007, requesting clarifications under completeness check.

4. Acknowledgement letter, dated 1 February 2008, from applicant to EFSA.

5. Letter from applicant, dated 3 March 2008, providing EFSA with an updated version of the application EFSA-GMO-UK-2007-48 submitted by Syngenta under Regulation (EC) No 1829/2003.

6. Letter from EFSA to applicant, dated 12 March 2008, delivering the 'Statement of Validity' for application EFSA-GMO-UK-2007-48, maize MIR604 x GA21 submitted by Syngenta Seeds S.A.S. on behalf of Syngenta Crop Protection AG under Regulation (EC) No 1829/2003.

7. Letter from EFSA to applicant, dated 12 March 2008, stopping the clock.

8. Letter from applicant to EFSA, dated 1 April 2008, providing the valid application.

9. Letter from applicant to EFSA, dated 9 July 2008, requesting public access to Member States comments on Syngenta applications submitted under Regulation (EC) No 1829/2003.

10. Letter from applicant to EFSA, dated 4 November 2008, providing an update on the progress made to address the questions of the EFSA GMO Panel (cf., safety of PMI).

11. Letter from applicant to EFSA, dated 7 April 2009, providing additional information on application EFSA-GMO-UK-2005-11.

12. Letter from EFSA to applicant, dated 24 June 2009, restarting the clock.

13. Letter from EFSA to applicant, dated 9 July 2009, requesting the authorisation to re-use additional information submitted in the frame of other applications.

14. Letter from applicant to EFSA, dated 14 July 2009, permitting to re-use additional information submitted in the frame of other applications.

15. Letter from EFSA to applicant, dated 5 October 2009, requesting additional information and stopping the clock.

16. Letter from applicant to EFSA, dated 18 November 2009, providing additional information.

17. Letter from EFSA to applicant, dated 11 January 2010, requesting additional clarifications and maintaining the clock stopped.

18. Letter from applicant to EFSA, dated 8 February 2010, providing additional information.

19. Letter from EFSA to applicant, dated 3 March 2010, restarting the clock.

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