SCIENTIFIC OPINION

Scientific Opinion on application (Reference EFSA-GMO-UK-2008-56) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize Bt11 x MIR604 x GA21 , for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2, 3]

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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 Bt11 x MIR604 x GA21 for food and feed uses, import and processing. Conventional crossing methods were used in the production of maize Bt11 x 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 Cry1Ab, PAT, mCry3A, PMI and mEPSPS proteins in maize Bt11 x 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 Bt11 x MIR604 x GA21 with its conventional counterpart except for the newly expressed proteins which provided resistance to certain lepidopteran and coleopteran target pests and tolerance to glufosinate-ammonium- and/or glyphosate-based herbicides. The safety assessment identified no concerns regarding potential toxicity and allergenicity of maize Bt11 x MIR604 x GA21. A feeding study on broiler chickens confirmed the nutritional equivalence of this GM maize to its conventional counterpart and a commercial non-GM maize variety. Considering the intended uses of maize Bt11 x MIR604 x GA21, which excludes cultivation within the European Union, no scientific assessment of potential environmental effects associated with cultivation of maize Bt11 x MIR604 x GA21 was required. In case of accidental release of viable maize Bt11 x 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 the

1 On request from the Competent Authority of The United Kingdom for an application (EFSA-GMO-UK-2008-56) submitted by Syngenta Seeds, Question No EFSA-Q-2008-375, adopted on 29 April 2010.

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glufosinate-ammonium- and/or 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 the information available for maize Bt11 x MIR604 x GA21 addresses the scientific comments raised by Member States and that the maize Bt11 x 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 Bt11 x MIR604 x GA21 is unlikely to have an adverse effect on human and animal health and the environment, in the context of its intended uses.

Key words

GMO, maize (*Zea mays*), Bt11 x MIR604 x GA21, insect resistant, herbicide tolerant, 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-2008-56) 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 Bt11 x MIR604 x GA21 (Unique Identifier SYNBTØ11-1xSYN-IR6Ø4-5xMON-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-2008-56, 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 MIR604 x GA21, Bt11 x GA21 and Bt11 x MIR604 on the market under EU regulatory procedures was taken into account. The scope of application EFSA-GMO-UK-2008-56 is for food and feed uses, import and processing of maize Bt11 x MIR604 x GA21 and all derived products, but excludes cultivation in the EU. The EFSA GMO Panel evaluated maize Bt11 x 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 new proteins, as individual proteins and in combination and the whole food/feed were evaluated with respect to toxicity, allergenicity and nutritional quality. An evaluation of environmental impacts and the post-market environmental monitoring plan was undertaken.

Maize Bt11 x MIR604 x GA21 has been produced by conventional crossing methods between lines containing the single maize events Bt11, MIR604 and GA21 to combine the lepidopteran resistance trait and tolerance to glufosinate-ammonium herbicides in maize Bt11, with the coleopteran resistance trait and the ability to use mannose as a sole carbon source in maize MIR604 and with the tolerance to glyphosate-based herbicides in maize GA21. These single maize events and the double stacked maize events MIR604 x GA21, Bt11 x GA21 and Bt11 x MIR604 have been the subject of separate assessments by the EFSA GMO Panel. No new genetic modifications were introduced in maize Bt11 x MIR604 x GA21.

Molecular analysis of the DNA inserts present in maize Bt11 x MIR604 x GA21 confirmed that maize Bt11, MIR604 and GA21 inserts are present and that their structures are retained. The expression levels of the Cry1Ab, PAT, mCry3A, PMI and mEPSPS proteins were comparable to those of the respective single maize events.

Based on the results of comparative analysis the EFSA GMO Panel concludes that maize Bt11 x MIR604 x GA21 is compositionally, phenotypically and agronomically equivalent to its conventional counterpart, except for the presence of Cry1Ab, PAT, mCry3A, PMI and mEPSPS proteins in maize Bt11 x MIR604 x GA21. Based on the assessment of data available, including the additional information provided by the applicant in response to the EFSA GMO Panel's request for maize Bt11 x MIR604 x GA21, its conventional counterpart and for the single maize events, the EFSA GMO Panel is of the opinion that crossing of maize Bt11, MIR604 and GA21 results in no interactions between the single maize events which causes compositional or agronomic changes. The Cry1Ab and PAT expressed in maize Bt11, the Cry3A and PMI expressed in maize MIR604 and mEPSPS expressed in maize GA21 have been assessed previously and no safety concerns were identified. Given all the information provided, the EFSA GMO Panel concludes that interactions between the single maize events that might impact on food and feed safety are unlikely and that the nutritional properties of Bt11 x MIR604 x GA21 maize would be no different from those of the conventional counterpart. The EFSA GMO Panel considers that it is unlikely that the overall allergenicity of the whole maize Bt11 x

MIR604 x GA21 has been changed. The nutritional value of maize Bt11 x MIR604 x GA21 has been studied in a feeding study with broilers which confirmed that the nutritional properties of maize Bt11 x MIR604 x GA21 would be no different from those of its conventional counterpart and a commercial non-GM maize variety.

The application EFSA-GMO-UK-2008-56 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 Bt11 x 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 Bt11 x MIR604 x GA21 grains during transportation and processing, except in the presence of glufosinate-ammonium- and/or 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 Bt11 x 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 Bt11 x MIR604 x GA21 addresses the scientific comments raised by Member States and concludes that the maize Bt11 x 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 Bt11, MIR604 and GA21 results in no interactions between the single maize events which would affect the safety of maize Bt11 x 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 Bt11 x MIR604 x GA21is 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 21 May 2008, the European Food Safety Authority (EFSA) received from the Competent Authority of the United Kingdom an application (Reference EFSA-GMO-UK-2008-56), for authorisation of genetically modified (GM) maize Bt11 x MIR604 x GA21 (Unique Identifier SYNBTØ11-1xSYN-IR6Ø4-5xMON-ØØØ21-9) for food and feed uses, import and processing, submitted by Syngenta Seeds within the framework of Regulation (EC) No 1829/2003 on genetically modified (GM) food and feed. After receiving the application EFSA-GMO-UK-2008-56 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 24 July 2008, EFSA received additional information (requested on 4 July 2008) and declared the application as formally valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003 on 19 August 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 19 November 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 Bt11 x 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 the 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 MIR604 x GA21, Bt11 x GA21 and Bt11 x MIR604 on the market under EU regulatory procedures were also taken into account (EFSA, 2005, 2007b, 2009a,b,c, 2010a,b).

The EFSA GMO Panel requested from the applicant additional information on 20 August 2008, 09 July 2009 and 05 October 2009. The requested information was provided by the applicant on 07 April 2009, 14 July 2009 and 05 October, respectively. The risk assessments of single maize events Bt11, MIR604 and GA21 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 its intended uses (EFSA, 2005, 2007b, 2009a,b)

- 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, 2000a). 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).

- 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, 2009b). Recently, for food and feed uses, import and processing have been 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, for 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, 2000b).

In giving its scientific opinion on maize Bt11 x MIR604 x GA21 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 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 Bt11 has been developed to provide protection against certain lepidopteran target pests (such as the European corn borer, *Ostrinia nubilalis* and other species belonging to the genus *Sesamia*) through the introduction of a truncated *cry1Ab* gene from *Bacillus thuringiensis* subsp. *kurstaki* and to be tolerant to glufosinate-ammonium herbicides by the introduction of a gene encoding a phosphinothricin-N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes*.

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 isomerise (PMI) protein 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.

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 Bt11 x 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 Bt11 x MIR604 x GA21 (Unique Identifier SYN-BTØ11-1 x 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 Bt11 x MIR604 x GA21 submitted in the EU, including 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 on the market under EU regulatory procedures was taken into account, (EFSA, 2005, 2007b, 2009a,b,c, 2010a,b).

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 Bt11 x MIR604 x GA21

Conventional crossing methods were used to produce maize Bt11 x MIR604 x GA21 and no new genetic modification was involved. The three inserts that are present in maize Bt11 x MIR604 x GA21 were derived from lines containing the single maize events Bt11, MIR604 or GA21. Each of these GM maize events was the subject of an earlier safety evaluation and separate opinions for each of them have been published (EFSA, 2005, 2007b, 2009a,b).

3.1.2. Summary of the evaluation of the single maize events

Maize Bt11

Maize Bt11 was developed by electroporation and regeneration of maize protoplasts. As a result of the genetic modification, the Bt11 event contains an insert bearing both a variant *cry1Ab* gene to confer resistance to specific lepidopteran pests and a *pat* gene as a selectable marker providing tolerance to glufosinate-containing herbicides.

Molecular analysis showed that maize Bt11 contains a single copy of the insert in the nuclear genome of the GM plant. . There is no evidence for the presence of partial insertions of *bla* gene sequences or non-coding vector backbone sequences. The nucleotide sequence of the entire Bt11 insert in maize was determined which enabled a direct comparison between the previously reported sequences (EFSA, 2005, 2009a). A total of eight nucleotide differences were identified when the Bt11 insert sequence of sweet maize was compared to the previously reported Bt11 sequence in field maize. The applicant attributed this discrepancy to sequencing errors in the original datasets. The GMO Panel considers this to be a reasonable

[4] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2008-375

assumption, which is further confirmed by an updated sequence analysis of both the insert and the original plasmid used for transformation.

Updated bioinformatic analysis supports the conclusion that the genomic sequences in both 5' and 3' regions flanking the insert of Bt11 event show homology to highly repetitive, *knob*-associated sequences. The data do not indicate any safety concerns with regard to the interruption of known genes or from the potential production of new toxins or allergens.

The genetic stability of the inserted DNA in event Bt11 was demonstrated and segregation data for PAT and Cry1Ab were shown to follow Mendelian genetics.

Maize MIR604

Maize MIR604 was developed by using *Agrobacterium*-mediated transformation and as a result expresses a modified version of a *cry3A* gene (m*cry3*A) derived from *Bacillus thuringiensis* subsp. *tenebrionis* conferring resistance to certain coleopteran target pests and a *man*A gene encoding phosphomannose isomerase (PMI) from *E.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 the MIR604 event have been determined and BLASTN analysis of the 5' and 3' flanking sequences has revealed no significant similarities with any known maize sequences. Analysis of putative open reading frames (ORFs) at the 5' and 3' flanking regions indicated no sequence similarities to known toxins or allergens.

Southern, PCR and ELISA analyses of MIR604 maize 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 towards glyphosate-containing 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 GA21 maize and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations.

3.1.3. Transgene constructs in maize Bt11 x MIR604 x GA21

Maize Bt11 x MIR604 x GA21 has been obtained by conventional crossing methods between lines containing the single maize events Bt11, MIR604 and GA21. No new genetic modification has been introduced in the stacked maize line. The integrity of the individual inserts present in this maize was investigated using Southern analyses. This involved the use of DNA probes specific for the Bt11, MIR604 and GA21 inserts and enzymatic digestions informative of the structure of the three events, including the junctions with the host genomic DNA. The predicted DNA hybridisation patterns from

each single maize event were retained in the stacked maize events Bt11 x MIR604 x GA21, demonstrating that integrity of the inserts was maintained.

3.1.4. Information on the expression of the inserts

The levels of newly expressed proteins Cry1Ab, PAT, mCry3A, PMI and mEPSPS in forage and grain of maize Bt11 x MIR604 x GA21 were assessed by enzyme-linked immunosorbent assay (ELISA). Tissue samples for analysis were collected from plants containing the single maize events Bt11, MIR604 and GA21 and from maize Bt11 x MIR604 x GA21, having a similar genetic background and grown in a field trial conducted in the major maize-growing region of the USA in 2006. The scope of the application covers food and feed uses, import and processing, therefore protein expression data related to the grains is considered the most relevant. These data are summarised in Table 1. Levels of proteins in the stacked line are comparable to levels in plants containing the single maize events.

Table 1. Protein expression levels in maize Bt11 x MIR604 x GA21, Bt11, MIR604 and GA21 grains (µg / g dry weight)

	Bt11 x MIR604 x GA21	Bt11	MIR604	GA21
Cry1Ab mean range	1.6 0.6 – 1.9	1.4 0.8 – 1.8	--	--
PAT	< 1.6 (LOQ)	< 1.6 (LOQ)	--	--
mCry3A	< 0.6 (LOQ)	--	< 0.6 (LOQ)	--
PMI mean range	3.1 2.1 – 4.9	--	2.9 1.4 – 4.8	--
mEPSPS mean range	6.3 5.3 – 7.1	--	--	6.8 5.2 – 8.5

LOQ: limit of quantification

3.1.5. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in events Bt11, MIR604 and GA21 was demonstrated previously (EFSA, 2005, 2007b, 2009a,b). In the maize Bt11 x MIR604 x GA21 the three inserts are combined. The Southern data presented show that all three events are present and that the structure of each insert is retained. Furthermore, each of the traits has been conserved in this maize.

3.2. Conclusion

As conventional crossing methods were used in the production of maize Bt11 x MIR604 x GA21, no additional genetic modification was involved. Southern analyses demonstrated that the structures of the Bt11, MIR604 and GA21 inserts were retained in maize Bt11 x MIR604 x GA21. The expression levels of Cry1Ab, PAT, mCry3A, PMI and mEPSPS proteins in the grains of maize Bt11 x MIR604 x GA21 have been demonstrated to be comparable with those of the single maize events. The EFSA GMO Panel concludes that the molecular characterisation does not indicate safety concerns.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

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

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).

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 USA 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 USA in 2006, following an EFSA GMO Panel's request. 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 varieties except for the presence of the PMI and mCry3A proteins (EFSA, 2009b).

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 was equivalent to that of the non-GM comparators, except for the introduced traits (EFSA, 2009b).

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 herbicides 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).

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

For the comparative analysis of the compositional characteristics of forage and grain of maize Bt11 x MIR604 x GA21 and its conventional counterpart were grown in 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/or glyphosate-based herbicides. Given the fact that the previous assessments of the herbicide-tolerant single maize events Bt11 and GA21 considered both plants treated with the target herbicides 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 analyzed for composition.

Field trials for comparative agronomic analysis were carried out at ten locations in the US in 2006 using a randomised complete block design with five replications per location.

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 analyzed 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 antinutrients (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, assessed in this application, 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

During field trials in 2006 at ten locations in the US (five replications per site), extensive data on phenotypic characteristics, agronomic performance (e.g., grain yield, number of emerged plants, plant population at harvest, plant height, ear height, root lodging) and disease susceptibility were collected for the maize Bt11 x MIR604 x GA21 and its conventional counterpart .

A statistical analysis on agronomic and phenotypic characteristics on a per-location basis was provided by the applicant at the EFSA GMO Panel's request, complementing the across-location analysis already provided by the applicant. Across locations, statistically significant differences were observed in grain yield, grain moisture, and plant yield. The statistical analysis showed additional statistically significant differences at individual field trial sites. However, when data from all locations were considered there were no consistent statistically significant differences that occurred in each separate location. In addition, the differences in the average values for plant height and grain moisture across locations were of minor magnitude.

In the absence of consistent unexpected differences between the studied maize plants, the EFSA GMO Panel concludes that no biologically relevant agronomic differences specific for maize Bt11 x MIR604 x GA21 as compared to its conventional counterpart are expected except for the introduced herbicide tolerance and insect resistance traits.

4.2. Conclusion

The EFSA GMO Panel concludes that forage and grain from the maize Bt11 x MIR604 x GA21, assessed in this application, are compositionally equivalent to those of its conventional counterpart except for the presence of the newly expressed proteins. The outcome of the phenotypic and agronomic analysis of maize Bt11 x MIR604 x GA21 did not show biologically relevant differences compared with its conventional counterpart except for the new traits. Based on the assessment of the data available, the EFSA GMO Panel is of the opinion that crossing Bt11, MIR604 and GA21 to produce maize Bt11 x MIR604 x GA21 results in no interactions between the single maize events which cause compositional or agronomic changes. The EFSA GMO Panel concludes that the maize Bt11 x MIR604 x GA21, assessed in this application, is compositionally and agronomically equivalent to its conventional counterpart except for the presence of the proteins Cry1Ab, PAT, mCry3A, PMI and mEPSPS in maize Bt11 x MIR604 x GA21.

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

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

Maize Bt11

Bioinformatics-supported studies showed that the amino acid sequences of the newly expressed Cry1Ab and PAT proteins do not show any significant similarity with the sequences of known toxins or allergens. For the safety testing the respective proteins produced in recombinant *E. coli* strains were used after it had been demonstrated experimentally that these proteins were equivalent to those produced in maize Bt11. The microbially produced Cry1Ab and PAT proteins were rapidly degraded in simulated gastric fluid. The Cry1Ab protein did not induce adverse effects in an acute oral toxicity study in mice. There were no indications of adverse effects after repeated-dose oral administration (14 days) of the PAT protein to rats.

With regard to animal studies with the whole product, feeding studies with maize Bt11 grain using different target animals, such as broiler chickens and laying hens fed grains, as well as dairy cows and beef cattle (steers) fed silage, indicated nutritional equivalence between transgenic Bt11 maize and the non-GM control (EFSA, 2005).

The EFSA GMO Panel also evaluated data, which were submitted after the first evaluation of maize Bt11, and concluded that the new information from an updated literature review and additional studies did not prompt the EFSA GMO Panel to change its previous opinion that maize Bt11 is as safe and as nutritious as the non-GM maize counterparts (EFSA, 2009a).

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 similarity 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 in 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 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 identical structural characteristics that would indicate potential 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 was 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, 2009b).

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 event 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-2008-56 includes the import and processing of maize Bt11 x MIR604 x GA21 and its derived products for use as food and feed. Thus, the possible uses of maize Bt11 x MIR604 x GA21 include the production of animal feed, but it also includes valuable food products, such as starch, syrups and oils.

The genetic modification of maize Bt11 x 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 Bt11 x MIR604 x GA21 is compositionally equivalent to its conventional counterpart , except for the newly expressed proteins (see Section 4.2), the effect of processing on maize Bt11 x 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 Bt11 x MIR604 x GA21

The Cry1Ab and PAT expressed in maize Bt11, 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, 2005, 2007b, 2009a,b) 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 Bt11, MIR604 and GA21 have been introduced in maize Bt11 x 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 Cry1Ab, PAT, mCry3A, PMI and mEPSPS in maize Bt11 x 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 Cry1Ab, PAT, mCry3A, PMI and mEPSPS and known proteins toxic to mammals.

Determination of the levels of the newly expressed proteins in grains of maize Bt11 x MIR604 x GA21, Bt11, 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 Cry1Ab, PAT, 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 Cry1Ab, PAT, mCry3A, PMI and mEPSPS proteins have been identified in maize Bt11 x MIR604 x GA21, and relevant changes in the composition of maize Bt11 x MIR604 x GA21 are unlikely.

5.1.4.3. Toxicological assessment of the whole GM food/feed

Maize Bt11, MIR604 and GA21 have previously been found as safe as their conventional counterpart for human and animal consumption (EFSA, 2005, 2007b, 2009a,b). In the present assessment, it was found that the structural integrity of the inserts in maize Bt11 x MIR604 x GA21 was not changed in comparison with the single maize events Bt11, MIR604 and GA21, respectively, and expression analysis of the proteins revealed that the overall levels of the proteins Cry1Ab, PAT, mCry3A, PMI and mEPSPS in maize Bt11 x MIR604 x GA21 were generally similar to the levels in the respective single maize events Bt11, MIR604 and GA21 (see section 3.2). Moreover, the composition and phenotypic and agronomic characteristics of maize Bt11 x MIR604 x GA21 were found equivalent to those of its conventional counterpart. The EFSA GMO Panel considered all the data available for maize Bt11 x MIR604 x GA21 and the newly expressed proteins Cry1Ab, PAT, 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 Bt11 x 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 (Cry1Ab, PAT, mCry3A, PMI and mEPSPS) present in maize Bt11 x MIR604 x GA21 have been evaluated previously and it was found unlikely that they are allergenic (EFSA, 2005 2007b, 2009a,b). 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 Cry1Ab, PAT, 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 the 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 Bt11 x MIR604 x GA21, as compared to the single maize events Bt11, 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 Bt11 x MIR604 x GA21 would significantly increase the intake and exposure to maize.

5.1.6. Nutritional assessment of GM food/feed

A 49-day feeding study using broiler chickens was performed by the applicant according to the ILSI (2003) recommendations. Groups consisting of 90 male and 90 female animals (6 pens with 15 male and 6 pens with 15 female animals per group; initial body weight: ca. 41g/chick) were fed with diets containing grain from maize Bt11 x MIR604 x GA21, a non-GM maize counterpart with comparable genetic background or a commercial non-GM maize variety (maize NC2007). The inclusion rate of maize grain in the starter (day 1-16), grower (day 16-35) and finisher diets (day 35-49) was approximately from 49 to 51%, from 55 to 57% and from 60 to 63%, respectively. The diets were adjusted for their contents in proximates, amino acids and metabolisable energy according to NRC (1994) and CVB (2002). Birds were provided feed and water *ad libitum*. Animal performance on the various diets was evaluated by measuring mortality, body weight gain (overall final weight of males: 3359; of females: 2764 g/animal), feed consumption, feed conversion ratio (cumulative FCR of males 1.69; of females 1.77 g/g) and carcass yields (fat pad, drums, thighs, wings and breasts). There were no statistically significant differences in body weight gain, carcass yield and mortality between the groups, and the overall survival was >97%. Statistically significant differences in feed conversion during the grower period were observed between broilers fed diets containing maize Bt11 x MIR604 x GA21 and maize NC2007; however they were not significantly different between broilers fed diets containing maize Bt11 x MIR604 x GA21 and its conventional counterpart. The broiler feeding study supported the results of the comparative compositional analysis and confirmed that maize Bt11 x MIR604 x GA21 is nutritionally equivalent to grain from its conventional counterpart and a commercial non-GM maize variety when used in adjusted 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 Bt11 x MIR604 x GA21 is any less safe than its conventional counterpart. In addition, maize Bt11 x MIR604 x GA21 is, from a nutritional point of view, equivalent to its conventional counterpart and a commercial non-GM maize variety. Therefore, and in line with the guidance document (EFSA, 2006a), the EFSA GMO Panel is of the opinion that post-market monitoring of the food/feed derived from maize Bt11 x MIR604 x GA21 is not necessary.

5.2. Conclusion

The Cry1Ab and PAT proteins expressed in Bt11, 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 Bt11 x 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 Bt11 x 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). The safety and nutritional properties of whole food and feed products derived from Bt11 x MIR604 x GA21 have also been considered. Maize Bt11 x MIR604 x GA21 was tested in a nutritional chicken feeding study, which shows that this maize is nutritionally equivalent to its conventional counterpart and a commercial non-GM maize variety. The EFSA GMO Panel concludes that the outcomes of the chicken feeding study further support the findings of the comparative analysis of composition confirming the nutritional equivalence of maize Bt11 x MIR604 x GA21 to its conventional counterpart and a commercial non-GM maize variety. In addition, the EFSA GMO Panel considers it unlikely that the overall allergenicity of maize Bt11 x MIR604 x GA21 has been altered. The EFSA GMO Panel concludes that the maize Bt11 x MIR604 x GA21 assessed in this application is as safe and nutritious as its conventional counterpart. The EFSA GMO panel concludes that maize

Bt11 x 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 Bt11 x MIR604 x GA21 and does not include cultivation. Considering the proposed uses of maize Bt11 x MIR604 x GA21, the environmental risk assessment is concerned with the exposure through manure and faeces from gastrointestinal tracts of animals fed maize Bt11 x MIR604 x GA21 and with the accidental release into the environment of maize Bt11 x MIR604 x GA21 grains during transportation and processing.

As the scope of the present application excludes cultivation, environmental concerns related to the use of glufosinate-ammonium- and/or glyphosate-based herbicides on maize Bt11 x 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 and the stacked maize events

In its previous scientific opinions, the EFSA GMO Panel was of the opinion that the single maize events Bt11, MIR604 and GA21 and the stacked maize events MIR604 x GA21, Bt11 x GA21 and Bt11 x MIR604 are as safe as their conventional counterparts, and that the placing on the market of maize Bt11, MIR604, GA21, MIR604 x GA21, Bt11 x GA21 and Bt11 x MIR604 for food and feed uses, import and processing is unlikely to have an adverse effect on human or animal health, or on the environment (EFSA, 2005, 2007b, 2009a,b,c, 2010a,b). Furthermore, post-market environmental monitoring plans, including general surveillance, were proposed by the applicant and considered in line with EFSA GMO Panel opinion on PMEM by the EFSA GMO Panel for maize Bt11, MIR604, GA21, MIR604 x GA21, Bt11 x GA21 and Bt11 x MIR604.

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 shown 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 Bt11, MIR604 and GA21 and the stacked events MIR604 x GA21, Bt11 x GA21 and Bt11 x MIR604 as compared to their conventional counterparts. In addition to the field trials carried out with the single maize events and stacked maize events (EFSA, 2005, 2007b, 2009a,b,c, 2010a,b), a

series of field trials with maize Bt11 x MIR604 x GA21 were conducted across ten US corn belt locations in 2006. Information on phenotypic and agronomic characteristics was provided to assess the agronomic performance of maize Bt11 x MIR604 x GA21 in comparison with its conventional counterpart. These field trial data showed enhanced biomass production when glufosinate-ammonium- and/or 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 Bt11 x 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 over-wintering of maize Bt11 x MIR604 x GA21 or maize with comparable properties such as single maize events.

The herbicide tolerance traits can only be regarded as providing a potential agronomic advantage for maize Bt11 x MIR604 x GA21 plants where and when glufosinate-ammonium- and/or glyphosate-based herbicides are applied. Similarly, insect resistance against certain lepidopteran and coleopteran target pests provides a potential agronomic advantage in cultivation under infestation of target pests. However, survival of maize plants outside cultivation or other areas where glufosinate-ammonium- and/or 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 Bt11 x 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 Bt11 x 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 Bt11 x MIR604 x GA21 has no altered survival, multiplication or dissemination characteristics, except when glufosinate-ammonium- and/or 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 Bt11 x MIR604 x GA21 grains will not differ from that of the single maize events Bt11, MIR604 and GA21, the stacked maize events MIR604 x GA21, Bt11 x GA21 and Bt11 x MIR604, or from 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 Bt11 x MIR604 x GA21 is expected (see section 5 of the scientific opinion).

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, 2009d 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, the 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 (*cry1Ab*, *pat*, m*cry3A* and *pmi* (*man*A)), as expressed in maize Bt11 x MIR604 x GA21 are of bacterial origin. Thus, in theory, the *cry1Ab*, *pat*, 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 hypothesized 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 Bt11 x MIR604 x GA21 would be expressed. The *cry1Ab* and *pat* genes are regulated by plant virus promoters. The activity of these plant virus promoters in unrelated organisms such as bacteria cannot be excluded, but in the unlikely event that the above mentioned genes and regulatory elements are taken up by bacteria, no selective advantage is anticipated, because *cry*, *pat* and *pmi* genes are already occurring in various bacterial species in the environment. Thus, the hypothesized low level exposure of environmental bacterial communities to the maize Bt11 x MIR604 x GA21 *cry1Ab*, *pat*, 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*, *pat* 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 hypothesized 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, 2009a).

(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 successful establishment and subsequent flowering of this GM maize plant. 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 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).

Herbicide tolerance and insect resistance provide agronomic and selective advantages in areas where glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation of target pests. 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 Bt11 x MIR604 x GA21, herbicide tolerance and insect resistance are not likely to provide selective advantages outside cultivation or other areas where glufosinate-ammonium- and/or

glyphosate-based herbicides could be applied in Europe, and/or under infestation of target pests. 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 Bt11 x 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 Bt11 x 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 Bt11 x 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 Bt11 x MIR604 x GA21 has no altered survival, multiplication or dissemination characteristics, except when glufosinate-ammonium- and/or 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 as a consequence of spread of genes from this maize in Europe will not differ from that of the single maize events Bt11, MIR604 and GA21, the stacked maize events MIR604 x GA21, Bt11 x GA21 and Bt11 x MIR604, or from that of conventional maize varieties.

6.1.2.3. Interactions of the GM plant with target organisms

The intended uses of maize Bt11 x MIR604 x GA21 specifically exclude cultivation and the environmental exposure to maize Bt11 x 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 Bt11 x 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 Bt11 x MIR604 x GA21 specifically exclude cultivation and the environmental exposure to maize Bt11 x 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 Bt11xMIR604xGA21 plants to enable any significant interaction with non-target organisms, which is very unlikely.

In addition, the EFSA GMO Panel evaluated whether the Cry1Ab and mCry3A proteins might potentially affect non-target organisms by entering the environment through manure and faeces from the gastrointestinal tracts of animals fed maize Bt11 x MIR604 x GA21. Due to the specific insecticidal selectivity of Cry proteins, non-target organisms most likely to be affected by the Cry1Ab and mCry3A proteins 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 Cry1Ab and mCry3A proteins enter the environment due to low expression in grains. Moreover, these Cry proteins are degraded by enzymatic activity in the gastrointestinal tract of animals fed on GM maize or derived feed products (see section 5 of the scientific opinion), meaning that only low amounts of these proteins 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 Cry proteins in the manure and faeces due to intrinsic microbial proteolytic activity. Therefore, exposure of soil and aquatic environments to the Cry1Ab and mCry3A proteins 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 a certain degree 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 Bt11 x MIR604 x GA21, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1Ab and mCry3A proteins 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 Bt11 x 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 Bt11 x MIR604 x GA21 would be mainly through manure and faeces from gastrointestinal tracts of animals fed maize Bt11 x 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 Bt11 x 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 Bt11 x 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.

The EFSA GMO Panel advises that appropriate management systems should be in place to restrict seeds of maize Bt11 x MIR604 x GA21 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) 1829/2003

6.2. Conclusion

The scope of the application includes food and feed uses, import and processing of maize Bt11 x MIR604 x GA21 and excludes cultivation. Considering the intended uses of maize Bt11 x MIR604 x GA21, the environmental risk assessment is concerned with indirect exposure mainly through manure and faeces from gastrointestinal tracts of animals fed maize Bt11 x MIR604 x GA21 and with the accidental release into the environment of maize Bt11 x 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 Bt11 x MIR604 x GA21 grains during transportation and processing, except in the presence of glufosinate-ammonium- and/or glyphosate-based. Taking into account the scope of the application, both the rare occurrence of feral maize plants and low levels of Cry1Ab and mCry3A protein exposure in maize Bt11 x 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 Bt11 x 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 Bt11 x MIR604 x GA21 for food and feed uses, import and processing. The EFSA GMO Panel evaluated maize Bt11 x MIR604 x GA21, which has been produced by conventional crossing methods between maize lines containing the single maize events Bt11, MIR604 and GA21, for food and feed uses, import and processing. All single maize events Bt11, MIR604 and GA21 and the stacked maize events MIR604 x GA21, Bt11 x GA21 and Bt11 x MIR604 have been evaluated by the EFSA GMO Panel (EFSA, 2005, 2007b, 2009a,b,c, 2010a,b). In evaluating maize Bt11 x MIR604 x GA21 the EFSA GMO Panel considered the application EFSA-GMO-UK-2008-56, 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 double stacked maize events MIR604 x GA21, Bt11 x GA21 and Bt11 x MIR604 on the market under EU regulatory procedures was taken into account(EFSA, 2005, 2007b, 2009a,b,c, 2010a,b).

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for maize Bt11 x MIR604 x GA21 produced by conventional crossing are sufficient to conclude on this part of the evaluation. The bioinformatic analyses of the inserted DNA and the flanking regions of the single maize events Bt11, MIR604 and GA21 do not raise safety concerns. The expression of Cry1Ab, PAT, mCry3A, PMI and mEPSPS proteins in maize Bt11 x 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 results of the comparative analysis indicated that the maize Bt11 x MIR604 x GA21 assessed in this application is compositionally, phenotypically and agronomically equivalent to its conventional counterpart, except for the presence of the proteins Cry1Ab, PAT, mCry3A, PMI and mEPSPS in maize Bt11 x MIR604 x GA21. Based on the evaluation of the data available, including additional information provided by the applicant in response to requests from the EFSA GMO Panel for maize Bt11 x MIR604 x GA21, for the single maize events and for its conventional counterpart(s), the EFSA GMO Panel is of the opinion that crossing of maize Bt11, MIR604 and GA21 results in no interactions between the single maize events which causes unexpected compositional or agronomic changes. The proteins Cry1Ab and PAT expressed in maize Bt11, the proteins mCry3A and PMI expressed in maize

MIR604 and the mEPSPS protein expressed in maize GA21 have been evaluated previously and no safety concerns have been identified in both the previous and current assessments. Given all the information provided, the EFSA GMO Panel concludes that interactions between the single maize events that might impact on food and feed safety are unlikely, the nutritional properties of maize Bt11 x MIR604 x GA21 would be not different from those of its conventional counterpart, and that it is unlikely that the overall allergenicity of the whole plant is changed. In conclusion, the EFSA GMO Panel considers that maize Bt11 x MIR604 x GA21 is as safe and as nutritious as its conventional counterpart, and that the overall allergenicity of the whole plant is not changed.

Considering the intended uses of maize Bt11 x 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 Bt11 x 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 glufosinate-ammonium- and/or glyphosate-based herbicides. Also, the low levels of environmental exposure to these GM maize plants and the Cry1Ab and mCry3A proteins through other routes indicate that the risk to non-target organisms is extremely low. It is highly unlikely that recombinant DNA would 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 Bt11 x MIR604 x GA21.

The EFSA GMO Panel recommends that appropriate management systems should be in place to restrict seeds of maize Bt11 x 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 Bt11 x MIR604 x GA21 addresses the scientific comments raised by Member States and concludes that the maize Bt11 x 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 Bt11, MIR604 and GA21 results in no interactions between the single maize events which would affect the safety of maize Bt11 x 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 Bt11 x MIR604 x GA21is 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, dated 21 May 2008, concerning a request for placing on the market of maize Bt11 x MIR604 x GA21 in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 28 May 2008, from EFSA to the Competent Authority of the United Kingdom.

3. Letter from EFSA to applicant, dated 4 July 2008, requesting additional information under completeness check

4. Letter from applicant to EFSA, dated 24 July 2008, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 19 August 2008, delivering the ‘Statement of Validity’ for application EFSA-GMO-UK-2008-56, maize Bt11 x MIR604 x GA21submitted by Syngenta under Regulation (EC) No 1829/2003.

6. Letter from EFSA to applicant, dated 20 August 2008, requesting additional information and stopping the clock.

7. Letter from applicant to EFSA, dated 7 April 2009, providing the additional information requested.

8. Letter from EFSA to applicant, dated 26 June 2009, restarting the clock.

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

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

11. Letter from EFSA to applicant, dated 8 March 2010, requesting additional information and maintaining the clock stopped.

12. Letter from applicant to EFSA, dated 22 April 2010, providing the additional information requested.

13. Letter from EFSA to applicant, dated 23 April 2010, restarting the clock.

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(notified under document number C(2008) 1112). Official Journal of the European Union L87, 19-22, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:087:0019:0022:EN:PDF

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