SCIENTIFIC OPINION

# Scientific Opinion on application (EFSA-GMO-UK-2007-49) for the placing on the market of the insect resistant and herbicide tolerant genetically modified maize Bt11xGA21 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Syngenta Seeds[1]

**EFSA Panel on Genetically Modified Organisms**[2]

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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 Bt11xGA21 for food and feed uses, import and processing. Conventional breeding methods were used in the production of maize Bt11xGA21 from inbred lines of the respective parental events. The structural integrity of the inserts in the single maize events as well as the phenotypes were retained in the stacked maize event. The expression levels of the Cry1Ab, PAT and mEPSPS proteins in maize Bt11xGA21 were demonstrated to be comparable with those of the single maize events. The comparative analysis of phenotypic, agronomic and compositional characteristics of this GM maize indicated equivalence with its non-GM maize counterpart and conventional maize, except for the expression of the target proteins Cry1Ab, PAT and mEPSPS, providing resistance to certain lepidopteran pests and tolerance to glufosinate-ammonium- and glyphosate-based herbicides. The safety assessment identified no concerns regarding potential toxicity and allergenicity of maize Bt11xGA21. A feeding study on broiler chickens confirmed the nutritional equivalence of this GM maize to its non-GM maize counterpart and conventional maize. Considering the intended uses of maize Bt11xGA21, which excludes cultivation within the European Union, no scientific assessment of potential environmental effects associated with cultivation of maize Bt11xGA21 was required. In case of accidental release of viable maize Bt11xGA21 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 glufosinte-ammonium- and/or glyphosate-based herbicides. The EFSA GMO Panel concludes that maize Bt11xGA21 is as safe as its non-GM maize counterpart with respect to effects on human and animal health and the environment, and is unlikely to have any adverse effect on human and animal health or on the environment in the context of its intended uses.

## KEY WORDS

GMO, maize, Bt11xGA21, insect resistance, herbicide tolerance, stacked event, risk assessment, food and feed safety, environmental safety, food and feed uses, import, processing, Regulation (EC) 1829/2003

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1 On request from the Competent Authority of the United Kingdom for application (EFSA-GMO-UK-2007-49) submitted by Syngenta Seeds, Question No EFSA-Q-2007-195, adopted on 15 September 2009.

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## SUMMARY

Following the submission of an application (Reference EFSA-GMO-UK-2007-49) 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 the insect resistant and herbicide tolerant genetically modified (GM) maize Bt11xGA21 (Unique Identifier SYNBTØ11-1xMON-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-2007-49, additional information provided by the applicant and scientific comments submitted by the Member States. Further information from applications for placing the single maize events Bt11 and GA21 on the market under EU regulatory procedures was taken into account, where appropriate. The scope of application EFSA-GMO-UK-2007-49 is for food and feed uses, import and processing of maize Bt11xGA21 and all derived products, but excludes cultivation in the European Union (EU). The EFSA GMO Panel evaluated maize Bt11xGA21 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 (EFSA, 2006a) and for the risk assessment of GM plants containing stacked transformation events (EFSA, 2007a). The scientific risk assessment evaluation 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 Bt11xGA21 has been produced by crosses between maize inbred lines containing the single events Bt11 and GA21 to combine the resistance trait against certain lepidopteran target pests and tolerance to glufosinate-ammonium-based herbicides in maize Bt11 with tolerance to glyphosate-based herbicides in maize GA21. These single maize events have been the subject of earlier risk assessment evaluations by the EFSA GMO Panel. No new genetic modifications were introduced in maize Bt11xGA21.

Molecular analysis of the DNA present in maize Bt11xGA21 confirmed that both maize Bt11 and GA21 inserts are present and that their structures are retained. With regard to protein expression for Cry1Ab and PAT, the overall protein levels were generally similar between maize Bt11xGA21 and Bt11. For the mEPSPS protein, the overall concentrations were also generally similar between maize Bt11xGA21 and GA21. The proteins Cry1Ab, PAT and mEPSPS have been evaluated previously and no safety concerns were identified. The EFSA GMO Panel found no evidence of any interactions between the newly expressed Cry1Ab, PAT and mEPSPS proteins.

The results of the compositional analysis of grain and forage material of maize Bt11xGA21, collected at field trials in the United States (US), indicated that, with the exception of the newly expressed proteins, maize Bt11xGA21 is compositionally and agronomically equivalent to its non-GM maize counterpart and conventional maize. Based on the comprehensive data available, including responses of the applicant to questions posed by the EFSA GMO Panel, the Panel concluded that there was no indication that crossing maize Bt11 with maize GA21 results in an interaction between the newly expressed proteins affecting composition and agronomic characteristics. Furthermore, the nutritional properties of maize Bt11xGA21 do not differ from those of its non-GM maize counterpart, whilst the overall allergenicity of the whole plant is not changed.

The application EFSA-GMO-UK-2007-49 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 Bt11xGA21. 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 Bt11xGA21 grains during transportation and processing

for food and feed uses. Taking into account the scope of the application, both the rare occurrence of feral maize plants and the low levels of exposure through other routes indicate that the risk to target and non-target organisms is extremely low. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize Bt11xGA21. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

In conclusion, the EFSA GMO Panel considers that the information available for maize Bt11xGA21 addresses the scientific comments raised by the Member States and that maize Bt11xGA21 is as safe as its non-GM maize counterpart with respect to effects on human and animal health and the environment. Therefore, the EFSA GMO Panel concludes that maize Bt11xGA21 is unlikely to have any adverse effect on human and animal health or 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-2007-49) for authorisation of genetically modified (GM) maize Bt11xGA21 (Unique Identifier SYNBTØ11-1xMON-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-2007-49 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission, and made the summary of the dossier available to the public on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 21 January 2008, EFSA received additional information (requested on 19 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 19 February 2008.

EFSA made the valid application available to the Member States and the European Commission, and consulted nominated risk assessment bodies of the 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 May 2008) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out a scientific risk assessment evaluation of maize Bt11xGA21 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 and GA21 on the market under EU regulatory procedures was taken into account, where appropriate.

The EFSA GMO Panel requested from the applicant additional information on 24 June 2008, 05 September 2008, 20 February 2009, 01 April 2009, 20 May 2009 and on 03 August 2009. The requested information was provided by the applicant on 06 August 2008, 30 September 2008, 27 February 2009, 14 April 2009, 17 June 2009 and on 24 August 2009. After receipt and evaluation of the full data package, the EFSA GMO Panel finalised its risk assessment evaluation on maize Bt11xGA21.

The single maize events Bt11 and GA21 have been the subject of earlier risk assessment evaluations and have received EFSA GMO Panel scientific opinions in favour of their authorisation (EFSA, 2005, 2007b, 2009a).

- 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 existing products of maize Bt11 made under Articles 11 and 23 of Regulation (EC) No 1829/2003 has been evaluated by the EFSA GMO Panel (EFSA, 2009a).

- Applications EFSA-GMO-UK-2005-19 and EFSA-GMO-RX-GA21, both submitted under Regulation (EC) No 1829/2003, concerning, respectively, the import and processing for food and feed uses of, and the renewal of existing products of maize GA21 have been evaluated by the EFSA GMO Panel (EFSA, 2007b). Recently, import and processing for food and feed uses have 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), whilst 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 Bt11xGA21 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-based herbicides by the introduction of a gene encoding a phosphinothricin-N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes*.

Maize GA21 has been developed to be tolerant to glyphosate-based herbicides by the introduction of a gene coding for a modified 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS) protein.

## TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific risk assessment evaluation of maize Bt11xGA21 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.

## ACKNOWLEDGEMENTS

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## ASSESSMENT

### 1. Introduction

The GM maize Bt11xGA21 (Unique Identifier SYNBTØ11-1xMON-ØØØ21-9) was evaluated with reference to its intended uses, taking 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 risk assessment evaluation presented here is based on the information provided in the application relating to maize Bt11xGA21 submitted in the EU including additional information from the applicant and information on the single maize events, as well as scientific comments that were raised by the Member States.

### 2. Issues raised by the Member States

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

### 3. Molecular characterisation

#### 3.1. Evaluation of the relevant scientific data

The EFSA GMO Panel guidance documents (EFSA, 2006a, 2007a) state that when single events have been combined by the interbreeding of existing approved GM plant events, the need for further molecular analysis will depend, on a case-by-case basis, upon the nature of the genetic modifications involved. Having considered the information provided in the application and scientific comments of the Member States, the EFSA GMO Panel requested clarification from the applicant with regard to the molecular characterisation of maize Bt11xGA21.

#### 3.1.1. Method of production of maize Bt11xGA21

Traditional breeding methods were used to produce maize Bt11xGA21 and no new genetic modification was involved. The two inserts that are present in maize Bt11xGA21 were derived from maize lines containing two independent events: Bt11 and GA21. Each of these single maize events was the subject of earlier safety evaluation and separate opinions for each of them have been published (EFSA, 2005, 2007b, 2009a). Maize Bt11xGA21 combines the insect resistance and glufosinate-ammonium tolerance traits from maize Bt11 with the glyphosate tolerance in maize GA21.

#### 3.1.2. Summary of the previous evaluation of the single events

##### 3.1.2.1. Maize Bt11

Maize Bt11 was generated by transformation of *Zea mays* protoplasts using a DNA fragment obtained by a restriction digest of the plasmid pZO1502 with the enzyme *Not*I. Regenerated plants were backcrossed to a selected line resulting in a plant which is called Bt11. The DNA fragment used for transformation carried two expression cassettes; a selectable marker gene *pat*, encoding phosphinothricin-N-acetyl transferase and a trait gene encoding a variant *Bacillus thuringiensis cry1Ab* gene encoding Bt endotoxin. Both the *cry1Ab* and *pat* gene cassette are controlled by the 35S promoter from the *Cauliflower mosaic virus* (CaMV), supplemented with the intron sequences to enhance gene expression. The polyadenylation signals are derived from the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens.*

Southern analyses of the single maize event Bt11 used a variety of DNA probes that included the *pat* and *cry1Ab* genes as probes for the genes intended to be inserted and the *amp* gene and the entire plasmid as probes to detect genome wide unintended insertions. The data obtained demonstrated that maize Bt11 contains a single DNA insertion with one copy of both the *cry1Ab* and the *pat* cassettes. There was no evidence that small non-coding sequences from the vector backbone sequence were inserted, including the *amp* gene.

The nucleotide sequence of the entire Bt11 insert in sweet maize was determined which enabled a direct comparison to the previously reported sequence (EFSA, 2005, 2009a). A total of eight nucleotide differences were identified when the Bt11 insert sequence was compared to the previously reported Bt11 sequence. The applicant attributed this discrepancy to sequencing errors in the original datasets. The EFSA GMO Panel supports this assessment which is validated by an updated sequence analysis of both the insert and the original plasmid used for transformation.

DNA sequences at the junctions between the insert and the maize genome were determined. At the 5' flank, approximately 350 bp of the plant DNA adjacent to the insert was sequenced and 540 bp at the 3' flank. No novel open reading frames (ORFs) were identified that spanned either the 5' or the 3' junctions between the Bt11 insert and maize genomic sequences. No fusion proteins are therefore expected. Bioinformatic analysis of the 5' and 3' flanking regions revealed homology with a maize 180 bp knob-associated tandem repeat. The insertion of the *Not*I fragment in the maize genome did not disrupt any maize endogenous ORF. Sequencing also confirmed the absence of vector backbone fragments, including partial *amp* coding sequences. An updated bioinformatic analysis (2008) confirmed the original analysis carried out by the applicant and supports the conclusion that the genomic sequences in both 5' and 3' regions flanking the insert of maize Bt11 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 maize Bt11 was demonstrated over several generations by Southern analysis. Segregation data for glufosinate-ammonium tolerance and insect resistance also demonstrated the traits are stable and inherited according to Mendel's laws of genetics. These data also support the presence of a single insertion locus.

#### 3.1.2.2. Maize GA21

Maize GA21 expresses a modified version of the EPSPS protein (mEPSPS), derived from wild type maize EPSPS and rendering maize GA21 tolerant to glyphosate-based herbicides. The action of glyphosate triggers disruption of the shikimate pathway (biosynthesis of aromatic amino acids) by inhibition of the EPSPS enzyme, causing death of the plants (Comai and Stalker, 1996). The mEPSPS is only different from the naturally present EPSPS protein by two amino acids.

Suspension culture cells of maize were transformed with a 3.49 kb *Not*I restriction fragment of the plasmid pDPG434 (derived from pUC19) using particle bombardment. The DNA fragment used for transformation consisted of the following m*epsps* cassette: the rice actin promoter (5' region of the rice actin 1 gene containing the promoter and first non-coding exon and intron), an optimised transit peptide containing sequences from maize and sunflower, a modified maize *epsps* coding sequence (m*epsps*), and the 3' nos terminator from *Agrobacterium tumefaciens*. The mutations in the coding sequence of the maize *epsps* gene led to amino acid changes at positions 102 (threonine to isoleucine) and 106 (proline to serine). As a result of these mutations, the m*epsps* containing maize line GA21 is tolerant to glyphosate-based herbicides. The vector backbone contained the origin of replication (*ori* ColE1), the *lac* sequence as present in pUC19, and the bacterial *bla* gene conferring resistance to ampicillin in bacteria.

Southern analyses showed that the insert in maize GA21 consists of six contiguous complete or truncated versions (fragments 1 to 6) of the 3.49 kb *Not*I restriction fragment. The insertions are located at a single locus. The absence of vector backbone sequences in GA21 plants has been demonstrated using a probe specific for the pDPG434 vector backbone. Therefore, the *bla* gene has not been transferred to maize GA21.

The nucleotide sequence of the insert introduced into maize GA21 has been determined in its entirety. Fragment 1 contains the rice actin promoter with a deletion of 696 bp at the 5' end, the actin first exon and intron, the optimized transit peptide, the m*epsps* gene and *nos* terminator. Fragments 2, 3 and 4 are complete versions of the 3.49 kb NotI fragment. Fragment 5 contains the complete rice actin promoter, the actin first exon and intron, the optimized transit peptide, and 288 bp of the m*epsps* gene which ends in a stop codon. Fragment 6 contains the rice actin promoter and the actin first exon truncated but no other elements. A single base pair change was observed in the *nos* terminator in fragments 1 and 2 (nucleotide C instead of G). In addition, a single base pair deletion is observed in the actin promoter of fragment 6. The observed mutations do not have an impact on the amino acid sequence of the newly expressed protein.

The sequences of 1 kb of the plant genome adjacent to the 3' and 4.2 kb at the 5' end were also determined and bioinformatic analysis gave no indication that the sequence was inserted in a functional maize gene. The 3' sequence shows homology to repetitive sequences in the maize genome. The 5' flanking sequence was shown to be of chloroplast origin. The five putative ORFs found at the junction between the insert and the plant DNA show no significant sequence homology to any known toxic proteins and allergens. One potential new ORF was apparently created at the junction between fragment 5 and 6 but lacked the necessary components to be transcribed. This ORF does not show homology to known or putative allergens or toxic proteins. Updated (2008) bioinformatic analysis of the 5' and 3' flanking regions of the GA21 insert provided data which were similar to that previously reported and 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 inheritance of the introduced glyphosate tolerant phenotype follows a Mendelian segregation pattern and the mEPSPS protein is stably expressed in maize GA21 across multiple generations. Southern analysis demonstrated that the insert in maize GA21 is stably inherited over three backcross generations.

### 3.1.3. Transgenic constructs in maize Bt11xGA21

Molecular analysis was performed to assess the integrity of the DNA inserts in the single maize events Bt11 and GA21 during conventional breeding to produce the stacked maize event Bt11xGA21. Data from Southern analyses of maize Bt11xGA21 demonstrated the predicted molecular organization of the *cry1Ab* and *pat* genes from maize Bt11 and the m*epsps* gene from maize GA21. The predicted DNA hybridisation patterns from each single event were retained in maize Bt11xGA21, demonstrating that integrity of the DNA inserts was maintained.

### 3.1.4. Information on the expression of the inserts

ELISA was used to compare the concentrations of Cry1Ab, PAT and mEPSPS proteins produced in the plants of maize Bt11xGA21 grown alongside the single maize events Bt11 and GA21 in a single field trial (2005) in the United States (US). The concentrations of these proteins were determined in several plant tissues (leaf, root, kernel, pollen) at three different growth stages.

#### 3.1.4.1. Cry1Ab protein

There were no statistically significant differences between the mean concentrations of protein in maize Bt11 and Bt11xGA21 plant tissues, except for roots at the anthesis stage. No statistical analysis of Cry1Ab concentrations in pollen was possible because the pollen samples were collected as pooled samples yielding a single sample for each hybrid. However, the Cry1Ab concentration in the maize Bt11xGA21 pooled pollen sample (0.12 µg/g dry weight) was very similar to that of the maize Bt11 pooled pollen sample (0.10 µg/g dry weight). Data for all non-GM maize samples were below the limit of detection.

#### 3.1.4.2. PAT protein

There were no statistically significant differences between the mean concentrations of protein in maize Bt11 and Bt11xGA21 plant tissues. No statistical analysis of PAT concentrations expressed in leaves and kernels at seed maturity and pollen collected at anthesis was possible due to the low levels of the PAT (below detection limits). Data for all non-GM maize samples were below the limit of detection for PAT.

#### 3.1.4.3. mEPSPS protein

The endogenous maize EPSPS protein is expressed at a significantly lower concentration than the mEPSPS protein in maize GA21. Although the antibodies used in the ELISA are capable of detecting the endogenous EPSPS, the EPSPS concentrations in all non-GM maize samples were below the limit of detection. Therefore, the ELISA values presented for transgenic plants represent the concentrations of mEPSPS. There were no statistically significant differences between the mean concentrations of protein in maize GA21 and Bt11xGA21 plant tissues, except for kernels at the seed maturity stage. In this case, the difference between the two means (6.08 µg mEPSPS/g dry weight for maize GA21 kernels and 5.35 µg mEPSPS/g dry weight for maize Bt11xGA21 kernels) is small (*ca.*, 12%). No statistical analysis of pollen mEPSPS concentrations was possible because the pollen samples were collected as pooled samples yielding a single sample for each hybrid. However, mEPSPS concentration for the maize GA21 pooled pollen sample (65.32 µg/g dry weight) was approximately 20% different from that of the maize Bt11xGA21 pooled pollen sample (80.53 µg/g dry weight). Data for all non-GM maize samples were below the limit of detection.

### 3.1.5. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in the single maize events Bt11 and GA21 was demonstrated previously (EFSA, 2005, 2007b, 2009a). The Southern data indicate that the structure of the inserts in the single maize events is retained in the stacked maize event Bt11xGA21. Furthermore, each of the traits has been conserved in the stacked maize event.

## 3.2. Conclusion

Molecular analysis of the DNA present in maize Bt11xGA21 confirmed that both maize Bt11 and GA21 inserts are present and that their structures are retained. For Cry1Ab and PAT, the overall protein levels were generally similar between maize Bt11xGA21 and Bt11. For the mEPSPS protein, the overall concentrations were also generally similar between maize Bt11xGA21 and GA21. Although some statistically significant differences were seen, these differences were small or not consistent across the growing season.

# 4. Comparative analysis

## 4.1. Evaluation of relevant scientific data

Having considered the information provided in the application and the scientific comments of the Member States, the EFSA GMO Panel requested from the applicant an additional comparative compositional analysis using the double stacked maize event Bt11xGA21 instead of the triple stacked maize event Bt11xMIR604xGA21.

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

#### 4.1.1.1. Maize Bt11

Maize Bt11 was compared with an appropriate non-GM maize counterpart with a genetic background comparable to maize Bt11. Forage and kernels 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 kernels 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).

#### 4.1.1.2. Maize GA21

Maize GA21 was compared with an appropriate non-GM maize counterpart with a genetic background comparable to maize GA21. Forage and maize tissues, including kernels, were collected for compositional analysis from field trials conducted during 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-based 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 kernels of maize GA21 are compositionally equivalent to those of conventional maize, except for the presence of the mEPSPS protein in maize GA21.

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

Maize Bt11xGA21 was compared with a non-GM maize counterpart during field trials in six locations in the US in 2005. The pedigree information provided by the applicant showed that the non-GM maize counterpart used as control had a genetic background comparable to that of maize Bt11xGA21. The non-GM maize counterpart was thus considered an appropriate comparator for maize Bt11xGA21 in the field trials.

The six locations are considered representative of the range of environmental conditions under which maize would normally be grown. At each location, maize Bt1xGA21 and the corresponding non-GM maize were grown in a randomised complete block design with three replicates for each genotype. Maize Bt11xGA21 plants were treated with both glyphosate- and glufosinate-ammonium-based herbicides.

Forage and grain derived from maize Bt11xGA21 and the non-GM maize counterpart were collected from field trials for compositional analysis.

In the context of previous applications, analytical data on materials obtained from field trials with the single maize events and the respective appropriate non-GM maize comparators were provided by the applicant (see section 4.1.1). The EFSA GMO Panel previously evaluated these data and concluded that the maize events Bt11 and GA21 (treated and untreated with the respective target herbicide) were compositionally and agronomically equivalent to their respective comparators, except for the newly introduced traits (EFSA, 2005, 2007b, 2009a). The EFSA GMO Panel noted the fact that treatment of the single maize events Bt11 and GA21 with the target herbicides to which they are tolerant did not affect their agronomic and compositional characteristics compared to untreated maize plants (EFSA, 2005, 2007b, 2009a) and, therefore, the EFSA GMO Panel accepts the design of field trials with maize Bt11xGA21.

Field trials for comparative agronomic analysis were carried out at nine locations in the US in 2005 using a randomised complete block design with four replications per location.

The EFSA GMO Panel considered the studies and the derived spectrum of data which was available for the comparative agronomic and compositional assessment as sufficient.

### 4.1.3. Compositional analysis

Compositional data were obtained by analysis of forage and grain harvested from field trials performed in maize growing regions of the US in 2005. The selection of compounds followed the recommendations of OECD (2002). The statistical analysis for each constituent was performed on the overall data and on data on maize material from each individual field trial site.

Forage from maize Bt11xGA21 and its non-GM maize counterpart used as control were analysed for proximates, fibre and minerals (moisture, protein, fat, ash, total carbohydrates, acid detergent fibre (ADF), neutral detergent fibre (NDF), phosphorus and calcium). The compositional analysis of grain of maize Bt11xGA21 and its non-GM maize counterpart included proximates and fibre (moisture, protein, fat, ash, total carbohydrates, total dietary fibre (TDF), ADF, NDF), starch, fatty acids, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, selenium and zinc), vitamins and vitamin precursors (vitamin B1, vitamin B2, niacin, vitamin B6, folic acid, β-carotene, vitamin E), phytic acid, raffinose, anti-nutrients (trypsin inhibitor) and other constituents (inositol, furfural, p-coumaric acid, and ferulic acid). Data obtained for these constituents in forage and grain were compared with the ranges reported in the literature for commercial maize varieties (OECD, 2002; ILSI, 2006).

The across location analysis of the composition of forage from maize Bt11xGA21 and its non-GM maize counterpart used as control did not reveal any statistically significant differences. The mean values obtained for maize Bt11xGA21 were within the literature ranges reported for commercial maize varieties.

The analysis of data on grain from maize Bt11xGA21 and its non-GM maize counterpart used as control across locations revealed statistically significant differences in some parameters: i.e., the levels of fat, TDF, vitamin E, palmitic, oleic and linoleic acid. On request of the EFSA GMO Panel, the applicant provided a statistical analysis on a per-location basis, showing that none of these

differences was statistically significant at each location. In addition, the levels of those constituents which were different from the level in the corresponding control were within the ranges reported in the literature for commercial maize varieties.

The EFSA GMO Panel considered the fact that maize Bt11xGA21 combines two traits conferring tolerance to different herbicides targeting amino acid metabolism. Amino acid levels (given in mg/g dry weight) and crude protein were not different compared with its non-GM maize counterpart used as control in the across location analysis.

The EFSA GMO Panel concludes that expression of the newly introduced genes in maize Bt11xGA21 does not result in any effect on the chemical composition and that maize Bt11xGA21 is compositionally equivalent to its non-GM maize counterpart and conventional maize, except for the presence of the Cry1Ab, PAT and mEPSPS proteins.

#### 4.1.4. Agronomic traits and GM phenotype

During field trials in 2005 at nine locations in the US (four 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, percent snapped plants, root lodging) and disease susceptibility were collected for maize Bt11xGA21 and its non-GM maize counterpart having a comparable genetic background.

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. This analysis showed statistically significant differences for yield, grain test weight, ear height, plant emergence and harvest population at individual field trial sites. However, when data from all locations were considered there were no consistent trends and no statistically significant differences in the analysis across locations.

The EFSA GMO Panel concludes that expression of the newly introduced genes brought together in maize Bt11xGA21 does not result in any unexpected agronomic effect and that the agronomic performance and phenotypic characteristics of maize Bt11xGA21 are comparable to those of its non-GM maize counterpart, except for the introduced traits.

### 4.2. Conclusion

The results of the comparative analyses indicated that maize Bt11xGA21 is compositionally and agronomically equivalent to its non-GM maize counterpart and conventional maize, except for the presence of the Cry1Ab, PAT and mEPSPS proteins in maize Bt11xGA21. Based on the evaluation of data available for maize Bt11xGA21 and for its appropriate non-GM maize counterpart, the EFSA GMO Panel has found no indication that stacking of the single maize events Bt11 and GA21 results in compositional or agronomic changes.

## 5. Food/Feed safety assessment

### 5.1. Evaluation of relevant scientific data

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

##### 5.1.1.1. 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 *Escherichia 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 using 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 maize Bt11 and its non-GM maize counterpart (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).

#### 5.1.1.2. 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 *Escherichia 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 indicated nutritional equivalence of maize GA21 to its non-GM maize counterpart. 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 uses

The scope of application EFSA-GMO-UK-2007-49 includes the import and processing of maize Bt11xGA21 and its derived products for use as food and feed. Thus, the possible uses of maize Bt11xGA21 include the production of animal feed and food products, such as starch, syrups and oils.

The genetic modification of maize Bt11xGA21 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, processing characteristics and overall use of maize Bt11xGA21 as a food/feed plant.

### 5.1.3. Effect of processing

Since maize Bt11xGA21 is compositionally equivalent to conventional maize, except for the newly expressed proteins (see section 4.2), the effect of processing on maize Bt11xGA21 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 Bt11xGA21

The proteins Cry1Ab and PAT expressed in maize Bt11 and the mEPSPS protein expressed in maize GA21 have been evaluated for their safety previously (EFSA, 2005, 2007b, 2009a) 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 present in the parental maize varieties have been introduced in maize Bt11xGA21. The EFSA GMO Panel considered all the data available for maize Bt11xGA21, and the newly expressed proteins Cry1Ab, PAT 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 Bt11xGA21 are unlikely.

#### 5.1.4.2. Toxicological assessment of new constituents other than proteins

No new constituents other than the Cry1Ab, PAT and mEPSPS proteins are expressed in maize Bt11xGA21 and no relevant changes in the composition of maize Bt11xGA21 were detected by the compositional analysis.

#### 5.1.4.3. Toxicological assessment of the whole GM food/feed

Maize Bt11 and GA21 have previously been found as safe as their non-GM maize counterpart for human and animal consumption (EFSA, 2005, 2007b, 2009a). A molecular characterisation undertaken on maize Bt11xGA21 identified no altered stability of the single maize events (see section 3.1.5) when these were brought together by crossing, and expression analysis of the proteins Cry1Ab and PAT revealed that the overall levels of the proteins Cry1Ab and PAT, as well as mEPSPS in maize Bt11xGA21 were generally similar to the levels in the single maize events Bt11 and GA21, respectively (see section 3.2). As the composition of maize Bt11xGA21 is equivalent to that of non-GM maize varieties and since no indication for interaction between the single events was found, the EFSA GMO Panel is of the opinion that no additional animal safety studies are required.

### 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 of 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 proteins Cry1Ab, PAT and mEPSPS present in maize Bt11xGA21 have been evaluated previously and it was found unlikely that they are allergenic (EFSA, 2005, 2007b, 2009a). 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

The issue of a potential increased allergenicity of maize Bt11xGA21 does not appear relevant to the EFSA GMO Panel since maize is not considered a common allergenic food. Food allergies to maize are of low frequency and mainly occur in populations of specific geographic areas. Rare cases of occupational allergy to maize dust have been reported. There is no reason to expect that the use of maize Bt11xGA21 will significantly increase the intake and exposure to maize. Therefore, a possible overexpression of any endogenous protein, which is not known to be allergenic, would be unlikely to alter the overall allergenicity of the whole plant or the allergy risk for consumers.

### 5.1.6. Nutritional assessment of GM food/feed

A 44-day feeding study using broiler chickens was performed. Groups consisting of 90 male and 90 female animals (6 pens with 15 male and 6 pens with 15 female animals per group) were fed with diets containing grain from maize Bt11xGA21, a non-GM maize counterpart with comparable genetic background or a conventional non-GM maize variety. The inclusion rate of maize grain in the starter, grower and finisher diets was approximately 51%, 57% and 63%, respectively. The diets were adjusted for their contents in specific amino acids, protein and metabolisable energy. Animal performance on the various diets was evaluated by measuring mortality, weight gain, feed conversion ratio and carcass yields (fat pad, drums, thighs, wings and breasts).

There were no statistically significant differences in mortality between the groups, and overall survival was >97%. Mean feed intake and body weight development did not differ between the test and control groups. Males fed diets containing grains from maize Bt11xGA21 had slightly, but statistically significantly increased cumulative (day 0-44) feed conversion ratios compared with the control group and the reference group. Since there were no differences in feed intake, body weight gain as well as carcass yields these differences in feed conversion ratios were not considered relevant by the EFSA GMO Panel.

Thus, the broiler feeding study supported the results of the comparative compositional analysis, which showed that grain from maize Bt11xGA21 is compositionally and therefore nutritionally equivalent to grain from the non-GM maize counterpart and conventional maize.

### 5.1.7. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that maize Bt11xGA21 is any less safe than its non-GM maize counterpart and conventional maize. In addition, maize Bt11xGA21 is, from a nutritional point of view, equivalent to conventional maize. Therefore, in line with the EFSA GMO Panel guidance document (EFSA, 2006a), the Panel is of the opinion that post-market monitoring of the food/feed derived from maize Bt11xGA21 is not necessary.

## 5.2. Conclusion

The proteins Cry1Ab and PAT expressed in the maize event Bt11 and the mEPSPS protein expressed in the maize event GA21 have been evaluated 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 maize Bt11xGA21 would be no different from those of conventional maize.

In conclusion, the EFSA GMO Panel considers that maize Bt11xGA21 is as safe and as nutritious as its non-GM maize counterpart and that it is unlikely that the overall allergenicity of the whole plant is changed. The EFSA GMO Panel concludes that maize Bt11xGA21 is unlikely to have any adverse effect on human and animal health in the context of its intended uses.

# 6. Environmental risk assessment and monitoring plan

## 6.1. Evaluation of relevant scientific data

The scope of the application is for food and feed uses, import and processing of maize Bt11xGA21 and does not include cultivation. Considering the proposed uses of maize Bt11xGA21, the environmental risk assessment is concerned with the exposure through manure and faeces from gastrointestinal tracts of animals fed maize Bt11xGA21 and with the accidental release into the environment of maize Bt11xGA21 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 Bt11xGA21 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 Bt11 and GA21

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

### 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 cultivation. 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.

The herbicide tolerance traits can only be regarded as providing a potential agronomic and selective advantage for this GM maize plant where and when glufosinate-ammonium- and/or glyphosate-based herbicides are applied. Similarly, insect resistance against certain lepidopteran 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 and cold climate conditions. Since these general characteristics are unchanged in maize Bt11xGA21, 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 Bt11xGA21 will differ from conventional maize varieties in their ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

Applicant's field trials have shown that there are no indications of an altered fitness of the single maize events Bt11 and GA21 as compared to conventionally bred hybrids with similar genetic background. In addition to the field trials carried out with the single maize events Bt11 and GA21 (EFSA, 2005, 2007b, 2009a), a series of field trials with maize Bt11xGA21 were conducted across nine US corn belt locations in 2005. Information on phenotypic and agronomic characteristics was

provided to assess the agronomic performance of maize Bt11xGA21 in comparison with non-GM maize. These field trial data showed enhanced biomass production when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation of target pests, but do not show changes in plant characteristics that indicate altered fitness and invasiveness of maize Bt11xGA21 plants. In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased establishment and spread of maize Bt11xGA21 and any change in survival capacity, including over-wintering.

Since maize Bt11xGA21 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 GM maize event will not differ from that of maize Bt11 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

Current scientific knowledge (see EFSA, 2009b for further details) suggests that horizontal gene transfer from GM plants to microorganisms under natural conditions is extremely unlikely, and its establishment would occur primarily through homologous recombination in microorganisms. With the exception of the m*epsps* gene from *Zea mays* expressed in maize GA21, all other inserted genes (*cry1Ab* and *pat*), as expressed in maize Bt11xGA21 are derived from bacteria. As the functional genes are already present in microorganisms in the natural environment, homologous recombination and acquisition of these genes by microorganisms will not alter the gene pool of the natural microbial community.

In addition, the *cry1Ab*, *pat* and m*epsps* genes in maize Bt11xGA21 are under the control of eukaryotic promoters with limited activity in prokaryotic organisms (see section 3.1.2; EFSA, 2009b).

Transgenic DNA is a component of many food and feed products derived from GM maize. Therefore, microorganisms in the digestive tract of humans and animals (domesticated and other animals feeding on fresh and decaying GM plant material) may be exposed to transgenic DNA, although DNA becomes degraded in the human or animal digestive tract.

Taking into account the origin and/or nature of the *cry1Ab*, *pat* and m*epsps* genes and the lack of selective pressure in the intestinal tract and/or the environment, the likelihood that horizontal gene transfer would result in increased fitness on microorganisms or other selective advantages is very small. For this reason, it is very unlikely that genes from maize Bt11xGA21 would become established in the genome of microorganisms in the environment or in the human and animal digestive tract. In the very unlikely event that such a horizontal gene transfer would take place, no adverse effects on human and animal health or the environment are expected, as no principally new traits would be introduced or expressed in microbial communities.

(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).

Herbicide tolerance and insect resistance provide agronomic and selective advantages in areas where herbicides containing at least one of the two specific active substances 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., 2009), 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 and frost. Since these general characteristics are unchanged in maize Bt11xGA21, 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. 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.

In conclusion, since maize Bt11xGA21 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 GM maize in Europe will not differ from that of maize Bt11 and GA21, or of other maize varieties.

#### 6.1.2.3. Interactions of the GM plant with target organisms

The intended uses of maize Bt11xGA21 specifically exclude cultivation and the environmental exposure to maize Bt11xGA21 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 Bt11xGA21 to enable any significant interaction with target organisms, which is very unlikely.

Environmental exposure to Cry1Ab protein is otherwise limited to manure and faeces from the gastrointestinal tracts of animals fed maize Bt11xGA21. Data supplied by the applicant suggest that only very low amounts of the Cry1Ab protein enter the environment due to low expression in kernels. Moreover, most Cry proteins are degraded by enzymatic activity in the gastrointestinal tract (see section 5.1.1), meaning that only low amounts of Cry proteins would remain intact to pass out in faeces (e.g., for Cry1Ab: Einspanier et al., 2004; Lutz et al., 2005, 2006; Wiedemann et al., 2006; Guertler et al., 2008). It can thus be concluded that the level of exposure of target organisms to the Cry1Ab protein is likely to be extremely low and of no biological relevance.

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

The EFSA GMO Panel evaluated whether the Cry1Ab protein might potentially affect non-target organisms by entering the environment through manure and faeces from the gastrointestinal tracts of animals fed maize Bt11xGA21. Due to the selectivity of Cry proteins, non-target organisms most likely to be affected by the Cry1Ab protein are those belonging to a similar taxonomic group as that of the target organisms.

Data supplied by the applicant suggest that only low amounts of the Cry1Ab protein enter the environment due to low expression in kernels. Moreover, most Cry proteins are degraded by enzymatic activity in the gastrointestinal tract (see section 5.1.1), meaning that only low amounts of Cry proteins would remain intact to pass out in faeces (e.g., for Cry1Ab: Einspanier et al., 2004; Lutz et al., 2005, 2006; Wiedemann et al., 2006; Guertler et al., 2008). There would subsequently be further degradation of the Cry1Ab protein in the manure and faeces due to microbial processes.

Exposure of soil and water environments to the Cry1Ab protein from disposal of animal wastes or accidental spillage of maize kernels is likely to be very low and localized. While Cry proteins can bind to clay minerals and humic substances in soil, thereby reducing their availability to microorganisms for degradation, a number of studies revealed that there is no persistence and accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky, 2008).

Considering the scope of the application (that excludes cultivation) and the intended uses of maize Bt11xGA21, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1Ab protein is likely to be very low and of no biological relevance.

#### 6.1.2.5. Interactions with the abiotic environment and biochemical cycles

Considering the scope of the application and the intended uses of maize Bt11xGA21 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 Bt11xGA21 would be mainly through manure and faeces from gastrointestinal tracts of animals fed maize Bt11xGA21 or through accidental release into the environment of GM maize grains during transportation and processing.

No specific environmental impact of maize Bt11xGA21 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 newly 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 Bt11xGA21 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 Bt11xGA21 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

### 6.2. Conclusion

The scope of the application includes food and feed uses, import and processing of maize Bt11xGA21 and excludes cultivation. Considering the intended uses of maize Bt11xGA21, the environmental risk assessment is concerned with indirect exposure mainly through manure and faeces from gastrointestinal tracts of animals fed maize Bt11xGA21 and with the accidental release into the environment of maize Bt11xGA21 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 Bt11xGA21 grains during transportation and processing for food and feed uses. Taking into account the scope of the application, both the rare occurrence of feral maize plants and low levels of Cry1Ab protein exposure in maize Bt11xGA21 grains or through other routes indicate that the risk to target and non-target organisms is considered extremely low.

The scope of the monitoring plan provided by the applicant is in line with the intended uses of maize Bt11xGA21, 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 evaluated maize Bt11xGA21, which has been produced by a cross between inbred maize lines containing the single events Bt11 and GA21, for food and feed uses, import and processing. Both single maize events Bt11 and GA21 were evaluated previously by the EFSA GMO Panel (EFSA, 2005, 2007b, 2009a). In evaluating maize Bt11xGA21, both single events and the stacked event were considered. The EFSA GMO Panel concluded that it was acceptable to use data for single maize events Bt11 and GA21 in support of the safety evaluation of the stacked maize event Bt11xGA21, and that the information available for maize Bt11xGA21 addresses the scientific comments raised by the Member States.

The EFSA GMO Panel is of the opinion that appropriate molecular analysis has been performed on maize Bt11xGA21 produced by conventional breeding with the single maize events Bt11 and GA21. Southern analyses demonstrated the predicted molecular organization of the *cry1Ab* and *pat* genes from maize Bt11 and the m*epsps* gene from maize GA21. The predicted DNA hybridisation patterns from each single event were retained in maize Bt11xGA21, demonstrating that the integrity of the DNA inserts was maintained. With regard to protein expression for Cry1Ab and PAT, the overall protein levels were generally similar between maize Bt11xGA21 and maize Bt11. For the mEPSPS protein, the overall concentrations were also generally similar between maize Bt11xGA21 and maize GA21. Although some statistically significant differences were seen, these differences were small or not consistent across the growing season.

The results of the comparative analysis indicated that maize Bt11xGA21 is compositionally and agronomically equivalent to its non-GM maize counterpart and conventional maize, except for the presence of the proteins Cry1Ab, PAT and mEPSPS in maize Bt11xGA21. Based on the evaluation of data available for maize Bt11xGA21, the single maize events and for the respective non-GM maize counterparts used as controls, the EFSA GMO Panel has found no indication that stacking of the single maize events Bt11 and GA21 would result in an interaction which might cause compositional or agronomic changes. The proteins Cry1Ab and PAT expressed in maize Bt11 and the mEPSPS protein expressed in maize GA21 have been evaluated 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, the nutritional properties of maize Bt11xGA21 would be not different from those of its non-GM maize counterpart, and that it is unlikely that the overall allergenicity of the whole plant is changed. The EFSA GMO Panel concludes that maize Bt11xGA21 is unlikely to have any adverse effect on human and animal health in the context of its intended uses.

Considering the intended uses of maize Bt11xGA21, 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 Bt11xGA21 grains during transportation and processing, there are no indications of an increased likelihood of establishment and spread of feral maize plants. Also, the low levels of environmental exposure to these GM maize plants and the Cry1Ab protein through other routes indicate that the risk to target and non-target organisms is extremely low. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize Bt11xGA21.

The EFSA GMO Panel advises that appropriate management systems should be in place to restrict seeds of maize Bt11xGA21 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, the EFSA GMO Panel considers that the information available for maize Bt11xGA21 addresses the scientific comments raised by the Member States, and concludes that maize Bt11xGA21 is as safe as its non-GM maize counterpart with respect to effects on human and animal health and the environment, and is unlikely to have any adverse effect on human and animal health or on the environment in the context of its intended uses.

## DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the United Kingdom (FSA), dated 14 November 2007, concerning a request for placing on the market of maize Bt11xGA21 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 (ref SR/KL/shv (2007) 2518814).

3. Letter from EFSA to applicant, dated 19 December 2007, with request for clarifications under completeness check (ref SR/KL/shv (2007) 2588054).

4. Letter from applicant, dated 21 January 2008, providing EFSA with an updated version of the application EFSA-GMO-UK-2007-49 submitted by Syngenta under Regulation (EC) No 1829/2003.

5. Letter from EFSA to applicant, dated 19 February 2008, delivering the 'Statement of Validity' for application EFSA-GMO-UK-2007-49, maize Bt11xGA21 submitted by Syngenta Seeds S.A.S. on behalf of Syngenta Crop Protection AG under Regulation (EC) No 1829/2003 (ref SR/KL/shv (2008) out-2695348).

6. Letter from applicant to EFSA, dated 26 February 2008, providing the valid application.

7. Letter from EFSA to applicant, dated 26 February 2008, with request for additional information from JRC-CRL (ref PB/KL/shv (2008) 2954586).

8. Letter from applicant to JRC-CRL, dated 03 March 2008, responding to request for additional information.

9. Letter from EFSA to applicant, dated 16 April 2008, restarting the clock after receipt of the requested additional information (ref PB/KL/shv (2008) 2954381).

10. Letter from EFSA to applicant, dated 24 June 2008, with request for additional information (ref PB/YD/shv (2008) 3117311).

11. Letter from applicant to EFSA, dated 09 July 2008, requesting public access to the scientific comments of the Member States on Syngenta applications submitted under Regulation (EC) No 1829/2003.

12. Letter from applicant to EFSA, dated 06 August 2008, responding to request for additional information.

13. Letter from applicant to EFSA, dated 30 September 2008, responding to request for additional information.

14. Letter from EFSA to applicant, dated 19 November 2008, restarting the clock after receipt of the requested additional information (ref PB/YD/shv (2008) 3472263).

15. Letter from EFSA to applicant, dated 20 February 2009, with request for additional information (ref PB/YD/shv (2009) 3693613).

16. Letter from applicant to EFSA, dated 27 February 2009, responding to request for additional information.

17. Letter from EFSA to applicant, dated 01 April 2009, with request for additional information and maintaining the clock stopped (ref PB/KL/YD/shv (2009) 3854530).

18. Letter from applicant to EFSA, dated 14 April 2009, responding to request for additional information.

19. Letter from EFSA to applicant, dated 20 May 2009, with request for additional information and maintaining the clock stopped (ref PB/KL/YD/ls (2009) 3980253).

20. Letter from applicant to EFSA, dated 17 June 2009, responding to request for additional information.

21. Letter from EFSA to applicant, dated 03 August 2009, with request for additional information and maintaining the clock stopped (ref PB/KL/YD/lg (2009) 4180491).

22. Letter from applicant to EFSA, dated 24 August 2009, responding to request for additional information.

23. Letter from EFSA to applicant, dated 09 September 2009, restarting the clock after receipt of the requested additional information (ref PB/KL/YD/mt (2009) 4247241).

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