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Opinion of the Scientific Panel on Genetically Modified Organisms on applications (references EFSA-GMO-UK-2005-19 and EFSA-GMO-RX-GA21) for the placing on the market of glyphosate-tolerant genetically modified maize GA21, for food and feed uses, import and processing and for renewal of the authorisation of maize GA21 as existing product, both under Regulation (EC) No 1829/2003 from Syngenta Seeds S.A.S. on behalf of Syngenta Crop Protection AG [1]

(Questions No EFSA-Q-2005-226 and EFSA-Q-2007-147)

Opinion adopted on 13 September 2007

SUMMARY

This document provides the opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on herbicide-tolerant genetically modified maize GA21 (Unique Identifier MON-ØØØ21-9) developed to provide tolerance to glyphosate by expressing a modified version of the EPSPS protein.

In delivering its opinion the GMO Panel considered the new application EFSA-GMO-UK-2005-19, additional information provided by the applicant (Syngenta Seeds on behalf of Syngenta Crop Protection AG) and the scientific comments submitted by the Member States. The scope of application EFSA-GMO-UK-2005-19 is for food and feed uses, import and processing of maize GA21 and all derived products, excluding cultivation. Information provided in the context of the application for renewal of the authorisation of maize GA21 as existing product, submitted under Regulation (EC) No 1829/2003 (Reference EFSA-GMO-RX-GA21), was also taken into account. The scope of application EFSA-GMO-RX-GA21 covers the continued marketing of existing food additives, feed materials and feed additives produced from maize GA21.

A single risk assessment for all intended uses of maize GA21 has been performed by the GMO Panel and one single scientific opinion for both applications submitted under Regulation (EC) No 1829/2003 is issued. The GMO Panel assessed maize GA21 with reference to the intended uses and the appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The scientific assessment included

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

Maize GA21 was transformed by particle bombardment of maize cells and expresses a modified EPSPS (5-enol pyruvylshikimate-3-phosphate synthase) protein. The molecular characterisation data established that maize GA21 contains a single insert having four intact and two truncated fragments of the introduced DNA. Appropriate analyses of the integration site including flanking sequences and bioinformatic analysis have been performed. Bioinformatic analysis of the insert and junction regions demonstrated the absence of any ORF potentially coding for known toxic or allergenic proteins. The expression of the genes introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations.

The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of maize GA21 does not raise safety concerns, and that sufficient evidence for the stability of the insert structure was provided.

Based on the results of compositional analysis of samples from a representative range of environments and seasons, the GMO Panel concludes that forage and kernels of maize GA21 are compositionally equivalent to those of conventional maize, except for the presence of the mEPSPS protein. In addition, field trials did not show changes in phenotypic characteristics and agronomic performance except for the introduced trait.

The mEPSPS protein did not induce adverse effects in a study on acute oral toxicity in mice. There were no adverse effects in a subchronic (90-day) feeding study with rats fed diets including kernels from maize GA21. A feeding study on broiler chickens provided evidence of nutritional equivalence of maize GA21 to conventional maize. In addition the overall allergenicity of the whole plant is not changed. The GMO Panel is of the opinion that maize GA21 is as safe as conventional maize. Maize GA21 and derived products are unlikely to have any adverse effect on human and animal health in the context of the intended uses.

The applications for maize GA21 concern food and feed uses, import and processing of maize GA21 and all derived products. There is therefore no requirement for scientific assessment of possible environmental effects associated with the cultivation of maize GA21. There are no indications of increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of GA21 seeds during transportation and processing. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize GA21.

In conclusion, the GMO Panel considers that the information available for maize GA21 addresses the scientific comments raised by the Member States and that maize GA21 is as safe as its non genetically modified counterparts with respect to potential effects on human and animal health or the environment. Therefore the GMO Panel concludes that maize GA21 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, GA21, glyphosate tolerance, EPSPS, MON-ØØØ21-9, human and animal health, environment, import, processing, food, feed, Regulation (EC) No 1829/2003, renewal, existing product.

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BACKGROUND

Application EFSA-GMO-UK-2005-19

On 8 August 2005 EFSA received from the Competent Authority of the United Kingdom an application (Reference EFSA-GMO-UK-2005-19), for authorisation of the glyphosate-tolerant genetically modified maize GA21 (Unique Identifier MON-ØØØ21-9), submitted by Syngenta Seeds S.A.S. on behalf of Syngenta Crop Protection AG within the framework of Regulation (EC) No 1829/2003 on genetically modified (GM) food and feed (EC, 2003) for food and feed uses, import and processing.

Maize GA21 has been previously evaluated by the Scientific Committee on Plants (SCP) and the Scientific Committee on Food (SCF). The SCP delivered its scientific opinion on the safety assessment of the genetically modified maize line GA21 with tolerance to the herbicide glyphosate submitted under Directive 90/220/EEC (SCP, 2000). Also, the SCF carried out an evaluation under Regulation 258/97/EC on novel foods and novel food ingredients and concluded that from the point of view of consumer health, grains from maize line GA21 and derived products were as safe as grains and derived products from conventional maize (SCF, 2002).

After receiving the application EFSA-GMO-UK-2005-19 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States as well as the European Commission and made the summary of the dossier publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 7 April 2006 EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC (EC, 2001), following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member State bodies had three months after the date of receipt of the valid application (until 7 July 2006) within which to make their scientific comments known.

The GMO Panel carried out a scientific assessment of maize GA21 taking account of the appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006).
On 19 May 2006, 6 July 2006, 7 December 2006 and 2 April 2007, the GMO Panel asked for additional information/clarifications on specific aspects of GA21 maize from the applicant. The applicant provided the requested information on 7 August 2006, 29 November 2006, 22 December 2006, 27 February 2007 and 27 April 2007. After receipt and assessment of the full data package, the GMO Panel finalized its risk assessment of maize GA21.

The GMO Panel carried out the scientific assessment of the genetically modified maize GA21 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into consideration the scientific comments of the Member States and the additional information provided by the applicant.

In giving its opinion on maize 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 receipt of the valid application. As additional information was requested by the GMO Panel, the time limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

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

Application EFSA-GMO-RX-GA21

On 29 June 2007, EFSA received from the European Commission an application for renewal of the authorisation of maize GA21 (EFSA-GMO-RX-GA21) (Unique Identifier MON-ØØØ21-9), submitted by Syngenta Seeds on behalf of Syngenta Crop Protection AG within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed (EC, 2003).

After receiving the application EFSA-GMO-RX-GA21 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), 5(5), 17(3), 17(5) as well as 8(2) and 20(2) of Regulation (EC) No 1829/2003. On 6 September 2007 EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

All data required for the risk assessment of the application EFSA-GMO-RX-GA21 have also been provided in application EFSA-GMO-UK-2005-19.

The GMO Panel performed one single comprehensive risk assessment for all intended uses of genetically modified maize GA21 and issued a single comprehensive scientific Opinion for both applications submitted under Regulation (EC) No 1829/2003.

TERMS OF REFERENCE

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

ASSESSMENT

The genetically modified (GM) maize GA21 (Unique Identifier MON-ØØØ21-9) was assessed with reference to its intended uses taking account of the appropriate principles described in the Guidance Document (EFSA, 2006). In its evaluation the GMO Panel also considered the comments that were raised by Member States on application EFSA-GMO-UK-2005-19. The risk assessment presented here is based on the information provided in the applications relating to maize GA21 submitted in the EU including additional information from the applicant.

1. Introduction

1.1. Description of the traits and mechanism of action

Maize GA21 expresses a modified version of the EPSPS protein (mEPSPS), derived from wild type maize EPSPS, and rendering maize GA21 tolerant to herbicides made of the active substance, glyphosate. The action of the 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 but this is sufficient to confer tolerance to glyphosate.

2. Molecular characterisation

2.1. Issues raised by the Member States

Comments were given regarding novel ORFs in the flanking regions and the stability of the insert.

2.2. Evaluation of relevant scientific data

2.2.1. Transformation process and vector constructs

Suspension culture cells of maize were transformed with a *Not*I restriction fragment of the plasmid pDPG434 using particle bombardment. This plasmid is derived from pUC19. The vector backbone contains the origin of replication (ColE1), the *lac* sequence from pUC19, and the bacterial *bla* gene conferring resistance to ampicillin in bacteria. Within this vector the region intended for insertion in the maize genome was cloned.

The region intended for insertion is the 3.49 kb *Not*I fragment consisting of the following *mepsps* 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 mutated maize *epsps* coding sequence (*mepsps*), 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 *mepsps* containing maize line GA21 is tolerant to glyphosate.

2.2.2. Transgenic constructs in the genetically modified plant

Southern analysis demonstrated a single insertion locus in maize GA21. At the request of the GMO Panel the applicant performed additional Southern analysis on genomic DNA using five restriction enzymes and two probes that cover the inserted *Not*I fragment. This was required to unravel the complex insert structure in maize GA21 and to allow comparisons with the obtained sequence data. This analysis demonstrated that the insert 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 by Southern analysis 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 *mepsps* gene and *nos* terminator. Fragments 2, 3 and 4 are complete versions of the 3.49 kb *Not*I fragment. Fragment 5 contains the complete rice actin promoter, the actin first exon and intron, the optimized transit peptide, and 288 bp of the *mepsps* 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. BLAST analysis of the 3' sequence 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 chloroplastidic origin. The integration of organellar DNA within the nuclear plant genome – being already present or acquired during the transformation – is established as a normal phenomenon in plant biology and the GMO Panel considered that this would not impact significantly on the present safety assessment.

2.2.3. Information on the expression of the insert

2.2.3.1. Expression of the introduced genes

Transcription of the *mepsps* gene was studied by Northern analysis in pooled leaf material from GA21 plants and isogenic non GM maize plants. By using an *epsps* specific probe a transcript of the expected size (1.8 kb) was detected in maize GA21. A hypothetical transcript of approximately 0.7 kb, which could have been the result of the truncated fragment, could not be detected, indicating that the truncated gene located on fragment 5 is not expressed.

Western analysis with six different polyclonal anti-EPSPS antibodies demonstrated an estimated 24-fold higher level of EPSPS protein in maize GA21 compared to the non GM control. Hybridisation with the antibodies in the control is due to the presence of the native EPSPS protein. No immuno-reactive EPSPS fragment in the range of 10 kDa or lower could be visualised in maize GA21. This indicates that the truncated fragment 5 does not result in the accumulation of protein.

Across all growth stages, mean mEPSPS concentrations were measured in leaves, roots and whole plants of maize GA21. The concentrations ranged from ca. <0.2 µg/g fw (fresh weight) to 15 µg/g fw (<0.3 to 70 µg/g dw [dry weight]). Mean mEPSPS concentrations measured in kernels at seed maturity and senescence ranged from ca. 4 to 7 µg/g fw (5 to 10 µg/g dw). Expressed in terms of biomass in the field the mEPSPS quantities in maize GA21 ranged from ca. 44 g mEPSPS/acre at whorl stage to ca 114 g mEPSPS/acre at seed maturity. The endogenous maize EPSPS protein is expressed at a significantly lower concentration than the transgenic mEPSPS protein in maize GA21.

2.2.3.2. Putative cryptic open reading frames (ORF) in maize GA21

Bioinformatic analysis was carried out to assess the potential for novel, putative ORFs created within the maize GA21 insert. A novel, putative ORF is defined as 1) beginning with an ATG and ending with one of the three stop codons, 2) encoding a minimum protein size of 50 amino acids, and 3) spanning consecutive fragment cassettes within maize GA21 or between an inserted fragment and plant DNA, or 4) beginning with a novel ATG created from a mutation upon transformation.

Using conservative search criteria it was concluded that 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.

In addition the applicant was asked to search for potential new ORFs created within the insert, between and within the fragments inserted. This revealed one novel, putative ORF created at the junction between fragment 5 and 6. On the basis of the analysis of the data it is concluded that this ORF lacks the necessary components to be transcribed and that the ORF does not show homology to known or putative allergens or toxic proteins.

2.2.4. Inheritance and stability of inserted DNA

The inheritance of the introduced glyphosate tolerant phenotype was studied in segregating backcross generations and follows a Mendelian segregation pattern.

The mEPSPS protein was analysed in leaf material over three backcross generations of the GM maize. Concentrations were not significantly different across these generations showing that 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.

These results indicated phenotypic and molecular stability of the insert present in maize GA21.

2.3. Conclusion

The molecular characterisation data establish that maize GA21 contains a single insert having four intact and two truncated fragments of the introduced cassette. The insert was analysed by Southern analysis and sequencing. No fragments from the vector backbone are present. In addition, bioinformatic analysis of putative novel ORFs spanning the two junction regions flanking the insert and analysis of putative novel ORFs within the insert spanning two adjacent fragments in maize GA21 were performed. These analyses showed that none of the six putative ORFs are likely to be expressed. Moreover none of these putative ORFs show significant sequence homology with known toxic proteins or allergens.

The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of maize GA21 does not raise any safety concerns, and that sufficient evidence for the stability of the insert structure was provided.

3. Comparative analysis

3.1. Issues raised by Member States

Comments were given concerning the experimental design of the comparative studies as well as the relevance of differences in agronomic properties and compositional parameters (in particular β-carotene) observed between maize GA21 and the non GM comparators.

3.2. Evaluation of relevant scientific data

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

Maize GA21 was compared with near-isogenic non GM controls. Whole crops and maize tissues, including kernels, were collected for compositional analysis from field trials. These field trials were performed during several seasons and at different locations (six locations during two seasons in the United States (2004 and 2005), five locations in the United States (1996), seven locations in the United States (1997) and four locations in Italy and Spain (1997)). In addition to the test and the near-isogenic non GM controls, five or six commercial non GM varieties were planted at each test site in 1997. As requested by the GMO Panel, the applicant justified the use of the respective non GM control as the most appropriate comparator in each

case. Maize GA21 plants treated with glyphosate as well as plants treated with conventional herbicides were included in the field trials.

3.2.2. Compositional analysis

From the field trials performed at six locations in the United States in 2004 and 2005, data on chemical composition were provided for material from each individual location and statistically analysed both for each location and all locations combined. The compounds analysed followed the recommendations of OECD (OECD, 2002). The data from field trials in 2004 and 2005 were used by the GMO Panel as the primary source for the comparative assessment of the composition of maize GA21. The GMO Panel is of the opinion, that this set of compositional data is in compliance with the principles described in the Guidance Document of the GMO Panel for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006).

The data from proximate and mineral analyses (fat, protein, total carbohydrate, acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, phosphorus, and calcium) of forage from maize GA21 (treated and non-treated with glyphosate) were compared to compositional data for forage from the non GM control and to typical ranges of the analysed constituents in commercial maize varieties reported in the literature (OECD 2002; ILSI, 2004). Statistically significant differences between maize GA21 and the non GM control were observed for some parameters, for example decreased overall levels of neutral detergent fibre and increased overall levels of phosphorous in forage of maize GA21 in the 2004 or 2005 seasons. There were no differences that were consistently observed over years and at each location.

The composition of kernels of maize GA21 and its control from harvests in 2004 and 2005 was analysed with respect to proximates (fat, protein, ash, moisture, total carbohydrates, starch), fatty acids (palmitic, stearic, oleic, linoleic and linolenic acid), amino acids (eighteen amino acids including aromatic amino acids), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, selenium and zinc), vitamins and provitamins (vitamin B1, vitamin B2, vitamin B_3, vitamin B_6, folic acid, vitamin E and β-carotene), anti-nutrients (phytic acid, raffinose and trypsin inhibitor) and other secondary metabolites (inositol, furfural, p-coumaric acid and ferulic acid).

The level of β-carotene was consistently statistically significantly higher in kernels of maize GA21 grown in 2004 and 2005 compared to the level in the corresponding non GM control. This difference was observed at each location, and it ranged from 12% to 25% across herbicide treatments and seasons. Furthermore, levels of cryptoxanthin, another carotenoid in the same metabolic pathway, were consistently higher in GA21 kernels compared to the non GM control in 2004 and in 2005. These differences ranged from 14% to 32% across herbicide treatments and seasons. Additional information provided by the applicant upon request from the GMO Panel demonstrated that there were no biologically relevant differences in β-carotene and cryptoxanthin levels between hybrids produced with maize GA21 and other GM maize, and the corresponding non GM control hybrids grown over one growing season at six locations in the USA. All β-carotene and cryptoxanthin levels observed in kernels of maize GA21 and non GM maize fell within the ranges reported for commercial maize varieties by the applicant and in scientific databases (ILSI, 2006). Therefore, the GMO Panel is of the opinion, that no further compositional analysis of carotenoids is required.

In addition, the compositional analysis of kernels from maize GA21 (glyphosate-treated and untreated) occasionally revealed statistically significant differences in the levels of some compounds compared to the non GM control. For example, kernels of treated maize GA21 contained lower overall levels of palmitic acid in the 2004 season. The overall levels of phosphorous were increased in kernels of untreated maize GA21

compared with control kernels in 2004. However, none of these differences were consistently observed over years and at each location. The levels of those compounds that differed from the levels in the corresponding non GM control were within the normal ranges reported in the literature for commercial maize varieties (OECD, 2002; Reynolds *et al*., 2005).

A scientific publication was provided summarising the results of the compositional analyses of forage and kernels from maize GA21 and the corresponding non GM comparator and commercial varieties obtained from the field trials performed in 1996 and 1997 (Sidhu *et al*., 2000). In these studies a more limited set of compounds (proximates, fiber, amino acids, fatty acids, minerals in grains; proximates, fiber, and minerals in forage) were analysed. Mean values and ranges calculated for the combined locations were given. Although some statistically significant compositional differences were detected across locations for some compounds, no consistent alterations were identified. Furthermore, all levels fell within the ranges observed for commercial varieties as reported in the application or within ranges reported in literature (OECD, 2002). The GMO Panel concludes that the results of the field studies performed in 1996 and 1997 do not indicate relevant compositional differences for forage and kernels derived from maize GA21 compared to the corresponding non GM comparator and the commercial varieties. This conclusion is in accordance with the previous opinion of the Scientific Committee on Food on maize GA21 assessing the field trials data from 1996 and 1997 (SCF, 2002). Furthermore, these conclusions are in line with the outcomes of the field studies performed in 2004 and 2005.

The GMO Panel considered the observed compositional differences between maize GA21 and its non GM comparators in the light of the field trial design, the biological variation and the levels of the compounds in conventional maize, and concludes that the composition of kernels and forage of maize GA21 falls within the normal ranges of conventional maize, except for the presence of the mEPSPS protein.

3.2.3. Agronomic traits and GM phenotype

During field trials over several seasons and at different locations (USA in 1999 and 2004, Brazil in 2003) extensive disease susceptibility and agronomic data (e.g. grain yield, number of emerged plants, plant population at harvest, ear height, plant height, percent snapped plants, stalk lodging, root lodging), as well as data on efficacy and selectivity of herbicide treatments were collected.

Statistically significant differences between maize GA21 and the corresponding non-GM comparator were observed for overall data on grain yield in the 2004 field studies. However, the differences were not consistently detected at each individual location, and all yield data fell within the range reported for non GM maize varieties as reported by the applicant. In addition, differences in the number of emerged plants, plant height, and percent snapped plants in 2004 were reported at some locations and were within the biological variation. No differences in the general appearance of the plants or any other phenotypic differences that could indicate unintended effects of the genetic modification were found.

The GMO Panel noted that in the course of the agronomic field trials conducted in Brazil in 2003, glyphosate treatment on GM plants resulted in phytotoxicity in up to 30 % of the plants at one out of the three sites. It is reported in the application that there was a high incidence of fungal disease in both maize GA21 and conventional maize in this tropical region of Brazil. Since phytotoxicity was also observed in up to 50 % of the non GM control plants treated with conventional herbicides, the GMO Panel accepts the explanation that the observed phytotoxicity resulted from the high incidence of fungi at this location.

The GMO Panel concludes that maize GA21 is equivalent to its non GM comparators with regard to phenotypic characteristics and agronomic performance except for the introduced trait.

3.3. Conclusion

Based on the results of compositional analysis of samples from a representative range of environments and growing seasons, it is concluded that that the composition of kernels and forage of maize GA21 falls within the normal ranges of conventional maize, except for the presence of the mEPSPS in maize GA21. In addition, field trials did not reveal changes in phenotypic characteristics and agronomic performance except for the introduced trait.

4. Food/feed safety assessment

4.1. Issues raised by Member States

Comments were given regarding the design of the toxicological and nutritional studies, the relevance of statistically significant differences found in the subchronic (90-day) rat feeding study, the mEPSPS protein used in the safety studies and the assessment of potential allergenicity.

4.2. Evaluation of relevant scientific data

4.2.1. Product description and intended use

The scope of application EFSA-GMO-UK-2005-19 is for food and feed uses, import and processing of maize GA21 and all derived products, excluding cultivation, and the scope of application EFSA-GMO-RX-GA21 covers the continued marketing of existing food additives, feed materials and feed additives produced from maize GA21.

Maize GA21 is intended to be processed like any conventional maize, and the applicant has provided information on the use of maize and derived products. The primary use of maize is for animal feed, but it is also processed into valuable food products, including e.g. starch, syrups and oils.

4.2.2. Effect of processing

The levels of the mEPSPS protein in wet and dry milled maize fractions as well as in oil and crisps derived from maize GA21 kernels were determined. The protein was not detectable by ELISA in the wet milled fractions, e.g. fibre, starch and germ meal (limit of detection 0.03 µg mEPSPS/g sample). However, it was quantifiable in all dry milled fractions analysed (e.g. approximately 10 µg/g flaking grits, 8 µg/g hulls and 5 µg/g flour). Partially refined oil derived from flaking grits and crisps produced from flour did not contain detectable levels of mEPSPS (limit of detection 0.02 µg mEPSPS/g sample).

The influence of temperature on the mEPSPS enzyme derived from a recombinant *Escherichia coli* strain (see Section 4.2.3.1) was studied *in vitro* by determining the specific activity after incubation of the enzyme at 25, 37, 65 and 95 °C for 30 minutes. After incubation at 25 and 37 °C there was no or only a slight influence on activity, whereas at 65 and 95 °C the enzyme was completely inactivated.

Based on the data of compositional analysis of raw agricultural commodities of maize GA21 and the non GM maize comparator (see Sections 3.2.2 and 3.3), the GMO Panel is of the opinion that there are no reasons to assume that the effects of processing of maize GA21 would be different from that of conventional maize.

4.2.3. Toxicology

4.2.3.1. Protein used for the safety assessment

Given the low expression level of the mEPSPS protein in maize GA21 and the very difficult task of isolating a sufficient quantity of purified protein from this maize for safety testing, an mEPSPS protein produced in a recombinant *Escherichia coli* strain was used. The microbial mEPSPS protein was compared with the EPSPS protein present in leaves of maize GA21. This maize contains both the newly expressed mEPSPS and the endogenous EPSPS enzyme. A comparison of the levels of mEPSPS in leaves of maize GA21 with the levels of the endogenous EPSPS in leaves of non GM maize (determined by ELISA) showed that the percentage of mEPSPS was approximately 96% of the total EPSPS protein in leaves of maize GA21.

The microbial and plant produced mEPSPS proteins had identical N-terminal amino acid sequences. SDS PAGE followed by Western analyses revealed a prominent band corresponding to the predicted molecular mass of 47.4 kDa for both proteins. MALDI-TOF mass spectrometry confirmed the predicted molecular mass of the microbial protein. In a study on protein glycosylation using a commercial glycan detection kit after SDS PAGE, the mEPSPS proteins from neither source were glycosylated. Using an EPSPS activity assay (determination of orthophosphate release from phosphoenolpyruvate) the proteins showed comparable enzymatic activities. The GMO Panel therefore accepts the *E. coli* derived mEPSPS protein as an appropriate substitute test material for the plant mEPSPS protein in the safety studies.

4.2.3.2. Toxicological assessment of expressed novel proteins in GA21 maize

The mEPSPS protein differs from the endogenous EPSPS in two of the total 445 amino acids constituting the protein (>99.3% identity). Threonine in position 102 of EPSPS has been replaced by isoleucine in mEPSPS, and proline in position 106 by serine, resulting in tolerance of the plants to glyphosate. Based on the DNA sequence information of the *epsps* and *mepsps* genes in maize GA21, the applicant expected the mEPSPS protein to have an additional methionine at the N-terminus. However, N-terminal sequencing of the mEPSPS protein in maize GA21 showed that this methionine is not present in the mEPSPS predominantly expressed in maize GA21.

EPSPS enzymes occur in conventional plants, fungi and microorganisms and are thus consumed as part of the normal diet by humans and animals. No adverse effects associated with the intake of these proteins have been identified (EFSA, 2003a, b). Other GM crops containing the EPSPS protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) have been previously evaluated and are regarded as being as safe as the respective conventional crops for human and/or animal consumption (ACNFP, 1994; SCP, 1998a, 1998b; EFSA, 2003a, 2003b, 2006).

Sequence homology

Bioinformatic analyses using the BLASTP search program and the National Center for Biotechnology Information (NCBI) Entrez Protein Database (NCBI, 2005) revealed no relevant homology between the mEPSPS protein and known toxic proteins.

In vitro digestibility

The stability of the mEPSPS protein isolated from leaves of maize GA21 as well as from a recombinant *E. coli* strain was tested *in vitro* in simulated mammalian gastric fluid (SGF). No intact protein (ca. 47.4 kDa) was detectable after incubation in SGF for 1 minute when the samples were analysed using SDS PAGE and protein staining. The GMO Panel notes that after incubation of the microbially produced mEPSPS in SGF for up to 60 minutes, diffusely stained regions (ca. 4-5 kDa) were visible. These regions were not present after analysis of mEPSPS samples incubated without pepsin.

Using Western analysis after SDS PAGE, no intact protein was detected after incubation in SGF for 1 minute. In the sample of plant-derived mEPSPS incubated for 1 minute, an immunoreactive fragment (ca. 6 kDa) was detected. This fragment was not detectable after incubation for 5 minutes or longer. The GMO Panel did not identify a safety concern regarding the potential presence of the fragment.

According to the opinion of the Scientific Committee on Food (SCF) in 2002, no fragments were detected in an earlier study using Western analysis after incubation of a protein preparation derived from maize GA21 in SGF for 15 seconds. Although no information was available on whether the protein was degraded to its constituent amino acids or to stable protein fragments, the SCF found no indication that for this type of protein stable fragments may be formed (SCF, 2002).

Acute oral toxicity

An acute oral toxicity study was performed in which a single dose of 2000 mg mEPSPS/kg bodyweight was administered to groups of 5 male and 5 female albino mice. In addition to the examinations normally carried out in this type of study (i.e. observation for clinical signs, determinations of body weight and food consumption during the observation period as well as gross pathology at necropsy), haematological and clinical chemistry parameters were analysed, the weights of selected organs were determined and histopathological examinations were conducted at the end of the observation period on day 15. This study did not reveal indications of adverse effects.

4.2.3.3. Toxicological assessment of constituents other than proteins

Since no new constituents other than the mEPSPS protein are expressed in maize GA21, and no biologically relevant alterations in the levels of endogenous compounds were detected in the comparative compositional analyses, no toxicological assessment of new constituents is required.

4.2.4. Toxicological assessment of the whole GM food/feed

The applicant has provided a subchronic (90-day) feeding study in rats using kernels of maize GA21 as a component of the diet. Groups of 12 male and 12 female Wistar-derived rats (Alpk:AP$_f$SD) were fed diets containing 10% or 41.5% (w/w) kernels from maize GA21 sprayed with glyphosate (treated), 10% or 41.5% kernels from maize GA21 sprayed with other herbicides (untreated) or 10% or 41.5% kernels from near isogenic non GM control maize treated with other selective herbicides.

No clinically relevant reactions were noted in the regular observations of the animals. In detailed examinations of the animals and quantitative assessments of body functions (including landing foot splay, grip strength and motor activity measurements), there were no biologically relevant differences between groups. Ophthalmoscopic examinations did not reveal relevant effects.

Food consumption was comparable in all groups and there were no relevant differences in food utilisation. Males receiving diets with 41.5% kernels from maize GA21 treated with glyphosate showed a reduced bodyweight compared with the controls in weeks 6, 10, 12, 13 and 14. These differences were not observed in males receiving diets with 41.5% kernels from untreated maize GA21. However, all values fell within the historical control ranges which were provided by the applicant on request of the GMO Panel. In the absence of indications of adverse effects, the GMO Panel does not consider the reduction in bodyweight as toxicologically relevant.

Several statistically significant differences in haematology and clinical chemistry parameters compared with the controls were noted: reduced mean cell volume in males of the low-dose groups (maize GA21 treated and untreated); reduced monocyte counts in males of the high-dose group (maize GA21 untreated); reduced neutrophil counts and plasma γ-glutamyl transferase in females of the low-dose group (maize GA21 untreated); reduced plasma phosphorous levels in males of the high-dose group (maize GA21 treated); reduced plasma creatinine in females of the low-dose groups (maize GA21 treated and untreated); reduced plasma glucose in females of the high-dose group (maize GA21 treated); reduced plasma chloride in females of the low-dose group (maize GA21 untreated). Single differences in organ weights were observed compared with the controls. In males, relative brain, heart and kidney weights were increased in the high-dose group (maize GA21 treated). Relative testes weights were increased in the low-dose group (maize GA21 treated). In females of the low-dose group (maize GA21 treated) the adrenal gland weights (relative and absolute) were reduced and brain weights (absolute and relative) and liver weights (relative) were increased. Liver weights (absolute) were increased in the low-dose group (maize GA21 untreated). These findings were generally not dose related, limited to one sex and/or no consistent pattern was identified when the herbicide-treatment of the plants was considered. Since, in addition, the findings were not accompanied by histopathological changes in the respective organs or tissues, the GMO Panel does not consider the observed statistical differences as toxicologically relevant.

The result of this study, which showed no indications of adverse effects, is in agreement with that of a previous subchronic feeding study in rats evaluated by the SCF (SCF, 2002).

4.2.5. 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 on 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 (EFSA, 2006; CAC, 2003).

4.2.5.1. Assessment of allergenicity of the newly expressed proteins

The wild-type *epsps* gene encoding the unmodified EPSPS protein was derived from maize, a source which is not regarded as commonly allergenic (see Section 4.2.5.2).

Bioinformatic analyses were conducted using an extended database (composed of entries identified as allergens or putative allergens in several databases). The overall similarity was examined by comparing sequential peptides of the mEPSPS protein to the allergen sequences using the FASTA search algorithm. No peptides having 35% identity in an 80-aa window to an allergen sequence were identified. In addition, when

the criterion of an identical 8-aa contiguous amino acid stretch was applied, the mEPSPS sequence yielded no positive outcomes. These analyses revealed no biologically relevant homology of the mEPSPS protein to known or putative allergenic proteins.

The potential allergenicity of the theoretical expression products of ORFs coding for putative fusion proteins in the regions flanking the inserts were considered in this dossier (see Section 2.2.3.2.). No resemblance with allergens was found. The studies on degradation of mEPSPS in simulated mammalian gastric fluid, which are also relevant for the assessment of potential allergenicity, have been described in Section 4.2.3.2. They showed that most of the protein was degraded by pepsin. The small amount of low molecular weight residual peptides that was detected in this experiment is unlikely to raise concerns regarding allergenicity.

Based on the information available the GMO Panel considers it unlikely that the mEPSPS protein in maize GA21 is an allergen.

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

Rare cases of occupational allergy to maize dust or maize pollen allergy, have been reported. Food allergy to maize is rare (Moneret-Vautrin *et al*., 1998), but IgE- binding proteins have been identified in maize flour (Pastorello *et al*., 2000; Pasini *et al*., 2002). Allergy to maize is detected in a minor fraction of the population of atopic patients. In addition, most individuals with a positive skin prick test (SPT) or having IgE antibodies against maize were suffering of respiratory allergy and only a few ones displayed a true food allergy upon oral challenge with maize products (Pasini *et al*., 2002; Jones *et al*., 1995). Therefore, oral sensitization to maize proteins is very rare.

The allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, for example through qualitative or quantitative modification of the pattern of expression of endogenous proteins. This issue does not appear to be a safety concern to the GMO Panel since maize is not considered a major allergenic food. A theoretically possible over-expression of any endogenous protein would be unlikely to alter the overall allergenicity of the whole maize GA21 plant.

4.2.6. Nutritional assessment of GM food/feed

A 49-day feeding study was carried out on broiler chickens (Ross 344 males crossed with Ross 308 females). Groups of 150 male and 150 female animals (in pens of 25 animals assigned in a randomised complete block design) were fed diets containing approximately 51-64% (w/w) of maize kernels depending on the growth status of the animals. The diets contained kernels from maize GA21 treated with glyphosate, from maize GA21 treated with conventional herbicides, from near-isogenic non GM control plants treated with conventional herbicides or from a commercial non GM maize.

There were no adverse effects in this study. Although the diets were not completely identical with regard to nutrient composition, animals fed diets containing kernels from maize GA21 showed no biologically relevant differences in mortality, body weight, feed conversion and carcass yield compared with animals receiving diets containing kernels from the non GM control plants and from commercial maize.

The broiler feeding study shows that kernels from maize GA21 are nutritionally equivalent to kernels from the non GM comparator and commercial maize.

4.2.7. Post-market monitoring of GM food/feed

The GMO Panel concluded that no information has emerged to indicate that maize GA21 is any less safe than its non GM counterpart and other conventional maize. Furthermore, this maize will be used as any other maize and no increased maize exposure is expected. Therefore, as laid down in the Guidance Document of the GMO Panel (EFSA, 2006), a post-market monitoring of the GM food/feed is not considered necessary.

4.3. Conclusion

The mEPSPS protein expressed in maize GA21 differs from the native maize protein in two amino acids (see Section 2.2.1). The amino acid sequence showed no homology to known toxic proteins and allergens. The mEPSPS protein was rapidly degraded in simulated mammalian gastric fluid and did not induce adverse effects in a study on acute oral toxicity in mice.

Based on the results of compositional analysis of samples from a representative range of environments and seasons, the GMO Panel concludes that forage and kernels of maize GA21 are compositionally equivalent to those of conventional maize, except for the presence of the mEPSPS protein.

There were no adverse effects in a subchronic (90-day) feeding study with rats fed diets including kernels from maize GA21. In addition, a feeding study with broiler chickens provided evidence of nutritional equivalence of maize GA21 to conventional maize.

The GMO Panel is of the opinion that maize GA21 is as safe as conventional maize and that the overall allergenicity of the whole plant is not changed. Maize GA21 and derived products are unlikely to have any adverse effect on human and animal health in the context of the intended uses.

5. Environmental risk assessment and monitoring plan

5.1. Issues raised by the Member States

Comments were given regarding potential effects on plant fitness and the need for clarifications on some agronomic characteristics as well as on potential effects of herbicides on biodiversity.

Further comments were raised regarding the need for more detailed post-market environmental monitoring measures as well as for specific management measures in case of accidental release of maize GA21 into the environment.

5.2. Evaluation of relevant scientific data

5.2.1. Environmental risk assessment

The scope of the applications on maize GA21 is for food (e.g. syrup, starch, oil) and feed (e.g. meal, oil) uses, import and processing of maize GA21 and all derived products. Therefore the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts

mainly of animals fed on the GM maize and with accidental release into the environment of GM seeds during transportation and processing.

The scope of both applications excludes cultivation; therefore concerns regarding the use of glyphosate treatments to maize GA21 apply only to imported and processed maize products that may have been treated with glyphosate in the countries of origin. However the regulation and risk assessment of glyphosate is within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market (EC, 1991).

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

Maize is highly domesticated and generally unable to survive in the environment without cultivation. Maize plants are not winter hardy in most regions of Europe, they have lost their ability to release seeds from the cob and they do not occur outside cultivated land or disturbed habitats in agricultural landscapes of Europe, despite cultivation for many years. In addition, there are no cross compatible wild relatives in Europe, and gene flow via pollen is largely restricted to neighbouring crops.

Tolerance to glyphosate provides an agronomic advantage in cultivation where and when glyphosate is applied. However survival of maize outside of cultivation in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, susceptibility to diseases and to cold climate conditions. Since these general characteristics of this GM maize are unchanged, herbicide tolerance is not likely to provide a selective advantage outside of cultivation in Europe. Therefore it is considered very unlikely that volunteers of this GM maize or its progeny will differ from conventional maize varieties in their ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

Field trials with maize GA21 have not shown any increased invasiveness, weediness or fitness characteristics, except in the presence of glyphosate. In addition to the information presented by the applicant, the GMO Panel is not aware of any scientific report of increased spread and establishment of the maize GA21 and any change in survival capacity, including over-wintering.

Since maize GA21 has no altered survival, multiplication or dissemination characteristics except in the presence of glyphosate, the GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this maize will not differ from that of conventional maize varieties.

5.2.1.2. Potential for gene transfer

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

(a) Plant to bacteria gene transfer

Based on present scientific knowledge and elaborated recently in more detail (EFSA, 2004; EFSA, 2007), gene transfer from GM plants to microorganisms under natural conditions is extremely unlikely, and its establishment would occur primarily through homologous recombination in microorganisms.

In the case of accidental release and establishment of maize GA21 in the environment, exposure of microorganisms to GM DNA derived from GM maize plants would take place during natural decay of GM plant material and/or pollen in the soil of areas where GM plants establish.

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

The modified *epsps* (*mepsps*) gene is under the control of an eukaryotic promoter (rice actin promoter) with little or no activity in prokaryotic organisms. Genes under control of prokaryotic regulatory elements conferring the same traits as expressed in the GM plants are widespread in microorganisms in natural environments. In addition, the *bla* gene coding for ampicillin resistance which was used as a selection marker during the construction of plasmid pDPG434 was not inserted in the maize GA21 genome. There is therefore no risk of transfer of the *bla* gene to microorganisms.

Taking into account the origin and nature of the m*epsps* gene, its natural occurring related genes and the lack of selective pressure in the intestinal tract and the environment, the likelihood that horizontal gene transfer of the m*epsps* gene would confer selective advantage or increased fitness to microorganisms is very limited. For this reason it is very unlikely that genes from maize GA21 would become transferred and established in the genome of microorganisms in the environment or human and animal digestive tract. In the very unlikely event that such horizontal gene transfer would take place, no adverse effects on human and animal health or the environment are expected, as no principally new traits would be introduced or expressed in microbial communities.

(b) Plant to plant gene transfer

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

The flowering of the sporadic 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.

Tolerance to glyphosate provides an agronomic advantage in cultivation where and when glyphosate is applied. However survival of maize outside of cultivation in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, susceptibility to diseases and to cold climate conditions. Since these general characteristics of this GM maize are unchanged, herbicide tolerance is not likely to provide a selective advantage outside of cultivation in Europe. Therefore, as for any other maize varieties, GM plants would only survive in subsequent seasons in the warmer regions of Europe and are not likely to establish feral populations under European environmental conditions (see Section 5.2.1.1).

In conclusion, since maize GA21 has no altered survival, multiplication or dissemination characteristics except in the presence of glyphosate, the GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this maize is considered to be extremely low.

5.2.1.3. Potential interactions of the GM plant with target organisms

This point was not considered an issue by the Member States or by the GMO Panel considering the intended uses of maize GA21, excluding cultivation, and consequently the low level of exposure to the environment.

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

This point was not considered an issue by the Member States or by the GMO Panel.

5.2.1.5. Potential interaction with the abiotic environment and biogeochemical cycles

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

5.2.2. Monitoring

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

Monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific quality of the monitoring plan provided by the applicant (EFSA, 2006). The potential exposure to the environment of maize GA21 would be through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM maize or through accidental release into the environment of GM seeds during transportation and processing.
No specific environmental impact of this GM maize was indicated by the environmental risk assessment and thus no case specific monitoring is required.

In the monitoring plan provided in the applications, the applicant describes i) the monitoring methodology focusing on existing networks made of i.e. grain traders and maize processors; ii) the types of information to be collected from the operators involved in the handling and use of viable maize GA21; (iii) the ongoing record keeping of the stakeholders network. The applicant will submit a general surveillance report on an annual basis.

The GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize GA21 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan. The GMO Panel advises that appropriate management systems should be in place to restrict seeds of maize GA21 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

5.3. Conclusion

The scope of the applications includes food and feed uses, import and processing of maize GA21 and all derived products and excludes cultivation. Considering the intended uses of maize GA21, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the maize GA21 and with accidental release into the environment of GA21 seeds during transportation and processing.

There are no indications of increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of maize GA21 seeds during transportation and processing.

The scope of the monitoring plan provided by the applicant is in line with the intended uses of maize GA21 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. Furthermore the GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel was requested to carry out a scientific risk assessment of the maize GA21 for food and feed uses, import and processing of maize GA21 and all derived products.

The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of maize GA21 does not raise safety concerns, and that sufficient evidence for the stability of the insert structure was provided.

Comparative analysis has shown that maize GA21 is compositionally and agronomically equivalent to conventional maize, except for the introduced transgenic trait. The risk assessment included an analysis of data from analytical studies, bioinformatics, and *in vitro* and *in vivo* studies. The GMO Panel concluded that the maize GA21 is as safe as its non-GM counterparts and that the overall allergenicity of the whole plant is not changed.

The application EFSA-GMO-UK-2005-19 concerns food and feed uses, import and processing of maize GA21 and all derived products. The application EFSA-GMO-RX-GA21 covers the continued marketing of existing food additives, feed materials and feed additives produced from maize GA21. There is therefore no requirement for scientific assessment of possible environmental effects associated with the cultivation of the GM maize. There are no indications of increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of GA21 seeds during transportation and processing. The scope of the monitoring plan provided by the applicant is in line with the intended uses of maize GA21.

In conclusion, the GMO Panel considers that information available for maize GA21 addresses the comments raised by the Member States and considers it unlikely that maize GA21 will have any adverse effect on human and animal health or on the environment in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of United Kingdom (FSA), dated 5 August 2005, concerning a request for placing on the market of maize GA21 in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 8 September 2005, from EFSA to the Competent Authority of the United Kingdom (ref. SR/KL/jq (2005) 1120).

3. Letter from EFSA to applicant, dated 6 March 2006, with request for clarifications under completeness check (ref SR/SM/jq (2006) 1407493).

4. Letter from applicant, dated 21 March 2006, providing EFSA with an updated version of the application EFSA-GMO-UK-2005-19 submitted by Syngenta Seeds S.A.S. on behalf of Syngenta Crop Protection AG under Regulation (EC) No 1829/2003:

   Part I – Technical dossier

   Part II – Summary

   Part III – Cartagena Protocol

   Part IV – Labelling and Unique Identifier

   Part V – Samples and Detection

   Part VI – Additional information for GMOs

5. Letter from EFSA to applicant, dated 7 April 2006, delivering the 'Statement of Validity' for application EFSA-GMO-UK-2005-19, maize GA21 submitted by Syngenta Seeds S.A.S. on behalf of Syngenta Crop Protection AG under Regulation (EC) No 1829/2003 (ref SR/SM/jq (2006) 1464611).

6. Letter from EFSA to applicant, dated 10 April 2006, with request for additional information from JRC-CRL (ref SR/KL/jq (2006) 1469338).

7. Letter from applicant to JRC, dated 12 April 2006, responding to request for additional information.

8. Letter from EFSA to applicant, dated 19 May 2006, with request for clarifications/additional information (ref. SR/KL/jq (2006) 1532464).

9. Letter from EFSA to applicant, dated 6 July 2006, with request for clarifications/additional information (ref. SR/SM/jq (2006) 1623129).

10. Letter from applicant to EFSA, dated 4 August 2006, providing additional information upon EFSA request.

11. Letter from JRC to applicant, dated 13 November 2006, reminding a previous request for clarifications (ref. JRC I06-BGMO/GVDE/SL/D(2006)(206) 27833).

12. Letter from applicant to EFSA, dated 27 November 2006, providing additional information upon EFSA request.

13. Letter from EFSA to applicant, dated 7 December 2006, with request for further information (ref. SR/SM/shv (2006) 1870382).

14. Letter from applicant to EFSA, dated 22 December 2006, providing additional information upon EFSA request.

15. Letter from EFSA to applicant, dated 22 January 2007, with request for further clarifications (ref. SR/SM/shv (2007) 1936329).

16. Letter from applicant to EFSA, dated 27 February 2007, providing additional information upon EFSA request.

17. Letter from EFSA to applicant, dated 2 April 2007, with request for further clarifications (ref. SR/SM/DC/shv (2007) 2069054).

18. Letter from applicant to EFSA, dated 27 April 2007, providing additional information upon EFSA request.

19. Letter from EFSA to applicant, dated 30 May 2007, about completeness of the data package for JRC-CRL (ref. SR/KL/shv (2007) 2165984).

20. Letter from EFSA to applicant, dated 6 June 2007, about additional data considered satisfactory (ref. SR/SM/shv (2007) 2178856).

21. Letter from the European Commission, dated 18 June 2007, concerning the request for renewal of the authorization for continued marketing of existing food additives and feed materials produced from maize GA21 in accordance with Regulation (EC) No 1829/2003, submitted by Syngenta Seeds S.A.S. on behalf of Syngenta Crop Protection AG (ref. SANCO/E1/SG/al/D(2007) 510444).

22. Letter from EFSA to applicant, dated 6 September 2007, delivering the 'Statement of Validity' for application EFSA-GMO-RX-GA21, maize GA21 submitted by Syngenta Seeds S.A.S. on behalf of Syngenta Crop Protection AG under Regulation (EC) No 1829/2003 (ref. SR/SM/shv (2007) 2361389).

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